Nos. 14-1418, 14-1453, 14-1505,
15-35, 15-105, 15-119 & 15-191

IN THE

Supreme Court of the United States

DAVID A. ZUBIK, ET AL.,
Petitioners,

v.

SYLVIA BURWELL, ET AL.,
Respondents.

[CAPTIONS CONTINUED ON INSIDE COVER]

On Writs of Certiorari to the
United States Courts of Appeals for the
Third, Fifth, Tenth, and D.C. Circuits

BRIEF OF THE OVARIAN CANCER RESEARCH FUND ALLIANCE, ITS PARTNER MEMBERS AND SCIENTIFIC ADVISORS AS *AMICI CURIAE* IN SUPPORT OF RESPONDENTS

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PRIESTS FOR LIFE, ET AL., *Petitioners,*

v.

DEPARTMENT OF HEALTH & HUMAN SERVICES, ET AL., *Respondents.*

ROMAN CATHOLIC ARCHBISHOP OF WASHINGTON, ET AL., *Petitioners,*

v.

SYLVIA BURWELL, ET AL., *Respondents.*

EAST TEXAS BAPTIST UNIVERSITY, ET AL., *Petitioners,*

v.

SYLVIA BURWELL, ET AL., *Respondents.*

LITTLE SISTERS OF THE POOR HOME FOR THE AGED, DENVER , COLORADO, ET AL., *Petitioners,*

v.

SYLVIA BURWELL, ET AL., *Respondents.*

SOUTHERN NAZARENE UNIVERSITY, ET AL., *Petitioners,*

v.

SYLVIA BURWELL, ET AL., *Respondents.*

GENEVA COLLEGE, *Petitioner,*

v.

SYLVIA BURWELL, ET AL., *Respondents.*

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STATEMENT OF INTEREST[1]

The Ovarian Cancer Research Fund Alliance, along with the partner members and scientific advisors listed on the last page of this brief (collectively, the "Alliance") respectfully submit this brief as *amici curiae*. The Alliance is a non-profit organization and the foremost advocate for women with ovarian cancer in the United States. To that end, the Alliance advocates for increased research funding for the

[1] No party or counsel for a party authored or paid for this brief in whole or in part, or made a monetary contribution to fund the brief's preparation or submission. No one other than *amici* or their members or counsel made a monetary contribution to the brief. All parties have consented to the filing of this brief in letters lodged with the Clerk.

development of an early detection test, improved healthcare practices, and life-saving treatment protocols. The Alliance also advocates for increased access to medicines and treatments that can help lower the risk of ovarian and other gynecologic cancers.

The Alliance, under its predecessor entity's title as the Ovarian Cancer National Alliance, has filed amicus briefs in other cases involving the Affordable Care Act's contraceptive coverage regulations in this Court and the courts of appeals. *See Burwell* v. *Hobby Lobby Stores, Inc.*, 134 S. Ct. 2751 (2014); *Eden Foods, Inc.* v. *Sebelius*, 733 F.3d 626 (6th Cir. 2013); *Gilardi* v. *Dep't of Health & Human Servs.*, 733 F.3d 1208 (D.C. Cir. 2013). It is participating here because the questions presented here are of tremendous importance to *amici*'s members. The Alliance believes that its expertise in the cancer-preventive benefits of oral and other contraceptives may aid this Court in addressing the far-reaching implications of the questions presented. Ovarian and endometrial cancers kill thousands of American women each year. And because there is currently no way to reliably detect ovarian cancer at an early stage, prevention remains the primary weapon against this devastating disease. An extraordinary amount of medical research shows that for many women at higher risk of developing ovarian cancer, oral contraceptive use can be the difference between developing this deadly cancer and not developing it.[2]

[2] For the convenience of the Court, the medical research discussed in this brief is included in the attached appendices.

The regulations and accommodation at issue take an important step toward increasing access to this critical preventive treatment in the battle against ovarian and other deadly gynecologic cancers. Petitioners' interpretation of the Religious Freedom Restoration Act (RFRA) would jeopardize that progress and needlessly prevent access to preventive care for thousands of women whose employers object to contraceptive use, even where the employer can opt out of the requirements to which they object simply by filling out a form stating their religious objections. That outcome is neither sound as a matter of health policy nor compelled by RFRA.

SUMMARY OF ARGUMENT

1. To succeed on their RFRA claims, Petitioners must prove that their exercise of religion is "substantially burden[ed]" by the regulatory accommodation at issue. For the reasons persuasively stated by each of the courts of appeals in these cases and in the Government's brief, Petitioners' substantial burden claim is unfounded. In addition, with respect to coverage for women who use contraceptives solely to secure the many preventive health benefits of these drugs that are unrelated to procreation, the government's accommodation imposes no burden at all on Petitioners' religious exercise.

2. Even if the accommodation regulations did impose a substantial burden on Petitioners' religious exercise, there is ample evidence that application of any such burden to Petitioners is in furtherance of a number of compelling governmental interests, including significant preventive health benefits of contraceptives that are entirely unrelated to procreation. Oral contraceptives and intrauterine devices (IUDs) are widely recognized preventive therapies

for reducing the risk of ovarian, endometrial, and other gynecologic cancers.

These cancers are particularly deadly. Ovarian cancer kills thousands of American women each year. More than one-half of the women diagnosed with the disease will die within five years. With no effective way to detect ovarian cancer at an early stage, prevention remains the most effective tool to combat the disease. Endometrial cancer—which forms in the tissue lining of the uterus—likewise kills thousands of American women each year. For these women, contraceptives are a potentially life-saving cancer-preventive treatment.

The medical practice of prescribing contraceptives to reduce a woman's risk of developing these cancers played a key role in the government's decision-making when it implemented the women's preventive-screening provision of the Affordable Care Act (ACA). The contraceptive coverage requirement, and the accommodation developed for employers that object on religious grounds, are thus based, in part, on the government's compelling interest in ensuring that women can reduce their risk of developing ovarian and other forms of cancer through having cost-free access to oral contraceptives. Assuring that all women have affordable access to such treatment—whether through employer-sponsored insurance or via the accommodation regulations—is critical to meeting this compelling interest. Petitioners' RFRA theory wrongly jeopardizes access to potentially life-saving preventive health benefits and should be rejected.

ARGUMENT

I. PETITIONERS' RELIGIOUS BELIEFS ARE NOT SUBSTANTIALLY BURDENED BY THE ACCOMMODATION REGULATIONS.

To succeed with a RFRA claim, Petitioners must first demonstrate that their religious exercise is "substantially burden[ed]" by the challenged regulations. 42 U.S.C. § 2000bb-1(a). The opinions of the courts of appeals in these cases, as well as the Government's brief, convincingly refute Petitioners' argument that the accommodation allowing them to opt out of the contraceptive coverage requirement substantially burdens their religious exercise. *See, e.g.*, *Geneva Coll.* v. *Sec'y U.S. Dep't of Health and Human Servs.*, 778 F.3d 422, 442 (3d Cir. 2015); *Little Sisters of the Poor Home for the Aged* v. *Burwell*, 794 F.3d 1151, 1180 (10th Cir. 2015); *E. Tex. Baptist Univ.* v. *Burwell*, 793 F.3d 449, 459 (5th Cir. 2015); *Priests for Life* v. *U.S. Dep't of Health & Human Servs.*, 772 F.3d 229, 237 (D.C. Cir. 2014); Br. for Resp'ts at 52-53.

The cancer prevention benefits of contraceptives, which this brief presents in detail,[3] offer yet another reason why the challenged regulation does not impose a substantial burden on Petitioners' religious exercise. Contraceptives provide significant medical benefits that do not fall within the scope of Petitioners' religious objections to their use, including prevention of many serious and deadly cancers. Many women, such as women who are not fertile or who are beyond child-bearing age, use contraceptives solely to obtain these substantial preventive benefits.

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[3] *See infra* Section II.

Although the most common reason women use oral contraceptives is to prevent pregnancy, 14 percent of users—1.5 million women—rely on them exclusively for non-contraceptive purposes.[4] A 2011 study analyzed, among other things, data from women who have never had sexual intercourse, totaling approximately 762,000 women, and confirmed that almost all of them reported using oral contraceptives for non-contraceptive reasons.[5]

When contraceptives are prescribed and taken for non-contraceptive purposes, the challenged regulations do not conflict with Petitioners' stated religious belief, which is based solely on the contraceptive effect of these products. For this reason, as well as those explained in the opinions below and in the Government's brief, the challenged accommodation imposes no substantial burden on Petitioners.

II. THE ACCOMMODATION REGULATIONS FURTHER A COMPELLING PUBLIC HEALTH INTEREST BY ENSURING ACCESS TO CANCER PREVENTIVE TREATMENTS.

Petitioners' RFRA claims also fail because of the abundant evidence that any "burden" to Petitioners furthers numerous compelling governmental interests, including significant preventive health benefits of contraceptives that are unrelated to procreation. It is well established based on decades of clinical research that oral contraceptives and IUDs can

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[4] Rachel K. Jones, Guttmacher Institute, *Beyond Birth Control: The Overlooked Benefits of Oral Contraceptive Pills* 3 (2011), http://www.guttmacher.org/pubs/Beyond-Birth-Control.pdf (last visited Feb. 17, 2016).

[5] *Id.* at 4.

reduce a woman's risk of developing ovarian and endometrial cancer and other forms of gynecologic malignancies by as much as 50 percent. The significant preventive association of the use of oral contraceptives with a lower risk of ovarian and endometrial cancer is a great discovery for women's health and public health. Contraceptives are potentially lifesaving preventative treatments that are particularly vital for women with a higher risk of developing the disease because of a family history of ovarian cancer or because they inherited the *BRCA1* or *BRCA2* gene mutations. Women need access to what currently is the only weapon available to fight ovarian and endometrial cancer that also allows them to preserve their ability to conceive. To be sure, contraceptives enable women to avoid unintended pregnancies and to safeguard their health when a pregnancy is hazardous or life threatening. But the scope of contraceptives' health services and benefits reaches well beyond the prevention of unwanted pregnancy.

The Health Resources and Services Administration (HRSA), an agency of the U.S. Department of Health and Human Services (HHS), specifically considered benefits to women's health other than their contraceptive uses when the agency issued the final regulations relating to coverage of women's preventive health services under the ACA. *See* Coverage of Certain Preventive Services Under the Affordable Care Act, 78 Fed. Reg. 39,870, 39,872 (July 2, 2013) ("[T]here are demonstrated preventive health benefits from contraceptives relating to conditions other than pregnancy (for example, prevention of certain cancers)").

In determining the scope of recommended coverage for preventive services for women as envisioned by

the ACA, HRSA relied in part on a report by the Institute of Medicine (IOM) analyzing the effectiveness of various preventive services for women.[6] The purpose of the IOM report was to "identify preventive services necessary for women's health and well-being and to identify specific services that could supplement the current list of recommended preventive services for women."[7] The IOM report recognized that contraceptive methods frequently have benefits that are separate from their use in pregnancy-prevention and, in particular, that "[l]ong-term use of oral contraceptives has been shown to reduce a woman's risk of endometrial cancer."[8] HRSA adopted the IOM's recommendation that preventive services for women include "[a]ll Food and Drug Administration approved contraceptive methods, sterilization procedures, and patient education and counseling for all women with reproductive capacity."[9]

The IOM recommendations make clear that the government has a compelling interest in ensuring that women have access to contraceptives as an essential preventive treatment to protect their health and well-being. A wealth of scientific evidence collected over decades consistently confirms the significant preventive association of oral contraceptives and IUDs with a lower risk of users develop-

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[6] *See* IOM, *Clinical Preventive Services for Women: Closing the Gaps* 1 (2011), https://iom.nationalacademies.org/Reports/2011/Clinical-Preventive-Services-for-Women-Closing-the-Gaps.aspx (last visited Feb. 17, 2016).

[7] *Id.* at 3.

[8] *Id.* at 107.

[9] HRSA, HHS, *Women's Preventive Services Guidelines*, http://www.hrsa.gov/womensguidelines/ (last visited Feb. 17, 2016).

ing certain deadly gynecologic cancers. The ACA contraceptive coverage regulations, including the religious objection accommodation, promotes women's health by ensuring that all women, regardless of employer, can access preventive treatments that significantly reduce the risk of some of the most prevalent and deadly cancers. Women are entitled to this access as a matter of federal law; the accommodation in the regulations allows Petitioners to avoid any obligation to provide it themselves.

A. Contraceptives provide significant medical benefits separate from prevention of pregnancies.

1. There is conclusive evidence that oral contraceptives provide access to potentially life-saving health benefits for women by reducing the risk of ovarian cancer. *Hobby Lobby*, 134 S. Ct. at 2799 (Ginsburg, J., dissenting) ("[T]he Government has shown that the contraceptive coverage for which the ACA provides furthers compelling interests in public health and women's well being . . . [and] secures benefits wholly unrelated to pregnancy, [such as] preventing certain cancers, menstrual disorders, and pelvic pain."). Ovarian cancer is the deadliest gynecologic cancer and the fifth leading cause of cancer deaths for women.[10] In 2015, the American Cancer Society (ACS) estimated that approximately 14,180 American women died from ovarian cancer—more than died from any other gynecologic cancer.[11] ACS

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[10] NCI, *A Snapshot of Ovarian Cancer*, http://www.cancer.gov/research/progress/snapshots/ovarian (last visited Feb. 17, 2016).

[11] American Cancer Soc'y, *Cancer Facts & Figures 2015*, at 4, http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf (last visited Feb. 17, 2016).

also estimates that approximately 21,290 women in the United States were diagnosed with the disease for the first time in 2015.[12] The high mortality rate reflects the lack of reliable early detection mechanisms and effective screening tests to detect ovarian cancer at an early stage. Most women receive the diagnosis at an advanced stage when it is often too late for any treatment to have a high probability of success.[13] Consequently, prevention is currently the most effective weapon to combat this deadly disease.

The landscape of preventive treatments is very limited. Treatment options include the removal of fallopian tubes and ovaries (known as prophylactic or salpingo-oophorectomy) and the closure of the fallopian tubes (tubal ligation), both of which require invasive surgeries and come with drastic consequences that irreversibly prevent a woman from ever conceiving a child.[14] A significantly less invasive method is the use of oral contraceptives. It is well established, through multiple clinical investigations over decades, that the use of oral contraceptives lowers risk of developing ovarian cancer. Indeed, as early as 1999, the scientific consensus was that "[t]he protection offered by oral contraceptives against ovarian cancer risk is one of the most consistent

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[12] *Id.* at 4.

[13] NCI, *A Snapshot of Ovarian Cancer*, http://www.cancer.gov/research/progress/snapshots/ovarian (last visited Feb. 17, 2016).

[14] NCI, *Ovarian, Fallopian Tube, and Primary Peritoneal Cancer Prevention (PDQ®),* http://www.cancer.gov/types/ovarian/patient/ovarian-prevention-pdq#section/all (last visited Feb. 17, 2016).

epidemiological findings."[15] The significant protective association between oral contraceptive use and the risk of ovarian cancer has been identified in retrospective "case-control" studies (which compare women diagnosed with ovarian cancer to women who did not develop the disease) and prospective "cohort" studies (which follow a sample group of women over time and later evaluate whether they develop ovarian cancer).[16]

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[15] C. La Vecchia & S. Franceschi, *Oral Contraceptives and Ovarian Cancer*, 8 Eur. J. Cancer Prevention 297, 297 (1999).

[16] *See, e.g.*, Aminah Jatoi et. al., *Prior Oral Contraceptive Use in Ovarian Cancer Patients: Assessing Associations with Overall and Progression-Free Survival*, 15 BMC Cancer 711 (2015); M. T. Faber et al., *Oral Contraceptive Use and Impact of Cumulative Intake of Estrogen and Progestin on Risk of Ovarian Cancer*, 24 Cancer Causes Control 2197 (2013); Laura J. Havrilesky et al., *Oral Contraceptive Pills as Primary Prevention for Ovarian Cancer*, 122 Obstetrics & Gynecology 139 (2013); V. Beral et al., *Ovarian Cancer and Oral Contraceptives: Collaborative Reanalysis of Data from 45 Epidemiological Studies Including 23,257 Women with Ovarian Cancer and 87,303 Controls*, 371 Lancet 303, 307-312 (2008); Julia B. Greer et al., *Androgenic Progestins in Oral Contraceptives and the Risk of Epithelial Ovarian Cancer*, 105 Obstetrics & Gynecology 731, 735 (2005); Roberta B. Ness et al., *Risk of Ovarian Cancer in Relation to Estrogen and Progestin Dose and Use Characteristics of Oral Contraceptives*, 152 Am. J. Epidemiology 233, 239 (2000); Harvey A. Risch et al., *Parity, Contraception, Infertility, and the Risk of Epithelial Ovarian Cancer*, 140 Am. J. Epidemiology 585, 589 (1994); Susan E. Hankinson et al., *A Quantitative Assessment of Oral Contraceptive Use and Risk of Ovarian Cancer*, 80 Am. J. Obstetrics & Gynecology 708, 712-714 (1992); Alice S. Whittemore et al., *Characteristics Relating to Ovarian Cancer Risk: Collaborative Analysis of 12 US Case-Control Studies – II. Invasive Epithelial Ovarian Cancers in White Women*, 136 Am. J. Epidemiology 1184, 1192 (1992); The Cancer and Steroid Hormone Study of the Ctrs. for Disease

A greater than 20 percent relative risk reduction occurs for every five years a woman takes oral contraceptives.[17] A 2008 study analyzed the public health effects of oral contraceptive use and concluded that oral contraceptives prevented approximately 200,000 cases of ovarian cancer worldwide since the drugs were first approved in 1960 and saved approximately 100,000 women who otherwise would have died from the disease.[18] That number is "likely to increase substantially in the future, with the further ageing of past users of oral contraceptives and the increasing numbers of new users."[19]

The need for preventive therapies such as contraceptives is even stronger for women who are at a higher risk of developing ovarian cancer and women with endometriosis. In particular, women with a family history of ovarian cancer, who have inherited mutations in the *BRCA1* and *BRCA2* genes, or who have hereditary nonpolyposis colorectal cancer (Lynch Syndrome), face an elevated risk of hereditary ovarian cancer.[20] The *BRCA1* and *BRCA2* genes collectively account for approximately 15 percent of

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Control and the Nat'l Inst. of Child Health and Human Dev., *The Reduction in Risk of Ovarian Cancer Associated with Oral-Contraceptive Use*, 316 New Eng. J. Med. 650, 654 (1987). *But see* Xiao Ou Shu et al., *Population-Based Case-Control Study of Ovarian Cancer in Shanghai*, 49 Cancer Res. 3670, 3673 (1989) (finding a slight increase in ovarian cancer risk associated with oral contraceptive use, although increase was not significant).

[17] Beral et al., *supra*, at 303-314.

[18] *Id.* at 307, 312.

[19] *Id.* at 312.

[20] NCI, *The Genetics of Cancer*, http://www.cancer.gov/about-cancer/causes-prevention/genetics (last visited Feb. 17, 2016).

ovarian cancers.[21] Roughly 1.3 percent of women in the general population will develop ovarian cancer during their lives.[22] However, according to recent estimates, approximately 39 to 46 percent of women who inherit a *BRCA1* mutation and 10 to 27 percent of women who inherit a *BRCA2* mutation will develop ovarian cancer during their lifetimes. There is a 24 percent lifetime risk of developing ovarian cancer for women with Lynch Syndrome.[23]

The benefits of contraceptives also appear to extend to women who have been diagnosed with ovarian cancer. A 2015 study evaluated the connection between oral contraceptive use and patients who ultimately develop the disease. The study concluded that oral contraceptive use prior to a diagnosis of ovarian cancer is associated with improved progression-free survival in patients, or the length of time patients lived with the disease without it worsening and improved survival.[24] Another 2015 study determined that ovarian cancer mortality rates decreased

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[21] A. Antoniou et al., *Average Risks of Breast and Ovarian Cancer Associated with BRCA 1 or BRCA 2 Mutations Detected in Case Series Unselected for Family History: A Combined Analysis of 22 Studies*, 72 Am. J. Hum. Genetics 1117, 1117-1130 (2003) (discussing strong evidence supporting association between BRCA 1 and BRCA 2 mutations and an increased risk for ovarian cancer); S. Iodice et al., *Oral Contraceptive Use and Breast or Ovarian Cancer Risk in BRCA1/2 Carriers: A Meta-Analysis*, 46 Eur. J. Cancer 2275, 2276 (2010) (same).

[22] NCI, *Surveillance, Epidemiology, and End Results Program, Stat Fact Sheets: Endometrial Cancer*, http://seer.cancer.gov/statfacts/html/corp.html. (last visited Feb. 17, 2016).

[23] J. Brian Szender and Shashikant B. Lele, *Fallopian Tube Ligation or Salpingectomy as Means for Reducing Risk of Ovarian Cancer*, 17 Am. Med. Ass'n J. Ethics 843, 844 (2015).

[24] Jatoi et al., *supra*, at 5.

by 23 percent from 1973 to 2011, due in part to the availability of oral contraceptives.[25] However, the investigators estimated that from 2010 to 2030, the annual number of cases of ovarian cancer in the United States will increase by 37 percent due to the aging baby-boomer generation and population growth.[26]

Oral contraceptives are the only viable preventive treatment to reduce the risk of ovarian cancer while preserving a woman's ability to conceive. But the number of annual diagnoses and mortality rates will only increase if employers can cut off access for women who need these preventive treatments.

2. Women who use oral contraceptives during their reproductive years also benefit from long-term protection against endometrial cancer. Endometrial cancer is the most common invasive gynecologic cancer among women in the United States. In 2015, the National Cancer Institute (NCI) estimated that there were approximately 54,870 new cases of endometrial cancer, and approximately 10,170 women diagnosed with endometrial cancer died during the year.[27] Cancer of the endometrium, which is the lining of the uterus, typically strikes women around age 60, after the end of their reproductive years; for that reason, the preventive benefits of contraceptive use are especially pronounced for these women. If a woman is diagnosed after the cancer has spread or

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[25] Victoria Sopik et al., *Why Have Ovarian Cancer Mortality Rates Declined? Part I. Incidence*, 138 Gynecologic Oncology 741, 746 (2015).

[26] *Id.* at 748.

[27] NCI, *supra* note 22.

metastasized, NCI estimates that her five-year survival rate is between 17 and 68 percent.[28]

According to a 2015 study, the protective effect of oral contraceptives on endometrial cancer lasts for over thirty years.[29] This large study examined more than 140,000 women from around the world, and the results confirmed that every five years of oral contraceptive use was associated with a 24 percent reduction in the risk of endometrial cancer.[30] The risk reduction of oral contraceptive use was even stronger, with a 31 percent lower lifetime risk of developing endometrial carcinoma as compared to 17 percent lower risk for less common sarcomas.[31] The study concluded that over the past fifty years, up to 400,000 cases (out of 3.4 million total cases) of endometrial cancers have been prevented by women's use of oral contraceptives, including approximately 200,000 cases in the past decade.[32] Women with hereditary nonpolyposis colorectal cancer syndrome have a markedly increased risk of endometrial cancer compared with women in the general population. Women with a family history of endometrial cancer in a first-degree relative are also at increased

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[28] *Id.*

[29] Collaborative Group on Epidemiological Studies on Endometrial Cancer, *Endometrial Cancer and Oral Contraceptives: An Individual Participant Meta-analysis of 27,276 Women with Endometrial Cancer from 36 Epidemiological Studies*, 16 Lancet Oncology 1061, 1061 (2015).

[30] *Id.* at 1065.

[31] *Id.* at 1067.

[32] *Id.* at 1068.

risk.[33] Access to contraceptives as a preventive treatment is critical for these women.

3. Studies also confirm that IUDs decrease the risk for endometrial cancer and that IUD use (at least short term) also decreases the risk of ovarian cancer.[34] These studies have demonstrated that women who have used an IUD at some point experienced a significant protective effect, reducing their risk of developing endometrial cancer by one-third to one-half compared to women who never used an IUD

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[33] NCI, *Endometrial Cancer Prevention – For Health Professionals (PDQ®),* http://www.cancer.gov/types/uterine/hp/endometrial-prevention-pdq (last visited Feb. 17, 2016).

[34] Robin M. Beining et al., *Meta-Analysis of Intrauterine Device Use and Risk of Endometrial Cancer*, 18 Annals Epidemiology 492, 492-499 (2008) (discussing decreased risk of endometrial cancer); D. Hubacher, *Noncontraceptive Health Benefits of Intrauterine Devices: A Systematic Review*, 57 Obstetrics & Gynecology Surv. 120, 120-128 (2002) (same); Abraham Benshushan et al., *IUD Use and the Risk of Endometrial Cancer*, 105 Eur. J. Obstetrics & Gynecology & Reprod. Biology 166, 167 (2002) (same); Deirdre A. Hill et al., *Endometrial Cancer in Relation to Intra-Uterine Device Use*, 70 Int'l J. Cancer 278, 279 (1997) (same); Susan Sturgeon et al., *Intrauterine Device Use and Endometrial Cancer Risk*, 26 Int'l J. Epidemiology 496, 498 (1997) (same); F. Parazzini et al., *Intrauterine Device Use and Risk of Endometrial Cancer*, 70 Brit. J. Cancer 672, 673 (1994) (same); Xavier Castellsagué et al., *Intrauterine Contraception and the Risk of Endometrial Cancer*, 54 Int'l J. Cancer 911, 915 (1993) (same); Roberta B. Ness et al., *Contraception Methods, Beyond Oral Contraceptives and Tubal Ligation, and Risk of Ovarian Cancer*, 21 Annals Epidemiology 188, 188-196 (2011) (discussing decreased risk of ovarian cancer); Daniel W. Cramer et al., *Conditions Associated With Antibodies Against the Tumor-Associated Antigen MUC1 and Their Relationship to Risk for Ovarian Cancer*, 14 Cancer Epidemiology, Biomarkers & Prevention 1125, 1125-1131 (2005) (same).

(after controlling for factors such as age, childbearing, and family history).[35] In addition, IUDs may significantly reduce the risk of cervical cancer. According to a 2011 study analyzing multiple international studies, women who used an IUD for at least one year reduced their risk of developing cervical cancer by 50 percent, compared to women who had never used an IUD.[36]

All of these data make clear that FDA-approved contraceptives are an important preventive tool to protect women's health. The coverage regulations ensure that women will have the opportunity to access these potentially life-saving preventive therapies, regardless of their employers' religious views.

B. The cancer-prevention benefits of contraceptives played a key role in the government's decision to include the contraceptive coverage provision in the ACA.

The reduced risk of ovarian cancer and other deadly cancers is a significant preventive health benefit of long-term contraceptive use. These preventive benefits, which are unrelated to the prevention of unintended pregnancies, were central to the government's interest in requiring coverage of contraception under the ACA.

Section 2713 of the Public Health Service Act, the statute on which the contraceptive coverage re-

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[35] Benshushan et al., *supra*, at 167; Castellsagué et al., *supra*, at 912.

[36] Xavier Castellsagué et al., *Intrauterine Device Use, Cervical Infection with Human Papillomavirus, and Risk of Cervical Cancer: A Pooled Analysis of 26 Epidemiological Studies,* 12 Lancet Oncology 1023, 1029 (2011).

quirement is based, does not itself require coverage of contraceptives. Rather, the statute requires group health plans and health insurance issuers offering group or individual health insurance coverage to cover and impose no cost-sharing on four specified categories of preventive medical treatment. The last of these categories is, "with respect to women, such additional preventive care and screenings not [otherwise recommended by the U.S. Preventive Services Task Force] as provided for in comprehensive guidelines supported by the Health Resources and Services Administration for purposes of this paragraph." 42 U.S.C. § 300gg-13(a)(4). Section 2713 makes clear that Congress's chief intent was to ensure that women have access to any medical treatment that effectively prevents illness and disease—which long-term use of contraceptives does by reducing women's risk of ovarian cancer and other deadly cancers.

This focus on promoting access to significant preventive benefits was carried through each step of Section 2713's implementation. When HHS issued rules to implement the statute, the agency explained that the law was needed because health plans lack the incentive to cover preventive services and individual patients lack the immediate incentive or ability to obtain them, resulting in avoidable illness and costly treatment down the road. Interim Final Rules Relating to Coverage of Preventive Services Under the Affordable Care Act, 75 Fed. Reg. 41,726, 41,731 (July 19, 2010). Likewise, when HHS engaged IOM to analyze what preventive services should be included in the guidelines envisioned by Section 2713, the agency's charge to IOM focused on identifying preventive services and screenings that "have been shown to be effective for women" and "are

needed to fill gaps in recommended preventive services for women."[37]

Under any of these standards, the cancer prevention benefits of contraceptives merit their inclusion among the preventive treatments for which coverage is required under Section 2713. Indeed, in identifying FDA-approved contraceptives as a preventive treatment that should be included in the HHS guidelines (and ultimately in the set of services for which coverage is required under Section 2713), IOM's report expressly cited preventive health benefits of contraception that are unrelated to pregnancy, including reduced risk of endometrial cancer and other serious diseases.[38] Long-term use of contraceptives offer women an opportunity to significantly reduce the risk that they will die of ovarian or endometrial cancer or suffer from other gynecologic diseases, and the government rightly recognized those benefits by including contraceptives in the preventive-services coverage regulations.

C. Ensuring access to cancer-prevention health benefits is a compelling government interest.

The government has "overlapping and mutually reinforcing compelling interests" in safeguarding public health, ensuring that women have equal access to health care, and assuring access to seamless, affordable contraceptive coverage. *Priests for Life*, 772 F.3d at 257, 263-264. These interests are furthered by the ACA contraceptive regulations and the accommodation process. *See* Coverage of Certain

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[37] *See* IOM, *supra*, at 2.

[38] *See id.* at 107.

Preventive Services Under the Affordable Care Act, 78 Fed. Reg. at 39,872, 39,887.

1. There is no dispute that the promotion of public health is a compelling government interest. *See, e.g.*, *Hobby Lobby*, 134 S. Ct at 2780; *Priests for Life*, 772 F.3d at 259-262; *Real Alts., Inc.* v. *Burwell*, __ F.3d __, No. 1:15-cv-0105, 2015 WL 8481987, at *27 (M.D. Pa. Dec. 10, 2015) ("comprehensive access to health care serves a compelling government interest" in promoting public health). The government also has a compelling interest in eliminating the gender discrimination that results from the fact that healthcare costs fall disproportionately on women. *Priests for Life*, 772 F.3d at 262-264 (recognizing government's compelling interest in gender equality); *Real Alts., Inc.*, __ F.3d __, 2015 WL 8481987, at *26 (certain "health obstacles" are "unique to women," and "eliminating the past practice of discrimination against women in the provision of health care coverage is sufficiently compelling to justify" the contraceptive coverage requirement).

2. The government furthers these compelling interests by requiring coverage for contraceptives as preventive health services necessary to protect the health and well-being of female employees. *Priests for Life*, 772 F.3d at 258 ("The government's asserted compelling interest here, writ large, is in a sustainable system of taxes and subsidies under the ACA to advance public health."). Five members of the Court determined as much in *Hobby Lobby*. 134 S. Ct. at 2785-2786 (Kennedy, J., concurring) (the contraceptive coverage requirement "serves the Government's compelling interest in providing insurance coverage that is necessary to protect the health of female employees"); *id.* at 2799 (Ginsburg, J., dissenting)

("[T]he Government has shown that the contraceptive coverage for which the ACA provides furthers compelling interests in public health and women's well being . . . [and] secures benefits wholly unrelated to pregnancy, [such as] preventing certain cancers, menstrual disorders, and pelvic pain."). The remaining members all presumed that "the interest in guaranteeing cost-free access to the four challenged contraceptive methods is compelling within the meaning of RFRA." *Id.* at 2780.

Petitioners argue that the government has no compelling interest in providing access to contraceptives because the ACA only requires access to "preventive care." Br. for Pet'rs in Nos. 14-1418, 14-1453 & 14-1505 at 62 (Jan. 4, 2016). But *Hobby Lobby* "shows that the government has a strong argument" that the ACA's contraceptive coverage regulations and associated accommodation further compelling government interests. *Univ. of Notre Dame* v. *Burwell*, 786 F.3d 606, 624 (7th Cir. 2015). And although some Petitioners object to only certain methods of contraception, a decision by the Court in Petitioners' favor would allow them to claim entitlement to exemption from the ACA's contraceptive coverage regulations and associated accommodation as to all FDA-approved contraceptives. That some Petitioners object to only certain contraceptive methods "does not lessen [the government's] compelling interests." *Hobby Lobby*, 134 S. Ct. at 2799-2800 (Ginsburg, J., dissenting).

3. Fundamental to *amici*'s interests, the cancer-preventive-health effects of contraceptives are critical to furthering the government's compelling interests in promoting public health and ensuring that women have equal access to health care. The signifi-

cant protective benefits of oral contraceptives and IUDs are supported by an extensive body of research demonstrating that contraceptives are one of the few evidence-based methods for the prevention of ovarian cancer and other deadly gynecologic cancers. *See supra* pp. 9-17. Contrary to Petitioners' argument that the government's compelling interests are "highly abstract" and too "broadly" framed,[39] this empirical evidence supports a specific, concrete, and identifiable government interest in reducing women's risk of lethal gynecologic cancers. *Hobby Lobby*, 134 S. Ct at 2799 (Ginsburg, J., dissenting) (the contraceptive coverage "secures benefits wholly unrelated to pregnancy, [such as] preventing certain cancers, menstrual disorders, and pelvic pain."); *Priests for Life*, 772 F.3d at 262 (under the ACA's contraceptive provision, "[t]he government further relied on the ways that contraceptive use can promote and improve women's health apart from their procreative health needs"); *Gilardi,* 733 F.3d at 1240 (Edwards, J., concurring in part and dissenting in part) (recognizing that contraceptives are prescribed to "reduce the risk of ovarian, endometrial, and gynecologic cancers," to prevent disease, and "preserve the health" of women with certain health conditions).

Given the high case-fatality rate associated with ovarian cancer and the lack of effective early detection techniques, prevention represents a critical opportunity to reduce morbidity and mortality rates of the disease. The government thus has a compelling interest in promoting public health by ensuring

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[39] Br. for Pet'rs in Nos. 14-1418, 14-1453 & 14-1505 at 66 (Jan. 4, 2016); Br. for Pet'rs in Nos. 15-35, 15-105, 15-119, & 15-191 at 57 (Jan. 4, 2016).

that women have access to contraceptives, without cost sharing, as preventive therapies.

4. To achieve this compelling interest, the government must be able to provide women access to affordable contraceptives. Indeed, the gravity of interests at stake, and the life and death nature of the cancers at issue, confirms that it is critical that contraceptives are both accessible and affordable. At the time the ACA became effective, twenty-eight states already had requirements that private insurance plans cover a full range of FDA-approved prescription contraceptive methods, and the ACA augmented those requirements by prohibiting cost sharing.[40] The prohibition on cost sharing is essential because women—particularly those at a high risk for ovarian cancer—must have access to *affordable* contraceptives over a long period of time so they can take full advantage of the preventive benefits detailed above, in consultation with their physicians.

Fortunately and as expected, the ACA's contraceptive coverage regulations have dramatically reduced the financial barriers to affordable contraceptives. Implementation of the regulations has already increased affordable access to contraceptive services for millions of women. According to a 2014 study, the percentage of women who did not pay any out-of-pocket expense for oral contraceptives increased from

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[40] Guttmacher Institute, *State Policies in Brief (as of February 1, 2016): Insurance Coverage of Contraceptives*, http://www.guttmacher.org/statecenter/spibs/spib_ICC.pdf (last visited Feb. 17, 2016).

15 to 67 percent between fall 2012 and spring 2014.[41] During that same time, the average out-of-pocket cost for oral contraceptives dropped from $14.35 to $6.48.[42] Studies showed similarly dramatic results for access to affordable IUDs: in March 2014, only 13 percent of women faced out-of-pocket costs for an IUD, compared to 58 percent in January 2012.[43] The average out-of-pocket costs for IUD insertion fell from $293.28 in June 2012 to $145.24 just one year later.[44]

The financial benefit to all women of the contraceptive coverage regulations cannot be overstated. Estimates show that women will save an average of $254.91 on the oral contraceptive pill and $248.30 on IUD insertions annually.[45] In 2013 alone, the reported savings in out-of-pocket costs on oral contraceptive pills was $483 million.[46] One study argues that the more accurate estimate, based on the 6.88 million privately insured women who use oral con-

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[41] Adam Sonfield *et al.*, *Impact of the Federal Contraceptive Coverage Guarantee on Out-of-Pocket Payments for Contraceptives: 2014 Update*, 91 Contraception 44, 45, 47 (2015).

[42] *Id.* at 47. The median out-of-pocket cost dropped from $10.00 to $0.00 in that same time-period. *Id.*

[43] *Id. See also* Jonathan M. Bearak et al., *Changes in Out-of-Pocket Costs for Hormonal IUDs After Implementation of the Affordable Care Act: An Analysis of Insurance Benefit Inquiries*, 93 Contraception 139, 141 (2016).

[44] Nora V. Becker & Daniel Polsky, *Women Saw Large Decrease in Out-of-Pocket Spending for Contraceptives After ACA Mandate Removed Cost Sharing*, 34 Health Aff. 1204, 1207 (2015).

[45] *Id.* at 1208.

[46] *Id.* at 1209.

traceptive pills, is an astonishing *$1.4 billion* per year.[47]

All women deserve access to affordable contraceptives. Indeed, data suggested that a "nontrivial portion of women with interest in an IUD but without any coverage"—between 8.8 and 37.9 percent—"worked for a religious employer that denies contraceptive coverage."[48] The cost savings that flow from the ACA's contraceptive coverage regulations are critical to furthering the government's compelling interest in promoting public health by providing access to affordable healthcare treatments. These massive cost savings should be available to all women, including those who work for religious nonprofits that need only complete self-certification forms to opt-out of providing contraceptive coverage themselves.

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[47] *Id.*

[48] Bearak et al., *supra*, at 142.

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The accommodation mechanism that Petitioners challenge here ensures access to critical preventive therapies—therapies that should be affordable and available to all women, regardless of the religious beliefs of their employers. The regulations at issue promote a compelling interest while providing a non-burdensome mechanism to accommodate religious objections. This Court should reject the Petitioners' challenge to that accommodation and allow all women access to this potentially life-saving preventive care.

CONCLUSION

For the foregoing reasons, as well as those in Respondents' brief, the judgments of the Third, Fifth, Tenth, and D.C. Circuits should be affirmed.

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APPENDIX

APPENDIX A

EUROPEAN JOURNAL OF CANCER PREVENTION 1999, 8, 297-304

ORAL CONTRACEPTIVES AND OVARIAN CANCER

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The protection conveyed by oral contraceptives against ovarian cancer risk is one of the best established and most important features of epithelial ovarian cancer on a public health scale. Ovarian cancer incidence and mortality rates have been declining in most developed countries for women born after 1920, and the decline was greater in countries where oral contraceptive use has been more widespread. Thus, data from descriptive epidemiology are consistent with a favourable effect of oral contraceptives on ovarian cancer risks. The overall estimated protection from cohort and case—control studies is approximately 40% in ever oral contraceptive users, and increases with duration of use to more than 50% for users of 5 years or longer. The favourable effect of oral contraceptives against ovarian cancer risk persists for at least 10-15 years after use has ceased, and it is not confined to any particular type of oral contraceptive formulation. However, available data do not provide definite evidence for more recent low-dose

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formulations and for longer periods of latency or recency of use. The protection is also observed on borderline malignancy ovarian neoplasms, and probably on benign epithelial cysts as well. There is suggestive evidence of some protection for sex-cord-stromal cancers, but not for germ cell neoplasms. In terms of biological mechanisms, oral contraceptives are thought to act on ovarian cancer risk by affecting the lifetime number of ovulations. The protection attributable to oral contraceptives on ovarian cancer risk is one of the major issues on any individual risk/benefit assessment and public health evaluation of this type of contraceptive use.

Key words: oral contraceptives, ovarian cancer, epidemiology, risk.

Introduction

The protection offered by oral contraceptives against ovarian cancer risk is one of the most consistent epidemiological findings, and one of the important examples — on a public health scale — of large-scale chemopreventive interventions.

In several developed countries young women showed substantial declines in ovarian cancer incidence and mortality, partly or largely attributable to the protection afforded by oral contraceptives (Para-zzini *et al.*, 1991a; La Vecchia *et al.*, 1992, 1998).

Cohort analyses based on data from Switzerland (Levi *et al.*, 1987), Britain (Villard-Mackintosh *et al.*, 1989), Sweden (Adami *et al.*, 1990), England and Wales (dos Santos Silva and Swerdlow, 1995) and the Netherlands (Koper *et al.*, 1996), as well as a systematic analysis of mortality trends in 16 major European countries (La Vecchia *et al.*, 1992, 1998), showed that women born after 1920, i.e. from the generations who had used oral contraceptives — had consistently reduced ovarian cancer rates. The downward trends were greatest in

countries where oral contraceptives have been more widely utilized (La Vecchia *et al.*, 1998).

Thus, descriptive data on ovarian cancer incidence and mortality are consistent with the hypothesis of a favourable impact of oral contraceptive use on subsequent ovarian cancer rates.

There are still a few open issues, including a clearer understanding of the biological mechan-ism(s), the potentially different role of various types of oral contraceptive formulations, and the very longterm implications of oral contraceptives, including potential interactions with hormone replacement therapy (HRT) and other exogenous hormones in the assessment of a woman's global exposure to exogenous hormones. These issues will be considered in the present paper, which will review the main results from cohort and case-control studies.

Cohort studies

The main results of cohort studies on oral contraceptive and ovarian cancer are summarized in Table 1.

Three cohort studies on oral contraceptives conucted in the US and Britain provided data on a total of about 100 cases of epithelial ovarian cancer. These included the US Walnut Creek Study (Ramcharan *et al.*, 1981), whose recruitment was made in 1968-72, including 10,638 women aged 18-54 years. Up to 1977 a total of 16 cases of ovarian cancer were reported, corresponding to an age-adjusted RR for ever oral contraceptive use of 0.4.

The Royal College of General Practitioners' study was based on 47,000 women recruited in 1968 in 1400 British general practices (Beral *et al.*, 1988); 30 cases of ovarian cancers were observed up to 1987, corresponding to multivariate RRs of 0.6 [95%, confidence interval (CI) 0.3-1.4] for ever pill users and of 0.3 for ≥10 years' use. Allowance in the analysis was made for age, parity, smoking, and social class.

The Oxford Family Planning Association study was based on 17,032 women enrolled between 1968 and 1976 from various family planning clinics in the UK (Vessey and Painter, 1995). Up to October 1993, 42 cases of ovarian cancer were registered, corresponding to RRs of 0.4 (95% CI 0.2-0.8) for ever oral contraceptive use and of 0.3 (95% CI 0.1-0.7) for >8 years of use. Adjustment was made for age and parity.

The results of these cohort studies on contraceptives are thus compatible with RRs of ovarian cancer around 0.5 for ever use and 0.3 for long-term use.

Furthermore, in the nurses' health study, based on 121,700 registered nurses aged 30-55 years in 1976, 260 cases of ovarian cancer were prospectively observed between 1976 and 1988 (Hankinson *et al.*, 1995). The multivariate RR for ever use, which essentially reflected former use, was 1.1 (95% CI 0.83-1.43), but declined to 0.6 (95% CI 0.32-1.07) for use ≥5 years. Adjustment was made for age, tubal ligation, age at menarche, age at menopause, smoking and body mass index.

Thus, the overall RR from cohort studies is around 0.8 for ever use and 0.5 for long-term use, on the basis of approximately 350 cases of ovarian cancer.

Although the total amount of data available from cohort studies on oral contraceptive and ovarian cancer is limited, and the possibilities of allowance for confounding are restricted, the consistency of available findings supports the existence of a real protective effect of oral contraceptive on ovarian carcinogenesis.

Case-control studies

Epidemiological evidence from case-control studies on oral contraceptive and ovarian cancer is well defined and consistent: at least 20 out of 21 studies published between 1980 and 1997 found relative risks below unity, the sole apparent outlier being a study conducted in China (Shu *et al.*,

1989). Table 2 gives the main results of case-control studies of ovarian cancer published between 1980 and 1997.

Willett *et al.* (1981), in a case-control study of 47 cases of ovarian cancer and 470 controls nested in the Nurses' Health Study cohort (based on 121,694 registered nurses aged 30-55 years in 1986 and residing in 11 larger US states) found an age-adjusted RR of 0.8 (95% CI 0.4-1.5) for ever oral contraceptive use, and of 0.2 (95% CI 0.1-1.0) for women aged 35 years or younger, who were more likely current or recent users.

Table 1.
Selected cohort studies on combined oral contraceptives (COC) and ovarian cancer, 1980-97

* * *

Hildreth *et al.* (1981) considered 62 cases of epithelial ovarian cancer and 1068 hospital controls aged 45-74 years from Connecticut, diagnosed between 1977 and 1978. The response rate was 71% for both cases and controls. The multivariate RR for ever pill use, after allowance for age and parity, was 0.5 (95% CI 0.2-1.5).

Weiss *et al.* (1981), in a population-based case—control study of 112 cases diagnosed between 1975 and 1979 from Washington and Utah, found an RR (adjusted for demographic factors and parity) of 0.6 for ever use and of 0.4 (95% CI 0.15-1.28) for longest use, of borderline statistical significance *(P* = 0.04). Response rate was 66% for cases and 92% for controls.

Franceschi *et al.* (1982) considered data on 161 cases of epithelial ovarian cancers and 561 hospital controls interviewed in Milan, Italy in 1979-80. The age-adjusted RR for ever pill use was 0.7 (95% CI 0.4-1.1).

Cramer *et al.* (1982) in a population-based case—control study of 144 cases and 139 population controls conducted during the period 1978-81 the greater Boston area found an RR, adjusted for age and parity, of 0.4 (95% CI 0.2-1.0) for

ever pill use, in the absence of a consistent duration risk relationship (RR = 0.6 for >5 years). However, this could be due to chance becasue of the small number of cases. The response rate was around 50% for both cases and controls.

Rosenberg *et al.* (1982), in a hospital-based case-control study of 136 cases and 539 controls collected between 1976 and 1980 from various areas of the USA and Canada, found an age-adjusted RR of 0.6 (95% CI 0.4-0.9) for ever pill use and of 0.3 for use of ≥5 years. The response rate was 94% for both cases and controls, and the results were not materially modified by multivariate analysis.

Risch *et al.* (1983) provided data from a case-control study of 184 cases and 705 controls from Washington and Utah diagnosed between 1975 and 1979, giving a significant multivariate RR estimate of 0.89 per year of oral contraceptive use. Response rate was 68% for both cases and 95% for controls.

In a case-control study conducted in 1980-89 on 150 cases and 250 hospital control from Athens, Greece, Tzonou *et al.* (1984) found a multivariate RR (adjusted for age, age at menopause and use of HRT) of 0.4 (95% CI 0.1-1.1). The lack of significance is explained through the low frequency of oral contraceptive use in that population.

The Cancer and Steroid Hormone (CASH) study (1987) was a population-based investigation conducted between December 1980 and December 1982 in eight areas of the USA on 546 women aged 20-54 years with ovarian cancer (492 epithelial) and 4227 controls. The response rate was 71% for cases and 83% for controls. The multivariate RR, adjusted for age and parity, for ever pill use was 0.6 (95% CI 0.50.2), and decreased to 0.2 (95% CI 0.1-0.4) for use >10 years. The results were consistent when specific formulations of oral contraceptives were considered separately. However, no meaningful protection was evident for very short-term use, i.e. 3-6 years (Gross *et al.*, 1992).

Harlow *et al.* (1988) provided information on oral contraceptive use on 92 cases of borderline malignancy epithelial ovarian cancers and 124 controls diagnosed between 1980 and 1985. The RR for ever use, adjusted for age and parity, was 0.4, in the absence, however, of a consistent duration—risk relationship.

Wu *et al.* (1988), in a hospital-based case-control study of 299 cases and 752 controls diagnosed in 1983-85 from the San Francisco Bay area found an RR, adjusted for parity, of 0.7 (95% CI 0.5-1.1) for ever OC use and of 0.4 (95% CI 0.3-0.7) for >3 years of use. The overall RR per year of use was 0.88 (95% CI 0.83-0.94). The response rate was about 70% for both cases and controls.

Shu *et al.* (1989) in a case-control study conducted in 1984-86 in Shanghai, China, on 229 ovarian cancer cases (172 epithelial) and an equal number of controls found an RR (adjusted for education, parity, ovarian cysts and age at menarche) of 1.8 (95% CI 0.8-4.1) for ever oral contraceptive use. However, only 21 cases and 12 controls had only ever used oral contraceptive. The response rate was 89% for cases and 100% for controls.

The World Health Organization (WHO) Collaborative Study of Neoplasia and Steroid Contraceptives (1989) included data on 365 cases of histologically confirmed epithelial ovarian cancer and 2397 hospital controls interviewed between 1979 and 1986 in seven (mainly developing) countries of the world. The response rate was 73% for cases and 94% for controls. The multivariate RR (adjusted for age, centre, year of interview and parity) for ever oral contraceptive use was 0.75 (95% CI 0.56-1.01), and decreased to 0.54 (95% CI 0.33-0.58) for 10 years' use or longer. The protection was of similar magnitude in developed and developing countries (Thomas, 1991).

In a case-control study conducted in 1978-81 in the Washington, DC area, on 296 patients with epithelial ovarian cancer and 343 hospital controls, Hartge *et al.* (1989) found

RRs (adjusted for age and race) of 1.0 (95% CI 0.7-1.7) for ever oral contraceptive use, and of 0.8 (95% CI 0.4-1.5) for ≥5 years' use. The response rate was 74% for cases and 78% for controls.

Booth *et al.* (1989) in a hospital-based case-control study of 213 cases and 451 controls interviewed between 1978 and 1983 in London and Oxford, England, found multivariate RRs of approximately 0.5 for ever use, and of 0.1 (95% CI 0.01-1.0) for >10 years' use, with a significant inverse trend in risk with duration of use. Allowance was made for age, social class, gravidity and duration of unprotected intercourse.

Parazzini *et al.* (1991b) provided data on 505 cases of epithelial ovarian cancer under 60 years of age and 1375 hospital controls interviewed between 1983 and 1989 in northern Italy. The multivariate RR (adjusted for sociodemographic factors, parity, age at menarche, lifelong menstrual pattern, menopausal status and age at menopause) for ever oral contraceptive use was 0.7 (95% CI 0.5-1.0), which decreased to 0.5 (95% CI 0.3-0.9) for ≥2 years' use, with a significant inverse trend in risk with duration. Response rate was 98% for both cases and controls.

Parazzini *et al.* (1991c) also considered 91 cases of borderline malignancy epithelial ovarian cancer and 237 hospital controls interviewed between 1983 and 1990 in northern Italy. The multivariate RR (adjusted for age, education, parity and age at menopause) for ever pill use was 0.3 (95% CI 0.2-0.6), and that for ≥2 years' use was 0.2 (95% CI 0.08-0.6). The response rate was 98% for both cases and controls.

In a case-control study of 189 cases and 200 controls conducted in 1989-91 in greater Athens, Greece (Polychronopoulou *et al.*, 1993), only three cases and seven controls reported ever pill use, corresponding to a multivariate RR of 0.80 (95% CI 0.11-3.67). The response rate for cases was about 90%.

Rosenberg *et al.* (1994) updated their 1982 report, providing data collected between 1977 and 1998 on 441 cases of epithelial ovarian cancer and 2065 hospital controls from various US areas. The response rate was 94% for both cases and controls. The multivariate RR for ever use was 0.7 (95% CI 0.6-1.0). No appreciable protection was observed up to 3 years' use, but the RR declined to 0.5 (95% CI 0.2-0.9) for ≥10 years'. The risk estimates were similar for various types of oral contraceptive formulations.

Risch *et al.* (1994, 1996) provided data on 450 cases of epithelial ovarian cancer aged 35-79 years and 564 controls diagnosed between 1989 and 1992 in Ontario, Canada. The response rate was 71% forcases and 65% for controls. The overall multivariate RR per each year of pill use, adjusted for age, parity, lactation, HRT use, tubal ligation, hysterectomy andfamily history of breast cancer was 0.90 (95% CI 0.86-0.94), and the protection was stronger for serous and endometrioid than for mucinous neoplasms.

Purdie *et al.* (1995) in a population-based study of 824 cases and 860 controls diagnosed between 1990 and 1993 in three Australian states found an RR around 0.6 for ever use, which declined to 0.26 (95% CI 0.18-0.38) for ≥10 years of use. Response rate was 90% for cases and 73% for controls. Allowance was made in the analysis for sociodemographic factors, family history of cancer, talc use, smoking, and reproductive and hormonal factors.

The findings of two meta-analyses of case-control studies on the issue are also included in Table 2.

These were conducted on 971 cases and 2258 controls from three European countries (Franceschi *et al.*, 1991) and on 2197 cases and 8893 controls in white women from 12 US studies (Whittemore *et al.*, 1992) (i.e. a total of over 3100 cases, and 11 000 controls).

In the European meta-analysis (Franceschi *et al.*, 1991) the multivariate RR was 0.6 (95% CI 0.4-0.8) for ever use, and

0.4 (95% CI 0.2-0.7) for longest use. Allowance was made for age and other sociodemo-graphic factors, menopausal status and parity.

In the US meta-analysis (Whittemore *et al.*, 1992) corresponding values were 0.7 (95% CI 0.5-0.6) for ever use and 0.3 (95% CI 0.2-0.4) for use >6 years. Adjustment was made for age, study and parity. The results were similar when hospital- or population-based studies were considered separately. The RRs were 0.7 for both types of studies for ever oral contraceptive use, 0.6 for hospital-based and 0.3 for population-based for longest use (>6 years), 0.95 and 0.90 (significant) per added year of use. An inverse association was also observed in a further analysis of 110 black cases and 251 black controls (RR = 0.7 for ever use and 0.6 for ≥6 years' use; John *et al.*, 1993). The US meta-analysis also included data on 327 borderline malignancy epithelial ovarian neoplasms in white women. The RRs were 0.8 (95% CI 0.6-1.1) for ever oral contraceptive use, and 0.6 (95% CI 0.40.9) for >5 years' use (Harris *et al.*, 1992).

Discussion

The most convincing aspect of the inverse relationship of oral contraceptive on ovarian cancer risk is given by the consistency of the results, independently from type of study (hospital- or population-based), geographic area (North America, Europe, Australia or developing areas of the world) and type of analysis, including allowance for covariates, which differed from study to study, but tended to include larger numbers of variables in most recent ones. Likewise, the inverse relationship between oral contraceptive and ovarian cancer was observed for most types of formulations considered, including low dose ones (CASH, 1987; Rosenblatt *et al.*, 1992; Rosenberg *et al.*, 1994).

The overall estimate of protection for ever use is approximately 40%, and a steady inverse relationship exists with duration of use. The protection was over 50%, and probably around 60%, for long-term use (i.e. >5 years).

Parity is a well-recognized protective factor for ovarian cancer (Parazzini *et al.*, 1991a), and is a correlate of oral contraceptive use, i.e. a potentially relevant confounder. The inverse relationship between oral contraceptive and ovarian cancer, however, also was observed after adequate allowance for parity in most studies, and was consistently reproduced in several studies across separate strata of parity, as well as of age and of other potential covariates, including marital status, education, menopausal status, other types of contraceptive use, and other selected menstrual and reproductive factors. Other potential confounding or indication bias, including selective exclusion of oral contraceptive use by smokers and by women at risk of liver and thromboembolic diseases (Fioretti *et al.*, 1997) were also unlikely to materially modify the inverse association observed between oral contraceptive use and ovarian cancer risk.

At least two studies (Harlow *et al.*, 1988; Parazzini *et al.*, 1991), and the meta-analysis of 12 US studies (Harris *et al.*, 1992), also considered borderline epithelial ovarian tumours. An inverse relationship was evident for these neoplasms too, suggesting that oral contraceptives exert a protection to the whole spectrum of epithelial ovarian carcinogenesis.

Limited information is available on different histological types of epithelial ovarian cancer. In a Canadian study (Risch *et al.*, 1996), the protection was apparently stronger for non-mucinous (OR = 0.89 per year of use) than for mucinous (OR = 0.98 per year of use) tumours. This observation, however, requires confirmation in other data-sets.

With reference to nonepithelial ovarian cancers, 38 germ cell neoplasms and 45 sex-cord-stromal neoplasms were considered from the collaborative analysis of 12 US case-controls studies (Horn-Ross *et al.*, 1992). The multivariate RRs among ever oral contraceptive users were 2.0 (95% CI 0.8-5.1) for germ cell cancers and 0.4 (95% CI 0.2-0.8) for sex-cord-stromal neoplasms. The data were inadequate for

evaluating duration of use, or any other time-risk relationship.

Likewise, the few available data indicate a consistent protection of oral contraceptive on benign epithelial tumours (ovarian cysts) (Parazzini *et al.*, 1989; Booth *et al.*, 1992), but not on benign ovarian teratomas (Westhoff *et al.*, 1988; Parazzini *et al.*, 1995).

The favourable effect of oral contraceptive on epithelial ovarian cancer seems to persist for at least 10-15 years after oral contraceptive use has ceased (Franceschi *et al.*, 1991; Whittemore *et al.*, 1992; CASH, 1987; Rosenberg *et al.*, 1994), and is not confined to any particular type of oral contraceptive formulation (Rosenblatt *et al.*, 1992; Rosenberg *et al.*, 1994). There is some suggestion that low-dose formulations may be slightly less protective: in the WHO Collaborative Study on Neoplasia and Steroid Contraceptives (Rosenblatt *et al.*, 1992), the RR of ovarian cancer for ever oral contraceptive use was 0.68 and 0.81 for high- and low-dose preparations, respectively.

Very little information is available on progestin-only oral contraceptives. In a hospital-based case-control study of 441 cases and 2065 controls recruited between 1977 and 1991 from various US states (Rosenberg *et al.*, 1994), 1% of cases versus 3% of controls had only ever used progestin-only oral contraceptives. The unadjusted OR was 0.3.

From a biological viewpoint, the beneficial effect of oral contraceptives on ovarian cancer risk has been interpreted chiefly within the framework of the incessant ovulation theory, i.e. a multistage theory of ovarian carcinogenesis (Casagrande *et al.*, 1979). Ovariostasis, induced by oral contraceptives as well as by pregnancy and menopause, avoids the exposure of ovarian epithelium to recurrent trauma and contact with follicular fluid (Parazzini *et al.*, 1991a).

Oral contraceptive use may also protect against ovarian cancer by reducing exposure to pituitary gonadotropins,

which stimulate the growth of cell lines derived from human ovarian carcinoma (Simon *et al.*, 1983). The lack of apparent protection by menopause replacement therapy (Parazzini *et al.*, 1991a; Whittemore *et al.*, 1992), however, does not support the existence of a favourable role of gonadotropin stimulation on ovarian carcinogenesis.

Since the incidence of ovarian cancer is already appreciable in middle age, and survival from the disease is unsatisfactory, the protection attributable to oral contraceptive use corresponds to a far from negligible number of deaths, and it is therefore one of the major issues in any risk/benefit and public health evaluation on the pill (Gross and Schlesselman, 1994; La Vecchia *et al.*, 1996). In such a sense, oral contraceptives represent one of the most important available chemopreventive agents.

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APPENDIX B

RESEARCH ARTICLE
OPEN ACCESS

PRIOR ORAL CONTRACEPTIVE USE IN OVARIAN CANCER PATIENTS: ASSESSING ASSOCIATIONS WITH OVERALL AND PROGRESSION-FREE SURVIVAL

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Abstract

Background: Prior studies have described a reduced risk of developing ovarian cancer with the use of oral contraceptives. In this context, we decided to examine if oral contraceptive use prior to a diagnosis of ovarian cancer is associated with better overall and progression-free survival.

Methods: This retrospective cohort study included ovarian cancer patients who were seen at the Mayo Clinic in Rochester, Minnesota from 2000 through 2013. Patients completed a risk factor questionnaire about previous oral contraceptive use, and clinical data were extracted from the electronic medical record.

Results: A total of 1398 ovarian cancer patients responded to questions on oral contraceptive use; 571 reported no prior use with all others having responded affirmatively to oral contraceptive use. Univariate analyses found that oral contraceptive use (for example, ever versus never) was

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associated with better overall survival (hazard ratio (HR) 0.73 (95 % confidence interval (0): 0.62, 0.86); p = 0.0002) and better progression-free survival (HR 0.71 (95 % CI: 0.61, 0.83); p < 0.0001). In multivariate analyses, contraceptive use continued to yield a favorable, statistically significant association with progression-free survival, but such was not the case with overall survival.

Conclusions: This study suggests that previous oral contraceptive use is associated with improved progression free survival in patients diagnosed with ovarian cancer.

Keywords: Ovarian cancer, Survival, Oral contraceptives

Decades of data show that oral contraceptive use reduces the risk of ovarian cancer. A greater than 20% relative risk reduction appears to occur for every 5 years a woman reports taking oral contraceptives [1]. This risk reduction is particularly salient among women who have used oral contraceptives for 10 years or longer at any point in their lives, and it also occurs in high-risk women, such as those with *BRCA1* and *BRCA2* germline mutations [2]. Continuous ovulation is thought to predispose to ovarian epithelial cell DNA damage, which in turn gives rise to carcinogenesis, thus providing mechanistic plausibility to how cessation of ovulation from oral contraceptives might lead to lower cancer risk [3].

Although large pooled analyses suggest that oral contraceptives could prevent 200,000 cases of ovarian cancer and 100,000 deaths from this malignancy over 20 years, such deductions have not spawned large-scale prevention trials [4, 5]. The many decades of follow up required to capture a small number of cancer cases, the enormous funding necessary to conduct prevention trials of sizable complexity, and the fact that oral contraceptives can also confer negative effects, such as an increased risk of thrombophlebitis and breast cancer, all lessen enthusiasm for the conduct of such prevention trials. Moreover, to date, the above robust

observation has not yet dramatically changed clinical practice.

In contrast to these data on ovarian cancer prevention, few studies have specifically sought to assess whether oral contraceptives prior to an ovarian cancer diagnosis is associated with better outcomes after contracting this malignancy. This possibility builds on previous data on the purported role of oral contraceptives in preventing ovarian cancer. Moreover, in contrast to primary prevention, establishing this observation could lead to prospective research aimed at improving outcomes in ovarian cancer patients. Thus, to further examine the effects of previous oral contraceptives on outcomes in ovarian cancer patients, we studied patients at the Mayo Clinic in Rochester, Minnesota. Our main aim was to determine whether oral contraceptive use prior to a diagnosis of ovarian cancer is associated with better overall and progression-free survival within the context of in depth multivariate analyses undertaken within a consecutively-recruited and monitored cohort of ovarian cancer patients.

Methods

Overview

This study focused on women with invasive primary epithelial ovarian, fallopian tube, or peritoneal cancer seen at the Mayo Clinic in Rochester, Minnesota. The study of all these tumors in aggregate has substantial precedent because these malignancies behave and are treated similarly. The Mayo Clinic Institutional Review Board (IRB) approved this study. As described previously, patients were consecutively recruited from 2000 through 2013 from the Mayo Clinic in Rochester, Minnesota [6]. All patients had to be 20 years of age or older and had to have provided written informed consent. Patients then completed a paper risk factor questionnaire (see below) that included queries on previous oral contraceptive use. Trained medical personnel extracted details on tumor histology, type of surgery, and

administration of chemotherapy from the electronic medical record.

Study endpoints

Outcome data were acquired through April 2014. Data on cancer recurrence were updated via the Mayo Clinic electronic medical record and included a mailed questionnaire to patients and medical record review. Vital status was gleaned from the Mayo Clinic electronic medical record, the Mayo Clinic Cancer Registry, and registration records. Death certificates were requested from the appropriate government bodies with the appropriate permissions to confirm dates of death.

This study analyzed overall survival, as defined as the interval from a histologic-or cytologic-confirmed cancer diagnosis to date of death. If vital status was unknown for a specific patient, that patient was censored on the date of last contact or at five years, whichever occurred first. The rationale for this approach rests in the fact that the majority of ovarian cancer-related deaths occur in the first five years after diagnosis. Progression-free survival was also assessed and was defined as the date from cancer diagnosis to the date of initiation of second-line cancer treatment or death. Although vital status was assessed in all patients, progression-free survival had been assessed in only a subset.

Definition of covariates

Oral contraceptive use was the main variable of interest, and it was assessed by means of a self-administered questionnaire. Patients were asked, “Have you ever used oral contraceptive pills (“the pill”)?” and were asked to mark the appropriate response of “yes” or “no.” If they answered “yes,” they were then asked to estimate duration of use in years, as summarized in this report as both a categorical variable (1-48 months and > 48 months) and a continuous variable. Other hormone-related variables were also assessed; these included age at menarche and menopause

status. Patients were also assessed for number of live births, coded as nulliparous versus one or two versus three or more. This grouping of parity was done because of efforts to maintain statistical power and because it appeared clinically reasonable.

A variety of clinical covariates, many of which have prognostic associations, were also considered. These consisted of 1) cancer stage; 2) cancer histology: high grade serous, low grade serous, endometrioid versus clear cell, mucinous, mixed epithelial, borderline invasive mixed epithelial, and other; 3) tumor grade; 4) outcome of initial surgery: no residual disease versus </= 1 cm of residual disease versus > 1 cm residual disease; 5) platinum-based chemotherapy administered within the first three months of surgery: yes versus no [7]; 6) patient age at cancer diagnosis; 7) smoking history: never versus former versus current; and 8) first degree family history of breast or ovarian cancer: yes versus no.

Analyses

Chi-square or Wilcoxon rank-sum tests were used, as appropriate, to compare all the covariates between never- and ever- oral contraceptive users. Univariate analyses were undertaken for all the variables described above. Oral contraceptive use was examined in two separate analyses: 1) based on a “ever” and “never” patient response and 2) based on duration of oral contraceptive use: never versus 1-48 months versus > 48 months or patient-reported years of use as a continuous variable. All variables were examined to assess their individual associations with overall survival and progression-free survival. Kaplan Meier curves were constructed to visualize unadjusted associations. Cox proportional hazards modeling accounting for left truncation was used for univariate and multivariate analyses with estimation of HRs and 95 % CIs. Left truncation is a standard method undertaken to limit sampling bias when one

is unable to consistently observe the time when an event might have occurred.

Table 1
Demograpics

* * *

Multivariate analyses were then conducted to identify the independent prognostic association of each of these variables and to estimate the effects of these variables on overall and progression-free survival endpoints. Three models were constructed with inclusion of 1) all variables except those with high rates of missing data; 2) variables that, in univariate analyses, had yielded a statistically significant association ($p < 0.01$) with overall and disease-free survival; and 3) variables that, in univariate analyses, had yielded a statistically significant association with overall survival and disease-free survival ($p < 0.01$) except those with notable missing data. These models were constructed in this manner to avoid biases that might arise from missing data. All statistical tests were two-tailed, and a p-value of < 0.05 is considered statistically significant. All statistical analyses were performed using Statistical Analysis Software version 9.3 (SAS Institute, Cary, North Carolina).

Results

Demographics

This study focused on 1398 ovarian cancer patients who had completed a questionnaire on oral contraceptive use at study entry. Within this cohort, 571 reported no prior oral contraceptive use. Among oral contraceptive users, the patient-reported median duration was 60 months (range: 1 to 444 months).

Baseline characteristics appear in Table 1. Patients who had used oral contraceptives were more likely to have had no residual disease from surgery but were less likely to have started platinum-based chemotherapy after surgery. Patients

who had used oral contraceptives were also diagnosed at an earlier age and had fewer live births.

Overall survival and progression-free

At the time of this report, 562 patients had died, and 656 had developed recurrent cancer or had died after accounting for left truncation. Univariate analyses, which do not take into account confounding factors, suggested that oral contraceptive use (ever versus never) was associated with better overall survival (HR 0.73 (95 % CI: 0.62, 0.86); p = 0.0002) (Fig. 1). Similarly, univariate analyses also suggest oral contraceptives (ever versus never) was associated with more favorable progression-free survival (hazard ratio (HR) 0.71 (95 % confidence interval (CI): 0.61, 0.83); p < 0.0001) (Fig. 2). These survival advantages were also observed when oral contraceptive use was further characterized based on duration of use. Compared to never users, patients who reported using oral contraceptive for one to 48 months manifested a more favorable overall survival and progression-free survival, as did patients who reported using them for more than 48 months (data not shown).

In the three constructed multivariate models, oral contraceptive use did not yield a statistically significant improvement in overall survival, but it did yield such an association with improved progression-free survival (Table 2).

Of note, the multivariate models pointed to patient age as a major confounder, as younger age was strongly associated with oral contraceptive use. For example, in the first model, with no adjustment for age, oral contraceptive use was, in fact, associated with better overall survival (HR = 0.70; p < 0.001) as well as with better progression—free survival. However, after adjusting for age, this association with overall survival lost its statistical significance, although the association with improved progression-free survival was maintained. Furthermore, we performed separate analyses on associations with oral contraceptive use and overall survival

and progression-free survival based on whether patients had residual disease postoperatively and found these prognostic associations with oral contraceptive use were sustained.

* * *

Fig. 1

* * *

Fig. 2

Discussion

This study examined whether oral contraceptive use prior to a diagnosis of ovarian cancer was associated with improved overall survival and progression-free survival. We observed this protective association in univariate analyses, but multivariate analyses yielded less consistent findings. In the latter, prior oral contraceptive use was associated with improved progression-free survival but not with overall survival. Younger patients reported greater use of oral contraceptives as well as longer survival. This study provides corroborative evidence that previous oral contraceptive use is associated with better clinical outcomes in patients diagnosed with ovarian cancer, at least with respect to progression-free survival.

Indeed, our findings are particularly noteworthy because of the detailed nature of our multivariate analyses. The fact that we were able to adjust for highly relevant clinical covariates such as the extent of the primary debulking surgery and the fact that we had detailed follow up information on consecutively-treated patients strengthen this report. Our study provides an important contribution to an emerging body of literature that indicates oral contraceptive use prior to a diagnosis of ovarian cancer is associated with better outcomes. Only a few studies have examined whether oral contraceptives appear to change outcomes in patients who develop ovarian cancer at a later date. First, using the Nurses' Health Study, the New England Case-control Study, the Australian Ovarian Cancer Study, and the NIH-AARP

Diet and Health Study, Poole and others examined numerous lifestyle factors and their effect on clinical outcomes in ovarian cancer patients [5]. Among 4,342 patients with ovarian cancer, previous oral contraceptive use was associated with a lower risk of death (five-year increase in relative risk 0.69 (95 % confidence interval (CI): 0.58, 0.82)). These investigators noted that their study design might not have captured patients with rapidly fatal malignancies and that limited clinical data were available to accommodate some of their analyses. Nonetheless, this observation appears plausible, particularly given the earlier-referenced studies that have focused on cancer prevention. Second, several investigators, including Vessey and others from the Oxford Family Planning Association Contraceptive Study, Hannaford and others from the Royal College of General Practitioners' Oral Contraceptive study, and those from the Collaborative Group on Epidemiological Studies of Ovarian Cancer have also reported decreased overall mortality among ovarian cancer patients who had used oral contraceptives prior to their cancer diagnosis [1, 8, 9]. The above two studies used a cohort design of oral contraceptive users and non-users and reported on death from ovarian cancer. These studies confirm the observation from Poole and others, although their primary goal was to understand cancer risk.

Table 2
Multivariate analyses for overall survival and progression-free survival

* * *

However, not all studies of oral contraceptive use and outcome have been consistent. For example, Nagle and others reported on 676 women diagnosed with ovarian cancer, and, although 310 women had used oral contraceptives, the latter did not demonstrate a protective association with respect to ovarian cancer mortality (adjusted hazard ratio (HR) 0.88 (95 % CI: 0.70, 1.11) [10]. This study examined a cohort of women with ovarian cancer and looked

at survival of cancer patients who were users of oral contraceptives and cancer patients who were not users of oral contraceptives. These authors concluded that "reproductive and hormonal exposures prior to diagnosis do not influence survival from invasive ovarian cancer, in contrast to their substantial effects on the etiology of this disease," and others have drawn similar conclusions [11]. Taken together, these studies provide justification for generating the study reported here.

Is this favorable association between prior oral contraceptive use and survival mechanistically plausible? It appears to be. First, as alluded to earlier, previous studies that have shown oral contraceptives protect against the development of primary ovarian cancer suggest that cessation of ovulation halts the repeated monthly trauma that occurs on the surface of the ovary, thereby limiting the possibility of epithelial cell mutation and subsequent carcinogenesis [3]. Similar mechanisms might be invoked to explain the favorable prognostic associations observed here. In an analogous fashion, epithelial ovarian cancer cells that undergo repeated, monthly trauma from ovulation are perhaps more likely to develop DNA mutations. The more frequent the trauma, the more apt these cells are to develop aberrant DNA mutations; the more numerous the DNA mutations, the more aggressive the cancer [12]. Although this line of thinking may contradict the hypothesis that ovarian cancer originates from fallopian tube fimbria, it nonetheless merits consideration, particularly because the fimbria are also exposed to hormones in the follicular fluid [13]. Second, in a preclinical model, Romero and others observed that contraceptive hormone exposure decreased matrix metalloproteinase-2 activity, invoking this observation to explain the effects of oral contraceptives on carcinogen-esis and perhaps also on the improved clinical outcomes observed by us and others [14]. One might speculate that the role of matrix metalloproteinase-2 proteins

in modifying the extracellular matrix confers long-term consequences that attenuate the malignant potential of ovarian cancers and provide greater susceptibility to cancer treatment. In view of a growing literature that underscores an inverse association between oral contraceptive use and poor outcomes from ovarian cancer, it appears important to probe into and delineate the mechanisms that underlie these observations, such as those posited above.

Our study has at least three limitations. First, the questionnaire we used did not capture detailed information on oral contraceptive product formulation, which may be informative, as oral contraceptives with high progesterone content appear to carry a more protective effect [15]. Second, the exact cause of death for many patients is still being curated and thus cause-specific mortality was not analyzed here, although our use of censoring data at date of last contact and limiting follow up to 5 years post-diagnosis are attempts to mitigate this limitation. Nonetheless, it remains possible that deceased older patients had died more frequently of non-cancer causes, a plausible scenario that might explain why our study did not reveal an improvement in overall survival with oral contraceptive use in multivariate analyses, despite having captured an improvement in progression-free survival. Finally, this study provides limited data on how recently oral contraceptives had been used, and such timing issues would likely have an important impact on the strength of this association. Despite such limitations, our study - coupled with several that preceded it points to a need to investigate mechanisms that explain how and why prior oral contraceptive use appears to improve clinical outcomes in ovarian cancer patients. Understanding such mechanisms might lead to more effective therapeutic interventions in patients diagnosed with this malignancy.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AJ, NF, KK, RV, ZZ, ML BF, and EG contributed to the conception, design, analysis, and interpretation of data. AJ, NF, KK, RV, ZZ, ML BF, and EG were all involved in drafting the manuscript or revising it critically for important intellectual content. All authors have given final approval of the version to be published. All agree to be accountable for all aspects of this work.

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APPENDIX C

ORIGINAL PAPER

ORAL CONTRACEPTIVE USE AND IMPACT OF CUMULATIVE INTAKE OF ESTROGEN AND PROGESTIN ON RISK OF OVARIAN CANCER

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Abstract

Purpose Oral contraceptive use decreases the risk of ovarian cancer, but no previous studies have assessed the impact of cumulative intake of estrogen and progestin on ovarian cancer risk.

Methods We used data from a population-based case-control study conducted in Denmark in 1995–1999 among women aged 35–79 years; 554 women with epithelial ovarian cancer and 1,564 age-matched controls were included in the analyses. Data were analyzed in multiple logistic regression models.

Results The use of combined oral contraceptives only and the mixed use of combined and progestin-only pills decreased the risk of ovarian cancer, while no association was found with exclusive use of progestin-only pills. No major differences in risk were found for users of combined oral contraceptives with high- and low-potency estrogen and progestin. There was no effect of cumulative progestin intake, but decreased risks of ovarian cancer with increasing cumulative intake of estrogen (OR = 0.82; 95 % CI 0.67–0.99, per 100 mg estrogen) and increasing duration of oral contraceptive use (OR = 0.95; 95 % CI 0.92–0.98, per year of use) were found. No effect of cumulative estrogen intake was found, however, after adjustment for duration of oral contraceptive use.

Conclusions The protective effect of oral contraceptives against ovarian cancer may be sufficiently explained by duration of anovulation. This suggests that if the estrogen and progestin doses are sufficient to cause anovulation, a higher intake of estrogen or progestin confers no extra protection against ovarian cancer.

Keywords Ovarian cancer - Oral contraceptives - Estrogen - Progestin - Potency - Duration - Case–control study

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Introduction

It is well known that oral contraceptive use decreases the risk of ovarian cancer [1, 2]. The protective effect is concordant with the "incessant ovulation" hypothesis, which proposes that repeated, uninterrupted ovulations during the reproductive years cause micro-trauma to the ovarian surface epithelium, leading to malignant transformation [3]. The decreased risk of ovarian cancer associated with oral contraceptive use may not be due to anovulation alone, however, as oral contraceptives are known to suppress secretion of gonadotropic hormones from the pituitary gland,

including follicle-stimulating hormone and luteinizing hormone, which are suggested to increase ovarian cancer risk by increased cell growth and inhibition of apoptosis [4]. Furthermore, oral contraceptives may reduce ovarian cancer risk by increasing progestin stimulation [5]. In experimental studies, progestin inhibited the growth of ovarian epithelial cells [6], and the synthetic progestin levonorgestrel induced apoptosis in ovarian surface epithelium [7].

Most oral contraceptive pills contain a combination of estrogen (ethinylestradiol or mestranol) and progestin, but progestin-only preparations exist as well. Since they became available, considerable changes have been made in the estrogen and progestin content of combined oral contraceptives with regard to generic substance, dose, and potency (i.e., the amount required to produce an effect of given intensity) [8]. The first oral contraceptives introduced contained 50 j.tg ethinylestradiol (or an equivalent 100 j.tg mestranol) and are referred to as "high-dose" oral contraceptives, whereas so-called "low-dose" oral contraceptives, which were introduced in the late 1970s, contained 20–40 j.tg ethinylestradiol [8]. At the same time as the reduction in estrogen dose, new types of progestins were developed [8]. Concern has been raised that the newer types of oral contraceptives do not protect against ovarian cancer to the same degree as the older high-dose formulations [9]; however, relatively few observational studies have addressed whether the specific hormone content of oral contraceptives affects the degree of protection against ovarian cancer, and the findings are equivocal [9–16]. Furthermore, to our knowledge, no studies have been published that assessed the possibility of an independent effect of cumulative intake of estrogen and progestin from oral contraceptives (i.e., lifetime doses of estrogen and progestin from oral contraceptives) on ovarian cancer risk and thus whether the protective effect may be explained by mechanisms other than anovulation.

Using data from a large Danish population-based case-control study on ovarian cancer, the MALignant OVArian cancer study (MALOVA), we examined the association between oral contraceptive use and risk of ovarian cancer. We collected comprehensive information on the estrogen and progestin doses derived from oral contraceptives and were thus able to examine the impact of cumulative intake of estrogen and progestin on ovarian cancer risk.

Materials and methods

The study is based on the data from the MALOVA study, which has been described in detail elsewhere [17].

Between January 1995 and May 1999, women aged 35–79 years who were scheduled for an explorative laparotomy or laparoscopy because of a suspicion of an ovarian tumor were asked to participate in the study. The women were recruited from 16 gynecological departments in Denmark. Women with ovarian tumors were interviewed personally as soon as possible after the diagnosis by trained nurses and were asked to give blood and tissue samples. A total of 959 women with histologically verified ovarian cancer were identified. Of these, 53 were considered too ill to participate, and 45 died before contact was made, leaving 861 eligible cases. Of these, 180 women did not wish to participate, leaving 681 women (79.1 %) who were included in the MALOVA study; 579 gave a personal interview and blood and tissue samples, and 102 gave only a blood sample. For the present study, women who gave only a blood sample and women with non-epithelial ovarian cancer (n = 25) were excluded, leaving 554 women with ovarian cancer for the final analyses: 343 with serous, 50 with mucinous, 75 with endometrioid, 44 with clear cell, and 42 with other histological types of ovarian cancer (including undifferentiated and papillary adenocarcinomas).

Using the unique Danish personal identification number as the key identifier, we drew a random sample of women aged 35–79 years from the general female population in the study

area. The controls were included simultaneously with cases and frequency matched in five-year intervals using the age distribution of women with ovarian cancer registered in the Danish Cancer Registry in 1987–1992. In all, 3,839 women were invited as controls with a personal interview and a blood sample. Contact could not be achieved with 301 women, 269 women were excluded because they had undergone bilateral oophorectomy, six women had moved out of the study area, and 126 women were too ill to participate, leaving 3,137 women as eligible controls. Of these, 1,021 women refused to participate in the study. There were therefore 2,116 (67.5 %) controls; 1,564 were interviewed personally and 552 by telephone. We included only the 1,564 women with a personal interview as controls, as the telephone interview did not include detailed information about oral contraceptive use.

During the interviews, the women were asked whether they had ever used combined oral contraceptives or progestin-only pills for at least one month. A life-event calendar and lists and color photographs of all brands on the market in Denmark between January 1, 1966, and December 31, 1994, were used to obtain detailed information about oral contraceptive use. Each woman was asked in which period and for how long she had used a specific brand. To help the woman recall the specific brand name, she was shown photographs of oral contraceptive brands marketed in the relevant period. If she could not recognize the brand from the photographs, her usage was recorded as "unspecified oral contraceptive use." As mestranol (100 j.tg) is approximately equipotent to ethiny-lestradiol (50 j.tg) [18], formulations containing more than 100 j.tg mestranol or 50 j.tg ethinylestradiol were categorized as "high-estrogen," whereas formulations containing <100 j.tg mestranol or 50 j.tg ethinylestradiol were categorized as "low-estrogen" formulations. There are no universally recognized standards for categorizing progestin potency; however, as in previous studies [14, 16], we

categorized formulations with a dose equivalent to 0.30 mg norgestrel or more as “high-progestin” and formulations with <0.30 mg norgestrel or its equivalent as “low-progestin” according to Grant’s glycogen deposition assay as described by Dickey and Stone [19]. Characterization of progestins not described in the paper by Dickey and Stone was performed using information from other sources [15, 16, 20]. Table 1 shows the different types of progestins that were contained in the formulations used and their categorization as either high or low dose.

Table 1
Characterization of progestins

* **

Statistical analysis

Associations between various measures of oral contraceptive use and risk of ovarian cancer were estimated in multiple logistic regression models and expressed as odds ratios (ORs) with corresponding 95 % confidence intervals (CIs). All analyses were adjusted for age in five-year categories corresponding to the sampling of controls. Furthermore, all analyses were adjusted for pregnancy (ever/never pregnant and number of pregnancies), family history of breast and/or ovarian cancer (yes/no), and hormone replacement therapy use (ever/never). These potential confounders were selected on the basis of a priori knowledge of their possible role in the development of ovarian cancer. We also considered other potential confounders (menopausal status, breast-feeding, maternal age at first and last pregnancy, tubal ligation, hysterectomy, body mass index, smoking status, and education) and included them in the final model if they altered the estimate for the association between ever/never use of any oral contraceptives and ovarian cancer risk by 10 % or more. However, this was not the case for any of the additional confounders, and therefore, none of them were included in the final logistic regression model.

Three analytical approaches were used. First, the associations with (a) the use of combined oral contraceptives only, (b) the use of progestin-only pills only, and (c) the mixed use of combined and progestin-only pills, respectively, and risk of ovarian cancer were investigated. Secondly, the association between the use of combined oral contraceptives according to hormone potency and ovarian cancer risk was examined. Finally, the associations between cumulative lifetime dose of estrogen and progestin and duration of oral contraceptive use, respectively, and ovarian cancer risk among women with a specified use of combined oral contraceptives and progestin-only pills were assessed. For the latter analysis, two models were fitted. Model 1 included adjustment for the selected potential confounders, while model 2 also included mutual adjustment between cumulative intake of estrogen, cumulative intake of progestin, and duration of oral contraceptive use.

Women with mixed use (i.e., women who used different kinds of combined oral contraceptives), women who did not remember the brand they had used, and women who used only progestin-only pills were excluded from the analyses concerning hormone potency. Among women with a specified use of oral contraceptives, the cumulative intake of estrogen and progestin from combined oral contraceptives and progestin-only pills was calculated by summarizing the intake (i.e., the specific dose multiplied by duration of use) for each period the woman had used oral contraceptives. Women who could not remember the name of the brand they had used were excluded from the analyses on cumulative intake of estrogen and progestin.

The linearity of the association with each of the quantitative variables (number of pregnancies, cumulative intake of estrogen and progestin, and duration of oral contraceptive use) was tested by comparison with a linear spline model with knots placed at the quartiles among cases by means of a likelihood ratio test. No significant deviation from linearity

was found for any of these variables (p [0.05), except for cumulative intake of progestin (p = 0.02). In model 2, however, with mutual adjustment between cumulative progestin intake, cumulative estrogen intake, and duration of oral contraceptive use, no significant deviation from linearity was observed for cumulative progestin intake (p = 0.53). Associations between cumulative estrogen and progestin intake and duration of oral contraceptive use, respectively, and ovarian cancer risk were illustrated graphically by fitting a linear spline with knots placed at the quintiles.

As the generic content of each contraceptive preparation varied, we tested whether the effect of different types of estrogens and progestins varied. The effect of the two artificial estrogens, ethinylestradiol and mestranol, in oral contraceptives was not statistically significantly different (p = 0.88). Progestins were divided into four major groups on the basis of their chemical derivation: estranes (norethisterone, lynestrenol, and ethynodiol), old gonanes (levonorgestrel and norgestrel), pregnanes (megestrol acetate, cyproterone acetate, and medroxyprogesterone acetate), and new gonanes (gestodene, norgestimate, and desogestrel) (Table 1). No statistically significantly difference was found in the association between the use of these four groups of progestins and ovarian cancer risk (p = 0.12). Because of a limited number of cases, we were unable to test differences in the effect of the progestins within the four groups.

All p values were two-sided, and a significance level of 5 % was used. All analyses were performed using the SAS software package (version 9.2; SAS Institute, Cary, NC, USA).

Results

Table 2 shows age-adjusted ORs for the associations between potential confounders and ovarian cancer risk. Ever being pregnant (OR = 0.38; 95 % CI 0.28–0.52) and ever breast feeding (OR = 0.52; 95 % CI 0.41–0.66) both decreased the risk of ovarian cancer. In contrast, an

increased risk of ovarian cancer was observed among women who had ever used hormone replacement therapy (OR = 1.26; 95 % CI 1.02–1.55), women with a family history of breast and/or ovarian cancer (OR = 1.44; 95 % CI 1.10–1.90), and women who had been hysterectomized (OR = 1.55; 95 % CI 1.15–2.08). The associations between menopausal status, tubal ligation, education, body mass index, smoking status, and risk of ovarian cancer were not statistically significant.

Table 2
Age-adjusted odds ratios (OR) and 95% confidence inervals (CI) for the association between various potential confounders and risk of ovarian cancer

* * *

Table 3 shows the characteristics of oral contraceptive use. Any use of oral contraceptives was reported by 46 % of cases and 57 % of controls. Combined oral contraceptive use only was reported by 40 % of cases (88 % of ever users) and 48 % of controls (84 % of ever users). Exclusive use of progestin-only pills was reported by 1.8 % of both cases and controls (4 % of ever users among cases and 3 % of ever users among controls), while the mixed use of combined and progestin-only pills was reported by 3.8 % of cases (8 % of ever users) and 7.5 % of controls (13 % of ever users). Among both cases and controls, most women had started using oral contraceptives when they were first introduced onto the Danish market, i.e., in 1966 or 1967. This was reflected in the time since first and last use of oral contraceptives, as most women in both the case and the control group had first used the products 25 years or more before diagnosis or interview and had last used them 20 years or more before diagnosis or interview. Most of the case women had used oral contraceptives for fewer than five years, whereas almost equal numbers of control women had used oral contraceptives for fewer than five years and for five years or more. The cumulative intake of estrogen varied from 0.63 to

732.6 mg (data not shown), and 35 women with ovarian cancer (25.4 % of specified oral contraceptive users) and 195 control women (32.0 % of specified oral contraceptive users) had a cumulative estrogen intake of 130 mg or more. Cumulative intake of the progestin component ranged from 3.15 to 46,879 mg (data not shown), and 37 cases (25.5 % of specified oral contraceptive users) and 156 controls (24.7 % of specified oral contraceptive users) had a cumulative progestin intake of 3,500 mg or more. In all, 145 case women (57.5 % of ever users) and 632 control women (71.0 % of ever users) provided information about estrogen and progestin dose.

Table 3
Characteristics of oral contraceptive use among ovarian cancer cases and controls

* * *

Exclusive use of combined oral contraceptives was associated with a statistically significantly decreased risk for ovarian cancer (OR = 0.68; 95 % CI 0.53–0.88), while no association was observed for the solitary use of progestin-only pills (OR = 0.97; 95 % CI 0.45–2.14). The mixed use of combined and progestin-only pills also decreased the risk (OR = 0.50; 95 % CI 0.28–0.87) (Table 4).

Table 4
Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the association between different measures of oral contraceptive use and risk of ovarian cancer

* * *

The risk of ovarian cancer associated with oral contraceptive use according to hormone potency was assessed in 395 case women and 1,039 control women with a specified use of combined oral contraceptives (Table 5). These women used the same brand of combined oral contraceptives in all the periods of oral contraceptive use. The risk decreased with the use of both high- and low-potency progestins compared with never use of combined

oral contraceptives, although the association was statistically significant only among users of high-potency progestins (OR = 0.59; 95 % CI: 0.42–0.83). Likewise, the associations between the use of both high- and low-potency estrogen formulations and ovarian cancer risk revealed a protective effect of estrogen, although the effect was statistically significant only for users of high-potency estrogens (OR = 0.60; 95 % CI: 0.44–0.83). In analyses for users of combined oral contraceptives only, no statistically significant difference in ovarian cancer risk was found between the use of high-potency progestin and the use of low-potency progestin or between the use of high-potency estrogen and the use of low-potency estrogen. When combined oral contraceptives were categorized into four groups based on different combinations of high- or low-potency estrogen and progestin, a statistically significantly lower risk of ovarian cancer was found for users of high-estrogen/high-progestin formulations than for never users (OR = 0.57; 95 % CI 0.41–0.80). For the other combinations of high- and low-potency estrogen and progestin, for which there were a limited number of cases and controls, no convincing associations with risk of ovarian cancer were seen. Furthermore, when users of high-estrogen/high-progestin formulations served as the reference group, no major differences were found among the four groups.

Table 5
Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of ovarian cancer among women with a specified use of combined oral contraceptives, according to hormone potency

* * *

Table 6 shows the associations between cumulative intake of estrogen and progestin and duration of oral contraceptive use, respectively, and ovarian cancer risk among 446 cases and 1,305 controls with a specified use of oral contraceptives. Cumulative estrogen intake was associated with a statistically significantly decreased risk of ovarian

cancer (OR = 0.82; 95 % CI 0.67–0.99, per 100 mg estrogen), while cumulative progestin intake did not influence the risk. Furthermore, increasing duration of oral contraceptive use decreased the risk (OR = 0.95; 95 % CI 0.92–0.98, per year of use). When the three variables were mutually adjusted, each extra year of oral contraceptive use decreased the risk of ovarian cancer by 6 % (94 % CI 0.89–0.98, per year of use), but the protective effect of cumulative estrogen intake was no longer present.

The last result is presented in more detail in Fig. 1, which depicts the risk of ovarian cancer (OR) as a function of (a) cumulative estrogen intake, (b) cumulative progestin intake, and (c) duration of oral contraceptive use among women with a specified use of oral contraceptives. The curves for cumulative estrogen and progestin intake show no apparent association with ovarian cancer risk, while the curve for duration of oral contraceptive use shows that the risk decreased with duration of use independently of the cumulative intake of estrogen and progestin.

As shown in Table 7, associations between cumulative estrogen and progestin intake and duration of oral contraceptive use, respectively, and risk of histological types of ovarian cancer were generally similar to those observed for overall ovarian cancer. Because of smaller numbers of cases, however, the risk estimates for the different histological types (mucinous, clear cell, and other ovarian cancers in particular) were less precise than those for overall ovarian cancer. The association with duration of use was strongest for ovarian cancer of serous and endometrioid origin, but absent for mucinous ovarian cancer.

Table 6
Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of ovarian cancer according to cumulative estrogen and progestin intake and duration of oral contraceptive use among women with a specified use of oral contraceptives

* * *

Fig 1

* * *

Discussion

In this population-based case–control study, we find that the use of combined oral contraceptives and the mixed use of combined and progestin-only pills decrease the risk of ovarian cancer; no association is found with exclusive use of progestin-only pills. In relation to the effect of hormone potency, no major differences in risk of ovarian cancer is found between the use of combined oral contraceptives with high- and low-potency estrogen and progestin. We find no effect of cumulative progestin intake but do observe a decreased risk of ovarian cancer with increasing cumulative estrogen intake and increasing duration of oral contraceptive use. When cumulative progestin intake, cumulative estrogen intake, and duration of oral contraceptive use are mutually adjusted, however, the effect of cumulative estrogen is no longer present, while the decreased risk associated with duration of oral contraceptive use persists. Thus, our results indicate that the protective effect of oral contraceptives against ovarian cancer is independent of the estrogen and progestin dose and may be sufficiently explained by duration of anovulation.

The decreased risk of ovarian cancer associated with the use of combined oral contraceptives observed in this study confirms previous findings [15, 16, 21]. Our observation that each extra year of oral contraceptive use decreased the risk of ovarian cancer by 6 % is in agreement with other studies, which showed risk reductions of 5–8 % per year of use [12, 14, 21, 22].

Few studies have examined the effect of high- and low-potency contraceptives on ovarian cancer risk, and the findings were ambiguous. In line with our results, some studies found that the protective effect was independent of the potency of estrogen [9, 11, 13]. In contrast, one case–

control study [10] found less protection against ovarian cancer with low-estrogen formulations than with high-estrogen formulations, while another case–control study [12] showed greater protection from low- than from high-estrogen pills. However, in none of these studies were the differences statistically significant. In a recent review, Cibula et al. [23] concluded that the protective effect of oral contraceptives against ovarian cancer risk did not differ between users of high- and low-estrogen formulations.

With regard to the progestin component of oral contraceptives, we found no difference in the protective effect of those with high- and low-potency progestins. Other studies have found associations between progestin potency and ovarian cancer risk. In an experimental study, Rodriguez et al. [7] found that progestin induced apoptosis in the ovarian epithelium of macaques, leading to the hypothesis that the progestin component may be the major factor in the protective effect of combined oral contraceptives. In line with this hypothesis, supported in a review by Risch [5], two case–control studies showed that oral contraceptives containing high-dose progestin conferred greater protection against ovarian cancer than those with low-dose progestin [14, 15]. Conversely, another case–control study found that the use of low-progestin formulations reduced the risk more substantially than the use of high-progestin formulations [16].

Table 7
Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of histological types of ovarian cancer according to cumulative estrogen and progestin intake and duration of oral contraceptive use among women with a specified use of oral contraceptives

* * *

The primary mechanism of action of combined oral contraceptives is prevention of ovulation [23] by inhibiting the production and secretion of follicle-stimulating and

luteinizing hormones by steroid feedback on the pituitary gland. The estrogen component inhibits the release of follicle-stimulating hormone and thus suppresses the development of the dominant ovarian follicle, while the progestin component inhibits the release of luteinizing hormone thereby preventing ovulation. Progestin-only pills partly suppress ovulation, but the anovulatory effect varies. However, the progestins also thicken the cervical mucus and decrease tubal motility, thereby creating a difficult passage for sperm. Furthermore, progestins thin the endometrium, resulting in tissue less receptive for implantation [24–26].

We are the first to report on the association between cumulative lifetime dose of estrogen and progestin and risk of ovarian cancer. The risk of ovarian cancer decreased with increasing duration of oral contraceptive use but not with cumulative estrogen and progestin intake, supporting the "incessant ovulation" theory, according to which chronic ovulation contributes to ovarian cancer by causing repeated trauma of the ovarian surface epithelium [3]. This suggests that, as long as the dose of estrogen and progestin is sufficient to cause anovulation, a higher intake of estrogen or progestin will not further protect against ovarian cancer. Therefore, the protective effect of oral contraceptive use on ovarian cancer risk is likely to be sufficiently explained by duration of anovulation. This conclusion is also supported by our finding that the risk was not decreased by exclusive use of progestin-only pills, although based on small numbers. Furthermore, Fig. 1 demonstrated that increasing duration of oral contraceptive use decreased the risk of ovarian cancer independently of the cumulative intake of estrogen and progestin. Thus, our results do not support the concern raised by Ness et al. [9] that oral contraceptives with low doses of estrogen and progestin might be less protective against ovarian cancer than high-dose formulations.

A potential role of gonadotropins in the pathogenesis of ovarian cancer has also been suggested. Gonadotropins are

synthesized in the anterior pituitary gland and may be involved in the transformation and progression of normal ovarian surface epithelium to neoplastic ovarian surface epithelium [4]. As oral contraceptive use results in reduced exposure to gonadotropins due to the steroid feedback on the pituitary gland, oral contraceptives may also protect against ovarian cancer by suppressing gonadotropin levels.

Previous studies have not examined the association between the hormone content of oral contraceptives and different histological types of ovarian cancer but only assessed the effect of ever/never use and duration of oral contraceptive use. In our study, separate analyses for the major histological types of ovarian cancer showed that duration of oral contraceptive use was associated with decreased risks of mainly serous and endometrioid ovarian cancer, with no reduction in risk of mucinous ovarian cancer. Compatible with our findings, four studies [27–30] found that ever use of oral contraceptives decreased the risk of non-mucinous ovarian cancers only. In contrast, three other studies [31–33] found no difference in the protective effect of oral contraceptive use against the major histological types.

Our study has several strengths, including the population-based design, the size of the study population, the higher response rates than in most other case–control studies, and the ability to control for known risk factors for ovarian cancer. In addition, we were able to assess for the first time the impact of cumulative intake of estrogen and progestin on ovarian cancer risk. The study also has some limitations. An important one is the retrospective nature of the study and the possible introduction of recall bias. Our information on oral contraceptive use was, however, based on personal interviews that included explicit questions, rather than self-completed questionnaires, and this may have reduced recall bias. Furthermore, the use of a life-event calendar and photographs increased the reliability of the information. Nevertheless, a large proportion of oral contraceptive users

were excluded from the analyses because they did not remember the brand they had used, which may have introduced some degree of differential misclassification, especially in the analyses concerning the risk of ovarian cancer associated with oral contraceptive use according to hormone potency, and these results should therefore be interpreted with caution. Although we had reasonably high participation rates, we cannot rule out the possibility that the exposure to oral contraceptives of the women who did not participate differed from that of the women who participated. Thus, some degree of selection bias cannot be excluded. Furthermore, as most of the formulations used by the women in our study contained both progestin and estrogen, we were unable to separate the effects of the two hormones on ovarian cancer risk completely. Some of the results in the present study were limited by imprecise risk estimates due to a low number of study subjects, e.g., the results concerning the use of progestin-only pills, the results concerning low-estrogen/high-progestin use and the results on low-estrogen/low-progestin use. Lastly, as the MALOVA study was conducted in the 1990s among women who had primarily used older-generation pills with high doses of estrogen and progestin, we were unable to evaluate the effect of more recent formulations. Nevertheless, as we found that the protective effect of oral contraceptives may be independent of the doses of progestin and estrogen, this is not likely to have affected the generalizability of our results.

In conclusion, we found no independent effect of cumulative intake of estrogen and progestin on ovarian cancer risk after taking into account duration of use; thus, the protective effect of oral contraceptives appears to be due mainly to inhibition of ovulation. This suggests that, as long as the doses of estrogen and progestin are sufficient to cause anovulation, a higher intake of estrogen or progestin will not add greater protection, although further studies are needed to confirm this finding. Lastly, the benefits of oral

contraceptive use should be balanced against possible side effects, such as venous thromboembolism [34] and potentially increased risks of cancers of the cervix and the central nervous system [35].

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Conflict of interest The authors declare that they have no conflict of interest.

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APPENDIX D

REVIEW

ORAL CONTRACEPTIVE PILLS AS PRIMARY PREVENTION FOR OVARIAN CANCER

A SYSEMATIC REVIEW AND META-ANALYSIS

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OBJECTIVE: To estimate the overall reduction in ovarian cancer risk associated with the use of oral contraceptive pills (OCPs) and whether reduction in risk is affected by specifics of OCP use, such as formulation or duration of use.

DATA SOURCES: We searched PubMed, Embase, the Cochrane Database of Systematic Reviews, and Clinical-Trials.gov for studies published from January 1990 to June 2012, with primary analysis of studies published since January 2000.

METHODS OF STUDY SELECTION: We reviewed 6,476 citations. We included English-language controlled studies with human participants reporting a quantitative association between exposure to OCPs (in which the explicit or implicit indication for OCP use was prevention of pregnancy or ovarian cancer) compared with no use of OCPs. Two investigators independently reviewed the title and abstract and full-text of articles for inclusion or exclusion decision; discordant decisions were resolved by team review and consensus.

TABULATION, INTEGRATION, AND RESULTS: Fifty-five studies met inclusion criteria. A random-effects meta-analysis of 24 case-control and cohort studies showed significant reduction in ovarian cancer incidence in ever-users compared with never-users (odds ratio 0.73, 95% confidence interval 0.66–0.81). There was a significant duration–response relationship, with reduction in

incidence of more than 50% among women using OCPs for 10 or more years. The lifetime reduction in ovarian cancer attributable to the use of OCPs is approximately 0.54% for a number-needed-to-treat of approximately 185 for a use period of 5 years.

CONCLUSION: Significant duration-dependent reductions in ovarian cancer incidence in the general population are associated with OCP use.

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Ovarian cancer is the eighth most common cancer in women (annual age-adjusted incidence 12.3/ 100,000) but is the fifth leading cause of cancer death (8.2/100,000).[1] Despite advances in

primary treatment, the mortality rate for ovarian cancer remains the highest among the gynecologic malignancies. Because ovarian cancer typically presents at a later stage (with concomitant higher mortality) than other common cancers,[1] there has been intense interest in developing effective screening strategies. Unfortunately, screening studies to date have not demonstrated reductions in mortality and false-positive rates have been high,[2–8] leading the U.S. Preventive Services Task Force to recommend against screening the general population for ovarian cancer (a "D" recommendation).[9]

Oral contraceptive pills (OCPs) represent a potentially promising primary prevention strategy for ovarian cancer. Several large pooled analyses suggest that OCPs confer a protective effect on ovarian cancer risk, with a risk reduction of up to 50% with long-term OCP use.[10–13] The largest pooled analysis to date estimates that OCP use already has prevented 200,000 cases of ovarian cancer and 100,000 deaths from this disease worldwide.[10]

We performed a systematic review and meta-analysis, sponsored by the Agency for Healthcare and Research Quality and the Centers for Disease Control and Prevention, to quantify the potential benefits and risks of OCP use for the purpose of reducing the incidence of ovarian cancer.[14] Although the current article deals with the use of OCPs for ovarian cancer prophylaxis in the general population, we also have examined the use of OCPs in subsets of women who are at elevated risk for development ovarian cancer (separate manuscript in preparation). In this article, we address the effect of OCPs on ovarian cancer risk in the general population and examine relationships between specific characteristics of OCP use and ovarian cancer incidence and mortality. Specifically, this article focuses on the following two key questions: 1) What is the effectiveness of combined (estrogen and progestin containing) and progestin-only OCPs for reducing the risk of ovarian cancer?; and 2) Do specifics of OCP use (e.g., dose or formulation, age at initiation, duration of use) affect the relative risk of development of ovarian cancer?

SOURCES

This systematic review and meta-analysis was compliant with Meta-Analysis of Observational Studies in Epidemiology guidelines (http://edmgr.ovid.com/ong/ accounts/moose.pdf). In collaboration

with an experienced librarian, we conducted searches of PubMed, Embase, the Cochrane Database of Systematic Reviews, and ClinicalTrials.gov to identify relevant literature published on or after January 1, 1990. Restricting the search to 1990 forward increases the likelihood that the types of OCPs used by the women in the studies we retrieved were similar to those currently available, and thus aids in maximizing the generalizability and clinical relevance of the results (complete search details are provided in Appendix 1, available at http://links.lww.com/AOG/A380). We supplemented the electronic searches with a manual search of citations from key review articles described in the full Agency for Healthcare and Research Quality report.[14]

STUDY SELECTION

Inclusion and exclusion criteria were developed based on population, intervention, comparators, outcomes, timing, and setting criteria. Study inclusion criteria were as follows: study includes women using OCPs for contraception or for primary prevention of ovarian cancer; study includes a comparison group consisting of no use of combination or progestin-only OCPs (either no contraceptive method or contraceptive methods other than combination or progestin-only OCPs); study reports a quantitative association between exposure to OCPs and ovarian cancer incidence or mortality; controlled studies (randomized trials, cohort studies, case-control studies) or pooled patient-level meta-analyses; sample size for nonrandomized studies was 100 or more participants; study is peer-reviewed and written in the English language; and study was published on or after January 1, 1990. Study exclusion criteria were as follows: study only reports outcomes related to the use of OCPs for postcoital contraception or in specialized populations such as women immediately after termination of pregnancy, or women receiving assisted reproductive technologies; or publication type is editorial, review, or letter to the editor.

Two investigators independently reviewed the titles and abstracts of retrieved articles for potential relevance to the key questions. Articles included by either reviewer were promoted to full-text screening, and two investigators independently reviewed each article and indicated an include or exclude decision for data abstraction. Based on clinical and methodologic expertise, pairs of

researchers were assigned to abstract data from the eligible articles. One researcher abstracted the data, and the second reviewed the completed abstraction form alongside the original article to check for accuracy and completeness. Disagreements at any stage were resolved by review and discussion among investigators.

We evaluated the quality of individual studies using the approach described in Agency for Healthcare and Research Quality's *Methods Guide for Effectiveness and Comparative Effectiveness Reviews*.[15] Summary ratings of good, fair, and poor were assigned to each study. Quality ratings for individual articles within study groupings could differ based on the quality of reporting, the evaluated outcomes, and the statistical and analytical methods used in the articles.

We determined the feasibility of completing a quantitative synthesis for a given outcome based on the volume of relevant literature, the conceptual homogeneity of the studies, and the completeness of the reporting of results. Meta-analysis was particularly challenging because all of the literature was observational. There was substantial heterogeneity in the types of exposures (e.g., OCP formulation), timing of exposures (e.g., intermittent use of OCPs over the course of a reproductive lifetime), and how exposures were measured and reported (ever-users compared with never-users or current users compared with noncurrent users, duration of use as a continuous or categorical variable). Outcome measures considered for the meta-analyses were disease-specific incidence, disease-specific mortality, and disease-specific survival. We performed meta-analyses on the following relationships: ever use of OCPs, duration of OCP use, age at first OCP use, time since last OCP use, and OCP formulation (estrogen, progestin).

To meta-analyze a specific association, we required at least three comparable individual studies. Studies also were required to report odds ratios (ORs) and 95% confidence intervals (CIs) or to provide sufficient data to allow us to calculate the 95% CI. We performed meta-analyses using Comprehensive Meta-Analysis 2.[16] All analyses were performed using a random-effects model. We included pooled analyses in our meta-analyses if all three of the following conditions were met: none of the individual studies

included in the pooled analysis already had been included for meta-analysis; at least half of the studies in the pooled analysis were published on or after January 1, 2000; and data in the pooled analyses were presented such that their inclusion in the current meta-analysis was feasible.

For the primary ever-use of OCP meta-analysis, we excluded studies that reported effects for only a particular subpopulation (e.g., studies reporting ORs only for women with a BRCA mutation) and not for the general population. Studies that reported ever-use of OCP ORs for mutually exclusive subpopulations (e.g., mucinous and nonmucinous tumors) were included in the meta-analysis, and results for the subpopulations were combined.

Evaluation of clinical relationships for which multiple temporal stratifications were possible, such as duration of OCP use, age at first OCP use, and time since last OCP use, required creation of the following additional simplifying assumptions: to facilitate identification of any existing duration–response effects, we included only studies that reported ORs for at least three different time intervals; and we required that the ORs were reported relative to no OCP use.

The challenge of performing a meta-analysis on duration of OCP use is that individual studies reported the ORs for different duration intervals. We assumed that the logarithm of each OR could be described by a linear model. The model included a random-effects term, sigma squared (o^2), as well as terms for the following time point intervals: 1–12 months; 13–60 months; 61–120 months; and more than 120 months. We then used independent variables to create the time period desired. For example, if the first interval had been from 1 to 36 months, the vector of independent variables would be (one third, two thirds, 0, 0, 0). This would reflect that one third of the patients in the interval were in the 1-month to 12-month interval and two thirds of the patients were in the 13-month to 60-month interval. Using this methodology, any interval could be described. The model was fitted using SAS PROC NLMIXED with “subject” set to the particular study.

Methods analogous to those used for the duration analyses were used for other temporal relationships. For age at first use, we assumed there were four different intervals: younger than 20 years

of age; 20–24 years of age; 25–30 years of age; and older than 30 years of age. For time since last OCP use, we used intervals of 0–10 years, 10–20 years, 20–30 years, and more than 30 years.

Studies were included in the meta-analysis examining the effect of different estrogen formulations if they reported the effect of low-dose estrogen-containing OCPs, high-dose estrogen-containing OCPs, or both on ovarian cancer incidence and included the definition of low-dose and high-dose estrogen.[17,18] For studies that presented estrogen dose results stratified by low or high progestin dose, ORs for groups with identical estrogen doses were combined across progestin arms using an inverse weighted meta-analysis. To compare high-dose with low-dose estrogen, we included those studies that had ORs for each with "never use" as a reference category and divided the high-dose OR by the low-dose OR. This has the effect of canceling out the never-use category. All analyses were performed using a random-effects model. Studies were included in the meta-analysis examining the effect of different progestin formulations if they reported the effect of low-dose progestin, high-dose progestin, or both on ovarian cancer incidence and presented an established reference for determination of progestin potency. These meta-analyses were analogous to those performed for estrogen dose.

To maximize the probability that members of the study populations used contemporary OCP formulations, we constrained the primary analyses to studies published from January 2000 to June 2012. We then conducted sensitivity analyses that included the older data from articles with publication dates beginning January 1990. This approach allowed us to compare the primary analysis results with those obtained from a longer date range and studies that may have included older formulations of OCPs. We conducted additional sensitivity analyses in which we repeated the meta-analyses excluding studies not conducted at least partially within the United States and excluding poor-quality studies.

We assessed the potential for publication bias using the following three methods: the funnel plot; which looks for an uneven number of studies falling to the left or right of the funnel; Begg and Mazumdar test based on the rank correlation between the observed effect sizes and observed standard errors; and Egger regression intercept, which is similar to that of Begg and

Mazumdar but uses actual values instead of ranks. We performed the calculations using Biostat Comprehensive Meta-Analysis 2.[16]

RESULTS

In the literature search (Fig. 1) conducted for the full Agency for Healthcare and Research Quality report, 6,476 unique citations were identified. After exclusions, 55 studies (92 articles) remained that reported ovarian cancer outcomes relevant to this article. The full list of included articles is provided in Appendix 2 (available at http://links.lww.com/AOG/A381); relationships between articles are detailed in Appendix C of the Agency for Healthcare and Research Quality report.[14]

Seventeen case-control studies (11 good quality, six fair quality, and one poor quality) representing 10,031 cases and 21,025 controls met criteria for the meta-analysis examining ever-use compared with never-use of OCPs (Appendix 3, available at http://links.lww.com/AOG/A382). See Appendix 4 (available at http://links.lww.com/AOG/A382) for ever-use compared with never-use data from all studies. The OR for meta-analysis of the case-control studies was 0.72 (95% CI 0.64–0.81), which demonstrates an almost 28% reduction in ovarian cancer risk in women who have ever used OCPs (Fig. 2A). The cohort meta-analysis included seven studies (three good quality, three fair quality, one poor quality) (Appendix 3, available at http://links.lww.com/AOG/A382), of which four included 625,999 participants and the other three included 3,981,072 person-years of follow-up. The OR for the cohort meta-analysis was 0.75 (95% CI 0.62–0.92), indicating a 25% reduction in ovarian cancer risk in women who have ever used OCPs (Fig. 2B). In a combined meta-analysis of all 24 case-control and cohort studies, the OR for ever-use compared with never-use of OCPs was 0.73 (95% CI 0.66–0.81). Based on an estimated lifetime risk of ovarian cancer of 1.38%,[19] an estimated lifetime prevalence of ever-use of OCPs of 83%,[20] and the estimates from our meta-analysis, the lifetime reduction in ovarian cancer attributable to the use of OCPs is approximately 0.54% for a number needed to treat of approximately 185. The duration of exposure to OCPs among ever-users is, by definition, the mean duration of use, for which the best estimate is approximately 4.5 years.[21]

* * *

Fig. 1. Literature flow diagram. *Description and length of oral contraceptive pill use not required for studies reporting ovarian cancer outcomes or conducted in a population using oral contraceptives for primary prevention of ovarian cancer. †Comparisons between oral contraceptive formulations acceptable for articles reporting venous throm-boembolism, stroke, or myocardial infarction.

Havrilesky. OCPs as Primary Prevention for Ovarian Cancer. Obstet Gynecol 2013.

Fifteen studies (seven good quality, seven fair quality, one poor quality) were included in a meta-analysis examining the effect of duration of OCP use on ovarian cancer incidence (Appendix 5, available at http://links.lww.com/AOG/A382). Of these, 10 were case-control studies (6,901 cases and 15,999 controls) and five were cohort studies (524,463 participants in three of the studies and 3,493,072 person-years in the other two studies).

* * *

Fig. 2. Forest plots describing the relationship between ever use compared with never use of oral contraceptive pills (OCPs) and ovarian cancer incidence. **A.** Case-control studies. **B.** Cohort studies. There was evidence of extreme heterogeneity for the analysis in (**A**), with a Q-value of 49.98 for 18 degrees of freedom (*P*<.001). There was evidence of extreme heterogeneity for the analysis in (**B**), with a Q-value of 23.63 for 6 degrees of freedom (P=.001). For the combined analysis of all study designs, there was evidence of extreme heterogeneity, with a Q-value of 77.79 for 26 degrees of freedom (P<.001). CI, confidence interval.

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Table 1 and Figure 3 show the ORs for the meta-analysis of duration of OCP use. These findings indicate a significant duration–response relationship between OCP use and ovarian cancer incidence, with a reduction in ovarian cancer incidence of more than 50% among women using OCPs for 10 or more years.

Six studies (four good quality, two fair quality) were included in the primary meta-analysis examining the effect of age at first OCP use on ovarian cancer incidence (Appendix 6, available at http://links.lww.com/AOG/A382). Of these, five were case-control

studies (3,552 cases and 4,713 controls) and one was a cohort study (103,552 participants). The results show a relatively strong relationship between younger age at first use and lower ovarian cancer incidence, although CIs overlap (Table 1). Most studies examining age did not control for duration of use, which is a potential confounder and reduces the strength of this finding. Two pooled analyses reported on age at first use, with none reporting significant trends.

Table 1. Estimated Odds Ratios for Ovarian Cancer

* * *

Eight studies (four good quality, four fair quality) were included in the meta-analysis examining the effect of time since last OCP use on ovarian cancer incidence (Appendix 7, available at http://links.lww.com/AOG/A382). Five were case-control studies (3,606 cases and 7,759 controls) and three were cohort studies (198,704 participants and 1,083,000 person years). None of the three pooled analyses reporting on time since last OCP use met inclusion criteria for meta-analysis.

* * *

Fig. 3. Relationship between duration of oral contraceptive pill use and ovarian cancer incidence. There is no evidence of heterogeneity. The estimated value of sigma (0) is 0.15.

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Table 1 lists the ORs for the meta-analysis of time since last OCP use. The individual ORs show significant associations between OCP use and ovarian cancer incidence among women who used OCPs within the past 20 years, but not for those with a longer time since last use. A test for differences between the four ORs was significant (P=.002). We then used the midpoint of each interval as the estimate of the time since last use for each subgroup. The slope from this model was highly significant (P=.001), indicating a stronger protective effect with a shorter time since last OCP use.

Six studies (five good quality, one fair quality) were included in the meta-analysis examining the effect of estrogen formulation on ovarian cancer incidence (Appendix 8, available at http://links.lww.com/AOG/A382). All were case-control studies, representing 2,607 cases and 6,400 controls. The definition of a low-estrogen OCP formulation varied among the six studies included in the

meta-analysis, with three studies using a cut-off of 35 micrograms of estradiol, two studies using a cut-off of 50 micrograms of estradiol, and one study reporting results for three separate doses of estradiol (20–34 micrograms, 35–44 micrograms, and 45 micrograms or more).

Five studies calculated ORs separately for high-dose or low-dose estrogen-containing OCPs compared with never use. Of these, two studies presented estrogen dose results stratified by low or high progestin dose. One study calculated a direct OR comparing high-dose with low-dose estrogen OCP use. When this was combined with the other five included studies, the OR comparing high-dose with low-dose estrogen was 1.25 (CI 0.95–1.64) (Fig. 4A). These results do not suggest a relationship between estrogen dose and ovarian cancer incidence.

Four studies (all good quality) were included in the meta-analysis examining the effect of progestin formulation on ovarian cancer incidence (Appendix 8, available at http://links.lww.com/AOG/A382). All were case-control studies, representing 2,049 cases and 5,479 controls. The four included studies classified progesterone potency based on a subnuclear vacuolation assay and a delay of menses test,[22] and defined low-dose progestin OCPs as those containing a relative potency cut-off of 0.2 mg norgestrel or less. Three of these studies also stratified progestin results based on low-estrogen or high-estrogen dose. One study calculated a direct OR comparing high-dose with low-dose progestin OCP use. The random-effects meta-analysis of all four studies reveals an OR for ovarian cancer incidence comparing high-dose progestin OCPs with low-dose progestin OCPs of 0.86 (95% CI 0.60–1.21) (Fig. 4B). These results do not support a relationship between OCP progestin dose and ovarian cancer incidence.

* * *

Fig. 4. Forest plots describing the relationship between high-dose compared with low-dose oral contraceptive pill formulations and ovarian cancer incidence. **A**. Estrogen formulations. There was some evidence of heterogeneity, with a Q-value of 10.611 for 5 degrees of freedom (*P*=.06). **B**. Progestin formulations. There was some evidence of heterogeneity, with a Q-value of 7.52 for 3 degrees of freedom (*P*=.057). CI, confidence interval.

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Three studies (all fair-quality cohort studies) were identified that examined the effect of OCP use on ovarian cancer mortality (Appendix 9, available at http://links.lww.com/AOG/A382). Two were population-based cohort studies (representing a total of 46,112 participants and 602,700 reported person-years) and assessed death from ovarian cancer as a primary outcome among ever-users compared with never-users of OCPs. Both reported a significant reduction in ovarian cancer mortality among OCP users that was similar in magnitude and direction to the reduction in incidence discussed previously. The third study identified a cohort of women with ovarian cancer and subsequently compared survival outcomes between OCP users (n=310) and nonusers (n=366), with nonsignificant findings.

We conducted sensitivity analyses in which we repeated the meta-analyses in three ways: including studies published from 1990 forward, excluding studies not conducted at least partially within the United States, and excluding poor-quality studies. None of our findings were changed substantively with these analyses.

The strength of evidence for each outcome is described in Appendix 10 (available at http://links.lww.com/AOG/A383). Because no randomized controlled trials were included in our analysis, the risk of bias was categorized as medium at best and high if other possible sources of bias were identified. With regard to directness of evidence, relationships between high and low steroid hormone doses and ovarian cancer incidence were considered to be indirect based on the use of "never use of OCP" as the reference category in those studies.

We graded the strength of evidence for relationships between ever use of OCPs and ovarian cancer incidence and mortality in the general population as moderate. The relationship between duration of OCP use and ovarian cancer incidence also was graded as moderate. The strength of evidence for the remaining relationships was graded as low.

We performed publication bias analyses as described in the methods of study selection (Appendix 11, available at http://links.lww.com/AOG/A384). We found no evidence of

publication bias for the Figure 2 meta-analyses assessing ever use compared with never use of OCPs and ovarian cancer incidence. For the meta-analyses examining the relationship between high-dose compared with low-dose OCP formulations and ovarian cancer incidence (Fig. 4), we identified a suggestion of publication bias for estrogen formulation and no evidence of publication bias for progestin formulation. The temporal analyses (age, duration, time since last use) are not amenable to these bias assessments.

CONCLUSION

In this systematic review and meta-analysis, we found that OCP use was associated with a decreased incidence of ovarian cancer (OR 0.73, 95% CI 0.66–0.81), with results from two large cohort studies showing a concomitant decrease in mortality. There is a positive relationship between the duration of OCP use and the degree of the protective effect. These findings are consistent with previous pooled analyses,10,12,13 which reported ORs for ever use compared with never use of OCP between 0.60 and 0.73; these previous analyses similarly identified a relationship between longer duration of OCP use and lower incidence of ovarian cancer. We estimate the lifetime reduction in ovarian cancer attributable to the use of OCPs to be approximately 0.54%.

The results of our meta-analysis show a strong relationship between duration of OCP use and ovarian cancer incidence (Fig. 3). Women who use OCPs for 10 or more years show a reduction in ovarian cancer incidence of more than 50%. Previous pooled analyses are consistent with these findings.[10,12,13] Although our reported OR comparing OCP use for less than 12 months with never use was not statistically significant, our duration analysis suggests that there is no time threshold for OCP effectiveness, and the duration– response relationship likely starts as soon as a woman commences OCP use.

Regarding age at first OCP use, the ORs also appear to show a clearly positive relationship. This suggests that the earlier a woman begins using OCPs, the greater the reduction in ovarian cancer incidence. However, it is not possible to differentiate the effects of age at first use from the effects of duration of use. Our findings are consistent with the largest pooled analysis[10] and are not unexpected, because the earlier a woman starts using OCPs, the longer the potential duration of use. The protective effect of OCPs appears to

attenuate with increasing time since last use, again consistent with the findings of the Collaborative Group.[10] Although the data available at the study level preclude estimation of the joint effect of duration and time since last use, stratified analysis of the pooled individual data by the Collaborative Group suggest that the magnitude of protection with increased duration is greater than the attenuation with time since last use.

In an effort to enhance the applicability of these findings to contemporary OCP formulations and dosages, we included only studies published since January 1, 2000, for the primary analysis and since 1990 for the sensitivity analysis. However, our primary meta-analysis produced an OR comparing ever use with never use (0.73) similar to ORs reported in the sensitivity analysis (0.72) and pooled analyses that included older studies. This suggests that current OCP formulations may have an effectiveness similar to older formulations in reducing the incidence of ovarian cancer. This is supported by our finding that the relative estrogen and progestin doses in OCPs do not appear to have an effect on ovarian cancer incidence. However, given that the age of peak incidence of ovarian cancer is in a woman's early 60s, even more recent publications do not capture the potential long-term effect of formulations introduced in the past 20 years.

Another limitation of the current analysis is the degree of generalizability of the included studies to clinical decision-making. The included studies almost never specifically reported the reasons for OCP use. It is likely that most women used OCPs for contraception or to treat conditions such as dysmenorrhea, whereas few used them for ovarian cancer prophylaxis.

The main limitation of our analysis is the lack of randomized, prospective trials examining the preventive effect of OCPs on ovarian cancer, raising the potential for bias. The most common study design within our primary analyses (ever compared with never use) was case-control (71%), with a minority being cohort studies (29%). The point estimate for case-control studies (0.72) was lower than for cohort studies (0.75), suggesting that there may be some residual confounding in the case-control studies. Likewise, although the majority of studies were rated as being of good or fair quality (92%), there was marked inconsistency across studies, particularly in the methods for adjustment of confounding.

The observed association between OCP use and reduced ovarian cancer risk fulfills many of the classic criteria for causal inference in epidemiology,[23] including strength of association, consistency across studies, temporality, a biological gradient, biological plausibility, and coherence. However, the potential for the limitations discussed to lead to biased estimates of the effects of OCP require considerable caution when using the results for clinical decision-making.

The current literature consistently shows a statistically significant reduction in ovarian cancer risk among women with a history of OCP use, with greater reductions in risk with longer duration of use. Although the overall body of evidence is supportive of the beneficial effects of OCPs on ovarian cancer, there remains potential for unmeasured bias. Continued evaluation of effects by dose of OCPs is warranted, especially because some of the older women included in studies published since 1990 would have used OCPs when higher doses were more commonly prescribed. Further research also is needed to sort out the relative importance of the duration and timing of use of OCPs. Understanding the combined effects of timing and duration is particularly important for making recommendations to women of mid-to-late reproductive age who are considering OCP use for ovarian cancer prevention but not necessarily for contraception. To facilitate future systematic reviews, one step would be to standardize the categories and descriptive statistics for reporting results. Although particular categorization choices may be best-suited for analyzing individual studies on the basis of study design and characteristics of a given population, reporting of standardized results— perhaps as an appendix to the main analysis—would greatly improve the ability to combine published results in meta-analysis.

This systematic review and meta-analysis confirms previous large studies in demonstrating a duration-dependent protective effect of OCP use on the incidence of ovarian cancer. The inherent limitations of our analysis prevent us from making recommendations regarding the preferred OCP formulation and dose or the optimal time period of use for ovarian cancer prevention. However, consideration of this benefit can be made along with careful consideration of the other known risks and benefits of OCP use.

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Obstetrics & Gynecology

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APPENDIX E

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OVARIAN CANCER AND ORAL CONTRACEPTIVES: COLLABORATIVE REANALYSIS OF DATA FROM 45 EPIDEMIOLOGICAL STUDIES INCLUDING 23 257 WOMEN WITH OVARIAN CANCER AND 87303 CONTROLS

Collaborative Group on Epidemiological Studies of Ovarian Cancer*

Summary

Background Oral contraceptives were introduced almost 50 years ago, and over 100 million women currently use them. Oral contraceptives can reduce the risk of ovarian cancer, but the eventual public-health effects of this reduction will depend on how long the protection lasts after use ceases. We aimed to assess these effects.

Methods Individual data for 23 257 women with ovarian cancer (cases) and 87 303 without ovarian cancer (controls) from 45 epidemiological studies in 21 countries were

checked and analysed centrally. The relative risk of ovarian cancer in relation to oral contraceptive use was estimated, stratifying by study, age, parity, and hysterectomy.

Findings Overall 7308 (31%) cases and 32 717 (37%) controls had ever used oral contraceptives, for average durations among users of 4.4 and 5.0 years, respectively. The median year of cancer diagnosis was 1993, when cases were aged an average of 56 years. The longer that women had used oral contraceptives, the greater the reduction in ovarian cancer risk ($p<0.0001$). This reduction in risk persisted for more than 30 years after oral contraceptive use had ceased but became somewhat attenuated over time—the proportional risk reductions per 5 years of use were 29% (95% CI 23-34%) for use that had ceased less than 10 years previously, 19% (14-24%) for use that had ceased 10-19 years previously, and 15% (9-21%) for use that had ceased 20-29 years previously. Use during the 1960s, 1970s, and 1980s was associated with similar proportional risk reductions, although typical estrogen doses in the 1960s were more than double those in the 1980s. The incidence of mucinous tumours (12% of the total) seemed little affected by oral contraceptives, but otherwise the proportional risk reduction did not vary much between different histological types. In high-income countries, 10 years use of oral contraceptives was estimated to reduce ovarian cancer incidence before age 75 from 1.2 to 0.8 per 100 users and mortality from 0.7 to 0.5 per 100; for every 5000 woman-years of use, about two ovarian cancers and one death from the disease before age 75 are prevented.

Interpretation Use of oral contraceptives confers long-term protection against ovarian cancer. These findings suggest that oral contraceptives have already prevented some 200 000 ovarian cancers and 100 000 deaths from the disease, and that over the next few decades the number of cancers prevented will rise to at least 30 000 per year.

Introduction

Use of oral contraceptives has long been known to reduce the incidence of ovarian cancer.[1].[2] Because ovarian cancer is not common in young women and the incidence increases with age, the public-health effect of this reduction depends mainly on how much the reduced risk persists decades after oral contraceptive use ceases. To investigate the relation between use of oral contraceptives and the subsequent risk of ovarian cancer, data for individual women from 45 epidemiological studies of ovarian cancers-[47] have been brought together, checked, and analysed centrally.

Methods

Identification of studies and collection of data

Epidemiological studies were eligible for this collaboration if they included at least 100 women with ovarian cancer (40 cases in cohort studies) and recorded information on each woman's reproductive history and use of oral contraceptives. Studies were identified from review articles, from computer-aided literature reviews up to January 2006, using Medline, Embase, and PubMed, and from discussions with colleagues. Principal investigators from each eligible study were invited to participate. Of the 48 eligible studies identified[1-50] (including one multicentre international study[25,31,36]) all but three[50] contributed to the collaboration. Individual data could not be retrieved by the investigators from two of these three studies[50] and investigators for the third[5°] could not be located.

Cases were defined as women with malignant epithelial or non-epithelial ovarian cancer and controls were women without ovarian cancer who had not undergone bilateral oophorectomy. Data for individual women were sought from principal investigators of every study on socio-demographic factors, reproductive and menstrual history, use of hormonal contraceptives, use of hormonal therapies for the menopause, height, weight, family history of breast and

ovarian cancer, and consumption of alcohol and tobacco. Cohort studies were incorporated using a nested case-control design, in which up to four controls were selected at random, matched for follow-up duration, age of the case at diagnosis, and, where appropriate, by broad geographical region. Data provided by investigators were checked and collated centrally so that analyses could be done using definitions as similar across studies as was possible. Apparent inconsistencies in the data were rectified, where possible, by correspondence with the investigators. After the records had been checked and corrected, investigators were sent summary tables and listings of the variables to be used in analyses for final confirmation.

Information on the histological classification of the ovarian cancers had been collected by principal investigators of all but 12 of the 45 participating studies[1,5,6,10,12,15,16,21,22,76,30,45] The classification system adopted in each study was used centrally to categorise tumours as epithelial or non-epithelial and, among the epithelial tumours, to categorise them further as dear cell, endometrioid, mutinous, serous, mixed, or other, according to the 10th revision of the International Classification of Diseases (ICD10).[51] Whenever possible epithelial tumours were further categorised as to whether they were borderline malignant or fully malignant.[51]

Table 1:
Details of studies and women included

* * *

Defining oral contraceptive use

Principal investigators of every participating study had collected information on whether individual women had ever used oral contraceptives, and most had also collected information on total duration of use, age at first and last use, and calendar year of first and last use. The cases had been diagnosed with ovarian cancer on average about 20 years after they had first used oral contraceptives. Validation

studies have shown that, although women were able to recall whether or not they took oral contraceptives in the past, their ability to recall reliably which preparations they used declined soon after use ceased.[52] There is, however, a strong relation between calendar year of use and the dose of oestrogen in the oral contraceptives typically used.[29,53,54] In the USA and UK, for example, the oral contraceptives prescribed before 1970 were typically high-dose preparations, often containing 100 pg or more of oestrogen; between 1970 and 1980 prescriptions were typically for medium-dose preparations containing about 5011g of oestrogen; and by 1980 most prescriptions were for low-dose preparations, containing 30 pg or less of oestrogen.[53,54] Calendar year of oral contraceptive use could therefore be taken, at least roughly, to be a proxy for oestrogen dose and women were classified according to the mid-year of use (before 1970,1970-79, and 1980 or after) to correspond to likely use of high-dose, medium-dose, and low-dose preparations. Sensitivity analyses were done, also classifying women by the calendar year of first and last use. Although most studies did not distinguish between oral contraceptives containing oestrogen-progestagen combinations and preparations containing progestagens only, more than 95% of oral contraceptives used in these populations would have been of the combined type.[55]

Statistical analysis and presentation of results

The statistical methods were similar to those used when analysing the worldwide data for the effects of oral contraceptives on breast cancer." Data from different studies were combined by means of the Mantel-Haenszel stratification technique, the stratum-specific quantities calculated being the standard "observed minus expected" (0—E) numbers of women with ovarian cancer, together with their variances and covariances.[55-57] Use of these simple stratified O—E values has the advantage of avoiding assumptions about the precise forms of any relations in the

data. The stratified O—E values, together with their variances and covariances, yield both odds ratios (subsequently referred to as relative risks) and associated p values. When two groups only are compared, relative risk estimates are obtained from the O—E value and its variance (V) by the one-step method[56,57] as are their standard errors (SE) and CIs. The actual formulae are: log relative risk=(O—E)/V; and its variance=1/V. When more than two groups are compared, variances are estimated by treating the relative risks as floating absolute risks (FARs).[58] This approach yields floated standard errors (F SE) and floated confidence intervals (FCI). The use of floating absolute risks rather than conventional methods does not alter the relative risks but slightly reduces the variances attributed to the relative risks that are not defined as 1.0. This method enables valid comparisons between any two exposure groups, even if neither is the baseline group. Any comparison between two log relative risks must, however, take the variation in each estimate into account (by summing their variances, as described elsewhere[55]). Because of the large number of relative risk estimates presented, 99% CIs are generally used in the figures; however, summary results in the text and figures use 95% CIs.

* * *

Figure 1:
Details of and results from studies contributing data for oral contraceptive use and ovarian cancer

To ensure that women in one study were compared directly only with similar women in the same study, all analyses were routinely stratified by study, by centre within study, by fine divisions of age (single years of age from 16 to 69, then 70-74, 75-79, 80-84, and 85-89), parity (0, 1, 2, 3, 4, 5, 6+; not known) and hysterectomy status (yes, no, unknown). These stratification variables were selected because they are related both to the use of oral contraceptives and to the risk of developing ovarian cancer. The effect on the main findings

of 12 other potential confounding factors (ethnic group, education, age at first birth, family history of breast cancer, age at menarche, menopausal status, use of hormone replacement therapy, height, weight, body-mass index, alcohol use, and smoking) was examined by comparing results before and after stratification for each variable separately and all simultaneously.

When results in the figures are represented by squares and lines the position of the square indicates the value of the relative risk (its area is inversely proportional to the variance of the logarithm of the relative risk, thereby providing an indication of the amount of statistical information available for that particular estimate) and the length of the line represents the CI. When appropriate, a trend in the relative risk of ovarian cancer with increasing duration of oral contraceptive use was calculated only among users (i.e., relative risks for ever users were compared with each other). For these calculations and for the graphical presentation of such results, the duration of oral contraceptive use associated with a particular category was taken to be the median duration within that category.

To estimate the absolute risk of ovarian cancer associated with 5, 10, and 15 years use of oral contraceptives, the relative risks obtained here were applied to published data for the age-specific incidence and mortality rates for ovarian cancer in high income countries [5][960] The age-specific rates were then used to estimate cumulative rates up to age 75. To illustrate the public-health effect of oral contraceptives on ovarian cancer, the numbers of cancers prevented in each of the five decades, starting with the 1960s, were estimated by applying the relative risks found here and statistics on oral contraceptive use in different generations of women to age-specific ovarian cancer incidence and mortality rates. In high-income countries the estimated proportions of ever-users of oral contraceptives in each successive 5-year birth cohort from 1916-20 to 1951-55

were: 5%, 15%, 29%, 40%, 51%, 65%, 76%, and 80%, respectively, with the average years of use among them being 3.6, 4.5, 5.1, 5.5, 5.8, 6.1, 6.3, and 6.3, respectively. These estimates were based on the pattern of oral contraceptive use recorded among controls in this collaboration and in a previous international collaboration:[55] for women born after 1955 use was assumed to be the same as for the 1951-55 birth cohort. In middle-income and low-income countries, oral contraceptive use was uncommon until recently.[61,62]

Figure 2:
Relative risk* of ovarian cancer by duration of use of oral contraceptives

* * *

Role of the funding source

The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation or writing of the report. The writing committee had full access to all the data and had final responsibility for the decision to submit for publication.

Results

Details of the 45 participating studies are shown in table 1. The studies are listed according to their design and, within each type of design, by the median year when the ovarian cancers were diagnosed in each study. Altogether the 45 studies were done in 21 countries, mostly in Europe or the USA, and they contributed a total of 23 257 women with ovarian cancer (cases) and 87 303 women without ovarian cancer (controls) to the analyses. The cancers were diagnosed in 1993, on average, and the mean age at diagnosis was 56 years; 7% were aged younger than 35 years, 11% were aged 35-44 years, 25% were aged 45-54 years, 30% were aged 55-64 years, and 27% were older.

Overall, 31% (7308) of the women with ovarian cancer and 37% (32717) of the controls had used oral contraceptives,

and the average duration of use was 4.4 and 5.0 years, respectively. Figure 1 shows the study-specific and combined relative risks of ovarian cancer in ever-users compared with never-users of oral contraceptives. (Studies with information content [var (0—E)] less than 20 are included in the "other" category for the relevant study design.) For each of the three types of study design there was a highly significant reduction in the relative risk of ovarian cancer in ever users of oral contraceptives. Overall, for ever vs never users the overall relative risk is 0.73, 95% CI 0.70-0.76, $p<0.0001$.

The longer that women had used oral contraceptives, the lower their risk of ovarian cancer (table 2). The overall relative risk decreased by 20% (95% CI, 18-23%, $p<0.0001$) for each 5 years of use (i.e., it was multiplied by a factor of 0.8). In women who had used oral contraceptives for about 15 years the risk of ovarian cancer was halved (figure 2). The effect of various potential confounding factors on the relation shown in figure 2 was examined by adjusting in turn for ethnic group, education, age at first birth, family history of breast cancer, age at menarche, menopausal status, use of hormone replacement therapy, height, weight, body-mass index, alcohol use, and tobacco consumption and also by adjusting for all the factors simultaneously. All these additional adjustments altered the estimated 20% decrease in relative risk per 5 years use by less than 1%.

Figure 3
Relative risk* of ovarian cancer by duration and time since last use of oral contraceptives

* * *

The women with ovarian cancer had stopped use of oral contraceptives an average of 18.6 years previously, and table 3 shows results by time since ceasing use. The relative risks for ovarian cancer were lower the more recently women had used oral contraceptives. The average duration of use was, however, greater in recent users than in those who had

stopped a long time previously (table 3). When the relation with duration of use was examined within categories of recency of use, there was some wearing off of the effect of oral contraceptives the longer ago use had ceased: the proportional declines in relative risk per 5 years use of oral contraceptives were 29% for those whose use had ceased less than 10 years previously, 19% for use ceased 10-19 years previously, and 15% for use ceased 20-29 years previously (test for heterogeneity, p=0.004, figure 3). These findings are unlikely to indicate misclassification of use long ago, since women recall reliably whether or not they took oral contraceptives in the past (but cannot recall the type used).[52] Despite this attenuation in proportional (but not in absolute) risk reduction after stopping use, the risk of ovarian cancer was still significantly reduced 30 or more years after use had ceased (table 3).

Table 4
Relative risk of ovarian cancer in ever-users of oral contraceptives compared with that in never users, by age at first use and duration of use of oral contraceptives

* * *

Table 5
Relative risk of ovarian cancer in ever-users of oral contraceptives compared with that in never users, by calendar year of use of oral contraceptives

* * *

Once duration of use and time since last use of oral contraceptives were taken into account, no other index of the timing of usage, women's ages at first and last use, and use before and after the birth of a child-had any material further effect on the relative risk of ovarian cancer. Table 4 shows results according to the women's ages at first use of oral contraceptives. There was no significant heterogeneity in the decline in relative risk with increasing duration of oral contraceptive use across women who started use at different

ages (test for heterogeneity, p=0.5). The distribution of women's ages at first use was 16% for those younger than 20 years, 34% for 20-24 years, 21% for 25-29 years, 14% for 30-34 years, 8% for 35-39 years, and 7% for older ages. Women's ages at last use were closely correlated with their age at first use, and the decline in ovarian cancer risk with increasing duration of use did not differ significantly by women's age at last use (3% were younger than 20 years at last use, 18% were 20-24, 24% were 25-29, 21% were 30-34, 15% were 35-39, 11% were 40-44, and 8% were older). The decline in ovarian cancer risk with increasing duration of use did not vary significantly by whether women had begun using oral contraceptives before or after the birth of their first child (decreases in relative risk per 5 years of use 26% vs 18%; test for heterogeneity, p=0.1).

As described in the methods section, the oestrogen dose in oral contraceptive preparations typically used in the 1960s was more than double that of preparations typically used in the 1980s.[29.5;.5'] Among women with ovarian cancer, almost 40% had a mid-year of oral contraceptive use in the 1960s and 13% had a mid-year of use in the 1980s or later (table 5). Those with a mid-year of use in the 1960s had, as expected, ceased use much longer ago than those with a mid-year of use in the 1980s or later (25 years vs 5 years previously). For a given time since last use, however, calendar year of use had little effect on the relative risk of ovarian cancer (table 5). Sensitivity analyses were done classifying women according to the calendar year of first use and calendar year of last use but, again, no obvious differences in ovarian cancer risk were found.

Figure 4
Percent reducing in ovarian cancer risk per 5 years oral contraceptive use for various subroups

* * *

The magnitude of the decline in the relative risk of ovarian cancer with duration of use did not vary significantly

according to 13 of the 15 personal characteristics examined (figure 4). Significant variation was seen only with age at diagnosis and menopausal status. However, the younger and pre-menopausal women had ceased use of oral contraceptives more recently than the older and postmenopausal women. When analyses were restricted to women whose use ceased 10-29 years previously (the only group with broadly similar recency of use and also with sufficient information to compare younger versus older women and pre-menopausal versus post-menopausal women), there was no significant heterogeneity by either age (p=0.1) or menopausal status (p=0.4). There was some variation in the magnitude of the decline in relative risk of ovarian cancer for each year of use of oral contraceptives across studies (x^2_2r49.1, p=0.006; figure 1) and by study design (x^2_2=10.4,p=0.006). This heterogeneity again reflects the variation in age and thus time since last use of oral contraceptives: cases in the prospective compared with the case-control studies were older (mean ages of 64 vs 52 years at diagnosis) and had ceased use longer ago (means of 24 vs 15 years since last use).

Data for histological subtype was available for 17099 women with ovarian cancer (74% of the total). Among these women the risk of ovarian cancer decreased by 21% for each 5 years of use of oral contraceptives (figure 5), similar to the 20% seen for all women (figure 1). The reductions in risk per 5 years of oral contraceptive use were broadly similar for epithelial and non-epithelial tumours. Among the epithelial tumours there was, however, heterogeneity across histological types (test for heterogeneity, p=0.0007), mainly because oral contraceptives seem to have little effect on mucinous tumours (12% of the total with histology). There was no significant heterogeneity in the trends with duration of use between the non-mucinous epithelial tumours (p=0.5). The findings were similar when the mucinous and serous

tumours were subdivided into whether they were of only borderline malignancy or were fully malignant (figure 5).

Figure 6 shows, for women in high income countries, the estimated cumulative incidence and mortality from ovarian cancer for never users of oral contraceptives and for those who used them for 5, 10, and 15 years, respectively, beginning at age 20 years. The percent decline in ovarian cancer rates for every 5 years of use was assumed to be 29% in current users and those who ceased use in the previous 10 years, 19% in those who ceased use 10-19 years previously, and 15% in those who ceased use 20 or more years previously. For women who never used oral contraceptives an estimated 1.2 in every 100 are diagnosed with ovarian cancer and 0.7 in every 100 die from the disease before the age of 75 years (in the absence of other causes of death). For 10 years use of oral contraceptives the estimated cumulative incidence was 0.8 per 100 and mortality was 0.5 per 100. As the reduction in risk is roughly proportional to duration of use, this means that for every 5000 woman-years of oral contraceptive use about two ovarian cancers and one death from the disease are prevented. (Ovarian cancers arising after age 75 years are not included in these or in subsequent calculations, nor are deaths from ovarian cancer after age 75 years that arose earlier.)

For women with background rates of ovarian cancer greater than the average, such as those with a family history of breast cancer or who are nulliparous, the reduction in absolute risk would be greater still. Conversely, for women with lower than average background rates the reduction in absolute risk would be less. For example, ovarian cancer rates in many middle-income and low-income countries are about half those in high income countries,[59,60] and so about two ovarian cancers and one death from the disease would be prevented for every 10 000 woman-years of oral contraceptive use.

Discussion

This worldwide collaboration has brought together and re-analysed data for over 23 000 women with ovarian cancer and 87 000 women without ovarian cancer from 21 countries. The results confirm that women who use oral contraceptives are at a reduced risk of ovarian cancer and show that substantial protection continues for decades. The reduction in risk is greater the longer that women used oral contraceptives and, although the relative (but not the absolute) risks are somewhat attenuated over time, there is still a significant reduction in risk more than 30 years after use has ceased. The relative decline in ovarian cancer risk with increasing duration of use does not vary substantially by women's ethnicity, education, age at menarche, parity, family history of breast cancer, use of hormone replacement therapy, body-mass index, height, or their consumption of alcohol and tobacco. The incidence of mucinous tumours (12% of the total) was little affected by oral contraceptives, but otherwise the proportional risk reduction did not vary much between different histological types.

Figure 5
Percent reduction in risk per 5 years use, by ovarian tumor histology

* * *

Figure 6
Absolute risk of ovarian cancer for women in high income countries, by duration of use of oral contraceptives

* * *

This collaboration includes individual data from 45 epidemiological studies, most of the eligible studies worldwide that have collected information on oral contraceptive use and ovarian cancer. Despite extensive efforts to identify studies with unpublished results, we cannot guarantee that none has been overlooked or that

information from continuing prospective studies is up to date, since such studies are accumulating data beyond the time when they contributed to this collaboration. Three published studies[48-50] could not contribute their data, and only some of the EPIC study centres[25,30,60] have done so. Nevertheless, these studies would have increased the number of cases by only about another 3%, and the published results from the studies not induded[48-50] do not differ from those reported. Thus, failure to include all the available data has not materially altered the overall findings.

Table 6
Estimated proportion of ovarian cancers prevented before age 75 years by past patterns of use of oral contraceptives in different countries

* * *

Substantial reductions in the oestrogen content of oral contraceptives have occurred over the 50 or so years that oral contraceptives have been in use. The ovarian cancers in this study were diagnosed, on average, almost 20 years after the women had last used oral contraceptives and, because the specific oral contraceptive preparations used are unreliably reported many years after use ceases," calendar year is used here as an indicator of the average oestrogen dose of the preparations. Typical oestrogen doses in the 1960s were more than double the typical doses in the 1980s and later,[29,53,54] but for a given pattern of usage there was no apparent variation in the relative risk of ovarian cancer between women whose oral contraceptive use was during the 1960s, 1970s, and 1980s (table 5), suggesting no appreciable differential effect of preparations typically used over the decades.

One of the main effects of oral contraceptives is to suppress ovarian activity, so some protection against neoplastic change is plausible. This makes it reasonable to infer that the associations seen here are chiefly causal—i.e., that previous oral contraceptive use decreases the age-

specific incidence of ovarian cancer in otherwise similar women. The exact mechanism by which oral contraceptives cause such a profound and long-lasting protection against ovarian cancer is, nevertheless, not well understood.

Oral contraceptives were first licensed almost 50 years ago. In the 1960s and 1970s most women who had used oral contraceptives were younger than 50 and so relatively few ovarian cancers would have been prevented (table 6). In subsequent decades the estimated proportion of cancers prevented increased, in part due to the increasing number of ever-users and in part due to the increasing age of past users, such that in the 2000s an estimated 13% of ovarian cancers before age 75 years were being prevented in women in high-income countries. In middle-income and low-income countries oral contraceptives have probably had little effect so far on ovarian cancer incidence, since use was uncommon until the 1980s.61,62

To illustrate the public-health implications of relative risks such as those reported here and the pattern of oral contraceptive use around the world, these results suggest that of the order of 200 000 incident cases and 100 000 deaths from ovarian cancer have already been prevented over the last 50 years. The number of cancers prevented each year is likely to increase substantially in the future, with the further ageing of past users of oral contraceptives and the increasing numbers of new users, especially in middle-income and low-income countries. In 2002 an estimated 80 million of a total of 120 million oral contraceptives users worldwide were in middle-income and low-income countries.[61] With this number of oral contraceptive users and current ovarian cancer incidence rates, the number of ovarian cancers prevented would rise over the next few decades to about 30 000 every year. However, the number prevented is likely to be still greater since the prevalence of oral contraceptive use in middle-income and low-income countries is predicted to increase."

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Conflict of interest statement

The writing committee declare no conflict of interest.

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APPENDIX F

AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS

ANDROGENIC PROGESTINS IN ORAL CONTRACEPTIVES AND THE RISK OF EPITHELIAL OVARIAN CANCER

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OBJECTIVE: Oral contraceptives (OCs) have been consistently linked to reduced risk of ovarian cancer. Oral contraceptive formulations display varying degrees of androgenicity. Data linking androgens to ovarian cancer suggest that OC androgenicity may impact efficacy in preventing ovarian cancer. The authors investigated whether OC efficacy might differ according to androgenicity by using data from a large, population-based, case-control study (the Steroid Hormones and Reproductions [SHARE] Study).

METHODS: Detailed data on OC formulation was obtained by an in-person interview for 568 cases and 1,026 controls. Multivariable logistic regression was used to assess the association of OC androgenicity with ovarian cancer while controlling for the known potential confounders of age, parity, family history of ovarian cancer, and tubal ligation.

RESULTS: Androgenic and nonandrogenic OCs conferred a similar and significant reduction in ovarian cancer risk (odds

ratio 0.52, 95% confidence interval 0.35–0.76 and odds ratio 0.59, 95% confidence interval 0.45–0.78, respectively). No differences in duration of use, age at first use, and time since last use were found between androgenic and nonandrogenic formulations.

CONCLUSION: In general, the androgenicity of an OC does not alter chemopreventive efficacy. (Obstet Gynecol 2005;105:731–40. © 2005 by The American College of Obstetricians and Gynecologists.)

LEVEL OF EVIDENCE: II-2

There were an estimated 25,400 new cancer cases and 14,300 deaths from epithelial ovarian cancer in 2003.[1] Oral contraceptives (OCs) have been consistently linked to reduced risk of ovarian cancer.[2–6] Cohort analysis of trends in mortality due to ovarian cancer demonstrate that women who were born after 1920, ie, from generations who had used OCs, consistently show decreased rates of ovarian cancer.[7] The contraceptive effect of OCs is hypothesized to derive from a suppressed mid-cycle gonadotropin surge and inhibited ovulation.[8] According to both the "incessant ovulation" and the "gonadotro-pin" hypotheses, OC use is anticipated to decrease the risk of epithelial ovarian cancer.[8] During the last 3 decades, OC formulations have changed with the introduction of new, lower-dose progestins that possess varying androgenic properties. Early progestins had a 21-carbon skeleton that made them estrogenic in nature.9,10 Subsequently, the 19-carbon nor-testosterones were developed, which possessed both androgenic and proges-togenic qualities. More recently, compounds that are less androgenic have gained in popularity.[10–12] A number of currently marketed OCs contain androgenic progestins, including Alesse, Triphasil, Levlen, Estrostep, Ortho-Cept, and Lo/Ovral. Over 10 million U.S. women and 100 million women worldwide are currently taking oral contraceptives, and approximately 20–25% of OCs contain androgenic progestins.[13]

We[3] and others[14] have explored whether estrogen and progestin dosages might alter the efficacy of OCs in preventing ovarian cancer by comparing risk reduction of OCs that contain various high- or low-dose combinations of estrogens and progestins.

However, OCs with high-dose progestins are not necessarily androgenic in nature, and the estrogen component is not known to influence androgenicity. We have found no studies to date that have examined ovarian cancer risk reduction solely in relation to the androgenicity of the synthetic progestins in OCs. A search of MEDLINE from 1966 to November 2004 with the search terms "androgenicity of progestins" and "androgenic progestins" and by combining the search term "ovarian neoplasms" with "progestins" resulted in 111 articles, none of which evaluated the androgenicity of progestins in relation to ovarian cancer risk.

Adverse effects of androgen excess, including acne, weight gain, and unwanted facial hair,[10,15,16] are experienced by many women taking androgenic OCs, and according to the literature, these androgenic adverse effects are more important than any other effects in explaining why women discontinue OC usage.[17–19] Polycystic ovarian syndrome, a condition characterized by increased circulating serum levels of adrenal androgens, may increase the risk for developing epithelial ovarian cancer.[20] Additionally, more than 90% of ovarian cancer tumors express androgen receptors,[21–24] and androgens play a role in follicular growth, maturation, and atresia.[25–27] Excess androgen exposure may be particularly important as a cancer risk factor for women with endometriosis,[26,28,29] a condition that is frequently managed with OCs. In this analysis, we explore whether the protection associated with OCs might be altered by the androgenicity of the progestin component among all women and, specifically, among women with endometriosis.

SUBJECTS AND METHODS

Women in this study were selected from a case-control study of contraceptive and reproductive risk factors for epithelial ovarian cancer (the Steroid Hormones and Reproductions [SHARE] Study). Cases, aged 20–69 years, with epithelial ovarian cancer diagnosed within the 6 months before interview were ascertained from 39 hospitals in the Delaware Valley surrounding Philadelphia between May 1994 and July 1998. Older subjects would have been unlikely to have been exposed to oral contraceptives. A total of 2,418 cases of histologically confirmed borderline or invasive epithelial ovarian cancer were initially identified. After excluding

women who were too young or too old (n = 640), resided outside the counties in which referral hospitals were located (n = 342), had a prior diagnosis of ovarian cancer (n = 158), or did not speak English or were mentally incompetent (n = 25), there were 1,253 potentially eligible women. After further excluding those who were diagnosed more than 6 months before interview (n = 296), were critically ill or dead (n = 69), or were untraceable (n = 15), 873 women remained who had incident cancer and were thus eligible for the study. Fourteen physicians did not consent to their patients' participation, and 92 women refused to participate. Thus, there were 767 completed case interviews (61% of potentially eligible cases and 88% of potentially eligible incident cases).

Controls, aged 65 or younger, were ascertained by random-digit dialing and frequency matched to cases by race, 5-year age groups, and 3-digit telephone exchange. Of the 14,551 telephone numbers screened for this purpose, 6,597 belonged to businesses or were not in service, and 5,640 had no female of eligible age in the household, leaving 2,314 households with potentially eligible participants. Of these, 1,928 households (83%) had a potentially eligible woman who was willing to be further screened. Upon screening, a further 291 either had no resident women eligible on the basis of age (n = 5), resided outside of the target counties (n = 11), had a prior diagnosis of ovarian cancer (n = 9), had a prior bilateral oophorectomy (n = 187), did not speak English or were mentally incompetent (n = 22), had critical illness or death (n = 6), or were untraceable (n = 51). Of the 1,637 screened and potentially eligible controls, 422 declined to be interviewed, and 1,215 (74%) completed interviews. Controls, aged 65–69 years, were ascertained through Health Care Financing Administration (HCFA) lists because we were concerned about reduced random-digit dialing response rates in this group. Four hundred twenty-three women, frequency-matched to cases by county of residence, were initially identified. One hundred sixty were ineligible for the reasons given above. Of the 263 potentially eligible women from HCFA lists, 111 refused to participate and 152 (58%) were interviewed. Therefore, of the total 1,900 screened and potentially eligible controls (1,637 from random-digit dialing and 263 from HCFA lists), 1,367 (72%) interviews were completed.

Cases included 616 women with invasive epithelial ovarian tumors and 151 women with borderline tumors. The diagnosis of ovarian cancer was confirmed by pathology in all cases. Central pathologic review was conducted on a random sample of 120 cases. The reference pathologist agreed with the original pathologic review for invasiveness in 95% of cases and for cell type in 82% of cases. The original pathologic diagnosis was then used for all cases.

Included in our study were women whom we could classify as having either exclusively used androgenic OCs, exclusively used nonandrogenic OCs, used both types of OCs, or reported never having used OCs. Women who could not be classified into 1 of these 4 categories because of missing information on OC formulations (n = 199 cases and 341 controls) were omitted from our analyses. Thus, the final analysis included 568 cases and 1,026 controls (78% of original SHARE cases and controls). Controls included in our analysis were more likely to be at the extremes of the study age ranges (< 40, ≥ 60) ($P = .001$) and were less likely to have had endometriosis ($P = .001$) than cases (data not shown). Number of live births, race, education, family history of ovarian cancer, tubal ligation, and hysterectomy did not differ statistically between included and excluded women.

A standardized 1.5-hour, in-person interview of cases and controls provided detailed demographic data as well as information on subjects' gynecologic and obstetric history, including menstrual history, pregnancy history, tubal ligation, lactation, hysterectomy, family history of breast and ovarian cancers, oral contraceptive use, and hormone replacement therapy use. Women were asked about endometriosis and were asked if this was diagnosed by a physician. A "life" calendar marked with important events that each participant recalled during her lifetime was used to enhance memory of distant events. The reference date was calculated as 6 months before diagnosis (cases) or interview (controls) to ensure that exposures occurred before ovarian cancer diagnoses in cases and within a similar time frame for cases and controls.

All contraceptive use was recorded, including the type of contraception, frequency of use, and duration of use. Additional details obtained for hormonal contraceptives included the brand,

reason for use, and reason for stopping use. Oral contraceptive use was categorized as use for contraception, for noncontraceptive uses such as to control abnormal bleeding or menstrual pain, or for both contraception and other uses. Picture books with photographs of oral contraceptives available in the United States (courtesy of Dr. Ruth Peters, University of Southern California, Los Angeles, CA) were used to help women specify the formulations used. For each combined OC preparation, we obtained information on active ingredients and doses by using a variety of existing databases and reference books. For discontinued medications, we made inquiries to pharmaceutical manufacturers. We tested the accuracy of the recall of OC formulation in a subset of 10% of the women participating in the SHARE study by conducting a second interview more than 6 months after initial interview. Agreement on OC use and formulation exceeded 90% between the 2 interviews.

The androgenicity of the progestin component of each OC formulation was determined by compiling data from various studies that assessed the androgenic potential of each progestin, while factoring in dose of progestin per OC formulation (Browning MC, Anderson J. Effect of oral contraceptives on plasma testosterone concentration [letter]. Br MedJ 1977;1:107).[1,9_11,16,30–52] Evaluations of androgenicity involve measuring the progestin's affinity for and binding to the androgen receptor, its effect on sex hormone binding globulin levels, its degree of binding to sex hormone binding globulin, and its effect on free testosterone.[9,10,38,47,53] The most androgenic pro-gestins used by cases in our study, levonorgestrel and dl-norgestrel, were originally derived from 19-nortestos-terone. They have a high affinity for sex hormone binding globulin and decrease free sex hormone binding globulin levels by binding it and displacing testosterone, consequently increasing free testosterone levels.

Table 1
Progestin Properties at One-Milligram Dose

* * *

We classified progestin androgenicity by pharmacokinetic actions of the progestin and dose (Table 1). Each progestin has a

different potency, milligram per milligram. A progestin that is considered to be high dose in terms of progestogenicity can be low in terms of androgenicity. A higher-potency progestin may be used in a much smaller dose and thus be equivalent to a larger dose of a less-potent progestin. For example, desogestrel is a very potent and androgenic progestin, but its usual oral contraceptive dose is 0.15 mg. Its progestin potency compared with 1.00 mg of norethindrone would be 0.15 X 9.0 = 1.35 times. For androgenicity, it would be 0.15 X 3.4 = 0.51 or half as androgenic as a pill containing 1 mg of norethindrone. As a cutoff value for progestin androgenicity, we classified as "androgenic" any drug with the equivalent of 1 mg of norethindrone or higher and as "nonan-drogenic" anything below the 1-mg equivalent. For example, high doses of norethindrone and norethindrone acetate in OCs gave them the classification of androgenic, such as the 10-mg dose of certain types of Norinyl or Ortho-Novum. Lower doses, such as 0.4 mg norethin-drone in Ovcon 35 (28 or 21 day) formulations, with a potency of 0.40, were categorized as nonandrogenic.

Among the 568 cases and 1,026 controls, a total of 125 different formulations of OCs were used. According to dose and potency, we were able to classify all of the progestins used in these 125 formulations in terms of their androgenicity. Table 2 gives an example of the comparative androgenicity of a sample of the OCs that study participants used.

Odds ratios (ORs), with corresponding 95% confidence intervals (CIs), were calculated as the primary measure of effect size. Because the SHARE Study used frequency rather than individual matching and matched on the basis of broad criteria, such as age within 5- to 10-year intervals, we used unconditional logistic regression models to adjust for any additional effects of potential confounding variables that had been determined a priori to affect ovarian cancer risk.[2,54–56] Included in the models were age and parity as continuous variables and tubal ligation and family history of ovarian cancer as dichotomous (yes/no) variables. Tests for trend (*P* value) were performed by coding OC duration as a grouped linear variable. Odds ratios for OC exposures were calculated from the estimated β coefficients and their standard errors. Maximum-likelihood ratios were obtained using the

STATA BLOGIT function to compare the calculated odds ratios and test for significant differences in their values. All tests of statistical significance were 2-tailed and considered significant at $P < .05$. All analyses were performed using STATA 8.0 (STATA Corporation, College Station, TX).

Table 2
Androgenic Effects of Selected Oral Contraceptives

* * *

RESULTS

Table 3 provides demographic information on ovarian cancer cases and controls for which complete OC data were obtained. As expected, cases were less likely to use oral contraceptives (40.0% versus 58.3%), to have had children (67.8% versus 86.2%), and to have had a tubal ligation (15.0% versus 32.4%) or hysterectomy (9.7% versus 13.0%). They were more likely to report a family history of ovarian cancer (3.5% versus 1.6%) and a personal history of endometriosis (7.9% versus 5.2%). Androgenic OCs were disproportionately used by younger women, whereas older women tended to have used nonandrogenic OCs, reflecting the chronology of the marketing of OC brands as new progestins were developed. Endometriosis was more commonly diagnosed among women who had used OCs (9.9% versus 2.1% of controls). However, the proportion of women diagnosed with endometriosis was not markedly different among androgenic versus nonandrogenic OC users (5.5% versus 7.5% of controls).

For androgenic-only OC users and users of both types of OCs, increasing duration of use was associated with a reduction in ovarian cancer risk (Table 4). In the nonan-drogenic group, longer duration did not appear to confer a significant dose-response relationship (OR 0.56 for < 5 years; OR 0.73 for ≥ 5 years).

Independent of the OC androgenicity, early OC use (before age 20) was associated with a greater reduction in risk than later age at first use (Table 4). The point estimate associated with androgenic-only OC use was nonsignificantly smaller than for the nonandrogenic-only use (OR 0.42 versus 0.54).[57] Oral

contraceptive use within the last 10 years appeared to be more strongly and inversely associated with ovarian cancer than use more than 10 years in the past, regardless of the androgenic content of the formulation. However, even past use that was discontinued more than 10 years previously was associated with a significant reduction in risk, and this observed relationship was independent of the androgenic potency of the particular OC formulation.

Compared to never-users, use of androgenic-only OC formulations was inversely associated with ovarian cancer (adjusted OR 0.52, 95% CI 0.35–0.76) (Table 4). A similar inverse association was observed with use of nonandrogenic-only OC formulations (adjusted OR 0.59, 95% CI 0.45–0.78) and with exposure to both androgenic and nonandrogenic OCs (adjusted OR 0.29, 95% CI 0.17–0.48).

Table 3
Demographic Description of Ovarian Cancer Cases and Controls

* * *

Among women with endometriosis (45 cases, 53 controls), androgenic formulations appeared to confer less protection than nonandrogenic formulations (OR 0.46 versus 0.23), although the sample size of these OC users was small (Table 5). Four cases and 10 controls with histories of endometriosis used both androgenic and nonandrogenic OCs and achieved significant risk reduction (OR 0.11, 95% CI 0.02–0.69, adjusted for age, number of live births, family history of ovarian cancer, and tubal ligation). Further adjustment for OC duration of all models in Table 5 did not alter any findings (data not shown).

Finally, the androgenicity of the OC formulations was not associated with invasiveness or with histologic subtype, and all our findings were similar when we limited cases to women with invasive disease (data not shown).

DISCUSSION

Independent of the androgenicity of its progestin component, OC use was associated with approximately a 40–50% overall decrease in ovarian cancer risk. Increasing duration of use, early age at first use, and recentness of OC use all provided increased protection against ovarian cancer, regardless of the androgenic potential of the progestin in the OC formulation used. In fact, longer duration of use of androgenic-only OCs appeared to confer increased protection against ovarian cancer when compared to nonandrogenic OC use. Among the limited number of women with endometriosis taking either androgenic-only or nonandrogenic-only OCs (27 cases, 34 controls), androgenic formulations appear to confer less protection than nonandrogenic formulations (OR 0.46 versus 0.23).[57]

Table 4
Odds Ratios for Characterists of Oral Contraceptive Progestin Type

* * *

Table 5
Ovarian Cancer Risk With Oral Contraceptive Type and Endometriosis

* * *

Although our results are reassuring regarding the lack of impact from the androgenicity of progestins in OCs overall, they are somewhat surprising in light of several findings linking androgens to increased ovarian cancer risk. In particular, we recently reported that, for women with endometriosis, danazol, a synthetic androgen that binds to androgenic receptors and sex hormone binding globulin resulting in a 3-fold increase in free testosterone levels, was an independent risk factor for ovarian cancer and was associated with a risk 3.2 times greater than among women who had never used the drug.[28] In the current study, among women with endometriosis, use of androgenic-only OCs was associated with somewhat diminished, but still apparent, protection. The small sample size limits the interpretation of this finding.

The most androgenic progestins are also the most progestogenic.[42] Possibly, the tumorigenicity induced by an androgenic progestin could be mollified by the apoptotic effect from its progestogenic nature. The change in the progestin component is important because a growing body of evidence suggests that it is the progestin portion of OCs that may provide some of its protective benefit against ovarian cancer. Progestin-only oral contraceptives, which do not totally suppress ovulation, are as protective against ovarian cancer as estrogen-progestin formulas.[58] In a study of cynomolgus macaques (*Macaca fascicularis*), animals randomized to receive estrogen plus progestin or progestin-only pills had a 4- to 6-fold increase in the proportion of apoptotic cells,[5] with the maximum 6-fold effect seen in the progestin-only group. These data suggest that progestins induce apoptosis and also support the observation that the protection against ovarian cancer afforded by OCs extends beyond that of ovulation suppression.[26] As further evidence, a recent case-control study showed that high-dose progestin OC formulations may be more protective than low-dose formulations,[14] although, in a separate study, we failed to find such a difference.[3]

It is also possible that, although progestins appear to have variable androgenicity, the net effects on androgenic hormones in vivo may be similar. A study comparing levonorgestrel to gestodene, a newer and less androgenic progestin, found that sex hormone binding globulin was elevated 2-fold in the levonorgestrel group and 3-fold in the gestodene group. After administering each preparation, serum levels of luteinizing hormone, follicle-stimulating hormone, estradiol, and progesterone were depressed, with greater reductions seen for gesto-dene. However, equal decreases were found in testosterone, androstenedione, and dehydroepiandrosterone sulfate with both preparations.[11] Thus, although each progestin possessed inherently different androgenic potencies, in the end, both of them reduced circulating levels of androgens similarly.

The strengths of our study include the population-based ascertainment of cases and controls; the large number of incident ovarian cancer cases; and the use of life-events calendars, comprehensive picture books, and structured interviews to

enhance the recollection of medical information and contraceptive preparations used. All of the methodological features limited the potential for selection bias and information bias. Moreover, because both participants and our interviewers were unaware of the research question addressed here, it is unlikely that recall bias or interviewer bias could account for our results.

Our study was limited by the small number of women who had taken androgenic progestin oral contraceptives. Only 224 women had ever used androgenic OCs, 108 exclusively and 92 in a combination of androgenic and nonandrogenic life OC use. Additionally, there were many women who did not know the exact formulation of oral contraceptive that they had been taking. Nonetheless, we were able to classify 78% of OC users according to the androgenic properties of the progestin formulation. The rest could not be classified because of insufficient information on the exact OC formulation. Other studies that have attempted to assess the relationship between OC formulations and ovarian cancer have also suffered from this limitation.[14,59] Although validation studies have found that recall of use and timing of OCs is quite accurate, recall for specific formulations is less so.[60–62]

A second potential limitation was that we did not adjust for polycystic ovary syndrome (PCOS) or the use of the medication Estratest in our analyses, which might have added a bias in androgenic exposures. However, a total of only 5 study participants had used Estratest in their lifetime, and 13 participants reported a history of PCOS, which should not have altered our results.

Another limitation of our study comes from the nature of the discovery and marketing of new progestins over the course of the past 40 years. The study was conducted in the late 1990s, and although the cohort of women who participated did contain present OC users, many of the women were past their childbearing years. The newer progestins, such as desogestrel and norgestimate, have not been available in OCs for nearly as long as older progestins, such as norethindrone or norgestrel. Of all of our pill users, only 15 had taken OCs containing low-dose, monophasic desogestrel, and 9 had taken low-dose, tri-phasic norgestimate. On the other hand, hundreds of women had taken

OCs containing norgestrel or norethindrone. However, we believe that our calculation of OC androgenicity was highly accurate and would apply to the newer progestins with equivalent validity. Norgestimate has an androgenic level of 1.9 when prescribed in a 1-mg dose. For the 9 women who had used norgestimate in our study in a tri-phasic form (0.18, 0.215, 0.25 mg), the highest dose (0.25 mg) had an androgenic potency of 0.47. Thus, it was categorized as nonandrogenic.

In summary, our findings indicate that, for women in general, the androgenicity of the progestin component does not alter the OC's protective effect. Given the many currently available OCs that contain androgenic proges-tins, our findings are reassuring. However, the possibility that androgenic OCs may be less effective than nonandrogenic OCs for women with endometriosis warrants further investigation.

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APPENDIX G

AMERICAN JOURNAL OF EPIDEMIOLOGY

RISK OF OVARIAN CANCER IN RELATINO TO ESTROGEN AND PROGESTIN DOSE AND USE CHARACTERISTICS OF ORAL CONTRACEPTIVES

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Although past studies have shown that oral contraceptives with 50 jig or more of estrogen reduce the risk of ovarian cancer, it is not clear whether newer, lower-dose formulations do as well. We conducted a population-based, case-control

study in the Delaware Valley to assess the impact of dose of oral contraception on risk of ovarian cancer. Cases aged 20–69 years with a diagnosis of epithelial ovarian cancer ascertained between May 1994 and July 1999 (n = 767) were compared with community controls (n = 1,367). Compared with never users, the adjusted risk of ovarian cancer was reduced by 40% for oral contraceptive users overall, with longer duration of use affording greater protection. The ovarian cancer risk reduction was similar for women who initiated oral contraception before 1972, when high-dose pills dominated the market; between 1972 and 1980; and after 1980, when newer, lower-dose pills dominated. Oral contraceptive estrogen and progestin content were compared for cases and controls after adjustment for current age, number of pregnancies, race, and family history of ovarian cancer. Use of low-estrogen/low-progestin pills afforded an estimated risk reduction (odds ratio = 0.5, 95% confidence interval: 0.3, 0.6) that was identical to that for high-estrogen/high-progestin pills (odds ratio = 0.5, 95% confidence interval: 0.3, 0.7). *Am J Epidemiol* 2000;152:233–41.

contraceptives, oral; estrogens; ovarian neoplasms; progestational hormones

Oral contraceptives are thought to be the most powerful known chemopreventative agents for ovarian cancer. A consistent body of research has shown that women who have taken oral contraceptives are about one-third less likely to develop ovarian cancer than are women who have never used them (1–4). These findings derive from studies involving women who primarily used older formulations of oral contraceptives containing higher doses of estrogen (≥50 jig) and progestins. During the past 3 decades, the amount of estrogen and progestin in oral contraceptives has steadily decreased, and new progestins have been introduced into the market (5). Although formulations containing <50 μg estrogen are equally effective in suppressing ovulation, they may be

less able to suppress gonadotropin levels than are higher-dose formulations (6–8). High gonadotropin levels may elevate the risk for ovarian cancer (9, 10), raising the concern that because lower-dose pills might not be as suppressive of gonadotropins, they also may not be as protective as higher-dose oral contraceptives.

Until now, few women taking the newer, lower-dose preparations had passed through the critical age window during which the incidence of ovarian cancer rises. Three previous studies evaluated ovarian cancer risk associated with specific oral contraceptive formulations, but all included a limited number of women who used lower-dose formulations (11–13). One reported somewhat less risk reduction for low- versus high-dose estrogen formulations (11). The other studies showed that various formulations of oral contraceptives (vs. nonuse) reduced the risk of ovarian cancer to various degrees, but did not directly assess whether lower-dose formulations were equivalent to higher-dose formulations in lowering ovarian cancer risk (12, 13). We report the results of a population-based, case-control investigation designed to address further the impact of dose of oral contraception on its association with ovarian cancer.

MATERIALS AND METHODS

Study subjects

Cases were women aged 20–69 years who had been diagnosed with epithelial ovarian cancer within the 6 months prior to interview. They were ascertained between May 1994 and July 1998 from 39 hospitals around the Delaware Valley, including contiguous counties in eastern Pennsylvania, southern New Jersey, and Delaware. All study subjects gave informed consent for participation, and Institutional Review Board approval was obtained from all hospitals from which subjects were recruited. A total of 2,418 cases of histologically confirmed, borderline or invasive epithelial ovarian cancer were initially identified. After exclusion of women who were not eligible for study because they were too

young or too old (*n* = 640), resided outside the counties in which referral hospitals were located (*n* = 342), had a previous diagnosis of ovarian cancer (*n* = 158), did not speak English or were mentally incompetent (*n* = 25), there were 1,253 potentially eligible women. After further exclusion of those who were diagnosed more than 6 months prior to interview (*n* = 296), were critically ill or deceased (*n* = 69), or were untraceable (*n* = 15), there remained 873 women who had incident cancer and were thus eligible for study. Fourteen physicians did not consent to their patients' participation, and 92 women refused to participate. Thus, our analyses are based on 767 completed case interviews (61 percent of potentially eligible cases and 88 percent of potentially eligible, incident cases). Our ascertainment of potentially eligible cases compared favorably with identified cases reported to the Delaware and Pennsylvania cancer registries for the counties of interest for one of the earlier study years. The median time from diagnosis to interview for cases was 90 days.

Table 1
Demographic and reproductive characteristics of ovarian cancer cases and controls, Delaware Valley area, May 1994 to June 1988

* * *

Controls aged 65 years or younger were ascertained by random digit dialing and were frequency matched by 5-year age groups and three-digit telephone exchanges to cases. Of the 14,551 telephone numbers screened for this purpose, 6,597 were businesses or were not in service, and 5,640 had no female of eligible age in the household, leaving 2,314 households with potentially eligible participants. Of these, 1,928 (83 percent) households had a potentially eligible woman who was willing to be screened further. Upon screening, a further 291 had no eligible resident woman on the basis of age (*n* = 5), residence outside of the target counties (*n* = 11), prior diagnosis of ovarian cancer (*n* = 9), a prior bilateral oophorectomy (*n* = 187), not speaking English or

being mentally incompetence (n = 22), being critically ill or deceased (n = 6), or being untraceable (n = 51). Of the 1,637 screened and potentially eligible controls, 422 declined to be interviewed, and 1,215 (74 percent) completed interviews. Controls aged 65–69 were ascertained through Health Care Financing Administration lists. A total of 423 women, frequency matched to cases by county of residence, were identified initially. Of these, 160 were ineligible for the reasons given above. Of the 263 potentially eligible women from Heath Care Financing Administration lists, 111 refused to participate, and 152 (58 percent) were interviewed. Therefore, of the total 1,900 screened and potentially eligible controls (1,637 from random digit dialing and 263 from Health Care Financing Administration lists), 1,367 (72 percent) are included in our analyses.

Table 1 (Continued)
Demographic and reproductive characteristics of ovarian cancer cases and controls, Delaware Valley area, May 1994 to June 1988

* * *

Cases included 616 women with invasive epithelial ovarian tumors and 151 with borderline epithelial ovarian tumors. The diagnosis of ovarian cancer was confirmed by pathology in all cases. Central pathologic review was conducted on a random sample of 120 cases. The reference pathologist agreed with the original pathologic review for invasiveness in 95 percent of cases and for cell type in 82 percent. The original pathologic diagnosis was then used for all cases.

Oral contraceptive use

Standardized 1.5-hour interviews were conducted by trained interviewers in the homes of participating women. A “life” calendar marked with important events that each participant recalled during her life was used to enhance memory of distant information. On the calendar, sexual activity, use of contraceptives, and reproductive events were coded for every

month from sexual debut until a reference date. The reference date was calculated as 6 months prior to the interview (for both cases and controls). Picture books with photographs of oral contraceptives available in the United States (courtesy of Dr. Ruth Peters, University of Southern California, Los Angeles, California) were used to help women specify the formulations used.

All contraceptive use was recorded, including the type of contraception, frequency of use, and duration of use. Additional details obtained for hormonal contraceptives included the brand, reason for use, and reason for stopping use. For each combined oral contraceptive preparation, we obtained information on active ingredients and doses (14) by using a variety of existing databases and reference books; for discontinued medications, we made inquiries to pharmaceutical manufacturers. Ethinyl estradiol and mestranol were the estrogens used in all combined oral contraceptives. Mestranol (100 μg) is approximately equipotent to estradiol (50 μg) (15, 16). Therefore, pills containing less than 100 μg of mestranol or less than 50 μg of ethinyl estradiol were categorized as low-estrogen dose formulations, and those containing 100 μg or more of mestranol or 50 μg or more of ethinyl estradiol were categorized as high dose.

Table 2
Oral contraceptive use characteristics, including estrogen and progestin does, among ovarian cancer cases and controls, Delaware Valley area, May 1994 to June 1988

* * *

There are no universally recognized standards for potency of progestins (17). Therefore, we used two alternative potency estimates, one based on the delay-of-menses test and the other based on the ability to induce subnuclear vacuolization, consistent with secretory function, in an estrogen-primed endometrium (18). Dickey and Stone (18) summarized potency data from two sources for each of these two assays.

To obtain a potency ranking for a given oral contraceptive preparation on the subnuclear vacuolization test, we multiplied the mean of the two potency estimates on the sub-nuclear vacuolization test for 1.0 mg of the progestin contained in that pill by the dose of progestin. Progestins were classified as low dose if their relative potency was less than 0.5 mg norgestrel. Alternatively, a mean potency estimate for each progestin was obtained from the delay-of-menses test. Again, the dose of the progestin was multiplied by the mean potency of 1.0 mg of each progestin. Progestins with a relative potency of 0.5 mg or more norgestrel were categorized as high dose. A relative potency of 0.3–0.4 mg norgestrel was considered intermediate, and a relative potency of 0.2 mg or less norgestrel was considered low dose.

Table 3
Odds ratios for characteristics of oral contraceptive use and dose adjusted for duration of oral contraception use, Delaware Valley area, May 1994 to June 1998

* * *

Covariates

Detailed demographic and reproductive information was obtained by interview. Demographic information included age, race, and education. Participants were asked about menstrual onset, regularity, and cessation. Each pregnancy, its length and outcome, as well as the length of breastfeeding, were recorded on the life calendar. Hysterectomy and its timing were recorded, as were women's reported weight and height. Detailed cancer histories for first-degree family members were also obtained.

Statistical analysis

Odds ratios, with corresponding 95 percent confidence intervals, were calculated as the primary measure of effect size. Because matching was based on frequencies for only two broad criteria, age within 5-year intervals and three-digit telephone exchange (or county of residence), we did not pre-

serve the "match" in the analyses. Odd ratios were adjusted for any residual effect of age and for gravidity (each as continuous variables), race (White/Black/other), and history of ovarian cancer in any first-degree relative (yes/no) in unconditional logistic regression models (19). Duration of oral contraceptive use was added to multivariable models in examining the relation between the risk of ovarian cancer and the following indicators of oral contraceptive use: time since last use, age at first use, year of first use, and dose. Statistical tests for trend in time since last oral contraceptive use, age at first use, calendar year of first use, and dose variables were based on evaluation of a continuous function for the use characteristic of interest among ever users; the model also contained all relevant adjustment covariates (age, gravidity, race, family history, and duration).

For analyses of combined oral contraceptive estrogen and progestin dose, only women who were taking combined oral contraceptive preparations with known estrogen and progestin content (n = 758) compared with women who never took oral contraceptives (n = 341) were included. We classified women's exposure to oral contraceptive formulations on the basis of the longest episode of use. Of the 1,366 women who ever used oral contraceptives, 78 used a tripha-sic or progestin-only preparation and so were excluded from these analyses. Among combined oral contraceptive users, 756 (59 percent) reported the brand name used for the longest episode, and 533 (41 percent) could not recall the brand name used. Of the 756 who recalled the brand name, 521 knew the specific formulation from which dose could be classified, and 235 used an unknown formulation of either Ortho-Novum (Ortho Pharmaceutical Corp, Raritan, New Jersey) or Norinyl (G. D. Searle & Co., Chicago, Illinois). Of the known Ortho-Novum and Norinyl formulations used by study participants, 68 percent were low estrogen/low progestin, and the remainder were high estrogen/low progestin. We analyzed the data, considering these unknown Ortho-Novum/Norinyl users first

as low estrogen/low progestin and then as high estrogen/low progestin, and it had no substantive effect on the interpretation of results. This report codes women using unknown Ortho-Novum/Norinyl preparations as low estrogen/low progestin in the analyses of oral contraceptive dose.

RESULTS

The 767 cases and 1,367 controls were predominantly in their fourth, fifth, and sixth decades of life; were White; and had completed high school (table 1). Since cases and controls were frequency matched on age, the crude odds ratios for age are reported to indicate the limited extent of residual confounding by age in the absence of adjustment. Pregnancies and livebirths were associated with a reduced risk for ovarian cancer; most of the effect occurred with the first reproductive event. Compared with White women, those in other racial groups were less likely to have ovarian cancer. Women who breastfed, particularly those who did so for 12 months or more, were somewhat less likely to develop ovarian cancer than were those who had a livebirth but did not breastfeed. Neither age at menarche nor age at menopause was associated with ovarian cancer risk in this study, nor was body mass index related to risk.

Table 4
Odds ratios for selected oral contraceptive use characteristics by invasiveness and histologic cell type, Delaware Valley area, May 1994 to June 1998

* * *

The risk of ovarian cancer was reduced by about 40 percent for oral contraceptive users overall after adjustment for age, gravidity, family history of ovarian cancer, and race (table 2). Longer duration of use afforded greater risk reduction. After adjustment for age, gravidity, family history of ovarian cancer, race, and duration of oral contraceptive use, the lowered risk was not significantly different for women who ceased oral contraceptive use 30 years or more previously compared with

10 years or fewer previously (odds ratio = 1.4, 95 percent confidence interval: 0.7, 2.6) (table 3). Similarly, oral contraceptives appeared to be protective, independent of age at initiation. Women who initiated use at or after age 35 years were afforded about the same protection as those who initiated use before age 20 (odds ratio = 0.9, 95 percent confidence interval: 0.5, 1.7) (table 3).

Dose of estrogens and progestins in oral contraceptive formulations did not substantially affect the reduction in ovarian cancer risk (tables 2 and 3). To evaluate the impact of dose, we first compared women who initiated pill use before 1972, when high-dose pills dominated the market; between 1972 and 1980, when a market transition from higher- to lower-dose formulations was underway; and after 1980, when new, lower-dose formulations predominated (17). The risk reduction associated with oral contraceptives did not differ by calendar period (table 2). We then estimated the odds of ovarian cancer by potency of estrogen and progestin among cases and controls who took oral contraceptives of known dose or who took unknown Ortho-Novum/Norinyl (see Materials and Methods) compared with those who never took oral contraceptives. Table 2 shows that the odds ratios for high-estrogen/high-progestin pills (odds ratio = 0.5, 95 percent confidence interval: 0.3, 0.7) was similar to that for low-estrogen/low-progestin pills (odds ratio = 0.5, 95 percent confidence interval: 0.3, 0.6) after adjustment for age, gravidity, family history of ovarian cancer, and race. Almost identical results were obtained after adjustment for age, education, parity, family history of ovarian cancer, race, tubal ligation, hysterectomy, and breastfeeding. Furthermore, table 3 shows that after adjustment for duration of oral contraceptive use, the odds ratio for low-estrogen/low-progestin compared with high-estrogen/high-progestin pills was identity (odds ratio = 1.0, 95 percent confidence interval: 0.7, 1.5). The results of these analyses are based on progestin dose categorized according to the subnuclear vacuolization test, but they were

consistent with those obtained when progestin dose was categorized according to the delay-of-menses test. Additionally, we analyzed the data according to estrogen potency independent of progestin and then according to progestin potency independent of estrogen (data not shown). These results indicated that high- and low-dose estrogen formulations (independent of progestin dose) were similarly protective and that high-, intermediate-, and low-dose progestin formulations (independent of estrogen dose) were similarly protective.

The impact of oral contraceptive use was not particularly variable by invasiveness of tumor (invasive vs. borderline) or by histologic type (serous, mucinous, endometrioid, clear cell, or other) as shown in table 4. In these subanalyses, the number of cases was smaller and confidence intervals were broader, but for all invasiveness/histologic types, the odds ratio among oral contraceptive users declined with longer duration of use. In addition, later age at initiation and lower-dose oral contraceptive formulations did not strongly reduce the protective effect of oral contraception in any invasiveness/histologic type. However, within broad confidence intervals, there was some suggestion that more than 10 years since oral contraceptive cessation was less protective for endometrioid and clear cell tumors.

We conducted a series of secondary analyses that served to show the robustness of our findings. First, we evaluated separately women less than age 55 years versus age 55 or older at interview. In both groups, low-estrogen/low-progestin and high-estrogen/high-progestin pills were similarly related to ovarian cancer risk in analyses after adjustment for age, pregnancies, family history, race, and duration of oral contraceptive use (odds ratios for low/low versus high/high doses were 0.7 and 1.0 for those aged less than 55 years and those aged 55 or older, respectively). Second, we restricted the analysis to women who used only one formulation of oral contraceptives, and this had almost no impact on the

comparison of low-estrogen/low-progestin with high-estrogen/high-progestin pills in relation to ovarian cancer (duration-adjusted odds ratio = 1.0, 95 percent confidence interval: 0.6, 1.6). Finally, we removed women who had used a formulation containing mestranol, again with no substantial effect on the comparison between low-estrogen/low-progestin and high-estrogen/high-progestin pills (odds ratio = 1.2, 95 percent confidence interval: 0.7, 2.1).

DISCUSSION

The protection afforded by oral contraceptives against ovarian cancer appears to be independent of the dose of estrogen or progestin. This observation is supported by analyses that show that women who initiated use of the pill before 1972, when pills generally contained 50 µg estrogen or more, were equally protected compared with women who initiated use of the pill after 1980, when pills generally contained less than 50 µg estrogen. As a consequence, oral contraceptive preparations commonly in use today appear to be equally as effective in reducing ovarian cancer risk as were higher-dose preparations of the past. Our data also indicate that the reduced risk of ovarian cancer from use of oral contraceptives continues for 30 or more years after discontinuation.

The effect of oral contraceptives in studies published prior to the mid-1980s has been primarily assessed in two meta-analyses, with summary odds ratios of 0.6 and 0.7 (2, 3). More recent case-control studies, in which a larger proportion of participants would have used lower-dose oral contraceptive preparations, generally support these estimates (20, 21), with the exception of one report by Hartge et al. (22).

To our knowledge, only three reports have specifically compared the effects on ovarian cancer risk of low-estrogen (<50 µg ethinyl estradiol) with high-estrogen dose (≥50 µg ethinyl estradiol) combined oral contraceptives (11–13). In a World Health Organization-sponsored case-control study, Rosenblatt et al. (11) compared 393 cases with 2,561 controls and found that the odds ratio for ovarian cancer was only

slightly higher for low-dose combined oral contraceptive preparations (odds ratio = 0.81) than for high-dose preparations (odds ratio = 0.68), a difference compatible with chance. A relatively small proportion of cases and controls used oral contraceptives: 30 cases used high-dose estrogen formulations, and 27 cases used low-dose formulations. The Cancer and Steroid Hormone Study evaluated a series of specific formulations, all of which were associated with relative risks of less than 1.0, some statistically significant and some not. The odds ratios associated with specific formulations ranged from 0.3 to 0.9. The relative reduction in ovarian cancer risk for higher- versus lower-dose formulations was not tested (12). Finally, Rosenberg et al. (13) showed odds ratios ranging from 0.4 to 1.3 for various formulations among women who used oral contraceptives for 3 or more years compared with controls. Given the small numbers of women using any given formulation, it was not possible to formally compare use of high- versus low-dose preparations.

In our study, oral contraceptives were protective long after stopping use (30 or more years) and were protective after relatively short durations of exposure (1–4 years). The long-term protection afforded by oral contraceptives has been shown in previous reports (1, 2, 23), although previous studies did not have the opportunity to observe as long a time interval between oral contraception cessation and incident ovarian cancer as did ours. These features (protection after short-duration use and long after cessation) enhance the attractiveness of oral contraception as a potential chemo-preventative for ovarian cancer. Recent evidence suggests that for women at elevated genetic risk for ovarian cancer, oral contraceptives may be protective (24). Further studies will be needed to evaluate the full benefit versus risk equation for such women, taking into account not only cancer at other sites but thrombotic risk as well (25–27).

Strengths of our study include the population-based ascertainment of cases and controls; the large number of

newly diagnosed cases; and the use of life-events calendars, comprehensive picture books, and structured interviews to enhance the recollection of medical information and contraceptive preparations used. All of these methodological features limited the potential for selection bias and information bias.

A weakness of our study was somewhat low participation rates among controls and cases. For cases, this was strongly influenced by whether women with prevalent ovarian cancer (diagnosed >6 months prior to interview) were included in the denominator when the response was calculated. In our design, we excluded such women to avoid survival bias. Excluding them from the denominator resulted in an 88 percent response rate; however, to the extent that the oral contraceptive use characteristics of these women may differ from those of women with ovarian cancer overall, we report the 61 percent response rate with them included in the denominator.

Another weakness is that, despite efforts to determine oral contraceptive preparations used over a lifetime, many women simply could not recall the exact formulation used. Nevertheless, we were able to classify estrogen and progestin dose for nearly 60 percent of users of combination oral contraceptives. Furthermore, we assumed unknown Ortho-Novum/Norinyl preparations to be low estrogen/low progestin. This resulted in confidence intervals that may have overestimated the precision of our estimates. Previous studies examining the relation between specific oral contraceptive formulations and ovarian cancer have also suffered from this limitation (11). Although validation studies have found that recall of use and timing of use of oral contraceptives is quite accurate, recall for specific formulations is less so (28–30). Because of this concern, we conducted an additional analysis using a surrogate measure of dose, i.e., year of initiation of oral contraceptive use. We nevertheless realize that this analysis may be influenced by cohort effects and time since last use.

In summary, our findings indicate that oral contraceptive formulations in common use today protect against ovarian cancer and that this effect continues long after use has stopped.

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APPENDIX H

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Abbreviation: CI, confidence interval.

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ORIGINAL CONTRIBUTIONS

PARITY, CONTRACEPTION, INFERTILITY, AND THE RISK OFEPITHELIAL OVARIAN CANCER

Harvey A. Risch,[1] Loraine D. Marrett,[2] and Geoffrey R. Howe[3]

A case-control study of reproductive factors and cancer of the ovary was conducted during 1989-1992 in metropolitan Toronto and nearby areas of Southern Ontario, Canada. In

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total, 450 women aged 35-79 years with histologically verified new primary epithelial ovarian cancers were interviewed concerning their reproductive histories. Over the same time period, 564 randomly selected population controls, frequency-matched to the cases according to three 15-year age groups, were also interviewed. Continuous unconditional logistic regression methods were used for analysis. It was found that childbearing and use of oral contraceptives were associated with significant decreasing trends in risk of ovarian cancer; the respective odds ratios were 0.78 for each full-term pregnancy ($p < 10^{-6}$) and 0.92 for each year of use ($p < 10^{-6}$). Hysterectomy was also associated with reduced risk, even after more than 20 years. Among parous women, infertility did not appear to affect risk; for nulliparous women, some evidence of increased risk was present, although fertility problems were reported by only a small fraction of nulliparae. It is suggested that the relatively lower parity of cases as compared with controls may be due to voluntary choices for having fewer children. *Am J Epidemiol* 1994;140:585-97.

contraception; infertility; ovarian neoplasms; parity; pregnancy; reproduction; retrospective studies

Cancer of the ovary is a highly fatal disease that affects, over a lifetime, almost 2 percent of women in the United States and Canada. During the last 15 years, various hypotheses regarding the etiology of this disease have been studied (1). Although mechanisms through which known exposure factors affect the risk of ovarian cancer development are still not particularly well understood, evidence exists that pituitary and/or sex hormones play an important role in the pathogenesis (2, 3). Perhaps best established is the *inverse* relation between ovarian cancer risk and parity. Par-ous women are at significantly lower risk than nulliparous women, and women who have had multiple full-term pregnancies are at even lower risk; the risk declines by approximately 15 percent for each additional full-term

pregnancy (12 reports were summarized by Whittemore et al. (4); see also 5-19). In addition, an inverse association with risk of ovarian cancer has been seen for use of oral contraceptives. For each year of use, risk seems to decrease by 5-10 percent, and this finding too has appeared in most work (10 of the 12 studies summarized by Whittemore et al. (4); also 6, 10, 17-21).

Low parity may be voluntary or involuntary. That is, it can arise from a lack of relevant sexual activity or from use of contraceptives ("voluntary"), or it can result from conditions causing decreased fertility or leading to hysterectomy ("involuntary"). Contraception, infertility, and hysterectomy may alter the risk of ovarian cancer development directly as well as through an effect on parity, and the degree to which each of these factors is independently associated with risk is unclear (22). Among nulligrav-idae, some studies have suggested an increase in risk for a history of 1 or more years of difficulty in conceiving (15, 17), although the same factor appears less related to risk for ever-gravid women (15, 17, 23). Interpretation of data from studies reporting on this factor for women in general, i.e., unstratified by or unadjusted for parity, is uncertain because of the association with low parity (5, 24, 25). A similar uncertainty holds for risk associations with contraceptive-free years of marriage (24, 26). In order to study voluntary and involuntary aspects of reproduction and the risk of ovarian cancer development, we carried out an exploratory case-control study, with a focus on fertility and contraception.

MATERIALS AND METHODS

Selection of subjects and data collection have been described in a previous report (27) and will be summarized here. All histologically confirmed, primary, malignant or borderline malignant epithelial ovarian tumors first diagnosed from November 1989 through October 1992 among Ontario, Canada, residents aged 35-79 years were identified from the province-wide pathology reports received

by the Ontario Cancer Registry. Those subjects living at the time of diagnosis in the regional municipalities of York, metropolitan Toronto, Peel, Halton, Hamilton-Wentworth, Waterloo, Brant, and Niagara and the city of Guelph were eligible to be cases. In total, we identified 631 eligible cases and interviewed 450 (71.3 percent); of the remainder, 55 had died (8.7 percent), 29 had physicians who refused consent (4.6 percent), 30 were too ill to be interviewed (4.8 percent), 17 were lost to follow-up (2.7 percent), and 50 refused to participate (7.9 percent). Because of the relatively short time from diagnosis to interview (approximately 12-14 weeks), fewer than 10 percent of eligible subjects had died; thus, no proxy interviews were conducted.

A sample of controls was obtained from the Enumeration Composite Record listing of individuals which is compiled by the Ontario Ministry of Revenue. These records are organized by census division, include all homeowners, tenants, and family members, and contain name, address, age, and sex. From this listing, the Ministry provided names and addresses of a random sample of women resident in the study area during the same 3-year period, frequency-matched within the age groups 35-49, 50 — 64, and 65-79 years to the expected distribution of cases based on incidence tabulations from the Ontario Cancer Registry. Controls were contacted by letter, with follow-up by telephone, to confirm suitability; arrangements were then made for interview. Control women who reported the removal of both (or an unknown number of) ovaries 1 year or more in the past were considered ineligible and were omitted from the study (n = 103). As with the cases, only living subjects were included. In total, 873 eligible control women were identified, of whom 564 (64.6 percent) were interviewed. The remainder either refused to participate (30.2 percent), were too ill (1.9 percent), or were lost to follow-up (3.2 percent).

A questionnaire was developed for ascertainment of medical and reproductive history. Detailed information was

obtained regarding menstrual characteristics, pregnancies, hormone and contraceptive use, and infertility factors. In the interview, a life events calendar was used to help organize the various reproduction-related behaviors and outcomes; thus, the ages at occurrence of and durations of time applicable to these factors were obtained. We asked about episodes of infertility in terms of periods of time without contraception when pregnancy was attempted without success. For tubal ligations, we assumed that the operations were irreversible; no subsequent pregnancies occurred among our subjects. All interview questions were the same for cases and controls, and for both groups, information pertaining to events or exposures that occurred within 1 year of interview was excluded from analysis. Interviews were conducted in person in the home of the subject.

To analyze the data, we used multivariate unconditional continuous logistic regression methods, which allow for the simultaneous examination of multiple exposure factors. The GLIM computer program (28) was employed. Both trends in risk odds with expokure (parameter estimates of slope) and relative odds by category were examined. We have reported trend effects for single units of exposure, e.g., 1 year of oral contraceptive use or one pregnancy. Tests of statistical significance were based on differences in log-likelihood; two-sided *p* values are given. For 95 percent confidence limits, we exponentiated the parameter estimate ± 1.96 standard errors. Each of the models in this paper included indicator terms for the age categories of the frequency matching (35-49, 50-64, and 65-79 years); we also included age as a continuous variable in order to adjust for residual age effects. Where not otherwise examined as variables of interest, total duration of oral contraceptive use and number of full-term pregnancies have been included as continuous terms in the models as well.

RESULTS

Both cases and controls were essentially white (96.4 and 96.1 percent, respectively), and as expected from the frequency matching, the mean ages were close, 57.2 and 57.5 years (table 1). A slightly greater number of controls than cases were born in Canada or the United States; however, mean years of education was almost identical for the two groups. Average reported height and weight at age 21 were virtually the same for cases and controls, giving very similar mean indices of body mass (weight (kg)/height^2 (m^2)). Consistent with many other studies of ovarian cancer, a smaller percentage of cases than controls were par-ous or had ever used oral contraceptives, and among those subjects, cases had had fewer full-term pregnancies than controls and had used oral contraceptives for shorter periods of time.

Parity and related factors

Table 2 shows the relative odds of ovarian cancer according to number of full-term pregnancies. Compared with nulliparae, parous women had a 61 percent lower risk overall, with relative odds ranging from 0.64 at one full-term pregnancy to 0.23 for five or more full-term pregnancies. No association with risk was present for ever having had a stillbirth (odds ratio = 1.19, 95 percent confidence interval (CI) 0.572.47). Figure 1 shows the percentages of cases and controls who reported having had a full-term pregnancy at any time in each 1-year interval of age during the reproductive years. Between the ages of 20 and 35, smaller fractions of cases than controls reported having been pregnant.

Trends in risk according to number of pregnancies are shown in table 3. Among all subjects, the odds of ovarian cancer dropped by about 22 percent with each successive full-term pregnancy, regardless of whether the pregnancy occurred before or after age 30, whether the subject was younger or older than 55 years at interview, or whether the subject had ever used oral contraceptives. Somewhat greater

protection per pregnancy was seen for women who had undergone hysterectomy or who reported having had an interval of infertility. When only parous subjects were analyzed, similar trends were seen, the relative odds declining by 16 percent for each additional full-term pregnancy. No association with risk was present for age at first or last full-term pregnancy or for average time span between pregnancies. There was slight evidence for an inverse association with total duration of lactation, one that was a bit stronger for average months of lactation per pregnancy ($p = 0.030$); pregnancies with lactation appeared to be slightly more protective than pregnancies without. No association was seen with number of miscarriages or with number of induced abortions among either parous subjects or nulliparae (for number of induced abortions, $p = 0.40$ and $p = 0.24$, respectively).

TABLE 1.
Sociodemographic and reproductive factors among ovarian cancer cases and controls, Southern Ontario, Canada, 1989-1992

* * *

TABLE 2.
Odds ratios for epithelial ovarian cancer according to number of full-term pregnancies, Southern Ontario, Canada, 1989-1992

* * *

Figure 1.

* * *

Contraception and hysterectomy

Results for use of oral contraceptives are given in table 4. Results are shown separately for nulliparous women, parous women, and all subjects. In general, a decreasing trend in risk was seen according to categories of increasing duration of oral contraceptive use. Compared with never use, the odds were close to one half at 1 year of use and approached one

third after 10 years of use. Little difference was found when parous women were subdivided into groups of parity 1-2 and ≥3 (not shown). Based on the trend estimates, the risk dropped by about 8 percent for each successive year of use, with somewhat greater reduction (13 percent) for nulliparae. Among women with a parity of 1 or 2, the odds ratio per year of use was 0.918 (95 percent CI 0.87-0.97), and for those with a parity of ˉ3, it was 0.945 (95 percent CI 0.89-1.01); effect modification (interaction) between duration of oral contraceptive use and parity was not statistically significant *(p* = 0.50). Similar reductions in risk with use of oral contraceptives were seen regardless of whether hysterectomy had been performed and regardless of whether intervals of infertility had ever been reported, as well as for oral contraceptive use before or after first full-term pregnancy. Age at first use and years since last use did not appear to be related to risk within any of the parity groups or among all subjects.

TABLE 3.
Trends in ovarian cancer risk according to parity-related factors, Southern Ontario, Canada, 1989-1992

* * *

Table 5 shows relative odds of ovarian cancer according to ever use of other forms of contraception. A small and nonsignificant decrease in risk was seen for women who had undergone tubal ligation, and essentially no association appeared with use of an intrauterine contraceptive device. In addition, there was no association found for having ever regularly used a contraceptive diaphragm, condoms, jelly, foam, rhythm or temperature methods, or a combination of the above. In total, from age 25 onward, about 20 percent more controls than cases had used some form of contraception.

Among our subjects, parous or nullip-arous, having had a hysterectomy was associated with significantly lower risk of ovarian cancer (table 5). The odds ratios were

similar for women of parity 1-2 (odds ratio = 0.53, 95 percent CI 0.32-0.90) and parity 3 (odds ratio = 0.57, 95 percent CI 0.34-0.95); effect modification by parity was not significant (p = 0.46). It has been suggested that patients undergoing hysterectomy may, as part of the operation, have their ovaries visually examined for the presence of malignancy (29). Where no cancer is found and the ovaries are not removed, such women will have a reduced risk of developing ovarian cancer, at least for the few years required for an occult tumor to produce signs or symptoms leading to diagnosis (29). However, among our study subjects, no difference was seen in risk according to years since hysterectomy (Appendix table 1); 15, 20, or more years following hysterectomy, the relative odds remained about 0.5.

TABLE 4.
Odds ratios and odd-sratio trends for epithelial ovarian cancer according to use of oral contraceptives (OC), Southern Ontario, Canada, 1989-1992

* * *

TABLE 5.
Odds ratios and odds-ratio rends for epithelial ovarian cancer according to contraception and infertility factors, Southern Ontario, Canada, 1989-1992

* * *

Infertility

Among parous women, having ever had an interval of time when pregnancy was attempted without success ("infertility") was not associated with increased risk of ovarian cancer (table 5). Little difference appeared between women of parity 1-2 and women of parity 3 (odds ratios were 0.53 (95 percent CI 0.27-1.05) and 0.51 (95 percent CI 0.18-1.46), respectively). For nulliparae, a 50 percent increase in risk was seen, though it was not statistically significant (p = 0.37). There were no trends in risk with total duration of reported infertility. Figure 1 shows that up to age 26, cases

were less likely than controls to report periods of infertility. Among nulliparous women who had had infertility intervals, later age at onset of the first interval was associated with increased risk *(p* = 0.0016); affected cases reported an average age at onset of 27.9 years, about 4.7 years later than that reported by affected controls. Little difference was seen in risk according to onset age for parous subjects. In total, 17 percent of nulliparous cases and 5.5 percent of parous cases reported ever having an interval of infertility. Between the ages of 15 and 45 years, more than 80 percent of subjects were not prevented by infertility (or hysterectomy) from becoming pregnant.

Finally, in the present study, two controls and no cases reported that they had ever used clomiphene citrate (Clomid®; Marion Merrell Dow, Inc., Kansas City, Missouri).

DISCUSSION

Prior to drawing conclusions from the present work, certain potential limitations should be considered. The response fraction of 71 percent of eligible cases suggests that our results may be slightly more representative of women in earlier disease stages than of all women with ovarian cancer. All of our cases were analyzed together, under the assumption that the various histologic types of epithelial tumors, including borderline tumors, have similar relations to the risk factors considered. The distribution of histologic types (serous, 50 percent; mucin-ous, 18 percent; endometrioid, 16 percent; clear cell, 6 percent; other and undifferentiated, 10 percent; and borderline malignant, 18 percent of all types) among cases was very similar to that seen elsewhere (21, 30). For the controls, with 65 percent participation of eligible subjects, it is possible that the recorded reproduction-related practices and outcomes may not have been completely representative of the female population of Southern Ontario. However, we have no evidence that the behaviors of the noninterviewed eligible

controls differed from those of our subjects. The usual reason given for refusal of the 2-hour interview was lack of sufficient time. With respect to parity, the average number of full-term pregnancies among our controls, 2.45, was very similar to that calculated from 1991 Ontario census age-specific data, 2.47 (31).

This study obtained infertility information only from subject reports, in terms of time intervals when subjects actually experienced difficulties in becoming pregnant. Thus, some misclassification could have occurred because infertile women who had never attempted to have children would not have reported any difficulties. In addition, the existence and length of reported periods of unsuccessful conception attempts may not closely reflect the underlying causes of the lack of success, although this information does bear on the behavioral choice to become pregnant. In our study, subjects were also asked whether they had ever been told by a physician that they had a fertility problem. Seven nulliparous subjects and eight parous subjects described such fertility problems but did not report any difficulties with respect to becoming pregnant. Inclusion of these 15 women with the subjects who had a history of infertility intervals made no difference in the results.

Consistent with virtually all other studies, the present work demonstrates that childbearing and oral contraceptive use are associated with significant and appreciable reductions in risk of ovarian cancer (4-21, 23). In addition, hysterectomy appeared to convey some protection, even after 20 or more years had elapsed since surgery, a finding supported by most, though not all, previous work (4, 17, 19, 29, 32-35). For both oral contraceptive use and hysterectomy, similar risk reductions were seen at each level of parity, suggesting that both factors may affect risk independently of the association between parity and risk. Likewise, the protection associated with increasing parity appeared among women who had undergone hysterectomy as well as among those with intact

uteri, and for both ever users and never users of oral contraceptives. Finally, oral contraceptive use seemed to be protective with or without a history of hysterectomy, and hysterectomy to be protective with or without ever use of oral contraceptives. It thus appears that parity, oral contraceptive use, and hysterectomy may have independent contributions in determining the risk of ovarian cancer development.

Evidence for a protective role of breast-feeding is less clear. Two reports have described trends of decreasing risk with longer durations of lactation (23, 36), though others have shown no relation (15, 17, 25, 37). Our study shows only a slight decreasing trend of borderline statistical significance.

We also found only a small, nonsignifi-cantly lowered risk for women who had undergone tubal ligation. Decreased risk has been seen in other work (12, 17, 19, 34, 35, 38), though not universally (13, 15). With regard to use of an intrauterine device or other nonpermanent forms of contraception, no studies, including this one, have demonstrated significant associations with risk of ovarian cancer (13, 15, 17, 18).

In general, we are unable to confirm a significant role for infertility over and above that for low parity in the risk of developing ovarian cancer. Although a possible increase in risk with infertility was observed for nulliparae, at other parity levels the risks did not exceed unity. Only two controls and no cases reported having ever used clomiphene citrate for their infertility. Among women with a history of infertility, parity was strongly protective. Less than 10 percent of the cases reported ever having an interval of infertility, which suggests that infertility does not account for a large fraction of disease occurrence. At any age during the reproductive years, more than 80-90 percent of subjects (including nul-liparae) were apparently not prevented from getting pregnant via infertility or hysterectomy. Thus, the relatively lower lifetime parity of cases as compared with

controls seems to be due to voluntary behavioral choices for having fewer children.

Among parous women, the age distribution of pregnancies (figure 1), as well as the lack of difference in age at first full-term pregnancy, indicate that in comparison with the controls, the cases did not put off childbearing to later years, when their fertility may have been lower. Some evidence for this delay is present for nulliparae (figure 1, ages 18 -25, and table 5), where initial observations by subjects about difficulty in conceiving appear about 3-5 years earlier for controls than for cases; i.e., cases who never gave birth started trying to become pregnant later than controls. However, even in the later reproductive years, the majority of subjects, including nulliparae, did not appear to be prevented from becoming pregnant.

For ever-gravid or parous women, a history of trouble conceiving has not generally been associated with significantly increased risk of ovarian cancer (4, 15, 37), nor has a history of 5, 10, or more years of unprotected intercourse (4, 17). One study, however, has shown a twofold increase in risk for a history of difficulty in conceiving among parous women (24). Among nullip-arous or nulligravid women, these aspects of infertility have shown relative risks in the 2-5 range in the same studies (4, 15, 17, 24, 37). In the Cancer and Steroid Hormone Study, neither having a medical diagnosis of infertility nor having a period of 2 or more years of unprotected sexual activity without conception was associated with risk, after adjustment for parity, oral contraceptive use, etc. (23). Thus, the evidence to date suggests that among women who ultimately succeed in having children, infertility per se has little to do with the risk of ovarian cancer; among women who remain childless, the great majority do so by choice, and the remainder may be at additional increased risk. These women could constitute a high-risk group worthy of special prevention efforts.

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APPENDIX TABLE 1
Odds ratios for epithelial ovarian cancer according to length of time since hysterectomy, Southern Ontario, Canada, 1989-1992

* * *

APPENDIX I

A QUANTITATIVE ASSESSMENT OF ORAL CONTRACEPTIVE USE AND RISK OF OVARIAN CANCER

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Objective: **To provide a quantitative assessment of the association between oral contraceptive (OC) use and ovarian cancer using results from the published literature.**

Data sources: **We conducted a MEDLINE literature search for all epidemiologic studies of OC and ovarian cancer published in English between 1970-1991. The reference list for each article was reviewed to locate additional published articles.**

Methods of study selection: **We included 20 studies in which a relative risk and either a standard error, confidence interval, or *P* value was reported, or sufficient**

data were presented to allow us to calculate these measures.

Data extraction and synthesis: **We summarized the findings using weighted averages and regression analyses. We found a summary relative risk of 0.64 (95% confidence interval 0.57-0.73) associated with ever-use of OC, indicating a 36% reduction in ovarian cancer risk. The risk of ovarian cancer decreased with increasing duration of OC use; we noted a 10-12% decrease in risk with 1 year of use and approximately a 50% decrease after 5 years of use. The reduced risk was present among both nulliparous and parous women and it appeared to last for at least 10 years after cessation of use. Although most studies assessed the use of OC formulations from the 1960s and 1970s, data from the Cancer and Steroid Hormone Study indicate that the decreased ovarian cancer risk may also be present with current lower-dose formulations.**

Conclusion: **The protective effect of OC against ovarian cancer risk should be considered in a woman's decision to use OC. (*Obstet Gynecol 1992;80:708-14*)**

Ovarian cancer is the fourth leading cause of cancer death in American women.[1] The lifetime risk of this disease is 1-2%,[2] and less than 40% survive 5 years after diagnosis.[1] Because ovarian cancer has a poor prognosis, prevention is important.

In 1977, Newhouse et a1[3] found that women who used oral contraceptives (OC) had a reduced risk of ovarian cancer. Since then, at least 20 additional epi-demiologic studies have addressed this issue. Two previous reviews[4,5] noted a reduction in ovarian cancer among women who had used OC, particularly for long durations. In this paper, we update these findings in a meta-analysis of 20 epidemiologic studies to estimate the impact of OC on reducing the risk of ovarian cancer. We also assess the effects of duration of use and time

since last use, and of potential modifiers such as age and parity.

Materials and Methods

We conducted a MEDLINE literature search for all epidemiologic studies of OC and ovarian cancer published in English between 1970-1991. The reference list of each article was reviewed to locate additional published articles. We did not include abstracts or unpublished studies because these data would not have been subject to peer review.

We included all studies in which a relative risk (RR) and either a standard error, confidence interval (CI), or *P* value was reported, or in which sufficient data were presented to calculate these measures. Reports without this information[6,7] were excluded. When 95% CIs were not presented, we calculated test-based intervals if the *P* value was reported, or if not, we estimated CIs from the crude data using Cornfield's method.[8]

For multiple reports from one study, mutually exclusive case and control series were included as separate studies.[9,10] Otherwise, only the more recent paper was used; the one exception was in the duration analyses, for which the earlier paper provided the necessary data.[11,12]

Only one study[13] assessed all ovarian cancers combined, rather than only those of epithelial origin. Because 80-90% of ovarian cancers are epithelial' and because the results were similar when including or excluding this study, we have included it. Three studies included only malignant ovarian tumors,[18-17] seven had malignant and borderline tumors,[6,12,13,18-21] and one addressed only borderline tumors; the rest did not indicate whether borderline tumors were includ-ed.3,9,10,23-213 Because the effect of OC is similar for the risk of both borderline and malignant tumors[6,19,20,22] and because we noted similar RRs for ever-use of OC in the four studies that provided estimates specifically for malignant tumors, we have included all studies in the analysis.

We used the term "relative risk" to denote an odds ratio or a risk ratio. Summary RRs for ever using OC were calculated within study design: hospital-based case-control studies, community-based case-control studies, and cohort studies. When a single study presented separate results for hospital and community controls,[3] we included only the results using community controls. A summary estimate across the three study designs was also calculated.

We calculated the summary RR associated with ever-use of OC using a method developed by DerSimonian and Laird,[29] which assumes a random-effects model and allows for sampling variation within and between studies. If there is variation in the results between studies, accounting for this in the analysis will result in appropriately wider CIs. When results between studies are similar, the DerSimonian-Laird estimate (and 95% CI) is comparable to a simple average weighted by the inverse of the variance. The Q statistic,[29] a test of homogeneity between studies, was calculated for each group of studies.

We calculated a summary RR associated with up to 1 year of OC use from the seven studies that reported these data.[16-21,26] We also calculated a summary RR from ten studies reporting OC use of at least 5 years.[11,13,17-21,23,26,28] When a report presented two duration categories of either 1 year or less[19] or 5 years or more,[19,28] we derived a single estimate by calculating a Mantel-Haenszel summary odds ratio.[8]

For each of the 15 studies that reported the RR by OC duration, we also fit a weighted regression line over the categories of duration to determine the average change in risk per year of OC use.[30] For each duration category, we used the inverse of the variance of the category-specific estimates as the category weight. We then computed an overall slope, weighting each individual slope by the inverse of its variance. This variance was somewhat underestimated as it does not account for the covariance resulting from the same non-OC users serving as the reference group in each duration category

within a study. In most studies, the referent group was large, minimizing this source of error. We used category midpoints to define the exposure duration in each category. Because the longest duration categories were open-ended, we conducted two analyses using the lower bound of that category plus 2 years and then the lower bound plus 4 years.

In alternative regression analyses, the regression line was either forced through zero or allowed to have a non-zero intercept. In calculating the latter summary estimate, we used the number of exposed cases to weight the individual slopes, because studies with only two duration categories had no variance in slope.

To assess whether the reduced risk of ovarian cancer persists after OC use has ended, we calculated a summary RR associated with 10 or more years since last use from seven studies.[10,18-21,26,28]

We calculated a summary RR for ever using OC separately for nulliparous and parous women and used a X^2 statistic[31] to test for significant differences in the RRs. We also reviewed the evidence for an effect of OC use by age at diagnosis. Although nine of the 20 reports addressed this issue,[9,10,13,18-20,26,28,32] the variability in age categories precluded a quantitative estimate.

Results

Table 1 presents the study characteristics and RRs associated with ever having used OC for the 20 studies. All RR estimates are at least age-adjusted (or are similar to the age-adjusted results according to the authors); the multivariate RRs are presented where possible. Of the 20 studies, 18 noted an inverse association between OC and ovarian cancer ranging from 0.25-0.8, although only six estimates were statistically significant.[12,15,18,19,22,28] One United States hospital-based case-control study was null (RR 1.0; 95% CI 0.7-1.7),[21] and a community-based case-control

study from China reported an increased risk (RR 1.8, 95% CI 0.8-4.1).[17]

The summary RR for the nine hospital-based case-control studies was 0.70 (95% CI 0.60-0.81), indicating a 30% reduction in risk (Figure 1).[9,10,16 20 21,25-28] The test of homogeneity between these studies was not significant *(P* > .50). Combining the eight community-based case-control studies yielded a similar 37% reduction in ovarian cancer risk (RR 0.63, 95% CI 0.490.80).[3,12,17-19,22-24] Here, the test of homogeneity was nearly significant *(P* = .10), reflecting the positive association reported in the study by Shu et al.[17] The summary RR from the three cohort studies was 0.43 (95% CI 0.25-0.75; homogeneity test, *P* > .25).[13,15,32] The summary RR from all 20 studies indicated a 36% decreased risk in ever-users of OC (RR 0.64, 95% CI 0.57-0.73).

Fifteen of the studies reported the effect of duration of OC use on ovarian cancer risk (Table 1). Nine studies showed a decreasing risk with increasing duration of use,[9,10,13,16,19,20,23,26,28] but the magnitude of the decrease varied.

We found a nonsignificant 12% reduction in ovarian cancer risk (summary RR 0.88, 95% CI 0.67-1.14) among the seven studies that reported an RR for up to 1 year of OC use. In four studies,[16,18,20,26] the relative risk ranged from 0.86-0.97. The Cancer and Steroid Hormone Study noted the largest reduction in risk (RR 0.6, 95% CI 0.4-0.9),[19] whereas two reports[17,21] documented an increased risk. The summary RR for 5 or more years of OC use was 0.46 (95% CI 0.36(159).11,13,17-21,23,26,28 The individual estimates ranged from 0.3-0.8 except in the study by Shu et a1,[17] which reported an increased risk.

Table 1.
Characteristics of Studies in Meta-Analysis and Relative Risk of Ovarian Cancer Associated With Ever-Use of Oral Contraceptives and Duration of Use

* * *

Figure 1.
Relative risks (RR) and 95% confidence intervals for ever use of oral contraceptives and ovarian cancer. Numbers at left indicate reference numbers

* * *

In the regression analysis (Table 2), the summary estimate was 0.89, indicating an 11% reduction in risk for each year of OC use. For 5 years of use, the estimated risk reduction was 46% (Table 2 and Figure 2). When we allowed for a non-zero intercept, the summary RR was unchanged. When we repeated the analysis, adding 2 years to the category of longest duration of use, the summary RR again changed very little (0.90).

Table 2.
Slope Per Year of Oral Contraceptive Use From a Regression Model and Predicted Relative Risks Associated With 1 and 5 Years of Use

* * *

The risk of ovarian cancer among women who had stopped using OC at least 10 years previously was at least 10% lower (range 10-70%) than among non-users in six of the seven studies.[10,18-20,26,28] In one study, a decrease in risk was noted even after 15 years since last OC use (RR 0.5, 95% CI 0.4-0.8).[19] Only Hartge et a1[21] reported an increased RR (RR 1.4, 95% CI 0.7-2.6) for those who quit using OC 10 or more years previously. Compared with non-users, women who stopped using OC 10 or more years before had a summary RR of 0.60 (95% CI 0.42-0.86), indicating persistence of a substantial protective effect of OC for at least 10 years after last use.

The association between OC and ovarian cancer among nulliparous women was reported in ten studies, but only eight provided data in a manner that we could use in our summary estimate.[9,10,18,20,22,24,26,28] In all eight studies, a decrease in ovarian cancer risk was noted among nulliparous women who used OC (RR 0.16-0.9); the summary RR was 0.55

(95% CI 0.380.80). The two studies not included in the summary estimate had similar findings.[16,19]

Seven studies presented results separately for pa-rous women.[9,18,20,22,24,26,28] The summary RR was 0.55 (95% CI 0.39-0.78), nearly identical to our findings for nulliparous women. In two of the four studies not included in our summary estimate,[19,16] a decreased risk of ovarian cancer was noted among OC users in every parity category. In the two remaining studies, an inverse association was noted in all but the highest parity category.[13,19] Only in the World Health Organization (WHO) study[2°] were the differences in the OC benefit statistically significant between parous and nulliparous women, with a stronger benefit among nulliparous women.

Figure 2.
Relative risk of ovarian cancer associated with different durations of oral contraceptive use: findings of 15 studies

* * *

In most reports that examined possible variations in the association between OC and ovarian cancer by age, an inverse association was noted in women of all ages.[9,10,13,19,20,26,28,32] All but two RRs were less than 1.0 (range 0.0-0.8), although the results were often not statistically significant. Thus, there are few data to suggest that the reduction in risk of ovarian cancer among OC users is altered substantially by differences in parity or age.

Discussion

The association between OC and ovarian cancer has been assessed in at least 20 studies and the findings have been remarkably consistent. We found a summary RR of 0.70 (95% CI 0.60-0.81) among hospital-based case-control studies, 0.63 (95% CI 0.49-0.80) among community-based case-control studies, and 0.43 (95% CI 0.25-0.75) among cohort studies. Our summary estimate from all three study designs, indicating a 36% risk reduction from ever-use of OC, is similar to that reported in two previous meta-

analyses[4,5] and in a pooled analysis of three hospital-based case-control studies.[33] We also found a 10-12% decrease in risk for each year of OC use and approximately a 50% decrease in risk after 5 years of use. Both nulliparous and parous women who use OC have a decreased risk of ovarian cancer, as do women of almost all ages.

Only one study reported an increase in ovarian cancer risk with OC use,[17] although the elevation was not significant and a modest protective effect could not be ruled out. This might reflect a differential effect of OC in a less developed country or in a low-risk population such as China. However, in the WHO study,' a decrease in risk was noted among OC users both in China (RR 0.88, 95% CI 0.17-4.67) and in the five less developed countries when results were combined (RR 0.77, 95% CI 0.56-1.01). The population-based design, high participation rates, and control for known ovarian cancer risk factors all reduce the likelihood of important bias. It seems most likely that this increase in risk was a chance finding.

Although our results reflect the limitations of the component studies, these are unlikely to account for the inverse association. Similar inverse associations are noted across study designs and populations, for which sources of bias differ. In the prospective studies, which are less prone to bias, the OC benefit was most pronounced. Because most publicity about OC has concerned adverse health effects and because women with ovarian cancer may remember past drug use better, recall bias would likely have resulted in an underestimate of the apparent benefit because of over-reporting of OC use by the cases. The consistency across studies and high statistical significance also make it unlikely that the strong inverse association between OC use and ovarian cancer results from chance.

We were unable to include the findings from two published reports because RRs or data to calculate CIs were not available. However, an inverse association was suggested in these studies.[5,7] In a meta-analysis using only published

reports, the possibility of publication bias must also be assessed. Studies with null findings are less likely to be submitted for publication and less likely to be published.' However, the consistency of the results across studies suggests that many null studies would be needed to dilute or negate this association.

Two primary mechanisms for the effect of OC use on ovarian cancer risk have been hypothesized. The "incessant ovulation" theory proposes that women have a greater risk of ovarian cancer with increasing number of ovulations, because of repeated injury to the ovarian epithelium (Fathalla MF. Incessant ovulation—a factor in ovarian neoplasm [letter]. Lancet 1971;ii:163). However, several authors have found that the degree of risk reduction varied per month of anovulation induced by OC use, parity, and lactation.[12,35] A second hypothesis is that the reduction in plasma gonadotropin levels in OC users decreases the risk of ovarian cancer. High gonadotropin levels have been associated with ovarian cancer in animal studies," and gonadotropins stimulate the growth of cell lines derived from human ovarian cancers.'

One study found a significant 40% reduction in risk after just 3-6 months of use.[19] It seems unlikely that such a short duration of use could reduce risk so markedly simply by suppressing ovulation or reducing gonadotropin levels. However, a more complex mechanism may be involved, or alternatively, the estimate of effect may have been biased. We observed a summary RR of 0.88 for OC use of 1 year or less. The results from the regression analysis were similar: The RRs were 0.89-0.90 per year of OC use. When we combined results from the ten studies presenting an RR for 5 or more years of OC use, we found a 54% reduction in risk, an estimate very similar to that predicted by the regression approach (RR 0.54 after 5 years of OC use). These results are also similar to those reported in a prospective study of OC use and ovarian cancer mortality.[38]

These analyses have several limitations. Among the studies that provided specific RRs for OC use of up to 1 year, the results are heavily weighted by the largest.[19] In addition, studies not presenting this category of use are perforce not included in the analysis (this limitation applies in all analyses using only a subset of the 20 studies). In the regression, we assumed that category midpoints represent exposure experience in that category, that the covariance between exposure categories was zero, and that there is a linear effect (on the log scale) of exposure on disease. Despite these limitations, we found remarkably similar reductions in risk in each analysis when we altered the assumptions. Overall, our results suggest a small decrease in ovarian cancer risk with even 1 year of OC use, but longer durations of use are needed for a substantial risk reduction.

The reduced risk of ovarian cancer appeared to last at least 10 years after quitting OC use. Most of the RRs in that analysis were not adjusted for duration of OC use; this would most likely lead to an underestimate of the true persistence of the beneficial effect, because women at a given age with longer durations since last use will have used OC for shorter durations. In the Cancer and Steroid Hormone Study[19] and when the results from two European studies[10,28] were pooled,[33] the protective effect of OC appeared to remain at least 15 years after last OC use.

These 20 studies assessed OC use almost exclusively in the 1960s and 1970s. Formulations have changed considerably over time: The estrogen dose has decreased from approximately 100 μg to as low as 30 μg in current formulations,"." and the types and dose of progestin have also changed. Gonadotropin levels are suppressed more with the high-dose than with the low-dose formulations.[41,42] Therefore, depending upon the mechanism of the protective effect of the earlier OC, the new formulations may or may not offer the same protection against ovarian cancer. Most studies provided insufficient data to address this issue in their

analyses. Only the Cancer and Steroid Hormone Study[19] assessed the effect of specific formulations, including two with 35 μg or less of ethinyl estradiol. The RRs for ever using these two formulations were 0.7 (95% CI 0.4-1.2) and 0.4 (95% CI 0.2-0.7), respectively, indicating that the protective effect may also be present with lower-dose formulations. Confirmation of this association with the use of current OC formulations is needed; however, the present evidence suggests at least some reduction in the risk of ovarian cancer.

The protection offered by OC against ovarian cancer, in addition to other known benefits (eg, contraception and reduced risk of endometrial cancer), must be weighed against any possible increase in the risk of other diseases associated with its use (eg, myocardial infarction). This issue is complex and has recently been considered in a risk-benefit analysis.'

The two factors most consistently associated with a decrease in ovarian cancer risk are parity and OC use. Because parity is usually not considered a modifiable risk factor, only OC use offers an opportunity for primary prevention. Women with a family history of ovarian cancer have a risk 3.3[21] to 18.2[25] times that of women with no family history. The decision to use OC must be made individually in consultation with a physician; the reduction in risk of ovarian cancer should be considered in that decision, especially for women at high risk of this disease.

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APPENDIX J

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Abbreviations: CI, confidence interval; OR, odds ratio.

CHARACTERISTICS RELATING TO OVARIAN CANCER RISK: COLLABORATIVE ANALYSIS OF 12 US CASE-CONTROL STUDIES

II. INVASIVE EPITHELIAL OVARIAN CANCERS IN WHITE WOMEN

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Data collected from 2,197 white ovarian cancer patients and 8,893 white controls in 12 US case-control studies conducted in the period 1956-1986 were used to evaluate the relation of Invasive epithelial ovarian cancer to reproductive and menstrual characteristics, exogenous estrogen use, and prior pelvic surgeries. Clear trends of decreasing risk were evident with Increasing number of pregnancies (regardless of outcome) and increasing duration of breast feeding and oral contraceptive use. Ovarian dysfunction leading to both infertility and malignancy is an unlikely explanation for these trends for several reasons: 1) The trends were evident even among the highly parous; 2) risk among nulliparous women did not vary by marital status or gravidity; and 3) risk among ever-married women showed little relation to length of longest pregnancy attempt or history of clinically diagnosed infertility. Risk was increased among women who had used fertility drugs and among women with long total duration of premenopausal sexual activity without birth control; these associations were particularly strong among the nulligravid. No consistent trends in risk were seen with age at menarche, age at menopause, or duration of estrogen replacement therapy. A history of tubal ligation or of hysterectomy with ovarian conservation was associated with reduced ovarian cancer risk. These observations suggest that pregnancy, breast feeding, and oral contraceptive use induce biological changes that protect against ovarian malignancy, that, at most, a small fraction of the excess ovarian cancer risk among nulliparous women is due to infertility, and that any

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increased risk associated with infertility may be due to the use of fertility drugs. Am J Epidemiol 1992;136:1184-1203.

estrogens; fertility agents, female; infertility; lactation; pregnancy

Ovarian cancer is the most common fatal vival rate of about 40 percent. Little is gynecologic malignancy, with a 5-year survival rate of about 40 percent. Little is knonw about the etiology of the disease. An altered risk of ovarian cancer has been iden-tified consistently with only two character-istics: a history of one or more full-term pregnancies and use of oral contraceptives. Both of these characteristics are associated with reduced risk.

A possible reason that the epidemiology of ovarian cancer is inconclusive is that no one study has enlisted enough cases to ex-amine separately, for women of different ages, races, and parity, the effects of personal characteristics at play during different times in life, the effects of highly correlated characteristics such as age at first pregnancy and number of pregnancies, and the variation in these effects with different subtypes of the disease. For example, it is unclear whether epithelial ovarian tumors of low malignant potential (also called borderline tumors) have the same etiology as invasive epithelial tumors because few studies have obtained enough subjects with borderline tumors to examine them separately.

In this article, we describe findings for invasive epithelial tumors in white women, based on a collaborative analysis of data from 12 case-control studies of ovarian cancer conducted in the United States (1-12). Findings for tumors of low malignant potential in white women are discussed in part III of the series (13). Part IV (14) relates these results to current hypotheses for the patho-genesis of epithelial ovarian cancer. Findings for black women and for women with non-epithelial cancers are reported elsewhere (15, 16).

MATERIALS AND METHODS

The present analysis is based on data for 2,197 white women with invasive epithelial ovarian cancer and 8,893 white controls, collected in 12 case-control studies conducted in the period 1956-1986 and involv-ing personal interviews with study subjects. Six studies (called hospital studies) involved hospital controls (1-6), and six (called population studies) involved random digit dial of neighborhood controls (7-12); hereafter, these studies will be referred to by reference numbers. All odds ratio estimates are ad-justed for study, year of birth, and reference age (i.e., age at diagnosis or interview). (See reference 17 for a more precise definition of reference age and a description of the 12 studies and the data processing, data man-agement, and statistical procedures.)

RESULTS

Infertility

Increased ovarian cancer risk among nul-liparous women could reflect an association between ovarian cancer and infertility, de-fined here as difficulty in conceiving or in carrying a conceptus to term. To evaluate this possibility, we first examine risk among nulliparous women according to marital status and gravidity (table 1). If childless women have increased ovarian cancer risk stemming from difficulty in conceiving, those who have gotten pregnant at least once should have lower risk than those who have not. Conversely, if increased risk reflects difficulty in carrying to term, childless women who have been pregnant should have the higher risk. Table 1 offers little support for either hypothesis: There are no consistent or statistically significant differences in risk between nulliparous women who have been pregnant and those who have not.

Among women who have never been pregnant, sexual intercourse without birth control is probably more common among those who have been married than among those who

have not. Thus, if childless women have increased ovarian cancer risk because of difficulty in conceiving, ever-married nulligravidae should have a higher risk than never-married nulligravidae. However, table 1 shows no consistent or statisti-cally significant differences in risk between these two groups of women.

Table 1.
Odds ratios (OR) for invasive epithelial ovarian cancer among nulliparous women according to gravidity and marital status

* * *

Among women who conceive but never carry to term, the failure is probably more commonly due to miscarriage among those who have been married and to induced abortion among the never-married. Thus, if increased ovarian cancer risk among the childless stems from difficulty in carrying to term, gravid nulliparous women who married should have higher risk than do their coun¬terparts who never married. Yet, among the gravid nulliparae, those who were ever mar¬ried had no greater risk than those who never married (table 1).

Additional analyses of nulliparous women separated by use of oral contraceptives (ever vs. never) produced results similar to those in table 1. In summary then, the data show little difference in risk among nulliparous women when divided into four subgroups according to presumed predominant reason for nulliparity: 1) never-married, nulligravid (lack of desire or opportunity for preg nancy); 2) never-married, gravid (induced abortion); 3) ever-married, nulligravid (ina-bility to conceive); 4) ever-married, gravid (inability to sustain a viable pregnancy). Thus, the data do not support either hypothesis of infertility as explanation for the increased ovarian cancer risk among nullipa-rous women.

However, marital status and gravidity in nulliparous women provide only crude in-dicators of infertility. Ovarian cancer risk may relate to more accurate measures de-rived

from self-reported histories of attempts to become pregnant or of long periods of sexual intercourse without birth control.

Table 2 shows odds ratios according to months of longest pregnancy attempt. The data provide little evidence of association for this measure of infertility. Relative to women who never tried to become pregnant for at least 1 full year, the odds ratio for women who tried for 2 or more years was 1.2 (95 percent confidence interval (CI) 0.82-1.8). The estimate was greater for the nulligravid (odds ratio (OR) = 1.7, 95 per-cent CI 0.67-4.4) than for the gravid (OR = 1.1, 95 percent CI 0.66-1.7), although not significantly so.

Table 2.
Odds ratios (OR) for invasive epithelial ovarian cancer among ever-married women according to length of longest pregnancy attempt and total duration of unprotected intercourse, by gravidity

* * *

Table 2 also shows odds ratios for invasive epithelial ovarian cancer in relation to total years of unprotected intercourse, defined as the number of years a woman was premen-opausal and sexually active but using no form of birth control, less any time spent pregnant or abstaining from intercourse. Women with a total of 15 or more years of unprotected intercourse experienced in-creased risk relative to women with fewer than 2 such years (OR = 1.6, 95 percent CI 1.2-2.2). This increased risk was present regardless of gravidity or parity; the odds ratio among the nulligravid was 2.4 (95 per-cent CI 1.0-5.5).

Long periods of unprotected intercourse without pregnancy may reflect fertility prob¬lems of the male or of the couple, such as female production of antibodies to semen. A more specific (if less sensitive) measure of female reproductive inadequacy is a clinical diagnosis of infertility that excludes involve¬ment of the male partner. Among ever-married women, history of physician-diagnosed infertility not

attributed to the male was associated with slightly increased risk among nulligravid (OR = 1.4, 95 per-cent CI 0.86-2.3) but not gravid (OR = 0.87, 95 percent CI 0.67-1.1) women, based on five studies (6, 8, 9, 11, 12). The overall odds ratio was higher for women diagnosed infertile after 1970 (OR = 1.5, 95 percent CI 0.67-3.3) than for those diagnosed during the period 1961-1970 (OR = 0.89, 95 per-cent CI 0.42-1.9) or earlier (OR = 0.92, 95 percent CI 0.61-1.4), based on studies 9, 11, and 12. Three studies (6, 11, 12) obtained more detailed information on the clinically assessed reason for infertility. Most women did not know why they were infertile. How-ever, those who attributed their infertility to an ovulatory abnormality had a higher risk than did women with no physician-diagnosed infertility (OR = 2.1, 95 percent CI 0.90-4.7). Fallopian tube dysfunction also was associated with increased risk (OR = 1.3, 95 percent CI 0.63-2.8), although the data were sparse. By contrast, women with other or unspecified types of infertility showed no increased risk (OR = 0.77, 95 percent CI 0.55-1.1).

Table 3 presents odds ratios for invasive epithelial ovarian cancer according to use of fertility drugs. The table shows increased risk associated with such drug use (OR = 2.8, 95 percent CI 1.3-6.1 relative to risk in women with no clinical history of infertility). By contrast, infertile women without fertility drug use experienced no increase in risk (OR = 0.91, 95 percent CI 0.66-1.3). The risk associated with the use of fertility drugs was higher among the nulligravid (OR = 27.0, 95 percent CI 2.3-315.6) than among the gravid (OR = 1.4, 95 percent CI = 0.52-3.6). Indeed, fertility drugs had been used by 12 of 34 nulligravid cases, compared with one of 23 nulligravid controls. There was no evidence of heterogeneity across studies in odds ratios associated with use of fertility drugs ($x = 1.4$, $p = 0.50$). The histologies of ovarian cancers among cases who had used fertility drugs were similar to those of cases who had not used drugs. Information on

specific fertility drugs used was too incomplete to provide meaningful analysis.

Pregnancies

Table 4 shows odds ratios for invasive epithelial ovarian cancer by number of term pregnancies. Parous women have lower risk than do nulliparous women (OR = 0.76, 95 percent CI 0.63-0.93 for hospital studies and OR = 0.47, 95 percent CI 0.40-0.56 for population studies). The odds ratio difference between hospital and population studies is largely due to differences in parity; the fitted odds ratios per term pregnancy are similar (OR = 0.87, $p < 0.001$ for hospital studies and OR = 0.81 ($p < 0.001$) for population studies). Moreover, risk decreases with increasing parity among the parous (not shown), with statistically signif-icant trends for both hospital ($p < 0.05$) and population ($p < 0.001$) studies. For population (but not hospital) studies, the greatest protection is associated with the first term pregnancy. The population data strongly support a model in which each additional pregnancy after the first confers the same percent risk reduction, estimated to be 14 percent. This reduction is smaller ($p < 0.001$) than the 40 percent reduction asso-ciated with the first term pregnancy. Restriction of analyses to ever-married women and adjustment for oral contraceptive use did not change this finding.

Table 3.
Odds ratios (OR) for invasive epithelial ovarian cancer among ever-married women according to use of fertility drugs, by gravidity

* * *

Table 5 gives odds ratios according to the number of failed pregnancies (defined as miscarriages, abortions, ectopic pregnancies, and stillbirths), adjusted for parity and oral contraceptive use. Risk decreases with in-creasing number of failed pregnancies in both hospital and population studies. Separate analysis (not shown) suggests that the protective

effects of failed pregnancies are consistent among parous women regardless of the number of term pregnancies, although such protection was not evident among the nulliparous (table 1).

The risk reduction per pregnancy is smaller in magnitude for failed pregnancies (OR = 0.93 and 0.94 for hospital and population studies, respectively) (table 5) than for term pregnancies (OR = 0.87 and 0.81) (table 4). Data on gestational length of each pregnancy (available from studies 1, 6, 11, and 12) suggest that the decreased protection associated with an incomplete pregnancy (miscarriage, abortion, or ectopic pregnancy) is due to its shorter length. Among the gravid, odds ratios per month of incomplete pregnancy and term pregnancy were similar: 0.980 versus 0.980, based on the hospital studies and 0.964 vs. 0.977 based on the population studies.

Table 6 shows odds ratios among parous women by age at first livebirth, adjusted for number of term pregnancies and duration of oral contraceptive use. Ovarian cancer risk was positively associated with increasing age at first livebirth in the hospital studies ($p = 0.46$), and negatively associated ($p = 0.01$) in the population studies. These observations were essentially unchanged by further adjustment for years of education. We found no clear associations between risk and age at first pregnancy.

Odds ratios associated with term pregnan-cies did not vary appreciably by study and showed no clear variation by "usual" level of body mass index. However, they decreased with reference age in both the hospital and population data, as discussed in the part IV of this series (17).

Table 4.
Odds ratios (OR) for invasive epithelial ovarian cancer according to parity

* * *

Table 5.
Odds ratios (OR) for invasive epithelial ovarian cancer according to number of failed pregnancies

* * *

Table 6.
Odds ratios (OR) for invasive epithelial ovarian cancer among parous women according to age at first livebirth

* * *

Breast feeding

Table 7 shows odds ratios among parous women according to months of breast feed-ing. Parous women who ever had breast-fed a child had lower risk than did those who never had done so (OR = 0.73, 95 percent CI 0.51-1.0 in the hospital studies, and OR = 0.81, 95 percent CI 0.68-0.95 in the pop-ulation studies). Each month of breast feed-ing was associated with an overall risk re-duction of 0.99 for both hospital ($p = 0.18$) and population ($p < 0.01$) studies. Odds ratios are adjusted for parity and oral contraceptive use and were not altered by further adjustment for years of education in those studies with data for this potentially confounding variable.

Pregnancy and breast feeding may protect against ovarian cancer by suppressing ovu-lation. Since the effectiveness of lactation in suppressing ovulation wanes with time since delivery, this hypothesis predicts that a month of lactation within, say, 6 months of delivery reduces risk more than does a month of subsequent lactation. The seven studies (2, 6, 8-12) with relevant data support this prediction: The percent risk reduction per month of breast feeding within 6 months of delivery exceeds that for subse¬quent breast feeding (2.5 vs. 1.4 percent for hospital studies and 1.2 vs. 0.9 percent for population studies).

Odds ratios associated with breast feeding did not vary among the parous by reference age or number of term pregnancies.

Age at menarche and age at natural menopause

Table 8 presents odds ratios for invasive epithelial ovarian cancer according to age at menarche. Both hospital and population studies show only weak trends of decreasing risk with increasing age at menarche. These trends were stronger in young women than in older women (data not shown). Table 8 also shows odds ratios according to age at natural menopause among women of reference age 55 years or more. No clear patterns are evident. Moreover, among premenopau-sal (586 cases and 2,314 controls) and nat-urally postmenopausal (206 cases and 1,180 controls) women of reference age less than 55 years, there was no trend in risk with increasing time since last menses (see table 2, part IV of this series (14)).

Exogenous estrogens

Women who had used oral contraceptives had a lower risk for invasive epithelial ovar-ian cancer than did nonusers, as seen in table 9 (OR = 0.70, 95 percent CI 0.52-0.94 in hospital studies and OR = 0.66, 95 per¬cent CI 0.55-0.78 in population studies). Among ever-users, risk decreased with in¬creasing years of use in both hospital and population studies. These odds ratios are adjusted for number of term pregnancies; further adjustment for breast feeding, based on a subset of the studies, gave similar results. The trend is stronger in population than in hospital studies and is stronger in women who had used the pill for 2-5 years than in users for 6 or more years. Indeed, there is little additional protection conferred by oral contraceptive use beyond 6 years: The odds ratios associated with each such additional year of use were 0.95 (p = 0.56) for hospital studies and 1.1 (p = 0.22) for population studies. This lack of association cannot be ascribed to paucity of long-term pill users, since women with 10 or more years of use numbered 139 (15 cases and 124 controls) in the hospital studies and 482 (34 cases and 448 controls) in the population studies.

Among oral contraceptive users, risk de-creased with increasing time since last use, after adjustment for parity and total duration of use. The risk reduction per year since last use was 0.97 ($p = 0.40$) for hospital data and 0.96 ($p = 0.03$) for population data. Compared with women who had used the pill within 5 years of their reference age, risk for those who had stopped more than 15 years earlier was 0.79 (95 percent CI 0.26-2.4) in hospital studies and 0.61 (95 percent CI 0.35-1.1) in population studies. The trends in risk with time since first use were similar but weaker.

Table 7.
Odds ratios (OR) for invasive epithelial ovarian cancer among parous women according to total duration of breast feeding

* * *

Table 8.
Odds ratios (OR) for invasive epithelial ovarian cancer according to age at menarce and age at natural menopose

* * *

Table 9.
Odds ratios (OR) for invasive epithelial ovarian cancer according to duration of oral contraceptive use

* * *

Odds ratios for oral contraceptive use did not vary by age at first use, by parity, or by usual level of body mass index. In the pop-ulation studies, however, oral contraceptive use was more protective to women who breast-fed for long periods than to women with little or no breast feeding ($p < 0.01$). Odds ratios for ever-use of oral contracep-tives showed statistically significant hetero-geneity among the population studies (xi = 17.1, $p < 0.01$): two studies found no difference in risk between users and nonu-sers. No such heterogeneity was seen among the hospital studies (xi = 5.4, $p = 0.25$).

Table 10 shows odds ratios for invasive ovarian cancer according to years of estrogen replacement therapy. Neither hospital nor population studies provided evidence of al-tered risk among those who used estrogen replacement therapy for more than 3 months relative to nonusers (OR = 0.93, 95 percent CI 0.68-1.3 and OR = 1.1, 95 percent CI 0.89-1.4, respectively) or among users for more than 2 years relative to nonusers (OR = 0.89, 95 percent CI 0.35-2.3 and OR = 1.1, 95 percent CI 0.59-2.0, respectively). The data in table 10 also fail to show clear trends in risk with duration of estrogen replacement therapy use. The overall trend per year of use is decreasing ($p = 0.37$) in the hospital studies and increasing ($p = 0.21$) in the population studies. Moreover, the individual, study-specific odds ratios were small in magnitude, statistically nonsignificant, and showed no systematic pattern. A decreased risk among current estrogen replacement therapy users compared with never-users achieved statistical significance in the population studies (OR = 0.52, 95 percent CI 0.33-0.84), but not the hospital studies (OR = 0.78, 95 percent CI 0.42-1.5). However, no clear trends were evident with time since last estrogen replacement therapy use.

Odds ratios associated with estrogen replacement therapy use did not vary significantly by reference age or by type of menopause (natural vs. surgical). Estrogen replacement therapy was associated with slightly reduced ovarian cancer risk among young (reference age less than 40 years) hys-terectomized women; the odds ratios for ever-use versus never-use in this group were 0.73 (95 percent CI 0.25-2.2) for hospital studies and 0.73 (95 percent CI 0.35-1.5) for population studies. Neither hospital nor population studies showed significantly altered risk of ovarian cancers of the endometrioid type associated with any estrogen re-placement therapy use or with duration of such use. Estrogen replacement therapy use may be correlated with level of education, which differed ($p < 0.001$) between cases and

controls in the population studies. However, analyses adjusted for years of education produced results similar to those in table 10.

Tubal ligation and hysterectomy

The overall odds ratios relating invasive epithelial ovarian cancer risk to history of tubal ligation were 0.59 (95 percent CI 0.38¬0.93) for the hospital data and 0.87 (95 percent CI 0.62-1.2) for the population data (table 11). Variation in odds ratios across individual studies achieved statistical signif-icance for the hospital ($p < 0.01$) but not for the population ($p = 0.49$) studies. Sparse data on timing of tubal ligation (available from only five studies) provided no evidence that associated odds ratios varied with age at surgery or time since surgery. However, the odds ratios differed by parity, being less than unity among nulliparous women and among women with two or more term preg¬nancies, but elevated among uniparous women in both hospital (OR = 3.1, $p = 0.04$) and population (OR = 1.8, $p = 0.16$) data.

As we were not able to distinguish hyster-ectomies performed as treatment for ovarian cancer from those performed for other rea-sons, we evaluated risk only in relation to hysterectomies performed at least 2 years prior to the reference date, on the grounds that these were not a consequence of ovarian cancer. The overall odds ratio associated with a history of hysterectomy with ovarian conservation was 0.66 (95 percent CI 0.50—0.86) for the hospital data and 0.88 (95 percent CI 0.72-1.1) for the population data (table 11). The latter showed some inter-study heterogeneity ($p = 0.11$); half of the study-specific odds ratios exceeded unity and half were less than one. Odds ratios among hysterectomized women in table 11 show no clear trends with time since hysterectomy.

Table 10.
Odds ratios (OR) for invasive epithelial ovarian cancer according to duration of estrogen replacement therapy

* * *

Table 11.
Odds ratios (OR) for invasive epithelial ovarian cancer according to tubal litigation and hysterectomy without bilateral oophorectomy

* * *

Tubal ligation and hysterectomy may pro-tect against ovarian cancer by impairing ovarian function and thereby causing anovulation (14). This hypothesis predicts greater protection to women hysterectom-ized during their reproductive years than to those undergoing such surgery later in life. The data in table 11 support this prediction. Relative to unhysterectomized women, those reporting hysterectomy before age 40 years have reduced risk (OR = 0.58, 95 percent CI 0.40-0.86 for hospital studies, and OR = 0.76, 95 percent CI 0.57-1.0 for population studies). In contrast, women who had hysterectomies at older ages experienced less risk reduction (OR = 0.73, 95 percent CI 0.51-1.0 in hospital studies and OR = 1.0, 95 percent CI 0.77-1.3 in population studies).

Alternatively, tubal ligation and hysterec-tomy may protect against ovarian cancer by preventing ovarian exposure to exogenous carcinogenic agents, such as talc, that enter the peritoneal cavity through the vagina. This hypothesis predicts that hysterectomy confers less benefit to women who had previously undergone tubal ligation than to those who had not. As predicted, the risk reduction associated with hysterectomy was greater among women without prior tubal ligation than among those with such prior surgery. However, few women had both a tubal ligation and a subsequent hysterectomy, and the differences, although evident in both hospital and population data, were small and failed to achieve statistical signif¬icance.

Other characteristics

The combined data provided only limited opportunity to examine the relation of adiposity to ovarian cancer risk because information about body size was obtained in the various studies for varying periods in a woman's life. Analysis of a variable representing usual body mass index gave conflicting re-sults in hospital and population studies, being negatively associated with risk in the hospital data and positively associated in the population data. Data on diet and exposures to talc, tobacco, alcohol, and coffee were limited to only a few of the original studies. Six of the studies gathered data on family history of cancers of the ovary, breast, and certain other sites; analyses of these data are currently under way.

DISCUSSION

Interpretation of these findings is limited by several potential sources of bias, some of which are discussed in part I (17). Nevertheless, several conclusions seem warranted.

Nulliparity is associated with increased ovarian cancer risk in each of the 12 studies. While large numbers of nulliparous women (510 rases and 1,397 controls) in the com-bined data permitted odds ratio estimation jointly by marital status and gravidity, nei-ther of these characteristics was associated with altered cancer risk among the childless. The absence of association could reflect poor specificity of marital status as a marker for opportunity to conceive and poor sensitivity of self-reported gravidity as a marker for inability to carry a conceptus to term. Yet other measures of infertility, such as length of longest pregnancy attempt or history of clinically diagnosed infertility, also failed to show strong association with invasive ovarian cancer risk.

Long periods of unprotected intercourse (totaling 15 or more years) were associated with increased cancer risk; the increase was stronger in the nulligravid than in the gravid. Moreover, women who had used fertility medications had

almost three times the risk of women with no history of infertility. This association, which was particularly strong among the nulligravid, could reflect more accurate recall of medication use among cancer patients than among controls. Yet, such recall bias seems unlikely in light of women's ability to recall their own gyneco-logic and obstetric histories accurately (18-20). The association, if not due to chance or bias, could reflect a causal relation between such medications and ovarian malignancy. A causal relation is supported by several anecdotal reports of benign and malignant tumors after treatment for infertility (21-24) and by similar findings for tumors of low malignant potential (13) and nonepithelial cancers (16). The association also could re-flect the use of fertility drugs by women with ovarian disorders that themselves lead to malignancy. Indirect implication of treat¬ment rather than condition is provided by the somewhat higher odds ratio associated with an infertility diagnosis after 1970, since fertility drugs were introduced in the United States in the 1960s. Interpretation is limited by small numbers (only three studies ob-tained relevant information) and by our in-ability to assess the specific fertility drugs used. The three medications most commonly used in the United States are clomiphene citrate, bromocriptine, and human menopausal gonadotropin. Their complica-tions include multiple gestations, karyotypic abnormalities in preovulatory oocytes, luteal phase defects, increased proportion of de-generated ova, and increased probability of spontaneous abortions and ectopic pregnan-cies (25, 26).

The data suggest that ovarian cancer risk is elevated in a subgroup of nulligravid women with refractory infertility, as measured by unresponsiveness to fertility medications and long periods of unprotected intercourse without pregnancy. Further research is needed to confirm these findings and to determine the specific medications and types of infertility associated with increased risk. Such research poses

challenging design problems in view of the infrequency of both fertility drug use and ovarian cancer.

The absence of association relating ovarian cancer risk to gravidity and marital status among nulliparous women suggests that their elevated risk may be largely, if not entirely, attributable to deprivation of some direct benefit associated with pregnancy.

This interpretation is supported by two other observations. First, strong trends of decreas-ing ovarian cancer risk were seen with in-creasing parity. If a common correlation of parity with some physiologic aspect of reproductive potency was responsible for the observed differences in risk, then additional pregnancies after the third or fourth would confer no protection (since such multiparous women are unlikely to terminate their childbearing because of infertility); yet, the data suggest that women with more than three or four children are at lower risk than are less parous women. Second, among the parous; even failed pregnancies were associated with reduced risk, with the risk reduction afforded by a month of pregnancy independent of the pregnancy's length or outcome. Both of these findings also were seen in a pooled analysis of three European case-control studies of epithelial ovarian cancer (27).

Ovarian cancer risk decreased with in-creasing duration of breast feeding and of oral contraceptive use. These trends also suggest a direct protective role for these char-acteristics. If the trends merely reflected a common correlation with reproductive potency, then lactation and oral contraceptive use would confer no benefit to women of high parity, and long periods of breast feed-ing and oral contraceptive use would confer little benefit above that of short periods. However, the data support neither of these predictions; rather, lactation and oral con-traceptive use appear to be as protective to women with four or more term pregnancies as to nulliparous women, and continued pill use appears to be as protective to users for 2-5 years as to users for shorter

durations. The data do show a waning of protection to users for 6 or more years, an observation that was not seen in a pooled reanalysis of two European case-control studies (28) and that needs confirmation in future studies.

Women who had used the pill in the distant past (15 or more years prior to the reference date) were at lower risk than were recent pill users, after adjustment for dura-tion of pill use and parity. Since oral contra-ceptives were introduced in the United States in the period 1960-1970 and since most of the 12 studies were conducted in the period 1970-1985, women who had ter-minated use for 15 or more years were likely to have used the higher potency formulations marketed during the 1960s (29). Thus, the data suggest that these early formulations provide enhanced protection against ovarian cancer. Alternatively, the effects of pill use may increase with time since last use. Further data are needed to distinguish these two possible explanations.

The data show no clear trends of invasive ovarian cancer risk with age at first livebirth, only weak trends of reduced risk associated with delayed menarche, and no consistent trends with delayed menopause. Such trends have been noted in some (30, 31), but not all (32), epidemiologic studies conducted in other countries. Menstrual characteristics are reported with error (33-35), which could obscure trends, as discussed in part IV of this series (14).

We found no clear association between estrogen replacement therapy and risk of invasive ovarian cancer, even when attention was restricted to cancers of the endometrioid cell type (36). This lack of association contrasts with positive associations between estrogen therapy and cancers of the endometrium and possibly the breast, and it underscores differences in the epidemiologies of these gynecologic malignancies. Other data also are equivocal or conflicting on the relation of estrogen replacement therapy to ovarian cancer. Hoover et al. (37) found a two- to threefold excess of ovarian cancer in a cohort of southern United States

white women who had used estrogens; however, the excess risk was confined to a small num¬ber of women who had used diethylstilbestrol (DES). Booth et al. (38) found no overall association between estrogen replacement therapy and ovarian cancer, an increased risk associated with the therapy among hys-terectomized women was based on few cases and could have been due to chance. It is not supported by the present analysis, which found a slightly reduced risk among young hysterectomized women.

A prior history of tubal ligation or of hysterectomy without bilateral oophorectomy was associated with reduced ovarian cancer risk in these combined data. Several sources of bias must be considered in inter-preting these observations. Weiss and Harlow (39) have hypothesized that the apparent protective effects of such pelvic surgery are artifacts due to selective removal of precan-cerous ovaries at surgery. This explanation predicts a decrease in the level of protection with increasing years since surgery. The data fail to support such a decrease, although we could not evaluate risk associated with hysterectomy within 2 years of the reference date. Unreported bilateral oophorectomy among hysterectomized controls might also produce spuriously reduced risk among hysterectomized women, although women tend to report accurately the number of ovaries removed (20). Yet another source of bias is overrepresentation of hysterectomized women among controls. Hysterectomy was associated with greater risk reduction in hos-pital than in population studies, which may merely reflect an increased prevalence of this surgery among hospital control women (40).

Several explanations have been proposed for the protective effects of tubal ligation and hysterectomy, if these effects were not due to chance or bias. They may protect by increasing the likelihood of anovulation, as discussed in part IV of this series (14). Alternatively, they may block access of ovarian carcinogens that enter the peritoneal cavity via the vagina. The latter hypothesis cannot be examined critically

without data on ex-posures to talc and other potential ovarian carcinogens. Both interpretations are sup-ported by the present finding of greater risk reduction associated with hysterectomy during the reproductive years than with such surgery later in life. Finally, hysterectomy without bilateral oophorectomy may protect against ovarian cancer because one ovary is removed at surgery. There is need for further research on the timing, context, type, extent, and sequelae of tubal ligation and hysterectomy as they may relate to ovarian cancer risk. When possible, self-reported histories should be verified by review of medical records.

In summary, the combined data suggest that a subgroup of infertile women with long periods of unprotected intercourse and/or prior use of infertility medications experience increased risk of invasive epithelial ovarian cancer. Pregnancy, breast feeding, and oral contraceptive use are associated with risk reductions that appear to be more than the consequences of a common corre-lation with reproductive potency. Age at first livebirth, age at menarche, age at menopause, and estrogen replacement use were not strongly associated with risk, while tubal ligation and hysterectomy were associated with reduced risk. Part IV (14) of this series explores the implications of these findings for the pathogenesis of epithelial ovarian cancer.

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APPENDIX K

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THE REDUCTION IN RISK OF OVARIAN CANCER ASSOCIATED WITH ORAL CONTRACEPTIVE USE

The Cancer and Steroid Hormone Study of the Centers for Disease Cntrol and the National Institute of Child Health and Human Development[1]

Abstract Although several studies have reported that the use of oral contraceptives decreases the risk of ovarian cancer, it is not clear whether the effect varies according to the oral-contraceptive formulation or the histologic type of cancer. To characterize this association more fully, we used data from a case—control study, the Cancer and Steroid Hormone Study. From 1980 to 1982, 546 women 20 to 54 years of age with ovarian cancer were enrolled from eight population-based cancer registries. The controls were 4228 women selected from the same areas. Women who had used oral contraceptives had a risk of epithelial ovarian cancer of 0.6 (95 percent confidence interval, 0.5 to 0.7) as compared with those who had never used them. This protective effect was seen in women who had used oral contraceptives for as little as three to six months, and it continued for 15 years after use ended; it was independent of the specific oral-contraceptive formulation and of the histologic type of epithelial ovarian cancer. (We could not adequately assess

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the association with nonepithelial ovarian cancers because of an insufficient number of cases.) We conclude that the use of oral contraceptives decreases the risk of epithelial ovarian cancer. (N Engl J Med 1987; 316:650-5.)

SINCE 1977, at least 11 published studies have suggested that the use of oral contraceptives protects women from ovarian cancer.[1-11] In 1983, results bearing on this association were reported from a preliminary analysis of data collected during the first 10 months of the Cancer and Steroid Hormone Study.[12] The main findings were that (1) the use of oral contraceptives decreased the risk of ovarian cancer, (2) the risk decreased as the duration of use and the interval since the first use increased, and (3) the effect persisted long after oral contraceptives had ceased to be used.

This report will update and expand on the initial report, using data collected during the entire study period. The large size of the study has enabled us to perform several analyses not previously reported from other studies. These include the assessment of risks associated with specific histologic types of ovarian cancer and with the use of specific oral-contraceptive formulations.

METHODS

The Cancer and Steroid Hormone Study is a population-based case—control study of cancers of the breast, endometrium, and ovary that is coordinated by the Division of Reproductive Health of the Centers for Disease Control (CDC). The general methods of this study have been described previously.[I3,14] Subjects were enrolled by Surveillance, Epidemiology, and End Results (SEER) centers of the National Cancer Institute in eight areas of the United States (the metropolitan areas of Atlanta, Detroit, Seattle, and San Francisco; the states of Iowa, Connecticut, and New Mexico; and the four urban counties of Utah).

Cases

The case group consisted of all the women 20 to 54 years of age who resided in any of the eight reporting areas and had ovarian cancer that was first diagnosed between December 1, 1980, and December 31, 1982. Although the SEER centers do not routinely collect information about women given a diagnosis of borderline epithelial ovarian cancer, for this study they attempted to enroll all women with ovarian cancer, regardless of tumor behavior (whether the tumor was borderline, malignant, or represented carcinoma in situ). Of the 816 women who satisfied our case definition, we interviewed 579 (71.0 percent). The others were not interviewed because of death (3.1 percent), illness (5.1 percent), refusal by the patient (5.2 percent), refusal by the physician (2.9 percent), and inability to conduct an interview within six months of diagnosis (12.7 percent).

Controls

The control group consisted of women 20 to 54 years of age who were identified during the period of case enrollment by an established method that involves telephoning randomly selected phone numbers of households in the geographic areas in which the cases live. [5,16] The proportion of controls in each five-year age group was selected to match the expected age distribution of the women with breast cancer enrolled in the study. Since breast cancer occurs more frequently than ovarian cancer, the selection of one control per woman with breast cancer provided more than adequate numbers of controls for the ovarian-cancer analyses. Of the 5698 women selected as controls, 4754 (83.4 percent) were interviewed. The others were not interviewed because they refused (11.9 percent) or because an interview could not be conducted within six months of selection (4.7 percent).

Interview

Female interviewers with a wide range of interviewing experience were recruited by the SEER centers. Before field

work began, all interviewers and supervisors participated in an intensive one-week training session at CDC. Between December 1980 and April 1983, cases and controls were interviewed in their homes with use of a standard questionnaire designed to collect information similarly from both groups. Detailed information on oral-contraceptive use, including dates and durations of use and the formulations used, was collected from the women who reported having used oral contraceptives for three or more consecutive months. A life calendar (a calendar on which to record major life events around which contraceptive use might be better remembered) and color photographs of oral-contraceptive packages were used to help women recall their contraceptive use up to the time of the interview.

Retrieval and Review of Histology Slides

Personnel at six of the SEER centers (Atlanta, Detroit, Seattle, San Francisco, Iowa, and Connecticut) attempted to retrieve histology reports and slides from the ovarian-cancer specimens of women who had been interviewed. Retrieval was successful for 517 (96.6 percent) of the 535 cases of ovarian cancer from the regions represented by these centers.

The slides were reviewed by a panel of three pathologists expert in the field of ovarian cancer (Drs. Robboy, Kurman, and Paris). The panel judged that 8 of the histology slides were inadequate to permit a diagnosis. Of the remaining 509, 496 (97.4 percent) were judged to show ovarian cancer. The conditions shown in the remaining 13 were diagnosed as metastatic cancer or benign processes. Each ovarian cancer was classified as epithelial or nonepithelial, assigned a histologic subtype, and characterized in terms of tumor behavior.' [7]

For cases from the six centers participating in the retrieval of histology slides, we examined how well the diagnoses of the local pathologists (obtained at the time of retrieval) agreed with the diagnoses of the pathology panel. Of the diagnoses of epithelial and nonepithelial ovarian cancer made

by local pathologists, 97.2 and 88.9 percent, respectively, were confirmed by the panel. However, local pathologists agreed with the panel less often (82.0 percent) when identifying the various histologic subtypes of epithelial ovarian cancer.

Since the pathology panel and local pathologists tended to agree on the general classification of epithelial and nonepithelial cancer, we used local pathologists' diagnoses (obtained from separate SEER data tapes) for the women with ovarian cancer from Utah and New Mexico, where histology slides were not retrieved. Diagnoses were available for 38 of the 40 cases from the two areas. We were also able to obtain SEER diagnoses for 14 of the 18 cases for which slide retrieval had been unsuccessful. Since there was less agreement between the panel and local pathologists on histologic subtype, for that analysis we used only cases and controls from the six centers that participated in slide retrieval. The SEER data tapes did not include complete information on tumor behavior.

The histologic diagnoses of the women with ovarian cancer are shown in Table 1.

Analysis

We excluded from the case group the 13 women who did not have a primary ovarian cancer, the 14 women whose histologic diagnoses were not known, and 4 women who either reported a previous history of ovarian cancer or did not know whether they had such a history. We excluded from the control group 516 women without ovaries or with an unknown number of ovaries. We also excluded 2 cases and 10 controls who did not know whether they had ever used oral contraceptives. This left 546 cases and 4228 controls for analysis.

For each woman who reported having used oral contraceptives for three or more consecutive months, we used information from the life calendar to characterize and

quantify her oral-contraceptive use until the date of diagnosis (for a case) or the date of interview (for a control). We determined the cumulative duration of each woman's oral-contraceptive use, the number of months since the first and last use, the age at first use, and whether the woman had ever used each specific oral-contraceptive formulation.

We considered that the following factors could possibly confound our results: the age at diagnosis or interview, race, education, income, parity, history of breast-feeding, menopausal status, number of ovaries, geographic region, history of infertility, history of smoking, history of alcohol use, frequency of pelvic examinations, and Quetelet's adiposity index (weight/height^2). We used Mantel—Haenszel[18] and logistic regression methods[19,20] to obtain odds ratios adjusted individually for each factor. Since parity and age were the only factors that distorted the risk estimates appreciably, we included these (as continuous variables) in a logistic regression model to obtain the relative-risk estimates presented here. We tested for the statistical significance of a trend in risk among exposed women by entering exposure in the model as a continuous variable. We used likelihood-ratio tests to determine whether there were different effects of oral-contraceptive use among specific subgroups.[19]

Table 1.
Histologic Diagnoses in the Women with Ovarian Cancer (Cases).

* * *

The tumor type was determined on the basis of separate SEER data.

RESULTS

First, we examined the relation between the use of oral contraceptives and epithelial ovarian cancer, using data from the 492 women with epithelial ovarian cancer and the 4228 controls. Table 2 shows various characteristics of the cases and controls. Because controls were frequency-matched to

the women with breast cancer according to age, we present the percentage distribution of characteristics of the controls standardized by the direct method[21] to the age distribution of the group with epithelial ovarian cancer. The cases were more likely than the controls to be white, to have lower parity, never to have breast-fed, to have had infrequent pelvic examinations, and to be obese.

Table 2.
Percentage Distribution of Selected Characteristics of Women with Epithelial Ovarian Cancer and Controls.

* * *

Compared with the women who reported never having used oral contraceptives, the women who reported ever having used them had a relative risk of 0.6 (95 percent confidence interval, 0.5 to 0.7) of having epithelial ovarian cancer. Women who had used oral contraceptives, but never for three or more consecutive months, had a relative risk of 1.1 (0.8 to 1.6). Because dates of use and formulation information were not collected for the 44 cases and 253 controls with such use, these women were excluded from the remaining analyses.

Women who had used oral contraceptives for at least three consecutive months had a lower risk of epithelial ovarian cancer than women who had never used them (Table 3). With five or more years of cumulative use, the relative risk decreased further. The trend of decreasing risk with increasing duration of use was statistically significant **(P =** 0.02, adjusted for age, parity, and interval since first use).

The risk reduction was most marked in women who had first used oral contraceptives at least 10 years before diagnosis or interview (Table 4). The trend of a decrease in risk with an increase in the length of time since the first use was also statistically significant **(P =** 0.02, adjusted for age, parity, and duration of use). When considered simultaneously, both the duration of use and the interval since first use appeared to play a part in the reduced risk

associated with oral-contraceptive use. However, because there were small numbers of cases in certain categories, we were unable to separate these effects completely.

Women who had used oral contraceptives had a decreased risk of epithelial ovarian cancer as compared with those who had not, regardless of the length of time since the last use. Women who had last used them 15 or more years previously had a relative risk estimate of 0.5 (0.4 to 0.8). Likewise, the age at the time of first use had no effect on the association, once the effect of the length of time since the first use was considered.

Women who had used a combination type of oral contraceptive exclusively had a relative risk of epithelial ovarian cancer of 0.5 (0.4 to 0.6) as compared with women who had never used any type of oral contraceptive. Women who had used a sequential oral contraceptive exclusively had a risk of 0.7 (0.5 to 1.1) as compared with women who had never used any oral contraceptives (based on 6 cases and 81 controls). Only I case and 8 controls had used a progestin-only oral contraceptive exclusively.

Table 3.
Cumulative Duration of Oral-Contraceptive Use by Women with Epithelial Ovarian Cancer and Controls.

* * *

A negative association was apparent for any use of each of the 11 most commonly reported combination oral contraceptives, regardless of the type or amount of estrogen or progestin in the formulation (Table 5). Women who had used 1 of these 11 formulations exclusively had risk estimates similar to those of women who had ever used that formulation.

There was a negative association between oral-contraceptive use and epithelial ovarian cancer for both borderline and malignant tumors, with risk estimates of 0.6 (0.4 to 0.9) and 0.5 (0.4 to 0.7), respectively. Likewise, the

observed reduction in risk was similar for each of the four major histologic subtypes of epithelial ovarian cancer, with relative risks ranging from 0.4 to 0.7.

At each parity level from zero through four, women who had used oral contraceptives had lower relative risks of epithelial ovarian cancer - ranging from 0.4 to 0.7 - than women who had not used them. This relation was not seen among women with a parity of five or more, who had a relative risk of 1.2 (0.6 to 2.4) associated with oral-contraceptive use. This interaction with parity was of borderline statistical significance **(P =** 0.08). The lack of an effect in women of higher parity was not explained by the effects of duration of use or length of time since the first oral-contraceptive use.

Table 4.
Relative Risk of Epithelial Ovarian Cancer According to Duration of Oral-Contraceptive Use and Interval since First Use.

* * *

A woman's age at diagnosis or interview did not affect the association between oral-contraceptive use and epithelial ovarian cancer. Likewise, menopausal status, adiposity, and frequency of pelvic examinations did not alter the association.

To determine whether the reduced risk associated with oral-contraceptive use was actually an indirect effect of fertility or of contraceptive use in general, we examined the association between oral-contraceptive use and epithelial ovarian cancer among women grouped according to various measures of fecundity, including gravidity, self-reported infertility, and physician-diagnosed infertility. In each instance, women without evidence of subfecundity had a reduced risk associated with the use of oral contraceptives that was similar to that of women who were possibly subfecund. We also examined the risks associated with hav-

ing used other specific methods of contraception as compared with never having used the method. The relative-risk estimates, adjusted for age, parity, and oral-contraceptive use, were as follows: diaphragm, 0.8 (0.7 to 1.0); condom or foam, 1.0 (0.8 to 1.3); intrauterine device, 1.1 (0.9 to 1.5); and vasectomy, 0.9 (0.7 to 1.2).

Table 5.
Any Use of Specific Combination Oral-Contraceptive Formulations by Women with Epithelial Ovarian Cancer and Controls.

* * *

We also studied the association between oral-contraceptive use and nonepithelial ovarian cancers. Most of the 54 women in this case group had pathology-panel diagnoses of germ-cell or sex cord-stromal tumors (Table 1). Women who had ever used oral contraceptives had a relative risk of 1.6 (0.5 to 4.7) of having a germ-cell type of ovarian cancer as compared with women who had never used them; among women whose use lasted five years or more, the relative risk of having a germ-cell tumor was 1.0 (0.2 to 4.0). Analysis of the relation between oral contraceptives and the sex cord—stromal type of ovarian cancer revealed a statistically significant interaction between oral contraceptive use and age. Women under 45 years of age who had ever used oral contraceptives had a crude odds ratio of having stromal tumors of 1.4 (0.2 to 11.9) as compared with those who had never used them. Among women 45 and older, no case had ever used oral contraceptives, which resulted in an odds ratio of zero. Using Cornfield's formula for exact confidence limits,[22] we calculated an upper 95 percent confidence limit of 0.4.

DISCUSSION

These results indicate that oral-contraceptive use even for a few months reduces the risk of epithelial ovarian cancer by 40 percent for women 20 to 54 years of age. The effect probably takes from 5 to 10 years to become apparent, but it

persists long after the use of oral contraceptives ends. Moreover, protection exists regardless of the formulation of oral contraceptive used.

Of the 816 women with ovarian cancer originally identified by the SEER centers, 29.0 percent were not interviewed. If the reasons for not being interviewed, such as the severity of ovarian cancer, were related to oral-contraceptive use, then our results could be biased. However, even if all the 67 women not interviewed because of illness or death had belonged to the group that had ever used oral contraceptives, the crude odds ratio compared with those who had never used them would be 0.8 — still a negative association.

We could not verify the information about exposure to oral contraceptives that was obtained from the study participants. The use of a life calendar and photographs of oral-contraceptive packages has been found in another study to improve women's recall of their histories of oral-contraceptive use.[23] If cases were more likely than controls to report the use of oral contraceptives, the magnitude of the negative association may have been underestimated.

Evidence of impaired fertility has been associated with an increased risk of ovarian cancer.[3,24,25] Infertile and subfecund women may be less likely to use any method of contraception. This situation could confound the relation between oral contraceptives and epithelial ovarian cancer and result in a spurious negative association. We did not find such confounding in our data. Controlling the results for measures of infertility did not alter the risk estimates. Moreover, all the women who were either relatively more fertile or relatively less so had reduced risks of epithelial ovarian cancer associated with oral-contraceptive use. Finally, the use of other contraceptive methods was not associated with reduced risks.

Our findings are generally consistent with previous investigations of the association between oral-contraceptive use and ovarian cancer, including the preliminary report from

this study.[I2] Other studies[2,6,8] have found that the relative risk of ovarian cancer decreased as the duration of use increased. We found that both the duration of use and the length of time since the first use had apparent effects, which persisted when we adjusted one for the other.

Because of the small numbers of cases with germ-cell or sex cord-stromal ovarian cancer, we could not adequately characterize the association between oral contraceptives and these cancers. Cramer and Welch[26] have predicted concordance in some risk factors for stromal and epithelial tumors. The finding of a statistically significant negative association between oral contraceptives and sex cord-stromal tumors in the older group is consistent with this prediction.

Suppression of ovulation and suppression of pituitary secretion of gonadotropins have both been postulated as mechanisms by which oral contraceptives protect against ovarian cancer.[27,28] If suppression of ovulation is the operating mechanism, then all combination oral contraceptives should have similar protective effects, since they all suppress ovulation at approximately similar rates.[29] That the commonly available combination oral contraceptives have similar associated risk estimates supports the ovulation-suppression theory. Combination oral contraceptives also cause suppression of pituitary gonadotropins"; however, the higher-dose formulations may suppress the pituitary response more than the lower-dose ones do.[31-33] If gonadotropin suppression is the operating mechanism, then the effect may vary according to the formulation. There were too few cases who had used each of the formulations to permit an assessment of the gonadotropin-suppres-sion theory.

Because women over 55 were unlikely ever to have used oral contraceptives, this study was limited to women 20 to 54 years of age. Since we found no evidence that the protective effect wanes many years after the last use, it may persist in older women. However, the association between oral

contraceptives and epithelial ovarian cancer in older women remains to be studied.

We have estimated that the use of oral contraceptives prevented over 1700 cases of ovarian cancer in 1982.[12] Age-adjusted incidence rates of ovarian cancer in the United States have changed little over the past 40 years.[34] However, as women who have used oral contraceptives move into the age groups that are at highest risk for epithelial ovarian cancer, we may witness a declining incidence of this serious disease.

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APPENDIX L

CANCER RESEARCH
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POPULATION-BASED CASE-CONTROL STUDY OF OVARIAN CANCER IN SHANGHAI

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ABSTRACT

A case-control study of 229 ovarian cancer cases (including 172 epithelial tumors) and an equal number of population-based controls was conducted during 1984 to 1986 in Shanghai, China, a low-risk area for ovarian cancer. Similar to studies in high-risk areas, the risk of epithelial tumors was high for nulliparous women (odds ratio, 1.6; 95% confidence interval, 0.8 to 3.2) and decreased with increasing number of

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livebirths ($P < 0.01$). Early menarche and late menopause were associated with increased risk, with the trend in risk for age at menarche being statistically significant. In contrast to other studies, oral contraceptive use was not associated with reduced risk, although there was some reduction in risk for those with a prior tubosterilizadon or intrauterine device use. Risk was also elevated among those reporting a prior ovarian cyst, medroxyprogesterone use, a first degree family history of cancer, and occupational exposure to paint. Risk factors for the nonepithelial tumors were similar to the other cancers, although the power to detect differences was limited.

INTRODUCTION

There is striking international variation in the incidence of ovarian cancer, with the highest age-adjusted rate (15.3/100,000 in Norway) being 5 times that of the lowest (3.2/100,000 in Miyagi, Japan) (1). Among Chinese women, ovarian cancer is relatively infrequent, with the documented incidence being 5.0/100,000 in Shanghai and 5.8 in Hong Kong (2). The incidence of ovarian cancer among Chinese in San Francisco, CA (8.5/100,000), although almost twice that of Chinese women, is still somewhat lower than American white women (12.9/100,000) (2).

These distributions suggest that there might be etiological differences between Chinese and American women. In other areas, reproductive factors have been found to play a major role in the etiology of ovarian cancer, with high parity relating to low risk (1, 3, 4). Oral contraceptive use has also been shown to have a protective effect on ovarian cancer risk (5-8). Other exogenous estrogens, for example, diethylstilbestrol, however, have been found to directly affect risk (1). Although other factors have been suggested to relate to the occurrence of ovarian cancer, their relationship to risk remains less clear. These factors include X-ray exposure (9, 10), viral infections (mumps, rubella, influenza) (1), chemicals (talc, asbestos) (5, 6), and animal fat intake (11).

Familial clustering of ovarian cancer has also been noted (12-14).

In order to clarify whether the varying incidence rates between Chinese and American women might relate to etiological differences, we conducted a population-based case-control study in Shanghai during 1984 to 1986.

MATERIALS AND METHODS

Cases consisted of all female residents of the urban Shanghai area, aged 18 to 70 yr, with ovarian cancer newly diagnosed between September 1, 1984, and June 30, 1986. Women with borderline-type ovarian tumors were excluded.

A total of 258 eligible cases was accrued from a population-based cancer register in Shanghai during the study period. Of these, 229 (88.8%) were interviewed, while 21 (8.1%) died before we could contact them, and 8 (3.1%) were untraceable. Clinical and histopathologic data at diagnosis, along with information on treatment and survival, were abstracted from hospital records. Nearly all (94.3%) of the cases were histologically confirmed, with the remainder being diagnosed either through ultrasound (3.1%) or clinical examination (2.6%). Of these tumors, 75.1% were epithelial, 7.4% were germ cell, 10.5% were sex cord, and 7.0% were other or undefined types.

One control was selected from the Shanghai general population by a standard random procedure to match each case within 5 yr of age. For each case, one neighborhood committee was selected from the 1457 committees in the Shanghai urban area, followed by the random selection of one household group (each usually containing 15 to 20 families). Two controls were randomly selected from a household group, with one serving as fast control and the other as an alternate. Women with bilateral oophorectomy were replaced with alternative controls. All eligible control women agreed to participate.

Information was collected through direct interviews by trained interviewers. The standard questionnaire covered demographic characteristics, reproductive history, medical history, familial cancer history, personal habits, occupation, and diet.

The measure of association used was the relative risk, as approximated by the OR.[2] Stratified analyses were first used to search for potential confounders, followed by conditional logistic regression techniques (15) to derive adjusted odds ratios and 95% CIs. Although a standard model was used for most analyses, alternative models with only pertinent confounding factors produced nearly identical point and interval estimates. A two-tailed test for trend in the logistic analyses was obtained by categorizing the exposure variable, assigning the score *j* to the *j*th exposure level of the categorical variable, and treating the scored variable as continuous. The variable, ovulation years, was calculated by both the methods of Risch *et al.* (16) and Casagrande *et al.* (17), but since the two methods provided similar results, only those derived by the former method are presented.

RESULTS

In order to control for effects of differing histological patterns, the majority of analyses focused on risk factors for the predominantly occurring epithelial tumors. The epithelial cancer cases and matched controls were found to be comparable in age distribution. The mean age was 49.1 yr for cases and 48.9 yr for controls. Cases tended to be better educated and have higher incomes than controls (Table 1); however, after adjustment for education, income failed to remain as a significant predictor of risk. No significant differences were noted between cases and controls with respect to number of household members, height, average weight, maximum weight, or body mass index (not shown).

[2] The abbreviations used are: OR, odds ratios; CI, confidence interval.

Fewer cases (93.5%) than controls (96.5%) were ever married.

Nulliparity was associated with a nonsignificantly elevated odds ratio of 1.6 (95% CI, 0.8 to 3.2). Women who had ever been pregnant but had had no livebirths had a slightly lower risk (OR, 1.3; 95% CI, 0.3 to 5.3). The protective effect of parity was significantly related to the number of livebirths (trend test, $P < 0.01$) but was not related to age at first livebirth (Table 2). A history of a miscarriage or stillbirth was not related to risk, but induced abortions were associated with slight reductions in risk.

Table 1
Distribution of selected demographic characteristics of epithelial ovarian cancer patients and matched controls, Shanghai, People's Republic of China 1984-1986

* * *

Table 2
Epithelial ovarian cancer risk by selected reproductive factors, Shanghai, People's Republic of China, 1984-1986

* * *

Table 3
Epithelial ovarian cancer risk by menstrual factors, Shanghai, People's Republic of China, 1984-1986

* * *

Age at menarche was significantly inversely related to risk, with women whose first menses occurred prior to the age of 14 having approximately 4 times the risk of those with menarche at ages 18 and older (Table 3). This effect persisted after adjustment for animal fat intake, a significant determinant of risk in this population (18). Risk increased with the usual length of the menstrual cycle, but the trend was not statistically significant. A total of 51.2% of the cases had ceased menstruating, compared with 48.8% of the controls, resulting in an adjusted odds ratio of 1.0 (95% CI,

0.4 to 2.2). Among the women with a natural menopause, late menopause was linked with a higher risk of ovarian cancer, although there was no distinct trend.

When queried regarding physician-diagnosed menstruation problems (at least 2 yr prior to diagnosis), more cases than controls reported irregular menstruation (OR, 2.7), amenorrhea or relative amenorrhea (OR, 2.6), menorrhagia (OR, 2.8), or dysmenorrhea (OR, 1.2). However, none of these excesses was statistically significant.

The relationship between ovarian cancer and various methods of birth control is summarized in Table 4. A total of 48.3% of the cases and 57.0% of the controls had ever used a method of birth control, resulting in an odds ratio of 0.8 (95% CI, 0.4 to 1.5). Separate analyses according to ever *versus* never use of various methods of birth control showed an elevated risk of ovarian cancer associated with oral contraceptive usage (OR, 1.8; 95% CI, 0.8 to 4.1) and a lower risk with tubosterilization (OR, 0.8; 95% CI, 0.4 to 1.6) and intrauterine devices (OR, 0.5; 95% CI, 0.2 to 1.1). There was, however, no obvious trend between the risk of ovarian cancer and the duration or years since first use of oral contraceptives, or the years since sterilization. Recomputation of risks using women who had never used any method of birth control as the referent group produced virtually the same point estimates for the various birth control methods but broader confidence intervals.

Since parity, menarche, menopause, and oral contraceptive use were all related to ovarian cancer risk, we attempted to summarize these events by calculating ovulation years (see Table 5 for method of computation). Risk increased with extended periods of ovulation, with women ovulating more than 30 yr having an odds ratio of 1.8 (95% CI, 0.6 to 5.4) compared to those with less than 17 yr. Since ovulation was related to the frequency of menstrual cycles, we also measured the effect of ovulation by calculating total times of ovulation, which was computed by dividing ovulating years

by usual length of the menstrual cycle. Neither the adjusted ORs nor the trend test was statistically significant

Table 4
Epithelial ovarian cancer risk by birth control methods used, Shanghai, People's Republic of China, 1984-1986

* * *

Table 5
Epithelial ovarian cancer risk by ovulatory patterns, Shanghai, People's Republic of China, 1984-1986

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Table 6
Epithelial ovarian cancer risk by selected female diseases and previous hormone usage, Shanghai, People's Republic of China, 1984-1986

* * *

A total of 14 female diseases and symptoms as well as 18 other diseases occurring at least 2 yr prior to ovarian cancer diagnosis was analyzed. No substantial differences between cases and controls were found with respect to childhood viral diseases (mumps, rubella, chicken pox), thyroid or adrenal diseases, hypertension, diabetes, allergies, and benign breast diseases (latter shown in Table 6). However, the risk of ovarian cancer was elevated in women with a history of pelvic infection (OR, 3.0; 95% CI, 0.3 to 30.2), operation for *myoma uteri* (OR, 3.0; 95% CI, 0.7 to 12.2), or ovarian cysts (OR, 12.0; 95% CI, 2.5 to 57.7). For ovarian cysts occurring less than or equal to 2 yr, 2 to 10 yr, and more than 10 yr prior to diagnosis of ovarian cancer, the odds ratios were 2.7 (95% CI, 0.2 to 30.4), 9.8 (95% CI, 1.2 to 81.0), and 15.3 (95% CI, 1.6 to 150.7), respectively. The association with ovarian cysts was stronger for premenopausal (10 exposed cases *versus* 0 controls) than postmenopausal women (OR, 5.6; 95% CI, 0.5 to 65.9). Given that women with ovarian cysts might be under better medical surveillance and, hence, have an earlier diagnosis of ovarian cancer, we computed the odds ratios according to the progressiveness of the ovarian cancer. Although the odds ratio for less progressive ovarian

cancer was higher (OR, 14.1) than for more progressive cancer (OR, 9.6), both were significant.

An increased risk of ovarian cancer was noted among women with a history of medroxyprogesterone usage (OR, 2.8; 95% CI, 0.9 to 8.5). This association did not appear to be explained by abnormal menstrual symptoms. No distinct trend was observed with years of use, although the number of reported users was limited. Six cases and one control reported using hormones to help them become pregnant (OR, 2.1; 95% CI, 0.2 to 22.7). Use of diethylstilbestrol was associated with an odds ratio of 5.4, although based on only 4 exposed cases. Other hormones, including corticosteroids and testosterone propionate, were not related to increased risk.

Similar proportions of cases and controls reported prior pelvic or chest X-ray exposure. Twelve cases and 2 controls had pelvic operations at least 2 yr prior to the diagnosis of ovarian cancer (OR, 5.9; 95% CI, 0.7 to 51.7). Three cases compared to 2 controls reported having a hysterectomy, resulting in an adjusted odds ratio of 1.1 (95% CI, 0.1 to 11.9).

A history of ever having smoked was associated with an odds ratio of 1.8 (95% CI, 0.7 to 4.8). There were no differences between cases and controls with respect to drinking or use of hair dyes.

Associations between ovarian cancer and occupation were examined by calculating odds ratios for jobs held for the longest period of time (Table 7). A total of 24.4% of cases *versus* 12.2% of controls were employed as professional/technical workers, scientists, and research workers, resulting in a crude odds ratio of 2.6 (95% CI, 1.5 to 4.6). However, after adjustment, the odds ratio was reduced to 1.4 (95% CI, 0.6 to 3.3). Chemical workers demonstrated a nonsignificantly elevated risk. No excess risks were found for any of the other occupations examined. Exposure to paint was associated with a nonsignificant elevation in risk (OR,

2.4; 95% CI, 0.9 to 5.9), but other occupational exposures were not related to ovarian cancer risk.

Cases reported familial cancer histories more often than controls. The odds ratio associated with a first degree relative having any cancer was 1.8 (95% CI, 0.8 to 4.0), and 1.9 (95% CI, 0.7 to 4.9) for a first degree female relative. The odds ratio associated with a familial female reproductive organ cancer history was 0.7 (95% CI, 0.2 to 2.8). Adjustment for the number of first degree relatives had virtually no effect on the observed risks.

Analyses also examined risk factors for the nonepithelial tumors (Table 8). Risk factors appeared similar to those identified for the epithelial cancers, although the power to detect associations was limited.

Table 7
Epithelial ovarian cancer risk by occupations and occupational exposures, Shanghai, People's Republic of China, 1984-1986

* * *

Table 8
Selected variables associated with nonepithelial ovarian cancer, Shanghai, People's Republic of China, 1984-1986

* * *

DISCUSSION

The present case-control study, conducted among a low-risk population for ovarian cancer, demonstrated several results for epithelial ovarian cancer consistent with studies in higher risk populations. The elevated risks associated with nulliparity and decreasing risk with increasing parity support previous findings (3-5, 16, 19-23). In line with several studies (3, 6, 24), but in contrast with others (7, 20-22), our study showed that neither age at first livebirth nor age at first pregnancy were significantly related to ovarian cancer. A history of a miscarriage or stillbirth was unrelated to risk, but

induced abortions were associated with slight reductions in risk.

The underlying mechanism by which pregnancy confers a protective effect on ovarian cancer risk is unclear. “Incessant ovulation” has received attention as a possible explanation (16,17, 25, 26), since ovulation exposes the ovarian epithelium to recurrent minor trauma and contact with follicular fluid (27).This hypothesis is supported by fmdings that an index of ovulatory years (the time from menarche to cessation of ovulation minus the time the ovary is anovulatory or protected) directly relates to ovarian cancer risk (17, 21, 22, 28). Our findings supported this notion, with women ovulating more than 30 yr having nearly twice the risk of those with less than 17 ovulatory yr. Total frequency of ovulation, however, did not appear to affect risk.

Abnormalities in endocrine function have also been hypothesized as an explanation for the association with nulliparity (29). It has been suggested that an unidentified abnormality in endocrine function may predispose women to both infertility and ovarian cancer (3). Of some support for this was our finding that cases more frequently reported irregular menstruation, amenorrhea, and menorrhagia. However, none of the elevations was statistically significant, and the possibility of recall bias cannot be dismissed.

Of note in this study was a strong relationship of risk with early age at menarche, with women having menses prior to age 14 having 4 times the risk of those first menstruating at age 18 or older. Age at menarche has not generally been found to be a risk factor for ovarian cancer (5, 20, 21), although one study did report that cases tended to have slightly earlier ages than controls (17). It would be of interest to examine the relationship in other populations where the range in ages at menarche was as wide as in this population of Chinese women.

We did not find a protective effect of oral contraceptives on ovarian cancer risk as reported in other studies (5, 6, 23,

30, 31). In our study oral contraceptive use was associated with a slight increase in risk, but neither the point estimate associated with ever use nor the trends with extended measures of use was statistically significant. Of note was the fact that excess risk was restricted to short-term (<1 yr) users, suggesting that the effect may relate either to the indications for use or an adverse reaction to use of oral contraceptives, as previously noted (17). Alternatively, the association might reflect the influence of recall bias, lifestyle correlates, or of chance, especially since the number of users was limited.

Our study disclosed that tubosterilization and intrauterine device usage reduced the risk of ovarian cancer. Although the reason for the protective effect associated with intrauterine device usage is unclear, the reduced risk associated with sterilization is consistent with an effect observed among Japanese women (32). However, two other studies did not find such an association (20, 33). It has been suggested that carcinogens can reach the ovary through the fallopian tube (34). Thus, tubosterilization might block the carcinogenic pathway by ligating the tube or terminating the blood supply to the ovary. Furthermore, the lowering of certain hormone levels following tubosterilization might be important (35, 36). Finally, the possibility that the operation allows for screening of abnormal ovaries should not be dismissed, although there was no relation of risk with years since sterilization.

Of note in this study was that a history of ovarian cysts was related to a 12-fold increased risk of ovarian cancer. Risk was highest for cysts diagnosed more than 2 yr prior to diagnosis and only significant in premenopausal women. It is possible that women with an ovarian cyst will have better medical surveillance and thus be diagnosed with ovarian cancer at an earlier stage. Although a significant association was found in this study even for those with more aggressive lesions, the possibility of selection bias cannot be totally eliminated.

Previous studies have suggested that oral contraceptives containing only progestogens may enhance the formation of ovarian cysts (37-40). In one study (41), the peripheral serum concentration of progesterone in ovarian tumor patients was found to be elevated, and experimental studies have demonstrated progesterone treatment can increase the incidence of ovarian cancer in mice (42). It was thus of interest that our study found use of medroxyprogesterone to be associated with a 2.8-fold increased risk of ovarian cancer, implying that progesterone might mechanistically relate to both ovarian cysts and ovarian cancer.

It has been suggested that women who work in rubber, electrical, and textile industries are at greater risk of ovarian cancer than women employed in other industries (43, 44). Asbestos and talc have also been suggested as risk factors on the basis of several epidemiological (5, 6) and pathological studies (45). The present study did not find any of the hypothesized occupations to relate to risk, but did note that chemical workers had a slightly increased risk of ovarian cancer. Women who were exposed to paint also had a 2.2-fold elevated risk.

A few familial clusters of ovarian cancer have been noted (12, 13). Cases in other studies frequently reported a familial history of cancer of female reproductive organs in general (20) or of cancer of the breast (14). In our study, cases more commonly reported a familial cancer history, although reproductive cancers did not predominate. In addition, the number of familial cancer cases was not large. Thus, it would appear that heredity is not a major contributing factor to the etiology of ovarian cancer in China.

In conclusion, our study showed that epithelial ovarian cancer risk factors among Chinese women and medical factors examined in this study do not solely appear to explain observed geographical patterns of ovarian cancer. Changing incidence rates upon migration support the notion that adopted environmental factors may be etiologically involved.

Relationships of ovarian cancer risk with dietary factors (11, 18) may be one possible explanation and should be pursued further as a possible explanation to the varying incidence rates between Chinese and American women.

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APPENDIX M

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AVERAGE RISKS OF BREAST AND OVARIAN CANCER ASSOCIATED WITH BRCA1 OR BRCA2 MUTATIONS DETECTED IN CASE SERIES UNSELECTED FOR FAMILY HISTORY: A COMBINED ANALYSIS OF 22 STUDIES

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Germline mutations in *BRCA1* and *BRCA2* confer high risks of breast and ovarian cancer, but the average magnitude of these risks is uncertain and may depend on the context. Estimates based on multiple-case families may be enriched for mutations of higher risk and/or other familial risk factors, whereas risk estimates from studies based on cases unselected for family history have been imprecise. We pooled pedigree data from 22 studies involving 8,139 index case patients unselected for family history with female (86%) or male (2%) breast cancer or epithelial ovarian cancer (12%), 500 of whom had been found to carry a germline mutation in *BRCA1* or *BRCA2*. Breast and ovarian cancer incidence rates for mutation carriers were estimated using a modified segregation analysis, based on the occurrence of these cancers in the relatives of mutation-carrying index case patients. The average cumulative risks in *BRCA1*-mutation carriers by age 70 years were 65% (95% confidence interval 44%–78%) for

breast cancer and 39% (18%–54%) for ovarian cancer. The corresponding estimates for *BRCA2* were 45% (31%–56%) and 11% (2.4%–19%). Relative risks of breast cancer declined significantly with age for *BRCA1*-mutation carriers (*P* trend .0012) but not for *BRCA2*-mutation carriers. Risks in carriers were higher when based on index breast cancer cases diagnosed at !35 years of age. We found some evidence for a reduction in risk in women from earlier birth cohorts and for variation in risk by mutation position for both genes. The pattern of cancer risks was similar to those found in multiple-case families, but their absolute magnitudes were lower, particularly for *BRCA2*. The variation in risk by age at diagnosis of index case is consistent with the effects of other genes modifying cancer risk in carriers.

Introduction

Mutations in the breast and ovarian cancer–susceptibility genes *BRCA1* (MIM 113705) (Miki et al. 1994) and *BRCA2* (MIM 600185) (Wooster et al. 1995; Tavtigian et al. 1996) are found in a high proportion of multiple-case families with breast cancer, especially if they also include one or more case patients with ovarian cancer (Ford et al. 1994). Screening for mutations in these genes for predictive genetic testing has become widespread, with 1750 protein-truncating mutations in these genes having been identified (see the Breast Cancer Information Core [BIC] Web site). Some women found to carry such mutations undergo prophylactic mastectomy and/or oophorectomy, because their cancer risk is extremely high. However, although it is very clear that mutations in these genes, segregating within these types of families, confer a substantial risk of both breast and ovarian cancer, the same may not apply to mutations detected in other settings, such as in families with less-extreme cancer histories or in incident cases, even those of early onset.

Table 1
Previously Published Breast and Ovarian Cancer Risks Associated with Mutations in *BRCA1* and *BRCA2*

* * *

Several approaches have been used to estimate the average age-specific cumulative cancer risks, or penetrance, associated with mutations in *BRCA1* and *BRCA2*. Early estimates applied the maximum-LOD-score (or linkage) method to multiple-case families collected for linkage studies for the identification of disease loci (Easton et al. 1993; Clerget-Darpoux 2001). Subsequent penetrance estimates have used the incidence of cancer in the relatives of mutation-carrying index case patients from case series unselected for family history. Analytically, these are the same method (i.e., a type of segregation analysis) applied with different corrections for family ascertainment. Both should give consistent estimates of penetrance, provided that the same penetrance function applies to all carriers. Different estimates will arise, however, either if the penetrance is mutation specific or if the penetrance is modified by other risk factors, genetic or environmental, that aggregate in families. Either of these phenomena would lead to a higher actual penetrance for mutations segregating in multiple-case families than for mutations segregating in the population as a whole. Some authors (e.g., Begg 2002) have described the penetrance estimates derived in this way as biased (Begg 2002). This is correct in the sense that they do not reflect the *average* risks to *all* carriers in the population. In practice, a counsellor is rarely interested in the risks to the "average" carrier. Virtually all genetic testing is conducted on women in families with multiple cases of the disease—the types of families from which the original penetrance estimates were derived. Some women are tested on the basis of weaker family histories or on the basis of having early-onset disease; risk estimates derived by studying the cancer incidence in relatives of population-based series of women with breast or ovarian cancer may then be more appropriate.

Published penetrance estimates are summarized in table 1. Breast and ovarian cancer risk estimates are generally higher in studies that are based on multiple-case families (Ford et al.

1994, 1998; Easton et al. 1995) than in those that are based on unselected series (Thor-lacius et al. 1998; Hopper et al. 1999; Warner et al. 1999; Anglian Breast Cancer Study Group 2000). Another study, based on the family histories of 120 Ashkenazi Jewish volunteers in whom one of three different founder mutations common to this population had been identified, also reported lower penetrance estimates than reports based on multiple-case families (Struewing et al. 1997). These penetrance estimates are averages over the mutations segregating in the families in which mutations have been identified. There are, however, data to support the hypothesis of allelic risk heterogeneity, such that different mutations confer different risks. Specifically, *BRCA2* mutations that occur in families with one or more cases of ovarian cancer tend to cluster in a central portion of the gene, termed the "ovarian cancer cluster region" (Gayther et al. 1997; Thompson and Easton 2001). A study of Breast Cancer Linkage Consortium (BCLC) families has shown that mutations in the ovarian cancer cluster region are associated with both a lower risk of breast cancer (relative risk [RR] 0.63) and a higher risk of ovarian cancer (RR p 1.9), as compared to mutations outside this region. Another study, based on probands with ovarian cancer, found that the *BRCA2*-associated breast cancer risk was associated only with mutations outside the ovarian cancer cluster region (Risch et al. 2001). Evidence for a genotype-phenotype correlation in *BRCA1* has also been found. Gayther et al. (1995) have found that the risk of ovarian cancer relative to the risk of breast cancer was higher in families with protein-truncating mutations in the first two-thirds of the gene than in families with protein-truncating mutations in the last one-third of the gene. More recently, Thompson and Easton (2002) have found that mutations in a central region of *BRCA1* were associated with a lower risk of breast cancer, and Risch et al. (2001) have reported that the risk of breast cancer increases with mutation position, from 5' to 3'.

Penetrance estimates based on multiple-case families may be inappropriate for the counselling of women without a strong family history of disease who have been found to carry a germline mutation in *BRCA1* or *BRCA2*. Although estimates based on mutation testing in case series unselected for family history are more appropriate in this context, published estimates from individual studies have lacked precision, with most studies having identified a few dozen mutations at most. To improve the precision of penetrance estimates based on unselected case series, we have combined data from a large number of such studies into a formal meta-analysis. This combined data set has also allowed us to examine variations in penetrance by type of mutation, type and age at diagnosis of index case, birth cohort, and study center.

Subjects and Methods

Studies

Studies were eligible for this meta-analysis if all of the following criteria were met: (1) The study was based on mutation testing of a series of index cases either of female or male breast cancer or of invasive epithelial ovarian cancer. (2) Index cases were sampled independently of family history (although they may have been selected by age at diagnosis or ethnic group). (3) Index cases had been tested for *BRCA1* and/or *BRCA2* mutations by a systematic screen conducted independently of family history (mutation screening may have been of the entire coding sequence, of some part of the sequence, or of specific founder mutations). (4) Enumeration of at least all first-degree relatives of identified mutation carriers was available, along with ages at diagnosis of breast and ovarian cancers and ages at last observation.

Potentially eligible studies were identified by a literature search using Medline (National Library of Medicine) and by personal contact through the BCLC. We contacted 21 research groups, in total, that we believed to have data from one or more relevant studies, and we received data from 15.

Participating investigators were asked to provide details on all recorded members of families in which the index case patient was found to have a *BRCA1* or *BRCA2* mutation. These details included date of birth, date of or age at diagnosis of any breast or ovarian cancer, and age at death or age at last observation. Data on cancers other than breast or ovarian cancer were sometimes also available but were not used in the present analysis. Investigators were also asked to provide details of each mutation identified.

Details of the studies included are given in table 2. Of the 22 studies included, 16 were conducted by ascertainment of female breast cancer index cases, 2 were conducted by ascertainment of male breast cancer cases, and 4 were conducted by ascertainment of ovarian cancer cases. Of the studies based on breast cancer index cases, 10 restricted ascertainment by age at diagnosis; in 9 of these, the upper limit for age at diagnosis was 50 years. None of the studies based on either male breast cancer index cases or ovarian cancer index cases imposed a restriction on age at diagnosis. Sixteen of the studies ascertained cases through a population-based cancer registry, whereas the remainder were based on unselected, hospital-based series. The recruitment method for the studies varied widely. Most studies obtained a blood sample and family-history data simultaneously, but some studies collected a blood sample first and then retrospectively obtained family-history data on mutation carriers whereas others obtained family history data first and collected a blood sample later. Furthermore, in some studies, not all available blood samples were analyzed.

Mutations were included in the present analysis if they were "pathogenic" according to the generally accepted criteria (see the BIC Web site)—that is, frameshift or nonsense mutations, splice-site mutations predicted to cause aberrant splicing, large deletions or duplications, and mis-sense mutations classified as such by BIC. In practice, the last group included only mutations in the ring-finger domain of *BRCA1*. In-frame deletions and

known poly-morphisms or "unclassified variants" were not included. A variety of mutation-screening techniques was used by the studies. Of the studies, 14 screened for mutations in both genes, 6 screened for mutations in *BRCA1* only, and 2 screened for mutations in *BRCA2* only. Six studies investigated specific founder mutations (in the Ashkenazi Jewish, Icelandic, and Polish populations), whereas the remaining 16 studies screened the coding sequence of either or both genes.

Table 2
Description of Studies Included in the Present Analysis

* * *

Statistical Methods

Kaplan-Meier estimation.—The cumulative probabilities of breast and ovarian cancer in mothers and sisters of *BRCA1*- and *BRCA2*-mutation carriers were estimated using the Kaplan-Meier product-limit method, using the program Stata (version 7). For this analysis, censoring age was the age at breast cancer diagnosis, age at ovarian cancer diagnosis, age at last follow-up, or age 70 years, whichever occurred first. SEs and confidence limits were obtained using Greenwood's formula.

Penetrance estimation.—We used the information on disease occurrence in relatives of mutation-positive index case patients to estimate age-specific breast and ovarian cancer incidences in mutation carriers by maximum likelihood, using modified segregation analyses implemented in Mendel (Lange et al. 1988; Antoniou et al. 2001). This is essentially the same methodology and software as that used for penetrance analysis in multiple-case families (but with a different ascertainment correction). Relatives were assumed to be followed from age 20 years and to be censored at the age at first cancer diagnosis, at the age at death, at the age at last follow-up, or at age 70 years, whichever came first. Information on mutation status in relatives was incorporated when available. Females born before 1890 were excluded from the analyses. Individuals with no age information (608 females from entire data set) or no year of

birth (53 females) were censored at age 0 years. To correct for ascertainment, we maximized the conditional likelihood of the pedigree given the phenotypic and genotypic information of the index case.

The main analyses were based on the fitting of fixed age-specific incidence rates for carriers. Initially, these rates were assumed to be independent of country of origin or year of birth, but we then explored variation in rates according to these covariates. Breast cancer incidence in carriers was assumed to follow $l(t) = l_0(t) \exp[g(t)]$, where $l_0(t)$ is the background incidence for England and Wales (1973–77) and $\exp[g(t)]$ is the age-specific RR of breast cancer in carriers as compared to population rates. The ovarian cancer incidences were assumed to follow a similar model. Conditional on the genotype, the probability of developing breast cancer was assumed to be independent of the probability of developing ovarian cancer. We estimated the age-specific log(RR) parameters $g(t)$ for five age groups: 20–29, 30–39, 40–49, 50–59, and 60–69 years. We then fitted models with carrier incidences parameterized in terms of rate ratios relative to country-, age-, and period-specific incidences. In all analyses, cancer incidences in noncarriers were assumed to follow country- and cohort-specific rates (Waterhouse et al. 1976, 1982; Muir et al. 1987; Parkin et al. 1992, 1997).

To test for differences in incidences among different subgroups, we fitted models in which we added a subgroup-specific log(RR) parameter. For example, to test for differences among centers, we fitted models including the five age-specific log(RR) estimates (for all centers) but also allowed an additional center-specific log(RR) (constant over age). A likelihood-ratio test was then used to test for heterogeneity of risk among centers. Similar tests were used to explore variations in incidences by year of birth, type of mutation, and type and age of index case patient. Trend tests were used to test whether the log(RR) estimates increased or decreased significantly with age.

To construct CIs for the log(RR) estimates, we assumed that the parameters were asymptotically normally distributed with the covariance matrix given by inverting the information matrix. Cumulative risk or penetrance and 95% CIs were calculated from the cumulative incidence λ (*t*), where $\Lambda(t) = \sum_{k=1}^{n} i_k t_k \exp(\beta_k)$, where i_k the incidence in noncarriers in the *k*th age band of length t_k and $*k* is the ln(RR) in the *k*th age band. The variance of the cumulative risk is given by the expression

$$\mathrm{var}[\Lambda(t)] = \sum_{k=1}^{n} i_k^2 t_k^2 \mathrm{var}(\beta_k) \exp(2\beta_k)$$

$$+2 \sum_{r<k,k=1}^{n} i_k i_r t_k t_r [\mathrm{var}(\beta_k)\mathrm{var}(\beta_r)]^{1/2} \exp(\beta_k) \exp(\beta_r) \mathrm{corr}(\beta_k, \beta_r) ,$$

and the cumulative risk *F*(*t*) is then given by *F*(*t*) = 1 - exp [-L(*t*)], with a 95% CI of 1 - exp {-L(*t*) ± . Uncertainty in RRs for factors with more than two categories (e.g., center) is presented as floating CIs (Easton et al. 1991).

Results

The 22 studies included in the present analysis screened a total of 6,965 female breast cancer cases, 176 male breast cancer cases, and 998 ovarian cancer cases and identified 289 *BRCA1*- and 221 *BRCA2*-mutation carriers (table 2). Table 2 also shows the number of individuals eligible for each study, the number enrolled, and the number of samples analyzed. However, estimation of a response rate that is comparable across all studies is not possible, because of the variety of protocols used in recruitment and data gathering (see the "Subjects and Methods" section). Family-history data were not available for 12 mutation carriers, leaving 280 families of *BRCA1*-mutation carriers and 218 families of *BRCA2*-mutation carriers in the present analysis. Among the first-degree relatives of *BRCA1*-mutation–positive index case patients, 125 breast cancers and 41 ovarian cancers were identified, and, among the first-degree relatives of *BRCA2*-

mutation–positive index case patients, 87 breast cancers and 13 ovarian cancers were identified.

Figure 1
Kaplan-Meier cumulative breast (*upper lines*) and ovarian (*lower lines*) cancer probability in sisters (*thick lines*) and mothers (*thin lines*) of BRCA1-mutation-carrying index case patients

* * *

Kaplan-Meier Estimates

Figure 1 shows the age-specific cumulative probabilities of breast and ovarian cancer in mothers and sisters of *BRCA1*-mutation carriers, using Kaplan-Meier estimation. The estimated cumulative risks of breast cancer by age 70 years were 29% (95% CI 23%–35%) in mothers and 42% (95% CI 30%–56%) in sisters; the corresponding cumulative risks of ovarian cancer were 15% (95% CI 10%–21%) in mothers and 14% (95% CI 7.5%–24%) in sisters. Although the estimated breast cancer risks are higher in sisters than mothers at all ages, the difference in risks was not statistically significant (log-rank $P = .056$ for breast cancer; log-rank $P = .75$ for ovarian cancer).

Figure 2 shows the corresponding Kaplan-Meier estimates for mothers and sisters of *BRCA2*-mutation carriers. The cumulative risks of breast cancer by age 70 years were 19% (95% CI 14%–26%) in mothers and 25% (95% CI 18%–34%) in sisters, whereas the cumulative risks of ovarian cancer were 5.1% (95% CI 2.7%–9.6%) in mothers and 4.5% (95% CI 1.7%– 12%) in sisters. Again, the differences in risks between mothers and sisters were not statistically significant for either cancer ($P = .12$ for breast cancer; $P = .53$ for ovarian cancer).

Average Penetrance Estimates

For the main analysis, we assumed that the age-specific incidences were the same for all mutation carriers and that incidences in noncarriers were country and birth-cohort

specific. The RRs of breast and ovarian cancer in *BRCA1*- and BRCA2-mutation carriers, compared to population rates for England and Wales in 1973–77, are shown in table 3. For *BRCA1,* the breast cancer RR increased to 33 in the 30–39-years age group and decreased with age thereafter (*P* trend .012), whereas the ovarian cancer RR estimates showed no apparent trend with age. The estimated breast cancer RR to *BRCA2*-mutation carriers was 19 in the 20–29-years age group and fell to -10 in older-age groups (*P* trend .98). Ovarian cancer RRs for *BRCA2*-mutation carriers were only estimated for ages 40 years, because there were no ovarian cancer cases diagnosed at >40 years of age in the first-degree relatives of *BRCA2*-mutation carriers. The RR increased to a maximum of 19 in the 50–59-years age group and then decreased.

The corresponding age-specific incidences are shown in table 4, and the cumulative cancer risks (penetrances) are shown in figures 3 and 4. The breast cancer incidence in *BRCA1*-mutation carriers increased with age up to age 45–49 years but remained roughly constant thereafter. The ovarian cancer rates were low below age 30 years and rose steeply with age thereafter, to -2% per annum, only slightly less than the breast cancer rates. The breast cancer incidence in *BRCA2*-mutation carriers increased progressively with age, whereas the ovarian cancer incidence increased up to age 55–59 years and then decreased slightly. The cumulative breast cancer risk by age 70 years in *BRCA1*-mutation carriers was estimated to be 65% (95% CI 51%–75%), and the ovarian cancer risk was estimated to be 39% (95% CI 22%–51%). For *BRCA2*-mutation carriers, the cumulative breast cancer risk by age 70 years was estimated to be 45% (95% CI 33%– 54%), and that for ovarian cancer was estimated to be 11% (95% CI 4.1%–18%).

Figure 2
Kaplan-Meier cumulative breast (*upper lines*) and ovarian (*lower lines*) cancer probability in sisters (*thick*

***lines*) and mothers (*thin lines*) of BRCA2-mutation-carrying index case patients**

* * *

Table 3
RRs of Breast and Ovarian Cancer in Mutation Carriers

* * *

Analysis by Center

We investigated potential heterogeneity of risk among centers by fitting models with additional center-specific RR parameters. For *BRCA1,* we grouped the U.K., Canadian, Polish, and other centers (43, 124, 48, and 65 families, respectively). RRs by center are shown in table 5. The estimated cancer risks were somewhat lower in the Canadian and Polish families than in the U.K. and other families, but there was no significant evidence of heterogeneity ($P = .32$).

For *BRCA2,* we grouped the U.K., Canadian, Icelandic, and other centers (44, 63, 69, and 42 families, respectively). Again, there was no significant evidence of heterogeneity ($P = .13$). The estimated breast cancer risks for Canadian centers were lower than for the U.K. and Icelandic centers (RR = 0.53), and the ovarian cancer risk was higher (RR = 3.1). There was also some suggestion of higher cancer risks for "other centers" as compared to the U.K. center (RR = 1.4 for breast cancer, and RR = 4.2 for ovarian cancer).

Effect of Year of Birth

We investigated the effect of birth cohort on breast and ovarian cancer risks by fitting models with additional parameters for birth cohorts (before 1920, 1920–39, 1940–59, and from 1960 onward for breast cancer; before 1920, 1920–39, and from 1940 onward for ovarian cancer). For *BRCA1*-mutation carriers, we found higher risks for both breast and ovarian cancer ($P = .011$ and $P = .0013$, respectively) in more-recent birth cohorts (table 5). The RR of breast cancer in *BRCA2*-mutation carriers also increased with more-recent

birth cohort (table 5), but not significantly (P = .16). There were too few ovarian cancer cases among the first-degree relatives of the index case patients to assess cohort effects on ovarian cancer risk in *BRCA2*-mutation carriers.

Effect of Type of Index Case

One hundred seventeen families were ascertained through a *BRCA1*-mutation–carrying index case patient with ovarian cancer, and 163 families were ascertained through a *BRCA1*-mutation–carrying index case patient with breast cancer. We fitted models, adding an RR parameter for type of index case (table 5). The breast cancer risk for *BRCA1*-mutation carriers ascertained through an ovarian cancer index case was lower than that for carriers ascertained through a breast cancer index case (RR = 0.60 [95% CI 0.38–0.94]; cumulative risk by age 70 years 56% vs. 72%). The ovarian cancer risks, however, did not differ significantly by type of index case (RR = 0.86 [95% CI 0.42–1.8]).

Families ascertained through a breast cancer case were subdivided further by age at diagnosis of the index case. The breast and ovarian cancer risk estimates were higher in the families of the early-onset index cases, although only the breast cancer effect was significant (table 5) (breast cancer RR = 2.2 [95% CI 1.4–3.3]; ovarian cancer RR = 1.8 [95% CI 0.82–4.0]). On the basis of this analysis, the breast cancer risk for *BRCA1*-mutation carriers for families ascertained through early-onset index cases was estimated to be 87% (95% CI 67%–95%) by age 70 years, and the ovarian cancer risk was estimated to be 51% (95% CI 9.1%–73%), compared to 61% (41%–74%) and 32% (11%–49%) for families ascertained through an older index case patient. The risk estimates for older index case patients with breast cancer were comparable to those for ovarian cancer index case patients (54% and 36%).

Fifty *BRCA2*-mutation–positive families were ascertained through an ovarian cancer index case, 148 were ascertained through a female breast cancer index case, and 20 were

ascertained through a male breast cancer index case. Of the female index case patients, 46 received a diagnosis at *!*35 years of age, and 102 received a diagnosis at 35 years of age. The estimated breast cancer risk in carriers ascertained through a breast cancer index case was higher than in those ascertained through an ovar ian cancer index case (RR = 0.42 [95% CI 0.20–0.88]). Conversely, the ovarian cancer risk was higher in the families ascertained through an ovarian cancer index case (RR = 2.4 [95% CI 0.74–8.1]). There was no evidence of a difference in risk according to whether the index case patient with breast cancer was a male or a female who received a diagnosis at 35 years of age (RR = 1.3 [95% CI 0.65–2.7]). Among carriers ascertained through a female breast cancer index case, there was no significant difference in the breast cancer risks according to whether the index case was diagnosed at <35 years of age or at a later age (RR = 1.2 [95% CI 0.57–2.5]; cumulative risks by age 70 years 55% [16%–76%] vs. 49% [32%–61%]), but there was some evidence of higher ovarian cancer risk for families ascertained through early-onset breast cancer cases (RR = 13 [95% CI 2.4–70]; cumulative risks by age 70 years 35% [0.61%] vs. 3% [0–7%]).

Table 4
Estimated Breast and Ovarian Cancer Incidence (%) in Mutation Carriers

* * *

Figure 3
Cumulative risk of breast and ovarian cancer in BRCA1-mutation carriers

* * *

Since ascertainment criteria varied by center, we also fitted models in which RRs for center and type of index case were fitted simultaneously. Under this model, the breast cancer risk for *BRCA1*-mutation carriers ascertained through index cases diagnosed as breast cancer at <35 years of age remained higher than that for carriers whose diagnosis was given at

later ages (RR = 2.2 [95% CI 1.2–4.2]). Some suggestion of a higher ovarian cancer risk among carriers ascertained through ovarian cancer index cases, not evident from the univariate analysis, emerged when center was taken into account (RR = 1.9 [95% CI 0.67–5.2]). We were unable to fit the effects of index case and center for *BRCA2.* These effects were confounded because all the ovarian cancer index cases were from one center (Canada).

We also fitted models allowing for both type of index case and year of birth. The estimated effects that type of index case had on both breast and ovarian cancer risk were similar to those estimated previously. Thus, the RR based on breast cancer cases diagnosed at <35 years of age, relative to those diagnosed at later ages, was 1.9 (95% CI 1.1–3.3), whereas the RR for mutation carriers ascertained through an ovarian cancer index case was 0.84 (95% CI 0.51–1.37). Adjustment for type of index case did not materially affect the year of birth effect: the estimated RRs for mutation carriers born in the 1920–39, 1940–59, and 1960-onward cohorts were 1.8 (95% CI 0.88–3.6), 2.5 (1.2–5.3), and 4.9 (1.4–18), respectively. We fitted similar models for *BRCA2*, but the RRs for both the type-of-index-case effect and the birth-cohort effect were similar to those when each effect was considered individually.

Center- and Cohort-Specific Incidence Models

The apparent variation in incidence by center and birth cohort may reflect variations in population-specific incidence rates. We therefore also performed analyses in which we estimated the age-specific RRs in carriers relative to population- and cohort-specific incidence rates. The RRs estimated in these models were very similar to those estimated in the analyses that assumed a constant background incidence rate (table 6). We then fitted models allowing for heterogeneity between center and birth cohort. The RR estimates by center were very similar to those for the fixed incidences models, with no significant

evidence for heterogeneity of risk by center for either gene.

The cohort effects were slightly less marked than the fixed incidence rate model. For *BRCA1*, the breast cancer RR estimates for the three later birth cohorts, relative to the before-1920 cohort, were as follows: 1920–39, 2.6 (95% CI 1.3–5.2); 1940–59, 3.1 (95% CI 1.5–6.5); and 1960 onward, 6.2 (95% CI 1.7–22.1). The ovarian cancer RRs for the 1920–39 and 1940–59 birth cohorts, compared to the before-1920 cohort, were estimated as 9.8 (95% CI 2.6–36.6) and 7.6 (95% CI 1.9–30.8), respectively. There were no ovarian cancer cases in relatives born after 1960, so no RR parameter was estimated for this cohort. Both effects remained highly significant. For *BRCA2*, the corresponding breast cancer RRs were 0.94 (95% CI 0.49–1.8), 1.4 (95% CI 0.65–3.0), and 3.6 (95% CI 0.65–19), respectively. As with the fixed incidence rate model, the cohort effects were not significant for *BRCA2*-mutation carriers ($P = .26$). There were too few ovarian cancer cases among the first-degree relatives of the index case patients to assess cohort effects on ovarian cancer risk in *BRCA2*-mutation carriers.

Figure 4
Cumulative risk of breast and ovarian cancer in BRCA2-mutation carriers

* * *

Table 5
RRs for Mutation Carries, as Compared to Baseline, for Models Allowing for Center, Type-of-Index Case, and Year-of-Birth Effects

* * *

Effect of Mutation Position

We investigated the possibility of allelic heterogeneity in risk by classifying mutations according to their position and by fitting models comparable to the previous BCLC analyses (Thompson and Easton 2001). Families with the *BRCA1*

C61G missense mutation were excluded from the present analysis. *BRCA1* mutations were categorized into three groups as defined previously: nucleotides 1–2400 (137 index cases), 2401–4184 (55 index cases), and 4185 onward (88 index cases). The RR of breast cancer for mutations in the central region as compared to that for mutations in the 5' region was estimated to be 0.93, and that for mutations in the 3' region was estimated to be 1.4; the corresponding risks for ovarian cancer were 1.8 and 1.1. These models did not fit significantly better than the null model.

BRCA2 mutations were divided into those within the ovarian cancer cluster region (nucleotides 3059–6629 [97 index cases]) and those outside the ovarian cancer cluster region (mutations at all other nucleotides [121 index cases]). We fitted models in which the breast and ovarian cancer risks were allowed to vary between the two regions. The estimated breast cancer risk was lower among carriers of mutations in the ovarian cancer cluster region as compared to mutations outside the ovarian cancer cluster region (RR p 0.57 [95% CI 0.32–1.0]). The corresponding ovarian cancer RR was 2.1 (95% CI 0.62–7.0).

Table 6
RR Estimates for Mutation Carriers, Based on Country- and Cohort-Specific Background Rates

* * *

Discussion

In this meta-analysis, we have used data from 22 studies that have tested patients with breast or ovarian cancer who were unselected for family history of germline mutations in *BRCA1* and/or *BRCA2* as a basis for the estimation of breast and ovarian cancer incidences and cumulative risks in mutation carriers. We are aware of a few studies that could not be included in the present analysis, and, undoubtedly, there are other such studies ongoing. Nevertheless, this overview represents the large majority of the available data,

and, especially given the costs of such studies, it seems unlikely that much greater precision will be available in the near future from studies of this design.

The major perceived strength of this approach, as compared with the linkage approach based on multiple-case families, is that it produces estimates that are less susceptible to the effects of other familial risk factors and mutation-specific differences in risk. Although this is true, it is important to note that the families that we have analyzed were still selected on the basis of an affected index case patient, so that, in the presence of modifying risk factors, the estimated risks will be higher than the risks to a completely unselected mutation carrier. Nevertheless, since one affected relative would usually represent an absolute minimum criterion for genetic testing, it seems unlikely that risk estimates that lie much outside this range will be needed in any practical situation.

There are two other important advantages of this approach. First, it provides estimates for site-specific cancer risks that are largely uncorrelated, whereas the breast and ovarian cancer risk estimates derived by the maxi-mum-LOD-score approach in multiple-case families tend to be strongly correlated. Second, relative to the maxi-mum-LOD-score approach, the estimates at early ages, when the risks are low, are more precise. A major disadvantage of this approach is that the prevalence of mutations in unselected case series is low and, therefore, very large numbers of cases need to be tested to provide precise estimates. Thus, the studies in the present analysis included 18,000 index cases, yielding 282 *BRCA1* and 218 *BRCA2* mutations. Despite this, the width of many of the confidence limits still exceeds 10%.

Another important issue is the accuracy of reporting of family history. Although some of the studies did attempt to confirm cancer diagnoses in relatives, this was not always possible, and only three studies were able to identify routinely all cancer diagnoses in relatives through national

records. In an attempt to minimize the effects of inaccurate reporting, we restricted our main analyses to first-degree relatives. Previous studies (e.g., Claus et al. 1998) have found that reporting of cancer in more-distant relatives is less accurate. Although data on more-distant relatives were easily incorporated in the analysis, we found that some of the penetrance estimates were higher. This suggests either inaccurate reporting of cancer diagnoses or incompleteness in the enumeration of relatives that correlated with the extent of family history.

The techniques used for mutation detection in the different studies—and, therefore, their sensitivity to detect mutations of different types—varied widely. Certain studies tested only for specific founder mutations (T300G, 185delAG, 4158delA, and 5382insC in *BRCA1;* 999del5 and 6174delT in *BRCA2*), but these mutations still represent a minority of the total set. Most of the groups used screening techniques that are most sensitive for small deletions and insertions, so these will be overrepresented in the data set; however, these are the mutations that account for the majority of mutations in families with linkage, and they represent the most important mutation type encountered in genetic testing. Since we restricted the present analysis to mutation types generally regarded as pathogenic, almost all the mutations are predicted to be protein truncating (the only exception being the T300G mutation in the ring finger of *BRCA1*). Thus, although these results are likely to be applicable generally to protein-truncating mutations, they will not be applicable to missense changes.

In a small number of families, carrier status of relatives was available, and we were able to incorporate this into the analysis. In most cases, however, carrier status had to be assigned probabilistically. The method relies on the assumption of Mendelian segregation of the mutation—which seems reasonable but also ignores the possibility of new mutation events. However, few new mutations in *BRCA1*

or *BRCA2* have been reported, and the new-mutation rate is generally assumed to be low.

Overall Estimates

The overall estimates confirm most of the qualitative features, of age-specific risks in *BRCA1*- and *BRCA2*-mutation carriers, that have been suggested from studies based on multiple-case families or individual population studies, but with much more precise quantification. Thus, the average risks of both breast and ovarian cancer are higher in *BRCA1*-mutation carriers than in *BRCA2*-mu-tation carriers, but the difference is much more marked for ovarian cancer and for breast cancer at earlier ages than for breast cancer at >50 years of age. The RR of breast cancer in *BRCA1*-mutation carriers, relative to general population rates, declines with age from >30-fold at <40 years of age to 14-fold at >60 years of age; by contrast, the RR in *BRCA2*-mutation carriers is -11-fold in all age groups at >40 years of age and is not significantly higher at earlier ages. As a consequence of this, the incidences in BRCA1-mutation carriers rise to a plateau of -3%–4% per annum in the 40–49-years age group and are roughly constant thereafter, whereas the *BRCA2* rates show a pattern similar to that in the general population, rising steeply up to age 50 years and more slowly thereafter.

Ovarian cancer risks in *BRCA1*-mutation carriers were low (in absolute terms) at <40 years of age (no cases at all were observed at <30 years of age). Thereafter, the incidences were -1% at 40–59 years of age and 2% at >60 years of age (the latter estimate is, however, particularly imprecise, since there are relatively few unaffected carriers in this age group). Ovarian cancer risks in *BRCA2*-mutation carriers are, in contrast, very low at <50 years of age but then increase sharply in the 50–59-years age group, perhaps declining somewhat thereafter. These differences in age-specific risks are mirrored by other important differences in the

pathological characteristics of tumors in carriers (e.g., the estrogen-receptor–negative status of most breast tumors in *BRCA1*-mutation carriers but not in *BRCA2*-mutation carriers) and must reflect some important functional differences between the two proteins.

Absolute versus Relative Risk Models—and Cohort and Center Effects

We chose to model the *BRCA1* and *BRCA2* penetranc-es primarily in terms of RRs compared to a single set of background rates (those for England and Wales), thus estimating a single set of incidences for carriers from all populations. We also performed an alternative analysis in which the penetrance was expressed in terms of RRs relative to the population-specific incidences (so that the absolute risks would be higher in populations with higher background incidence rates). Such a model may be more appropriate if risks in carriers were modified by important lifestyle risk factors to a similar (relative) extent as in noncarriers. In fact, we found little evidence to favor one model over the other. Although we did find some evidence of variation in penetrance among populations, this did not correlate directly with population rates—for example, breast cancer risks were lower in families from the Polish center than in those from the U.K. centers but were similarly lower in families from the Canadian centers.

We found that year of birth had a marked effect on breast cancer risk in *BRCA1,* with a slightly weaker (and nonsignificant) effect in *BRCA2*-mutation carriers and for ovarian cancer risk in *BRCA1*-mutation carriers. The breast cancer effect was slightly weaker when analyses were performed relative to cohort-specific background rates but was still highly significant. Most of this effect was due to a markedly lower risk in women born before 1920. A possible explanation for this effect is the incomplete reporting of cancers among women born in this generation. In practice, the before-1920 birth cohort is not relevant to

current genetic counselling, and exclusion of this birth cohort from the present analysis made little difference to the overall penetrance estimates (data not shown). There was also some evidence of a higher breast cancer risk in the 1960-onward birth cohort. This result seems less likely to be due to the underreporting of cancers in relatives. It could conceivably reflect changing patterns of reproductive risk factors, such as age at first pregnancy, breast feeding, or oral contraceptive use. Changes in screening practices may also account for some of the cohort effect. This will require more-detailed investigation.

Type of Index Case

We found some evidence of variation in penetrance estimates according to the type of index case. The breast cancer risk estimates for both *BRCA1*- and *BRCA2*-mutation carriers were higher when the index case was a breast cancer case, rather than an ovarian cancer case, and were markedly higher when the index case was a breast cancer case diagnosed at <35 years of age. A similar effect has been reported previously (Eccles et al. 1994). The ovarian cancer risks in *BRCA1*-mutation carriers were also highest in families selected on the basis of a breast cancer index case diagnosed at <35 years of age, but, for *BRCA2*-mutation carriers, the risks were higher when based on ovarian cancer index cases (albeit with wide confidence limits). Such differences in penetrance estimates are generally consistent with the hypothesis that other genes modify risks in carriers. Alleles conferring a higher risk of breast cancer will be more frequent among index cases diagnosed at earlier ages, leading to higher breast cancer risks in carriers' relatives. The more complicated pattern of ovarian cancer risk in *BRCA1*-mutation carriers may be explicable if some modifiers of breast cancer risk also modified ovarian cancer risk. No genetic modifiers have been definitively implicated yet, although several have been suggested; these include, for breast cancer, the lengths

of triplet repeats in the androgen-receptor (Rebbeck et al. 1999) and *AIB1* (Rebbeck et al. 2001) genes and polymorphisms in the progesterone-receptor (Runnebaum et al. 2001) and (for *BRCA2*) *RAD51* (Levy-Lahad et al. 2001) genes. Rare alleles at the *HRAS1* minisatellite locus have been suggested to be associated with ovarian cancer risk in *BRCA1*-muta-tion carriers (Phelan et al. 1996).

Mutation-Specific Risks

Mutation-specific differences in risk have been suggested for both *BRCA1* and *BRCA2* from differential cancer risks in multiple-case families, and we were able to test these hypotheses in this data set. In the case of *BRCA2,* Thompson and Easton (2001) have found a higher risk of ovarian cancer but a lower risk of breast cancer in carriers of mutations in the ovarian cancer cluster region. The RR estimates from the current data set were of a similar magnitude (0.57 for breast cancer and 2.1 for ovarian cancer), but neither was significant. Furthermore, the ovarian cancer effect disappeared once the 999del5 and 6174delT mutations were removed from the analysis. In the case of *BRCA1,* Thompson and Easton (2002) have found a lower risk of breast cancer associated with mutations in a central region of the gene (nucleotides 2401–4184), together with a lower risk of ovarian cancer for mutations 3′ of nucleotide 4184. The estimate RRs from our data set were in the same direction (and, in the case of the breast cancer risk, of a similar magnitude) as those reported by Thompson and Easton (2002), but neither effect was statistically significant. Although the consistency of these results is reassuring, they emphasize that detailed analyses of mutation-specific risks can be achieved only through studies of multiple-case families.

Conclusions

The present analysis has provided breast and ovarian cancer risks in *BRCA1*- and *BRCA2*-mutation carriers that are based on the majority of available data from studies of

mutation screening in series of patients with breast and ovarian cancer who were unselected for family history. It has confirmed that the lifetime risks based on this design are lower than those based on high-risk families, suggestive of some modification of risk by other factors, but the differences are smaller than has been suggested by some previous studies. The variation in risks by type of index case and by age at diagnosis of index case is also suggestive of risk modifiers. Risk estimates for counselling should take into account both mutation status and family history, as well as other risk factors once their effects become reliably known.

Acknowledgments

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Electronic-Database Information

URLs for data presented herein are as follows:

Breast Cancer Information Core, http://research.nhgri.nih.gov/ bic/

Online Mendelian Inheritance in Man (OMIM), http://www .ncbi.nlm.nih.gov/Omim/ (for *BRCA1* and *BRCA2*)

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Nos. 14-1418, 14-1453, 14-1505,
15-35, 15-105, 15-119 & 15-191

IN THE

Supreme Court of the United States

DAVID A. ZUBIK, ET AL.,
Petitioners,

v.

SYLVIA BURWELL, ET AL.,
Respondents.

[CAPTIONS CONTINUED ON INSIDE COVER]

On Writs of Certiorari to the
United States Courts of Appeals for the
Third, Fifth, Tenth, and D.C. Circuits

APPENDIX VOL. II OF II OF *AMICI CURIAE* THE OVARIAN CANCER RESEARCH FUND ALLIANCE AND ITS PARTNER MEMBERS AND SCIENTIFIC ADVISORS' BRIEF IN SUPPORT OF RESPONDENTS

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PRIESTS FOR LIFE, ET AL., *Petitioners,*

v.

DEPARTMENT OF HEALTH & HUMAN SERVICES, ET AL., *Respondents.*

ROMAN CATHOLIC ARCHBISHOP OF WASHINGTON, ET AL., *Petitioners,*

v.

SYLVIA BURWELL, ET AL., *Respondents.*

EAST TEXAS BAPTIST UNIVERSITY, ET AL., *Petitioners,*

v.

SYLVIA BURWELL, ET AL., *Respondents.*

LITTLE SISTERS OF THE POOR HOME FOR THE AGED, DENVER , COLORADO, ET AL., *Petitioners,*

v.

SYLVIA BURWELL, ET AL., *Respondents.*

SOUTHERN NAZARENE UNIVERSITY, ET AL., *Petitioners,*

v.

SYLVIA BURWELL, ET AL., *Respondents.*

GENEVA COLLEGE, *Petitioner,*

v.

SYLVIA BURWELL, ET AL., *Respondents.*

APPENDIX N

EUROPEAN JOURNAL OF CANCER
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ORAL CONTRACEPTIVE USE AND BREAST OR OVARIAN CANCER RISK IN BRCA1/2 CARRIERS: A META-ANALYSIS

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Abstract

Background: Women with BRCA1 or BRCA2 mutations are at increased risk of breast and ovarian cancer. Oral contraceptives (OC) use has been associated with a reduction in ovarian cancer risk and with a moderately increased breast cancer risk, which tends to level off in the few years after stopping. The association between oral contraceptive and BRCA1 or BRCA2 gene mutations carriers is unclear.

Methods: We performed a comprehensive literature search updated to March 2010 of studies on the associations between OC users and breast or ovarian cancer for ascertained BRCA1/2 carriers. We obtained summary risk

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estimated for ever OC users, for duration of use and time since stopping.

Results: A total of 2855 breast cancer cases and 1503 ovarian cancer cases, carrying an ascertained BRCA1/2 mutation, were included in our meta-analyses, based on overall 18 studies. Use of OC was associated with a significant reduced risk of ovarian cancer for BRCA1/2 carriers (summary relative risk (SRR) = 0.50; 95% confidence interval (CI), 0.33– 0.75). We also observed a significant 36% risk reduction for each additional 10 years of OC use (SRR: 0.64; 95% CI, 0.53–0.78; P trend < 0.01). We found no evidence of a significant association between OC and breast cancer risk in carriers (SRR: 1.13; 95% CI, 0.88–1.45) and with duration of use. OC formulations used before 1975 were associated with a significant increased risk of breast cancer (SRR: 1.47; 95% 1.06, 2.04), but no evidence of a significant association was found with use of more recent formulations (SRR: 1.17; 95% 0.74, 1.86).

Conclusions: OC users carrying an ascertained BRCA1/2 mutation have a reduced risk of ovarian cancer, proportional to the duration of use. There is no evidence that recent OC formulations increase breast cancer risk in carriers.

1. Background

There is clear evidence that germ line mutation in BRCA1 (MIM #113705) or BRCA2 (MIM #600185) account for a large proportion of familial breast/ovarian cancer and confer very high lifetime risks for both cancer sites.[1] Approximately 5–10% of all epithelial ovarian carcinomas result from genetic predisposition[2] and the great majority of these are associated with BRCA genes, as opposed to 25% of all hereditary breast cancers.[3,4] The lifetime risk of breast or ovarian cancer for women who inherited a BRCA mutation is highly variable and depends on the specific mutation, on the population studied and are extremely higher than the lifetime risk in the general population.[5–10] In addition, there is evidence that cancer patients with BRCA1 and BRCA2

mutation are characterised by different pathological and clinical features, some of which have prognostic value.[11] Some studies demonstrated that breast cancers in BRCA1 carriers more likely do not express oestrogen and progesterone receptors or Her-2/neu (triple-negative breast cancer), while breast cancers in BRCA2 carriers seem to share the same pathologic characteristics as non-carriers.[12] Moreover, oral contraceptives (OC) use was associated with an increased risk of cancer among triple-negative breast cancer, but not among non-triple-negative breast cancer.[13]

In the general population long-term exposure to oestrogen may increase a woman's chance of developing breast and ovarian cancer. The level of estrogens is associated with the repair capacity of breast and ovarian epithelial cells that may result in tumour formations, instead of apoptosis.[14,15] Oestrogen levels are high in ovulating women and any factor that limit the period of ovulation (pregnancy, late onset of menstruation or early onset of menopause) decreases the lifetime exposure to oestrogen and thus the risk for both types of cancer.

The measures for ovarian cancer prevention and early detection are limited as symptoms are frequently non-specific, patients are often diagnosed with advanced disease and family history of early-onset breast/ovarian cancer remains the single most important factor in determining individual ovarian cancer risk.[17–20]

Some studies suggest that non-genetic risk factors may differ in women with hereditary breast and ovarian cancer caused by alterations in the BRCA1/2 genes. Breast cancer typically occurs in these women at a much younger age, but the risk is not influenced by the age at menarche and it is also unclear whether the relationship between parity, age at menopause and breast cancer risk holds true in women who have BRCA mutations.[1,21]

OC use has been associated with a moderately increased breast cancer risk, which tends to decline progressively after

termination of use and with a reduction in ovarian cancer risk for women unselected for predisposing genetic mutations.[22,23]

The use of OC for mutation carriers could be controversial because of the increasing breast cancer risk, especially early-onset, and the contemporary protective effects for ovarian cancer.

The present meta-analysis was conducted to examine and clarify whether exogenous hormone in the form of OC might modify the risk of breast or ovarian cancer in BRCA mutation carriers. Furthermore we investigated the association between specific mutation (BRCA1 or BRCA2) and OC use for breast or ovarian cancers.

2. Materials and methods

2.1. Search strategy, inclusion criteria and data abstraction

We conducted a literature search updated to March 2010 using validated search strategies[23–25] on the following databases: PUBMED, EMBASE, Ovid MEDLINE®, using combinations of the following MeSH terms and keywords: 'oral contraceptives', 'cancer', 'ovarian' or 'breast', 'BRCA1' or 'BRCA2'. We also identified the most cited articles on the topic using ISI Web of Knowledge® Science Citation Index Expanded™ (Journal Citation Report). In addition we reviewed the references of all articles of interest and preceding reviews on the topic to identify additional relevant studies. The search was limited to human studies and no language or time restrictions were applied.

2.2. Meta-analysis on the impact of OC use on cancer risk in mutation carriers

Our aim was to study the association between OC use and the risk of breast/ovarian cancer in women carrying a BRCA1/2 mutation.

Published reports fulfilling the following inclusion criteria were included in the meta-analysis:

(1) Studies containing the minimum information to obtain an estimate of the relative risk (RR), with its uncertainty, of:

(a) breast and/or ovarian cancer associated with OC use in BRCA1/2 mutation carriers ascertained by a genetic test;

(b) ascertained BRCA1/2 mutation, in association with OC use, in patients with breast and/or ovarian cancer.

(2) Case–control, cohort studies and nested case–control studies, published as original articles.

(3) Independent studies. In case of multiple reports on the same population or sub-population, we considered the estimates from the most recent or most informative report.

(4) Study populations that were as homogeneous as possible. We excluded study performed on subjects all submitted to a surgical procedure (bilateral salphingo-oophorectomy), which could have modified the association between OC and cancer risk for affected.

(5) Case–controls studies with controls not directly tested for the mutation were excluded by the analyses evaluating cancer risk in BRCA1/2 carriers.

The exposure of interest was ever OC use, defined as any duration of OC use lifetimes. In Tables 1 and 2 we detailed definitions of the exposures as reported originally by authors.

Table 1
Features of the studies included in the meta-analysis on the impact of OC use on cancer risk in mutation carriers

* * *

Table 2
Features of the studies included in the meta-analysis on association between of OC use and mutation status in cancer patients

* * *

Presence of heterogeneous exposures was investigated in a sensitivity analysis. We also explored duration of OC use, time since last use and age at start use.

When available we used fully adjusted estimates. Articles were reviewed and data were extracted and crosschecked independently by two investigators (S.I. and S.G). Any disagreement was resolved by consensus among them.

The following information were extracted and coded from the original articles: adjusted risk estimates or crude data, year of publication, type of study, country of the study, features of populations, definition of the exposure, cancer site, mutation status, adjustments and matching variables used in the analysis and study design. When dose–response estimates on duration of OC use and time since last OC use were provided, we retrieved the study-specific dose response risk estimates and frequencies for each level of exposure.

Results from unpublished data obtained in our Institute were also added in the meta-analysis and evaluated in a sensitivity analysis.

2.3. Association between BRCA1/2 carrier status and OC use for breast or ovarian cancer patients

We also studied the magnitude of the association between BRCA1/2 mutation and OC use in patients with breast/ovarian cancer in a case–case approach.

Fig. 1
Flow chart of selection of studies

* * *

3. Statistical methods

When available, we retained estimates adjusted for the maximum number of confounders.

We always presented random effects models to evaluate summary relative risk (SRRs) obtained with maximum

likelihood estimates, in order to be more conservative.[42] Homogeneity of effects across studies was assessed using the Chi-square statistic (which we considered statistically significant when the P-value was 60.10)[43] and quantified by I^2, which represents the percentage of total variation across studies that is attributable to heterogeneity rather than chance.[44] When more than a single risk estimate was present in a study (i.e. separate estimates for BRCA1 and BRCA2), we adjusted the pooled estimates for intra-study variation. When possible we performed separate analyses for type of mutation by using a bivariate approach. Sub-group and meta-regression analyses were carried out to investigate potential sources of between-study heterogeneity.[45] Many studies reported estimates for first use of OC in or after 1975, when dose of oestrogen in OC formulation was reduced substantially. We performed meta-regression by year at start OC, assuming that women who started their OC after 1975 have used low-dose OC.

In the dose–response analysis, we considered duration of OC use and time since last use as explanatory variables. In pooling dose–response data, we took into account correlation between RRs categories within the same study, using Greenland and Longnecker method.[46]

We also studied the magnitude of the association between BRCA1/2 mutation and OC use in patients with breast/ovarian cancer with a case–case comparison. Following this approach, cancer patients with the mutation formed the ‘pseudo-cases’ and patients without the genotype formed the ‘pseudo-controls’ group. The two groups were then compared with respect to the prevalence of each exposure. The SRRs obtained reflects the association between the exposure (OC use) and the genotype (BRCA1/2 mutation), assuming the independence of genotype and exposure in the source population.

Sensitivity analysis was carried out in order to evaluate whether overall results were influenced by a single or a group

of studies.[47] Publication bias was evaluated by funnel plots and quantified by the Egger's test.[48,49] All analyses were performed with SAS Software using PROC MIXED (SAS, 8.02 for Windows, Cary, NC).[50]

Table 3
Summary risk estimates of the association between OC use and cancer risk in mutation carriers

* * *

4. Results

Details on the search strategy and the data extrapolation are described in Fig. 1. The main characteristics of the studies included in the analyses are shown in Table 1.

4.1. OC-associated breast cancer risk

The analysis was based on five studies (2855 breast cancer cases, 2954 healthy carriers). Breast cancer risk estimates for various categories of OC use are described in Table 3.

For BRCA1/2 carriers, we found that breast cancer risk was not significantly increased by OC use (SRR = 1.13; 95% confidence interval (CI): 0.88–1.45). Similarly, no significant association was found when we limited the analysis to BRCA1 or BRCA2 carriers (Fig. 2 left).

There was no evidence of a dose–response relationship with duration of OC use (P = 0.20).

The association between time since stopping OC use and breast cancer was assessed basing on three studies and overall 2109 cases. Compared to never users, BRCA1/2 carriers who stopped OC at least 10 years before diagnosis were at significant increased risk of breast cancer (SRR = 1.46; 95% CI, 1.07– 2.07). By contrast, no significant association was observed for women who stopped OC use within the last 10 years. Difference between the two estimates was statistically significant ($P = 0.03$).

We also found that OC formulations used before 1975 were associated with increased risk of breast cancer (SRR = 1.47;

95% CI, 1.06–2.04). On the contrary no evidence of an association was found with use of recent formulations (SRR = 1.17; 95% CI, 0.74–1.86).

Fig. 2
Association between oral contraceptive (OC) use and breast or ovarian cancer in BRCA1/2 carriers

* * *

4.2. OC-associated ovarian cancer risk

Overall the meta-analysis was based on five studies (1503 ovarian cancer cases, 6315 healthy carriers).

In Table 3, we present risk estimates for ovarian cancer associated with different exposures to OC. We found a significant protective association between OC use and the risk of ovarian cancer (SRR = 0.50; 95% CI, 0.33–0.75).

When we performed separate analyses by type of mutation, OC use was associated with a significant reduced risk of cancer for both BRCA1 (SRR = 0.51; 95% CI, 0.40–0.65) and BRCA2 mutations carriers (SRR = 0.50; 95% CI, 0.29–0.89) (Fig. 2 right).

We found a significant linear decrease in risk for carriers with increasing duration of OC use: each additional 10 years of OC use the risk decreased by 36% (95% CI, 22–47%, $P < 0.01$ for trend).

5. Sensitivity analysis, meta-regression and publication bias

In this meta-analysis, the term ‘ever OC use’ was referred to any use of OC reported during lifetime. This is a general definition, which includes all meanings considered by the authors: Haile[29] included in that definition OC users for at least 1 month, Heimdal[28] for at least 3 months, while Whittemore[33] evaluated OC users for at least 1 year. The influence of these different definitions of exposure was evaluated in sensitivity analyses with no substantial differences for breast/ ovarian cancers risk.

Among the studies included in the analysis on breast cancer, one study[27] has a very large weight. Similarly, McLaugh-lin[34] could drive the analysis on ovarian cancer and it is also the only study with no histological confirmation of cancer diagnosis. Testing whether the exclusion of these studies may have potentially biased the estimates, we did not observe any change in the overall results.

In order to prevent from inclusion of prevalent cases, two studies[31,34] reported separate estimates limiting the cohort to subjects with a diagnosis within 5 and 3 years since diagnosis, respectively, in order to prevent from survival bias. We investigated the possible effect of inclusion of prevalent cases performing the analysis including the estimates form the cohorts restricted to incidence cases, where the survival bias is likely to be smaller, without marked change in breast (SRR = 1.10; 95% CI: 0.93–1.29) or ovarian cancer estimates (SRR = 0.49; 95% CI: 0.32–0.75).

The core of our meta-analysis included case–controls, both hospital and population based, and cohort studies. However, we performed in a sensitivity analysis a separate analysis for case–controls and cohort studies, without any difference in the estimates. Our main analysis on the effect of OC on cancer for mutation carriers comprised only one cohort[31] for breast cancer. Excluding the latter from the analysis the summary estimate remains similar (SRR = 1.04; 95% CI: 0.79– 1.38).

Some studies included patients who had undergone salp-hingo-oophorectomy. Most of them presented estimates adjusted for this effect or used it as a matching variable. We performed separate analysis for studies taking into account this risk modifier, with lower estimates for studies taking into account this factor, but no differences in the estimates for both breast and ovarian cancer (P = 0.19 and P = 0.19; respectively).

No indication of publication bias was found when assessing OC effect on both cancer sites: P-values from weighted Eg-

ger's test for funnel plot were 0.90 for breast cancer and 0.73 for ovarian cancer.

Since our analysis includes studies based on familial cancer cases, we evaluated in breast cancer analyses whether there was any difference between estimates adjusted or not for family history. No difference was found between them through meta-regression (P = 0.41). The estimates used for ovarian cancer analysis were not adjusted for this factor.

6. Association between OC use and mutation status in cancer patients

Features of the studies included in the analysis are detailed in Table 2. We evaluated estimates from case–case approaches to study whether mutation carriers were more likely than non-carriers to use OC.

The estimates were based on a total of 241 breast cancer cases and 371 ovarian cancer cases with a BRCA1/2 mutation. We found no significant associations between BRCA1/2 mutation status and use of OC for breast/ovarian cancer, even separately investigating the cancer sites and mutations (Fig. 3).

7. Discussion

Our meta-analysis was based on 2855 breast and 1503 ovarian cancer cases with a BRCA1/2 mutation. We found no evidence of a significant increased breast cancer risk in OC users overall, for recent formulation of OC and in the first 10 years after cessation.

Fig. 3
Association between BRCA1 and BRCA2 combined carrier status and oral contraceptives (OC) use in cancer patients

* * *

Our outcomes differ from results obtained in a previous pooled-analysis, based on 54 studies. The authors investigated the association between OC use and breast

cancer risk in the general population, showing a significant association between OC use and breast cancer. However, the estimate in this pooled analysis was slightly above the unit (RR = 1.07; SD = 0.02)[22] and the risk progressively declines, disappearing during the 5 years after stopping. Our study on mutation carriers was based on ever OC users, and it suggests evidence of an increased risk of breast cancer of 46% only for women who ceased OC use more than 10 years before diagnosis. This increasing risk could be explained by the effect of age as women who ceased in more distant time are supposed to be older than recent quitters. To some extent these results could also be explained by differences in OC formulations: most women who stopped OC use 10 or more years before diagnosis tend to have used higher dose preparation. In fact, in our analyses OC formulations used before 1975 (when drugs were likely to contain high doses of hormones) were associated with a 46% increased risk of breast cancer, on the contrary no association was found with use of recent formulations.

We also confirmed that carriers who use OC are at a significant reduced risk of ovarian cancer. The reduction is associated with ovarian cancer in a dose–response relationship: risk is greater the longer women used OC.

The reduction in ovarian cancer risk of 50% for BRCA1/2 carriers ever OC users was consistent with, and higher than, the reduction observed in the general population: in a pooled meta-analysis, based on 45 epidemiological studies, the reduction observed for ever OC users was 27%. Similarly, in our results the overall risk decreased by a 20% for mutation carriers for each five years of use, consistent with the 20% reduction observed in the general population.[23]

We carried out a separate analysis by type of mutation, based on the rationale that cancer patients with BRCA1 and BRCA2 mutation are characterised by different cancer subtypes in terms of oestrogen, progesterone or Herb2 status. In fact we could suppose that the risk for triple negative

breast cancer, which is more frequent in BRCA1, due to hormonal risk factors, such as OC use, could be higher.[12,13] However, we did not find significant differences between BRCA1 and BRCA2 mutation carriers.

We also conducted a separate meta-analysis to determine whether OC use differs in breast/ovarian cancer cases with or without a mutation. Oral contraceptive use was not significantly more common for carriers compared with cases without any mutation.

Relative risk estimates of case–case approach are based on the assumption of independence between presence of a mutation and OC. This seems to be reasonable in all studies we included in the analyses, even if there may be a possibility of a violation of this assumption. If there were a positive association between genotype and exposure in the underline population, this could lead to some bias in the estimates, when compared to the ratio of the relative risk that the authors are attempting to estimate. Only analyses on case–controls and cohort studies would address this limitation. Therefore, we based our conclusions mainly on the latter results.

Studies included in the analyses are based on different study designs and analyses, different types of mutations and baseline cancer risk. We investigated how these aspects could have influenced the estimates through subgroup analyses and meta-regressions.

The studies that formed the basis of our meta-analysis included case–controls, both hospital and population based, and cohort studies. We found no difference in the estimates obtained from separate analyses on case–controls and cohort studies.

Some studies included patients who had undergone salp-hingo-oophorectomy, a cancer prevention strategy that could have an impact on the magnitude of the protection afforded by oral contraceptives use. Most of these studies presented

adjusted estimates for this effect or used it as a matching variable. We evaluated whether this could have overestimated the protective association, performing separate analysis for studies taking into account this risk modifier, with no differences in the estimates for both cancer sites.

One possible source of bias is that the studies we included in the analyses reported different definitions of exposure. In fact, the majority of the authors defined ever OC users as women with any duration of use. We investigated differences in the estimates by types of definitions reported by the authors and we found no substantial variations.

Another possible limitation of the present analysis could arise from the inclusion of prevalent cases which may result in survival bias. If OC use is associated with a higher mortality in women with breast or ovarian cancer, the selection of prevalence cases might operate to reduce the risk. However, the investigation of heterogeneity and sensitivity analyses did not show any substantial effect of this factor, suggesting that survival bias was limited.

Most of the published evidence related to BRCA1/2 was based on large families with many individuals affected by breast/ovarian cancer. Because family members share heritable and probably environmental factors, it is possible that an amount of cancer cases diagnosed in these families may be partly due to other genetic or environmental factors.

Moreover, the inclusion of studies conducted on members of families with multiple cases of cancer may bias the risk estimates as oral contraceptives use in these carriers may not pertain to the general population of carriers. However, the study with the highest weight, used for breast cancer analysis,[27] selected participants from previous trials and research protocols; therefore, cohort selection from clinical genetic centres should not be the main issue of this analysis.

There has been a change in the formulation of OC over the past several decades. In the recent formulations there is a

substantial reduction in the oestrogen content. Typical oestrogen doses in the 1960s were more than double the typical doses in the 1980s and later, so that recent formulations may be considered less hazardous than the older. Calendar year (before or after 1975) is used in many studies as an indicator of the average oestrogen dose of the preparations. We found that OC formulations used before 1975 were associated with increased risk of breast cancer. On the contrary no association was found with use of recent formulations.

This is the first meta-analysis addressing breast or ovarian cancer risk for OC users for BRCA1/2 carriers. The study involved overall 5809 and 7818 mutation carriers in the analysis on breast and ovarian cancer, respectively. The main strength of our meta-analysis is the large number of cases included, with a known mutation in one of the BRCA1 or BRCA2 genes, and the possibility to investigate the association with duration of use, age at start, time since quitting and calendar time.

Even if the ideal would be to present all the estimates of risk by types of mutation, we could not carry out all our analyses by BRCA status because many authors presented only estimates for BRCA carriers combined, presumably due to limited statistical power.

Another possible limitation of this meta-analysis is the lack of published prospective studies. In fact all but two retrospective cohort studies were case–controls, and even if we try to investigate the effect of study design, we were not able to completely address the issue of potential presence of recall bias. However, in the pooled analysis on observational studies, there was no difference in the association of OC use with breast cancer between prospective cohort studies and case– controls studies.

Our investigation of the potential effect of different study designs and adjusting factors did not show any impact on the

summary estimates, however, possible sources of unexplained bias could remain and influence our results.

Our meta-analysis provides evidence that OC reduces ovarian cancer risk and no evidence that recent formulation of OC increases breast cancer risk for women with a germ line mutation in BRCA1 or BRCA2.

Further prospective studies on carriers may have to confirm our results and could also evaluate the additive effect of post-hormone use or types of OC that we could not deeply investigate in this setting.

Conflict of interest statement

None declared.

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APPENDIX O

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FALLOPIAN TUBE LIGATION OR SALPINGECTOMY AS MEANS FOR REDUCING RISK OF OVARIAN CANCER

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The Problem of Ovarian Cancer

Ovarian cancer remains the most lethal gynecologic malignancy in the United States, both in rate of fatality (64 percent of patients ultimately die of their disease [1]) and in overall deaths (14,270 in 2014 [2]). Although 50–75 percent of patients treated with chemotherapy initially respond to the medications, most will have recurrences of the disease [1]. The driving force behind the poor survival rates is the stage at diagnosis. Approximately 65 percent of patients present with widespread (stages III or IV) disease, at which point

cure is uncommon [2]. For patients with stage I disease, on the other hand, five-year survival rates exceed 90 percent [2].

One reason that most patients are diagnosed at late stages is that the clinical symptoms of ovarian cancer usually do not become apparent until the disease has disseminated throughout the peritoneal cavity. Although multiple attempts have been made to develop screening programs aimed at detecting early-stage disease, current screening methods are fraught with low sensitivity and specificity, high false-positive rates, and an unfavorable balance between the risks of early intervention and the benefits of cancer risk reduction [2– 4].

Attempts at Ovarian Cancer Screening

Because the clinical symptoms of ovarian cancer are vague and often appear late in the course of disease, numerous attempts have been made to initiate screening programs to identify preclinical disease in asymptomatic women [3]. Some methods for screening include pelvic examination, ultrasound, and blood testing. The Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial found that screening did more harm than good with respect to ovarian cancer [3]. Specifically, study subjects underwent unnecessary surgeries that did not diagnose ovarian cancer and were associated with intraoperative and postoperative complications. The United Kingdom Collaborative Trial of Ovarian Cancer Screening, published in 2015, found that serial testing of the cancer antigen (CA) 125 protein, interpreted according to the Risk of Ovarian Cancer Algorithm (ROCA), and ultrasound were better at detecting ovarian cancer than a single threshold CA 125 test [5]. Ultimately, screening for ovarian cancer is not ready for application outside of clinical trials because the results have not been validated in independent cohorts. Clinicians must maintain a high index of suspicion, i.e., consider ovarian cancer a likely possibility, to clinically diagnose it.

Due to the absence of an effective screening algorithm for assessing risk or clinical symptoms that develop with early-stage disease, primary prevention strategies are crucial for reducing ovarian cancer-related deaths.

Experience from Hereditary Breast and Ovarian Cancer Syndromes

Identifying patients at increased risk for ovarian cancer is key to prevention, early detection, and, ultimately, improving survival. Those with BRCA1 mutations have a 39–46 percent lifetime risk of ovarian cancer, those with BRCA2 mutations have a 10–27 percent risk, and up to 24 percent of those with Lynch syndrome will develop ovarian cancer [6]. At this time, the best tools that clinicians have for ovarian cancer prevention are a thorough family history and testing appropriate patients for genetic susceptibility [7]. The Society of Gynecologic Oncologists (SGO) policy statement on genetic counseling says unaffected individuals with increased risk—i.e., relatives with ovarian cancer; a family history suggestive of Lynch syndrome based on Amsterdam Criteria or Bethesda Guidelines; known mutations in the family or a family member diagnosed with breast cancer before age 45; multiple breast cancers, male breast cancer, pancreatic cancer, or aggressive prostate cancer (with a Gleason score of 7 or above)—should be referred for genetic counseling and, potentially, testing for germline mutations in BRCA [7]. If BRCA mutations or Lynch syndrome are identified, the National Comprehensive Cancer Network (NCCN) recommends removal of both fallopian tubes and ovaries between the ages of 35 and 40, based on the particular mutation carried. CA 125 tests and pelvic ultrasound have been considered, but there is not sufficient evidence that these tests are sensitive or specific enough to obviate the need for surgery [8].

Fallopian Origin and Prevention of Ovarian Cancer

A proposed model for ovarian carcinogenesis arising in the fallopian tube has emerged over the last decade [9, 10]. This

tubal-origin hypothesis has gained traction with identification of pre-invasive lesions in the fallopian tubes of high-risk patients undergoing risk-reducing surgery [10]. Thus, bilateral salpingectomy with ovarian conservation was proposed as a "middle-ground" method of primary prevention, with the benefit of removing potential tissue of origin and without the risks of surgical menopause. This method has been proposed for clinical trials in high-risk patients, but results are not currently available [11]. The SGO in 2013 published a clinical practice statement recommending that a bilateral salpingectomy should be considered "at the time of abdominal or pelvic surgery, hysterectomy, or in lieu of tubal ligation" [12]. The American College of Obstetricians and Gynecologists (ACOG) had a more tempered statement, saying that salpingectomy should be considered for population-risk patients, i.e., those without increased risk based on personal or family history, but they were clear that the approach to pelvic surgery, hysterectomy, or sterilization should not change simply to increase the chances of completing bilateral salpingectomy [13]. Both of these statements were more conservative than the proposed plan of the British Columbia Ovarian Cancer Research Group program, instituted in 2010, which involved performing opportunistic salpingectomy with benign hysterectomy or in lieu of bilateral tubal ligation for permanent contraception. These authors suggested that this approach would yield a 20– 40 percent population risk reduction for ovarian cancer over the next 20 years [14].

The estimated risk reduction for any individual person undergoing opportunistic salpingectomy is up to 50 percent [14]. Although this is an appreciable benefit, it must be tempered with a reminder that women at population risk of ovarian cancer have only a 1:70 or 1.4 percent lifetime risk [14]. The significant benefits of opportunistic salpingectomy, besides the risk reduction, are the ease and speed of the procedure, the rarity of complications, the convenience of

removing the specimen, and the fact that surgical removal is theoretically the only way to permanently reduce the risk of ovarian cancer [15] (although bilateral tubal ligation without salpingectomy has also been associated with decreased risk [16]). Whether salpingectomy is more beneficial than tubal ligation has not been established.

Unresolved Questions

Despite the popularity of salpingo-oophorectomy as a method of reducing risk of ovarian cancer, data from the Nurses' Health Study suggest that oophorectomy before age 47.5 years may be associated with increased risk of death from other causes, such as cardiovascular disease [4], and that the actual permanent risk reduction with salpingectomy, as opposed to the theoretical 50 percent reduction [14], is not entirely clear.

Numerous questions remain regarding the optimal timing of salpingectomy, as the timespan during which the ovaries are susceptible to induction of cancer from the fallopian tubes is certainly not infinitely large. A bilateral salpingectomy at age 30 is logically more effective at risk reduction than the same surgery at age 60. Unfortunately, the relationship between time and risk reduction has not been not characterized, and prospective studies of the effect of age at salpingectomy on risk reduction would require prohibitively large cohort sizes and long follow-up periods. Similarly, there are other commonly accepted interventions associated with risk reduction, including oral contraceptive pill use and breastfeeding [2, 15, 16]. It is not known how salpingectomy and oral contraceptive pill use interact with one another, although presumably women with a history of bilateral salpingectomy will use birth control pills less frequently, given that the prevention of unintended pregnancy is no longer a concern.

Another unresolved question is whether salpingectomy should be used instead of tubal ligation for a "two birds with one stone" approach to sterilization and risk reduction.

Caution should be exercised when choosing salpingectomy over tubal ligation for sterilization, not because of the inability to reverse salpingectomy—tubal ligation also should not be performed on women who may desire future childbearing, and in vitro fertilization is a viable method of achieving pregnancy after salpingectomy or tubal ligation [17]—but because "low-risk" surgery does not equal "no risk." We should be cautioned by prior experience with opportunistic appendectomy at the time of cesarean section or hysterectomy [18]: with opportunistic appendectomy, stump leaks, bleeding, and infection were all possible. Furthermore, salpingectomy increases the length of the operation, and length of surgery has consistently been identified as an independent risk factor for postoperative morbidity [19–23], so even an opportunistic salpingectomy can increase some risks.

Another issue is that payers may be reluctant to authorize the charges for risk-reducing procedures, given the number needed to prevent a single case of ovarian cancer. The theoretical number needed reported by Kwon and colleagues in 2015 was 273 for salpingectomy at the time of hysterectomy and 366 for salpingectomy in lieu of other tubal occlusion methods for sterilization [14]. Although these numbers are on the same order of magnitude as the number needed to vaccinate with the human papilloma virus vaccine in the United States [14], the costs associated with vaccination are less than the costs of salpingectomy.

Conclusions

Ultimately, we think ACOG's recommendation of a discussion about risks and benefits of removing both fallopian tubes at the time of hysterectomy is reasonable. However, we cannot place enough importance on the statement, "the approach to hysterectomy or sterilization should not be influenced by the theoretical benefit of salpingectomy" [13]. In the absence of results from prospective studies, which will not be available for decades,

fallopian tubes should be removed when a convenient opportunity arises, but extensive surgery should not be attempted just for that purpose.

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APPENDIX P

WHY HAVE OVARIAN CANCER MORTALITY RATES DECLINED? PART I. INCIDENCE

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ABSTRACT

The age-adjusted mortality rate from ovarian cancer in the United States has declined over the past several decades. The decline in mortality might be the consequence of a reduced number of cases (incidence) or a reduction in the proportion of patients who die from their cancer (case-fatality). In part I of this three-part series, we examine rates of ovarian cancer incidence and mortality from the Surveillance Epidemiology and

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End Results (SEER) registry database and we explore to what extent the observed decline in mortality can be explained by a downward shift in the stage distribution of ovarian cancer (i.e. due to early detection) or by fewer cases of ovarian cancer (i.e. due to a change in risk factors). The proportion of localized ovarian cancers did not increase, suggesting that a stage-shift did not contribute to the decline in mortality. The observed decline in mortality paralleled a decline in incidence. The trends in ovarian cancer incidence coincided with temporal changes in the exposure of women from different birth cohorts to various reproductive risk factors, in particular, to changes in the use of the oral contraceptive pill and to declining parity. Based on recent changes in risk factor propensity, we predict that the trend of the declining age-adjusted incidence rate of ovarian cancer in the United States will reverse and rates will increase in coming years.

Contents

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I. Introduction

Ovarian cancer accounts for 3% of all cancers in women, but is overrepresented in terms of cancer deaths (5%). In 2014, in the United States, 21,980 women were diagnosed with ovarian cancer and 14,270 women died of it [1]. Ovarian cancer is primarily a disease of postmenopausal women; approximately 70% of cases and 85% of ovarian cancer deaths occur after age 55 [2]. A woman who is diagnosed with breast cancer at age 70 is likely to die of another cause [3] — in contrast, if a woman is diagnosed with ovarian cancer at age 70, there is an 80% chance that the cancer will cause her death [4]. This is because the fatality rate is high (70%) and because 80% of deaths occur within five years of diagnosis [4]. As the American population ages and expands [5], the annual number of ovarian cancer cases is expected to rise. In order to reduce the burden of ovarian cancer in the population, it is necessary to prevent deaths across the age spectrum, and in particular, deaths in older women.

The modern era of ovarian cancer therapy began in 1977 with the introduction of cis-platinum. Nowadays, over 60% of women with invasive ovarian cancer are treated with debulking surgery and with a combination of a platinum agent and a taxane [6]. Since 1975, the mortality rate for ovarian cancer in the USA has declined by 23% [7]; it is tempting to conclude that the decline was the consequence of chemotherapy, but before doing so, it is prudent to explore alternative explanations. In the first two parts of the three-part series, we examine SEER rates of ovarian cancer incidence, case-fatality and mortality, with reference to calendar year, age and tumour stage, and we consider possible reasons for the observed decline in mortality. In Part I, we consider if the decline was

due to a reduced number of cases (through changing trends in elective oophorectomy and/or in reproductive risk factors) or was due to a downward stage shift at presentation (through screening or better awareness). In part II, we consider if the decline in mortality was due to new and better treatments [8]. In part III, we discuss potential approaches for reducing ovarian cancer mortality in the future, through prevention, early detection and treatment [9].

Mortality rates describe the number of deaths from ovarian cancer in a given year, relative to the size of the population. A decline in mortality may reflect a reduction in the number of women diagnosed with ovarian cancer (incidence) or a reduction in the proportion of ovarian cancer patients who die from their disease (case-fatality). After a decline in incidence or in case-fatality, there will be a corresponding decline in mortality following a lag period of several years.

The Surveillance, Epidemiology, and End Results (SEER) registry has reported incidence, case-fatality and mortality data for 26% of the United States population since 1975 [7]. The use of standardized (versus crude) rates removes the effect of any changes in the age distribution of the underlying population in order to facilitate comparisons over time. All age-adjusted incidence and mortality rates are standardized to the 2000 United States population (the standard population) and are expressed in terms of cases per 100,000 women per year. We complement the SEER data analysis by cross-referencing other data sources which compile information on reproductive risk factors and oophorec-tomies. Information on the use of oral contraceptives, parity, breast-feeding and tubal ligations was abstracted from questionnaires that were completed by 2000 North American women without ovarian cancer who attended a clinic appointment for BRCA genetic testing at our research laboratory and were found to be negative for mutations in BRCA1/2. Oophorectomy data were obtained from the National Health Discharge Survey database maintained by the

Centers for Disease Control and the National Center for Health Statistics.

2. Trends in mortality

From 1975 to 2011, in the United States, the age-adjusted mortality rate from ovarian cancer declined by 23%, from 9.8 per 100,000 per year to 7.5 per 100,000 per year. The rate declined by 8% from 1975 to 2001 and by 17% from 2002 to 2011 (Fig. 1).

The 23% decline in the age-adjusted mortality rate is an indication that progress has been made; however, it does not reflect the actual burden of the disease in the United States. The total number of ovarian cancer deaths in a given year is influenced by the age-specific mortality rates, as well as by the age-distribution and the size of the population at risk The unadjusted (i.e. crude) mortality rate is calculated by dividing the total number of ovarian cancer deaths in a given year by the total number of women in the population. From 1975 to 2011, the crude mortality rate fell by only 2% (from 93 per 100,000 per year to 9.1 per 100,000 per year) (Fig. S1). That is, the aging of the female population between 1975 and 2011 has offset the decline in age-specific mortality rates. From 1975 to 2011, the total number of ovarian cancer deaths in the United States increased by 38%, from 10,367 deaths to 14,323 deaths, despite the 23% reduction in the age-adjusted mortality rate.

The trends in age-adjusted mortality differed for women in different age groups (Fig. S2). From 1975 to 2011, for women from ages 50 to 64, the mortality rate declined continuously (by 44.7%). For women between ages 65 to 74, the mortality rate first increased (by 9.2% from 1975 to 1991) and then declined (by 22.8% from 1991 to 2011). For women ages 75 and older, the rate increased by 43% from 1975 to 2002 and then declined (by 123% from 2002 to 2011).

Trends in age-specific rates may be attributable to period and/or cohort effects. A period effect results from the introduction of a change that affects the risk of an entire

population simultaneously, irrespective of age. A cohort effect compares the lifetime experiences of individuals grouped by year of birth. For example, women who were 50 years of age in 1975, 65 years of age in 1990 and 75 years of age in 2000 all belong to the same birth cohort — the first women exposed to the oral contraceptive pill, which was introduced in 1960 [10].

3. Trends in incidence

Incidence rates describe the number of women who are diagnosed with ovarian cancer in a given year, relative to the size of the population. Incidence rates are calculated by dividing the number of cases by the population at risk. Only people with ovaries are at risk for developing ovarian cancer (i.e. males are not included in the denominator of ovarian cancer rate calculations). Women who have had their ovaries removed are also, by definition, not at risk for ovarian cancer, but these women are not excluded from the population at risk in SEER incidence and mortality rates. Changes in the proportion of women in the population with intact ovaries may therefore influence trends in ovarian cancer incidence and mortality. Incidence rates differ from mortality rates because not all women who are diagnosed with ovarian cancer will die from it. If a particular factor affects the incidence of ovarian cancer, the impact on the number of ovarian cancer deaths will not be seen until several years later. The lag period between a change in incidence and a change in mortality reflects the survival times of the patients (i.e. from diagnosis to death).

The observed trends in ovarian cancer incidence parallel the trends in ovarian cancer mortality. From 1975 to 2011, the age-adjusted ovarian cancer incidence rate fell by 26%, from 163 per 100,000 women per year to 12.1 per 100,000 women per year (Fig. 2). Ovarian cancer incidence declined by 3.4% from 1975 to 1991 and by a further 23% from 1991 to 2011. The decline, which began in 1991, was followed by a decline in mortality about 10 years later.

The trends in incidence varied for women from different age groups (Fig. S3). From 1975 to 2011, ovarian cancer incidence

in women ages 50 to 64 years fell by 13.5 per 100,000 per year (a relative decline of 36%). Incidence in women ages 65 to 74 rose by 8.6 per 100,000 per year from 1975 to 1985 (a relative increase of 17%), and then fell by 18.4 per 100,000 per year from 1985 to 2011 (a relative decline of 31%). Incidence in women ages 75 and older rose by 14.8 per 100,000 per year from 1975 to 1993 (a relative increase of 31%) and 133 per 100,000 per year from 1993 to 2011 (a relative decline of 21%). The decline in incidence in women ages 65 and older suggests that the reduction in ovarian cancer deaths is the result of a reduction in cases of ovarian cancer (surprisingly, in 1984 and 1985, the age-specific incidence rates were higher in women ages 65 to 74 than in women ages 75 and older. This is unexpected, given that incidence rates for ovarian cancer typically increase monotonically with age (Fig. 3). This transient reversal in 1984 and 1985 may be an artifact of sampling error or small sample size rather than a true increase in incidence. It might also reflect changing constellations in risk factor propensity for ovarian cancer).

Fig. 1
Ovarian cancer mortality rates, United States, 1975 to 2011 (age-adjusted).

* * *

In 2011, the incidence rate of ovarian cancer in the United States peaked among women ages 80 and older (Fig. 3), whereas the incidence count of ovarian cancer (i.e. the actual number of new ovarian cancer diagnoses) peaked among women ages 60 to 64, and then declined (Fig. S4). Women who were 60 to 64 years old in 2011 were born between 1946 and 1950, and represent the first born of the baby boom generation. After age 80, women tend to die of other causes and the at risk population becomes smaller.

4. Early detection

If the decline in ovarian cancer mortality were attributable to improvements in early detection (i.e. through screening or better

awareness) we would expect to see a stage-shift in disease at presentation. Ovarian cancer may be diagnosed because of symptoms (e.g. abdominal pain) or signs of disease (e.g. distended abdomen), or as a consequence of a positive screening test in an asymptomatic woman (i.e. abnormal pelvic examination, serum CA125 concentration or trans-vaginal ultrasound). The definitive diagnosis of ovarian cancer requires histological confirmation; the conventional date of diagnosis is the date of surgery.

Fig. 2
Ovarian cancer incidence and mortality rates (age-adjusted).

* * *

Fig. 3
Age-specific ovarian cancer incidence rates, by age, 2011.

* * *

In the SEER database, between 1975 and 2011, ovarian cancers were classified as either localized, regional or distant, based on the extent of cancer present at the time of surgery (i.e. stage at diagnosis). Localized disease (stage I) refers to ovarian cancer that is confined to the ovary, regional (stage II) refers to ovarian cancer that is confined to the pelvic tissues (uterus, fallopian tubes, ovaries or other intra-peritoneal tissues), and distant (stage III/IV) refers to ovarian cancer that has spread beyond the pelvic tissues (i.e. retroperitoneal lymph nodes, peritoneal cavity, liver, spleen or pleural effusion). The goals of staging are to aggregate patients into groups who have a similar prognosis and who require a similar approach to treatment, and to facilitate comparisons over time.

Statistical cure is defined as the point in time following diagnosis when the mortality rate from ovarian cancer is the same as the mortality rate of women in the general population. Ovarian cancer patients who survive for 12 years

may be considered cured [11]. In the following pages, the term “cure rate” refers to the proportion of patients who are alive 12 years after diagnosis. The cure rate for patients with localized ovarian cancer is 88%; however, most patients (65%) present with distant-stage ovarian cancer, and for them the cure rate is 18% (SEER database).

It is hoped that the proportion of women who are diagnosed with early-stage ovarian cancer (and who are ultimately cured) might be increased through screening (i.e. by identifying pre-symptomatic ovarian cancer), through increased awareness (i.e. by reducing the time from first symptoms to doctor visit) or through better diagnostic methods (i.e. by reducing the time from first doctor visit to pathologic confirmation of ovarian cancer). If ovarian cancer screening has contributed to the observed decline in mortality, we would expect to see an increase in the incidence of localized ovarian cancer and a decrease in the incidence of distant ovarian cancer (i.e. a stage-shift). From 1975 to 2011, the incidence of localized ovarian cancer fell by 1.5 per 100,000 per year (a relative decline of 35%), the incidence of regional ovarian cancer fell by 0.1 per 100,000 per year (a relative decline of 8%), and the incidence of distant ovarian cancer fell by 2.1 per 100,000 per year (a relative decline of 22%) (Fig. 4). The incidence of ovarian cancer has declined at all stages; therefore it is unlikely that screening has had a significant impact on ovarian cancer rates.

An increase in the incidence of early-stage ovarian cancer without a proportionate decline in late-stage ovarian cancers is an indicator of overdiagnosis, i.e. the detection of low-risk cancers that might never become clinically apparent in the absence of screening (and rarely lead to death). For ovarian cancer, the detection of borderline tumours through screening may be considered examples of overdiagnosis; in general, these cancers do not progress into high-grade or advanced-stage tumours [12]. The absence of a significant increase in the incidence of localized ovarian cancer through screening

precludes overdiagnosis. Further, there is no evidence that invasive ovarian cancers, however small, regress spontaneously.

Several randomized control trials have shown that screening asymptomatic women using trans-vaginal ultrasound and CA125 can detect a significant proportion of ovarian cancers in pre-clinical and early stages [11,12]; however, no screening protocol has yet been shown to reduce the number of advanced stage diagnoses or the number of ovarian cancer deaths [13]. Other approaches to ovarian cancer screening that are being evaluated include the use of serial CA125 measurements (e.g. the Risk of Ovarian Cancer Algorithm) [14] and the addition of other bio-markers (e.g. Human Epididymis Protein 4) in combination with CA125 [15]. The United States Preventive Services Task Force currently recommends against screening for ovarian cancer in asymptomatic women at average risk [16].

The symptoms of ovarian cancer are non-specific (e.g. bloating, pelvic pain or bowel irregularities) and patients and doctors may overlook their potential significance. Retrospective studies have reported delays of four to six months from symptom onset to a diagnosis of ovarian cancer [17-19]. Delays attributable to the patient and the doctor are roughly equal; about 70% of patients present with symptoms to their doctor within two months of first symptom onset, and about 65% of patients are diagnosed with ovarian cancer within two months after presenting with symptoms to their doctor. There has recently been an impetus to increase awareness of ovarian cancer symptoms in an attempt to reduce the time from first symptoms to diagnosis with the hope of improving ovarian cancer survival rates [20].

If formal efforts to increase awareness are successful, there should be an increase in the proportion of cancers diagnosed at an early stage. However, from 1975 to 2011, the proportion of patients with localized ovarian cancer declined from 29% to 25% (Fig. S5). This indicates that early diagnosis through

better awareness has not contributed to the observed decline in mortality.

Fig. 4
Ovarian cancer incidence rates, by stage at diagnosis, 1975 to 2011 (age-adjusted).

* * *

It has recently been proposed that early detection of ovarian cancer should strive towards the diagnosis of low-volume advanced stage ovarian cancer, rather than the identification of early-stage (stages I and II) ovarian cancer [21]. The best predictor of long-term survival from advanced stage ovarian cancer is primary surgical resection to no residual disease (i.e. no visible tumour remaining in the abdomen) [22], and the lower the volume of tumour at presentation, the greater the probability that surgery will result in no residual disease [23]. Better awareness of ovarian cancer symptoms might result in an improvement in survival rates among patients with advanced stage ovarian cancer, rather than a stage shift per se. The premise for earlier diagnosis of ovarian cancer in symptomatic women is currently being investigated by the Diagnosing Ovarian Cancer Early (DOvE) study in Canada. In the preliminary report, prompt screening of symptomatic women with CA125 and trans-vaginal ultrasound identified a greater proportion of early-stage ovarian cancers compared with patients diagnosed through usual assessment (36% versus 23%) and a greater proportion of low-volume advanced stage ovarian cancers (35% versus 21% in clinic patients) [21]. Importantly, 73% of patients diagnosed through prompt screening based on symptoms had no residual disease after debulking surgery (versus 44% of clinic patients). In comparison, between 30% and 40% of women with advanced stage ovarian cancer in the United States currently achieve a status of no residual disease through primary debulking surgery [24]. This is discussed in greater detail in part II.

5. Ovarian cancer histology

Approximately 90% of all ovarian cancers arise from ovarian or fallopian tube epithelial cells. Ovarian carcinomas are of four main histologic types: serous (68%), endometrioid (20%), clear cell (8%) and mu-cinous (6%). The 12-year survival rates (all stages) of patients with endometrioid (57%), clear cell (64%) or mucinous carcinoma (58%) are superior to that of patients with serous ovarian carcinoma (27%) (Table S1). A shift in the histological distribution of ovarian carcinomas over time may therefore impact on mortality rates.

It has recently been proposed that the category of serous carcinomas be subdivided into two subcategories, which are distinguishable from each other (primarily) by grade. The largest category, high-grade serous carcinomas, comprises 90% of the total. It is proposed that the majority of high-grade serous carcinomas arise from the epithelium of the fallopian tube [25].

SEER does not distinguish between high-grade and low-grade serous carcinomas. The distinction has important implications for treatment; the smaller group (low-grade serous carcinomas) does not respond to chemotherapy. The distinction is also potentially important for screening and prevention. In principal, the greatest impact of any prevention program will be realized by reducing the number of high-grade serous cancers (discussed in part III). Also, screening must go beyond detecting non-serous and low-grade serous carcinomas if it is to be used to reduce ovarian cancer mortality.

6. Ethnic group

The incidence of ovarian cancer is higher in white women than in women from other racial or ethnic groups (Table S2). Ovarian cancer survival rates at 12 years are superior in white women (38%) compared with African-American women (32%) but they are inferior compared with Hispanic women (43%) and Asian women (52%). If the relative frequencies of the various

racial and ethnic groups in the United States population change appreciably over time, this might impact on ovarian cancer incidence and mortality rates. From 1970 to 2011, the proportion of females that were white dropped from 87% to 80% [26]. At the same time, the proportion of Asian women increased from 1% to 5%. From 1992 to 2011, ovarian cancer incidence fell by 19% in white women, by 8% in African-American and by 8% in Asian women.

7. Bilateral oophorectomy

Bilateral oophorectomy refers to the surgical removal of the ovaries. Elective bilateral oophorectomy may be undertaken for the prevention of ovarian cancer or for the treatment of benign conditions such as pelvic pain, ovarian cysts or endometriosis [27]. Approximately 90% of all elective oophorectomies in the United States are performed as an adjunct operation in women who undergo hysterectomy for a benign condition [28]. At the time of hysterectomy, about 45% of pre-menopausal women and 75% of post-menopausal women undergo a concomitant bilateral (salpingo-) oophorectomy [29]. Women who have had their ovaries (and tubes) removed have a 95% reduction in their risk of developing ovarian cancer [30,31]. The probability that a woman will have both ovaries intact (i.e. have not undergone an elective bilateral oophorectomy) at a given age can be calculated based on the age-specific rates of bilateral oophorectomy for each year since birth.

Between 1965 and 2005, the rates of elective bilateral oophorectomy fluctuated between 1.5 and 3.0 per 1000 women per year [32,33]. Following the Women's Health Initiative report on the adverse health effects associated with the use of hormone replacement therapy in 2002 [34], rates of oophorectomy in premenopausal women began to decline [35]. In 2008, the American Congress of Obstetricians and Gynecologists released a statement recommending against prophylactic bilateral oo-phorectomy in women below age 45 [36].

From 1975 to 2005, there was a steady decline in the proportion of women in the population without ovaries. Women from recent birth cohorts (i.e. born after 1950) have had fewer oophorectomies than older women (Fig. S6). In 2005, an estimated 19% of women ages 70 and older have previously undergone an elective bilateral oophorectomy (Fig. S7). We estimate that, in the absence of these oophorectomies, there might have been 25,155 cases of ovarian cancer in 2005 versus 21,557 observed (i.e. about 14% of ovarian cancers were prevented in 2005 as a result of elective bilateral oophorectomies).

8. Risk factors for ovarian cancer

The principal risk factors for ovarian cancer are oral contraceptives, pregnancy, breast-feeding and tubal ligation [37]. These factors are of particular importance as they are protective, ubiquitous, and they have significant and long-lasting effects. Temporal changes in exposure to these four risk factors are expected to impact upon ovarian cancer incidence and mortality rates. Few risk factors that increase the risk of ovarian cancer have been confirmed; these include hormone replacement therapy [38], talcum powder [39], high body mass index [40] and endometriosis [41] and are not considered here. The role of genetic predisposition in ovarian cancer is discussed in part III [9].

Fig. 5
Proportion of women in 2014 who have ever taken an oral contraceptive, by age.

* * *

We plotted the age-specific incidence rates for ovarian cancer by birth cohort (Fig. S8). The cumulative risk of ovarian cancer to age 70 was 1.1% for women born in 1920 and was 0.98% for women born in 1940 (a relative decline of 10.9%). The cumulative risk to age 50 was 0.29% for women born in 1940 and was 0.25% for women born in 1960 (a relative decline of 13.8%). (Because age-specific incidence data are only available beginning in 1975,

cumulative risk estimates for earlier birth cohorts are partially based on incidence rates from later birth cohorts, and will underestimate any difference in risk between birth cohorts.)

Using data abstracted from questionnaires that were completed by 2000 women from North America, we estimated the probability that women born in various birth cohorts (from 1920 to 1969) were exposed to each risk factor at some time (Table S3), and based on the estimates for each risk factor we generated relative risks for developing ovarian cancer at or above age 60 compared with a theoretical reference group with no exposure (Fig. 5).

8.1. Oral contraceptives

Oral contraceptives were introduced in the United States in 1960 by G.D. Searle and Company [10]. Women of reproductive age in 1960 (ages 15 to 44) were born between 1920 and 1945. The proportion of women who have ever taken an oral contraceptive increased from 18% for women born in 1920 to 84% for women born in 1945, and has remained stable at 83% to 86% thereafter (Table S3).

On average, women who have ever used oral contraceptives have a 25% reduced risk of ovarian cancer compared with women that have never used oral contraceptives [42]. The level of protection increases with the duration of use and attenuates with time since last use. Thirty years after discontinuation of an oral contraceptive, the relative risk for ovarian cancer is approximately 0.8 for less than five years of use, 0.7 for five to ten years of use and 0.6 for more than 10 years of use. Because most women with ovarian cancer are diagnosed after age 60, the full impact of exposure to oral contraceptives on ovarian cancer incidence and mortality has only recently been observed.

In the United States population in 2014, about 85% of women below age 70 have previously taken an oral contraceptive, whereas only 18% of women age 90 to 95 have

previously taken an oral contraceptive (Fig. 5). This indicates that between 1990 and 2015, the proportion of 70-year old women who had ever taken an oral contraceptive increased from about 20% to 85%.

82. Parity

On a population basis, parity is the second most important risk factor for ovarian cancer. The relative risk for ovarian cancer is estimated to be approximately 0.81 per child born (for practical purposes, we limit the protective effect of parity at five births, which corresponds to a 65% reduction in risk, compared with nulliparous women) [37]. In the United States, the average number of children per woman (mean parity) peaked at 3.8 children between 1946 and 1964 (during the post-World War II baby boom), and declined thereafter [43]. The mean parity of women born between 1920 and 1935 fell from 3.9 to 3.0 children (Table S3). This declined further to 1.8 children for women born in 1945 and to 1.5 children for women born in 1965.

8.3. Breast-feeding

Women who breast-feed their infants have a lower risk of ovarian cancer, compared with mothers who do not breast-feed. The relative risk for ovarian cancer among parous women that have ever breast-fed is approximately 0.85 (independent of parity) [44]. The extent of protection increases with duration of breast-feeding (i.e. the total number of months). 51% of mothers born between 1920 and 1924 breast-fed at some point. This fraction dropped to 44% of mothers born between 1935 and 1939, because of increasing numbers of women entering the workforce and because of the introduction and promotion of infant formula around 1970 [45]. In 1975, the proportion of mothers who breast-fed began to increase, stabilizing at 70% to 75% of mothers born in 1960 or later. The resurgence of breast-feeding has been attributed to increased knowledge about the benefits of breast-feeding and successful efforts to increase breast-feeding awareness, initiation and duration [45].

Breast-feeding is unique among risk factors in that the prevalence of ever-exposure is currently increasing (Table S3). However, the extent of protection is dependent on the total duration of breast-feeding (number of months), which in turn, depends on the number of children born (parity). Although the proportion of mothers who breast-feed their infants have increased in the United States, the mean parity of women in the population has decreased; in consequence, the average number of months of breast-feeding in the population has declined.

8.4. Tubal ligation

Tubal ligation is associated with a 15% to 25% reduction in the risk of ovarian cancer [46]. The magnitude of risk reduction is greater for endometrioid and clear cell carcinomas (50%) than for mucinous (30%) and serous carcinomas (20%). The protective effect appears to persist for 20 or more years; however, long-term studies are required to confirm the duration of protection. From 1975 to 1990, there was a shift in contraceptive use among women ages 30 to 44 from the oral contraceptive pill to tubal ligation [47]. The prevalence of tubal ligation increased from 4% of women born in 1920 to about 35% of women born between 1940 and 1949, and has declined thereafter (Table S3).

8.5. Relative risk of ovarian cancer from exposure to the four risk factors

Compared with a theoretical cohort of women who have never taken an oral contraceptive, the estimated proportion of cases prevented by the use of oral contraceptives was 3% for women born between 1920 and 1924 and increased to 25% for women born between 1945 and later (Fig. 6). Compared with nulliparous women, parity conferred a 56% reduction in ovarian cancer risk for women born between 1920 and 1924, after which the extent of protection from parity began to decline, with a 32% reduced risk for women born between 1945 and 1949, and a 27% reduced risk for women born between 1965 and 1969. Women born between 1920 and 1945 experienced a 22% reduction in ovarian cancer risk due to

oral contraceptives, and a 24% increase in ovarian cancer risk due to declining parity.

The impacts of breast-feeding and tubal ligation on ovarian cancer incidence rates in the United States are modest in comparison with the effects of oral contraceptives and parity. Compared with women who have never breast-fed, the percent of ovarian cancers prevented by breast-feeding is estimated to be 7% for women born in 1920, decreasing to 6% for women born between 1945 and 1954, and then increasing to 9% for women born in 1960 or later (Fig. 6). Compared with women who have not had a tubal ligation, the greatest protection against ovarian cancer from tubal ligations was for women born between 1940 and 1949 (5% risk reduction).

8.6. Cumulative effects

The probability that a woman will develop ovarian cancer in her lifetime depends to a large extent on her cumulative exposure to all risk factors. In the absence of any exposure to the protective factors described above, the lifetime risk of ovarian cancer is estimated to be approximately 2.7% (as opposed to the observed population risk of 1.4%). Fig. S9 shows the overall propensity for women in different birth cohorts to develop ovarian cancer, as a result of exposure to all risk factors. Compared with a theoretical cohort of women with exposure to none of the four risk factors, the percentage of ovarian cancers prevented rises from 66% for women born between 1920 and 1924 to 71% for women born between 1940 and 1944 (a 5% reduction in ovarian cancer risk), and subsequently declines to 63% for women born between 1965 and 1969 (an 8% increase in ovarian cancer risk).

Examination of the trends in reproductive risk factors can be used to predict future ovarian cancer incidence rates. Women born between 1920 and 1945 were below age 65 between 1975 and 2010, corresponding to the continuous decline in ovarian cancer incidence in the 20 to 49 and 50 to 64 age groups since 1975 (Fig. S3). Women born between 1920 and

1945 were between the ages of 65 to 74 years beginning in 1985 (and ending in 2019), coinciding with the decline in incidence in women ages 65 to 74 that also began in 1985. Women born between 1920 and 1945 were 75 years of age and older beginning in 1995. In 2025, these women will be 75 to 100 years of age, at which point the decline in incidence due to risk factors is expected to reverse. (We assume that the relative risk for ever-exposure to a given risk factor is constant with time. We did not account for differences in the duration of exposure or recency of risk factor exposure between birth cohorts. We assume that the relative risks attributable to each factor are independent and cumulative.)

9. Synopsis

From 1975 to 2011, ovarian cancer mortality fell by 23%. The greatest period of decline (18%) was between 2001 and 2011, when mortality fell from 9.0 per 100,000 per year to 7.5 per 100,000 per year. The decline in ovarian cancer mortality is a consequence of a decline in ovarian cancer incidence. The decline in incidence is largely due to the introduction of oral contraceptives in 1960, and the subsequent expansion in their use (from 0% to 85%) from 1960 to 1990. The introduction of oral contraceptives has previously been implicated in declining incidence and mortality rates among women younger than age 60 [48,49], but the impact in older women and on overall mortality is only now being captured.

The SEER database is a very useful resource due to its large size and long period of record; however, there are some intrinsic limitations of using SEER data which should be acknowledged. SEER does not have a centralized review. There may be some misclassification of the ovarian cancer diagnoses in terms of both primary site and histology. The staging classification of ovarian cancer has changed over time. We do not have information on stage for all women and it is possible that some women were classified incorrectly. Our risk factor analysis is based on prevalence data from 2000 North American women

and this may not be representative of the entire United States female population.

Fig 6
Relative risk of ovarian cancer from exposure to a given risk factor, by year of birth, compared with a theoretical cohort of women with no exposure to the risk factor. OCP - oral contraceptive pill.

* * *

10. Future trends

In 2025, it is estimated that 85% of women younger than age 80 will have taken an oral contraceptive at some time, and the mean parity will fall below two. The total duration of breast-feeding in the population and the proportion of women with a tubal ligation are also declining. As a result, after 2025 age-standardized ovarian cancer incidence rates will increase. Due to the aging of the baby boom generation (i.e. women born between 1946 and 1965), the mean age of the United States population is increasing. The population is also expanding in size. As a result, we estimate that from 2010 to 2030 the annual number of ovarian cancer cases diagnosed in the USA will increase by 37%, from 20,921 cases to 28,591 cases. The number of cases will increase by 18% (3698 cases) due to a shift in the age-distribution and by 19% (3972 cases) due to population growth. Based on changing risk factor propensity and changing population demographics, we expect to see an increase in the number of ovarian cancer cases over the next 15 to 30 years.

In part II, we examine SEER rates of ovarian cancer case-fatality, and we explore to what extent advances in ovarian cancer treatment contribute to the decline in ovarian cancer mortality [8]. In part III, we discuss future prospects for reducing ovarian cancer mortality, which incorporate genetic testing, preventive surgery, screening and treatment [9].

Conflict of interest

The authors have nothing to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/jygyno.2015.06.017.

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APPENDIX Q

ENDOMETRIAL CANCER AND ORAL CONTRACEPTIVES: AN INDIVIDUAL PARTICIPANT META-ANALYSIS OF 27276 WOMEN WITH ENDOMETRIAL CANCER FROM 36 EPIDEMIOLOGICAL STUDIES

Collaborative Group on Epidemiological Studies on Endometrial Cancer*

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Summary

Background Oral contraceptives are known to reduce the incidence rate of endometrial cancer, but it is uncertain how long this effect lasts after use ceases, or whether it is modified by other factors.

Methods Individual participant datasets were sought from principal investigators and provided centrally for 27 276 women with endometrial cancer (cases) and 115 743 without endometrial cancer (controls) from 36 epidemiological studies. The relative risks (RRs) of endometrial cancer associated with oral contraceptive use were estimated using logistic regression, stratified by study, age, parity, body-mass index, smoking, and use of menopausal hormone therapy.

Findings The median age of cases was 63 years (IQR 57-68) and the median year of cancer diagnosis was 2001 (IQR 1994-2005). 9459 (35%) of 27 276 cases and 45 625 (39%) of 115 743 controls had ever used oral contraceptives, for median durations of 3.0 years (IQR 1-7) and 4.4 years (IQR 2-9), respectively. The longer that women had used oral contraceptives, the greater the reduction in risk of endometrial cancer; every 5 years of use was associated with a risk ratio of 0.76 (95% CI 0.73-0.78; p<0.0001). This reduction in risk persisted for more than 30 years after oral contraceptive use had ceased, with no apparent decrease between the RRs for use during the 1960s, 1970s, and 1980s, despite higher oestrogen doses in pills used in the early years. However, the reduction in risk associated with ever having used oral contraceptives differed by tumour type, being stronger for carcinomas (RR 0.69, 95% CI 0.66-0.71) than sarcomas (0.83, 0.67-1.04; case-case comparison: p=0 .02). In high-income countries, 10 years use of oral contraceptives was estimated to reduce the absolute risk of endometrial cancer arising before age 75 years from 2.3 to 1.3 per 100 women.

Interpretation Use of oral contraceptives confers long-term protection against endometrial cancer. These results suggest that, in developed countries, about 400 000 cases of endometrial cancer before the age of 75 years have been prevented over the past 50 years (1965-2014) by oral contraceptives, including 200 000 in the past decade (2005-14).

Funding Medical Research Council, Cancer Research UK.

Introduction

Use of oral contraceptives is known to reduce the incidence of endometrial cancer.' Because endometrial cancer is uncommon in young women but its incidence increases sharply with age, the public health effects of this inverse association depend mainly on the extent to which the reduced risk of endometrial cancer persists long after use ceases. To

investigate the association between use of oral contraceptives and the subsequent risk of endometrial cancer, individual participant data from 36 epidemiological studies of endometrial cancer have been brought together and analysed centrally.

Methods

Identification of studies and collection of data

This collaboration was established in 2005. Since 2012, epidemiological studies were eligible for indusion if they collected individual data about use of hormonal contraceptives and reproductive history from at least 400 women with endometrial cancer in retrospective studies, and at least 200 women in prospective studies. Before 2012, retrospective studies with fewer than 400 cases of endometrial cancer had been eligible, so some studies with fewer cases are included in this analysis. Eligible studies were identified from review articles, computer-aided literature searches in PubMed and Medline (up to Jan 31, 2012), using combinations of the search terms “endometrial cancer risk”, “endo-metrium cancer risk”, “hormon*”, “oral contraceptive”, and “OC”, plus the additional terms “cohort”, “prospective”, “women”, and “cancer risk”, and from discussions with colleagues. Efforts were made to identify all studies that induded relevant information, irrespective of whether or not results about oral contraceptives had been published, and principal investigators from each eligible study were invited to participate.

Cases were defined as women with invasive cancer of any histological type of the body of the uterus who were without previous cancer (except non-melanoma skin cancer); controls were women without previous cancer who had an intact uterus. Prospective studies were incorporated by a nested case-control design, in which up to four controls were selected at random from cohort members, matched for exact year of birth, date of recruitment (within 6 months), duration of follow-up (at disease onset), and, when appropriate, other

matching criteria used by the principal investigators (eg, geographical region). Individual participant data on sociodemographic and reproductive factors, use of contraceptives, use of hormonal therapies for the menopause, reproductive history, height, weight, consumption of alcohol and tobacco, and family history of breast and endometrial cancer were sought from the principal investigators of every study. For prospective studies, reported information on the use of oral contraceptives was taken from the last record before disease onset, to calculate duration of use and time since last use (assuming no further use). Information about the use of menopausal hormonal therapy and hysterectomy was also that most recently recorded. Datasets provided by investigators were collated centrally and recoded using similar definitions, as far as possible. Apparent inconsistencies in the data were discussed with the study investigators and if they could not be rectified, decisions were made about which values to incorporate into the pooled dataset. After the records had been checked and corrected, investigators were sent summary analyses of the variables to be used for final confirmation that their data had been interpreted correctly.

44 eligible studies were identified[2-45] of which 36 are included in the current analysis.[2-37] Four groups of researchers declined to participate in this collaboration[38-41] and a further four groups agreed in principle to provide data at a future date.[42-45]

Principal investigators provided individual information about whether or not women had ever used hormonal contraceptives (as defined by each study) and most also provided information about the total duration of use and age or calendar year at first and last use. Only 13 studies collected information on the type of hormonal contraceptives;[7,17,19,21,25-30,33,35,36] women from the remaining 23 studies were assumed to be using combined oral contraceptives (ie, those containing both oestrogen and

progestin) because more than 95% of hormonal contraceptive users included in studies with such information reported using combined preparations. There were too few women with endometrial cancer who had used exclusively progestin-only oral contraceptives (56 cases), progestin-only injectable hormonal contraceptives (19 cases), combined injectable hormonal contraceptives (three cases) or sequential oral contraceptives (41 cases) for reliable analysis.

Statistical analysis

Statistical analyses were done with Stata version 13.0. Conditional logistic regression was used to calculate relative risks (RRs) of endometrial cancer in relation to the use of oral contraceptives and their corresponding 95% CIs. Where only two groups were compared, conventional CIs were used. When several groups were compared, with one taken as the reference group with an RR of 1, the variance of the log risk in the reference group and in each of the other groups was calculated from the variances and covariances of the log RRs in those other groups.[47] These group-specific variances yield the group-specific CIs for each group (including the reference group) that are plotted in the figures.

All analyses were stratified by study, centre (for multicentre studies), age group (16-19, 20-24 years, and so on up to 75-79, 80-84, and 85-89 years), parity (0, 1, 2, 3, 4, ≥5, or not known), body-mass index (BMI <25, 25-30, z30 kg/m^2, or not known), smoking (never, ever, or unknown) and type of menopausal hormone therapy used (never, oestrogen-only exclusively, combined exclusively, both oestrogen-only and combined, other types, or unknown use). The effect on the main findings of further stratification by ethnic origin, education, age at first birth, age at last birth, age at menarche, age at menopause, menopausal status, and family history of endometrial cancer was examined by comparing results before and after stratification for each variable separately. Women with missing information for any of these adjustment factors were assigned to a separate stratum for the relevant

variable to conserve total numbers analysed; sensitivity analyses excluded these women.

The RR of endometrial cancer per 5-year duration of oral contraceptive use was estimated by fitting a log-linear trend across categories of duration (never, <1, 1—<5, 5—<10, 10—<15, and ≥15 years), using the median value within each category.

The association of endometrial cancer risk and duration of oral contraceptive use was cross-classified by time since last use and by mid-calendar-year of use (grouped as 1960-69, 1970-79, and 1980-89) to assess the independent effect, if any, of these factors on risk. Although the composition of oral contraceptive pills has varied substantially over time, a strong association exists between calendar year of use and oestrogen dose in the oral contraceptives typically used.[48-50] In the USA and UK, for example, the oral contraceptives prescribed before 1970 were typically high-dose preparations, often containing 100 pg or more of oestrogen; between 1970 and 1980 prescriptions were typically for medium-dose preparations containing about 50 µg of oestrogen; and by 1980 most prescriptions were for low-dose preparations, containing 35 pg or less of oestrogen.[49,50] Thus, in these analyses, decade of use was taken as a correlate of oestrogen dose of oral contraceptives.

The classification system adopted in each study was used centrally to categorise tumours into three broad histological subtypes: type I (endometrioid carcinomas); type II (non-endometrioid carcinomas); and uterine sarcomas. Type I tumours, which were much the most common type, induded endometrioid tumours (International Classification of Diseases for Oncology [ICD]-0-3 morphology codes: 8380, 8381, 8382, and 8383), adeno-carcinoma tubular (8210 and 8211), papillary adenocarcinoma (8260, 8262, and 8263), adenocarcinoma with squamous metaplasia (8570), mutinous adeno-carcinoma (8480 and 8481), and adenocarcinoma not otherwise specified (8140). Type II tumours included serous

(8441), papillary serous (8460 and 8461), squamous cell (8050, 8070, 8071, and 8072), adenosquamous (8560), small-cell carcinoma (8041), mixed-cell adenocarcinoma (8323), and dear cell carcinoma (8310), as described elsewhere.[51]

***Table* 1**
Details of studies and women included

* * *

***Figure* 1**
Relative risk* of endometrial cancer by use of oral contraceptives in each of the contributing studies

* * *

Information about duration of use was available for 8873 cases and 43 783 controls across all studies combined. BCDDP=Breast Cancer Detection Demonstration Project. NHS=Nurses' Health Study. CNBSS=Canadian National Breast Screening Study. IWHS=lowa Women's Health Study. MEC=Multiethnic Cohort Study. NIH-AARP=NIH-AARP Diet and Health Study. EPIC=European Prospective Investigation into Cancer and Nutrition. PLCO=Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. MISS=Melanoma in Southern Sweden Cohort. CASH=Cancer and Steroid Hormone Study. ANECS=Australian National Endometrial Cancer Study. *Stratified by study (centre), age, parity, body-mass index, smoking, and type of menopausal hormone therapy used.

Uterine sarcomas were defined as sarcoma, not otherwise specified (8800-8806), fibrosarcoma (8810-8833), lipo-sarcoma (8850-8858), myosarcoma (8890-8896), rhabdo-myosarcoma (8900-8902, 8910-8912), endometrial stromal sarcoma (8930-8931), or cancer coded as sarcoma by study investigators. Significance tests for heterogeneity of the relative risks for oral contraceptive use by tumour subtype compared cases only (case-case comparisons), because controls provide no additional information. Analyses by histological subtype were based on smaller numbers than

those for all endometrial cancers. Hence, although they were still stratified by study (centre) and age, to retain sufficient statistical information within each stratum they were adjusted rather than stratified for parity, BMI, smoking, and type of menopausal hormone therapy used.

When results are presented in the form of plots, RRs are represented by squares and their corresponding CIs or group-specific CIs by horizontal lines. The position of the square indicates the point estimate of the RR, and the area of the square is inversely proportional to the variance of the logarithm of the RR (or, for multigroup analyses, log risk), thus providing an indication of the amount of statistical information available for that particular estimate. Where summary RRs have been calculated, these are shown as open diamonds. Because of the large number of RR estimates presented, 99% CIs are generally used in the figures; however, throughout the text 95% CIs are quoted.

Cumulative incidence rates of endometrial cancer (up to the age of 75 years) associated with different durations of use of oral contraceptives were estimated by application of RR estimates for endometrial cancer from the present analyses to age-specific incidence rates for women in 21 high-income countries in western Europe, North America, and Australasia (appendix p 8).” Absolute numbers of cancers prevented were estimated from birth cohort-specific prevalences of oral contraceptive use.[53]

Figure 2

Relative risk of endometrial cancer in users of oral contraceptives compared with never-users, by (A) duration of use, and (B) duration of use and time since last use of oral contraceptives.

* * *

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, writing of

the report, or the decision to submit for publication. The writing committee had full access to all the data, could request any analyses, and had final responsibility for the decision to submit for publication.

Results

Table 1 presents the details of the 36 participating studies. The studies are listed by their design and, within each type of design, by the median year when the endometrial cancers were diagnosed in each study. Most studies were done in Europe or North America, with three from Asia, one from Australia, one from South Africa, and one multinational study. Together, the analyses induded 27 276 women with endometrial cancer (cases) and 115 743 women without endometrial cancer (controls). The median year of cancer diagnosis was 2001 (IQR 1994-2005) and the median age at diagnosis was 63 years (IQR 57-68), with 847 (3%) of women diagnosed before 45 years of age, 3743 (14%) at 45-54 years, 11 287 (41%) at 55-64 years, and 11 399 (42%) at 65 years or older.

Overall, 9459 (35%) of 27 276 women with endometrial cancer and 45 625 (39%) of 115 743 controls had ever used oral contraceptives, with a median duration of use of 3.0 years (IQR 1-7) and 4.4 years (2-9), respectively. The prevalence of ever having used oral contraceptives was substantially lower in controls from Asia (899/11180; 8%) than in controls from Europe and North America (39 050/86 293; 45%).

Figure 1 shows the study-specific and combined relative risks of endometrial cancer in ever-users compared with never-users of oral contraceptives and, in the ever-users, the RR per 5 years of use. Results are presented according to study design. Studies with a low information content (defined as 1/var[ln RR] <20) are induded in the “other” category for each relevant study design. Overall, the risk of endometrial cancer was significantly lower in women who had ever used oral contraceptives than in women who had

never used them (RR 0.69, 95% CI 0 .67-0 .72), with no significant heterogeneity between the three types of study design (heterogeneity test; p=0.15).

The longer women had used oral contraceptives for, the lower their risk of endometrial cancer was, with each 5 years of use associated with an RR of 0.76 (95% CI 0.73-0.78, p<0.0001), based on 8873 cases and 43 783 controls who were ever-users (figure 1). In women who had used oral contraceptives for a duration of 10-15 years (median 11.8 years) the relative risk of endometrial cancer was 0.52 (95% CI 0 .48-0 .57; figure 2A). These analyses were stratified by study (centre), age, parity, BMI, smoking, and type of any menopausal hormone replacement therapy used. Similar results were obtained when the analyses were stratified by age and study alone (RR per 5 years use of oral contraceptives 0.75 [95% CI 0.73-0.77]), and further stratification for each of ethnic origin, education, age at first birth, age at last birth, age at menarche, age at menopause, menopausal status, or family history of endometrial cancer likewise changed the RR per 5 years of use by 0.01 or less (appendix p 4). The proportional reduction in risk of endometrial cancer per 5 years of oral contraceptive use varied slightly by age at diagnosis (heterogeneity test; p=0.004), with RR 0.71 (95% CI 0.67-0.75) for women diagnosed before 60 years of age and RR 0.79 (0.75-0.82) for women diagnosed at 60 years of age or older. The association did not vary by BMI, parity, use of menopausal hormone therapy, menopausal status, smoking status, age at menarche, ethnic origin, or alcohol use (figure 3). The exdusion of women with missing values for any of these stratification variables also made a negligible difference to the risk estimates (making the fully stratified RR per 5 years use of oral contraceptives 0.75, 95% CI 0.72-0.77).

***Figure* 3**

Relative risk of endometrial cancer per 5 years use of

oral contraceptives, by various lifestyle and reproductive characteristics.

* * *

Most women with endometrial cancer had stopped using oral contraceptives many years before their cancer diagnosis (median time since last use 29 years [IQR 22-34]). Women who had used oral contraceptives more recently had also, on average, used them for a longer duration (eg, women who had used oral contraceptives less than 15 years previously had a median duration of use of 4.7 years [IQR 1.3-9.9], whereas women who had last used oral contraceptives 30 years or more previously had a median duration of use of 3.0 years [1.0-5.3]). For a given duration of use, the reduction in risk was slightly greater in women with more recent use, although a significant protective effect remained more than 30 years after use had ceased (figure 2B and appendix p 5).

In 7452 women with endometrial cancer for whom information about the timing of their oral contraceptive use was available, 3235 (43%) had a mid-year of oral contraceptive use in the 1960s and 371 (5%) had a midyear of use in the 1980s (appendix p 6). The RRs per 5 years duration of use of oral contraceptives in the 1960s, 1970s, and 1980s did not vary significantly (heterogeneity test; p=0.15, appendix p 6). There was also no significant heterogeneity in the RR per 5 years of use by age at first use or age at last use (appendix p 7).

However, there was some evidence that the RR depended on the histological subtype of endometrial cancer (table 2). Compared with women who had never used oral contraceptives, ever-users had an RR of 0.69 (95% CI 0.66-0.71) for carcinomas, based on 26 877 cases, which was similar for type I and type II carcinomas. Based on relatively few cases, ever-use of oral contraceptives was not significantly associated with the risk of uterine sarcoma (RR 0.83 [95% CI 0.67-1.04], based on 399 cases; heterogeneity, from direct case-case comparison of sarcomas vs carcinomas

p=0.02). Analyses were also done in women with information about duration of oral contraceptive use. For carcinoma, the RR per 5 years use of oral contraceptives was 0.75 (95% CI 0.73-0.77, based on 8701 cases); for uterine sarcoma, the corresponding RR was 0.88 (95% CI 0.74-1.03, based on 172 cases; heterogeneity, from direct case-case comparison of sarcomas vs carcinoma p=0.24).

Based on the RRs presented in figure 2 and age-specific rates of endometrial cancer for women in high-income countries, cumulative incidence rates of endometrial cancer were estimated for never-users of oral contraceptives and for women who had used them for different durations, beginning at 20 years of age. For women who never used oral contraceptives, an estimated 2.3 in every 100 would be diagnosed with endometrial cancer before the age of 75 years. The corresponding cumulative incidence rate for women who had used oral contraceptives for 5, 10, and 15 years was estimated to be 1.7, 1.3, and 1.0 per 100 users, respectively (figure 4). The annual incidence of endometrial cancer is low in women still young enough to be using oral contraceptives, but it is much higher in those aged 60-70 years. In this age range, the number of women who were ever-users of oral contraceptives has grown steeply over the past 50 years, from essentially zero in the 1960s to about three-quarters in high-income countries today.[53] Hence, the annual number of endometrial cancers prevented by ever-use of oral contraceptives has also increased steeply over the past 50 years. Using birth cohort-specific prevalences of oral contraceptive use in western developed countries,[53] we estimate that over the past 50 years (1965-2014) in 21 countries in western Europe, North America, and Australasia, oral contraceptive use has prevented a total of about 400 000 endometrial cancers, including 200 000 in the past 10 years (2005-14), at ages 30-74 years (appendix p 8). Because these results are based on population incidence rates, they

automatically allow for the different rates of hysterectomy in those populations.

Figure 4

Absolute risk of endometrial cancer incidence per 100 women up to 75 years of age in high-income countries by duration of oral contraceptive use (population-weighted rates, 2003-07, for 21 countries in Western Europe, North America, and Australasia)

* * *

Discussion

This international collaboration has brought together and re-analysed almost all of the available epidemiological evidence on the reduction in endometrial cancer incidence associated with oral contraceptive use, and indudes data from 27 000 women with endometrial cancer from 36 studies. Overall, the longer women had used oral contraceptives, the greater the reduction in the risk of endometrial cancer. On average, every 5 years of oral contraceptive use was associated with a relative risk of 0.76, so about 10-15 years of use halves the risk. A protective effect persists for at least 30 years after use ceases, and does not seem to depend much on the dose of oestrogen in the contraceptive formulations or on personal characteristics such as parity, adiposity, or menopausal status.

Combining results from many studies has the obvious advantage of yielding a large sample size, which reduces random errors, and it also avoids the biases that could be produced by undue emphasis on particular studies with extreme results. Only a third of the eligible studies have published on oral contraceptives and endometrial cancer,[4,7,8,10,17,18,21,24,29-31,33,35] so a review based solely on these studies could be affected by publication bias. Despite extensive efforts to identify all studies with unpublished results, it is impossible to guarantee that others do not exist; furthermore, it is not possible to have completely up-to-date

information from the continuing prospective studies. However, the eight eligible studies that were identified but did not contribute data to this collaboration together contain only about 12% as many women with endometrial cancer as the included studies. Hence, failure to indude these studies probably had no material effect on the main findings. Only one of these eight studies has published results on oral contraceptives and endometrial cancer, and its reported findings are broadly similar to ours." The 36 induded studies were of varied design and were done in different settings, with wide variation in the duration of use and time since last use of oral contraceptives. However, the effects of a given duration of use did not vary significantly between women with different characteristics or between studies with different designs.

The main analyses were stratified simultaneously by study, centre within study, age at diagnosis, parity, BMI, smoking, and use of menopausal hormone therapy. This fine stratification was feasible because of the large sample size. It meant that the analyses of the association between oral contraceptive use and risk of endometrial cancer are based on comparisons between women in the same study who were of the same age and who had a similar history of other risk factors for endometrial cancer.

Although few studies provided information about hormonal constituents of the preparations used, the oral contraceptives of the 1960s would generally have contained much higher doses of oestrogen than those of the 1980s. Overall, however, there was no apparent decrease between use in the 1960s and 1980s in the relative risk associated with a given duration of use. These results show that the amount of oestrogen in the lower-dose pills is still sufficient to reduce the incidence of endometrial cancer, which is consistent with findings from two studies that have assessed individual dosages of the hormonal constituents.[41,54] The numbers of women who reported using anything other than combined

oral contraceptives (eg, sequential oral or progestin-only oral contraceptives and/or injectable hormonal contraceptives) were too small for reliable analysis.

The decline in endometrial cancer risk with increasing duration of use does not seem to vary substantially with parity, BMI, use of menopausal hormone therapy, menopausal status, smoking status, age at menarche, ethnic origin, or alcohol intake. The reduction in risk associated with 5 years use of oral contraceptives was slightly greater in women diagnosed before 60 years of age than in women diagnosed at an older age, but given the number of significance tests done, this could be due to chance. The reduction in endometrial cancer risk with increasing duration of use does not seem to vary much with factors related to the timing of use, such as age of first or last use, time since last use, or calendar period of use.

The effect of oral contraceptives does, however, seem to vary by histological subtype, with ever-use strongly associated with a reduced risk of type I and probably of type II endometrial carcinoma, but somewhat less strongly associated with a reduced risk of uterine sarcoma—a much rarer type of cancer. Another pooled analysis that included 15 studies, most of which contributed to the current analysis, also reported a similar reduction in risk of both type I and type II endometrial carcinoma for ever use of oral contraceptives[51] but no significant association with uterine sarcoma.[55]

Taken together, it is reasonable to infer that the associations recorded here are causal (ie, that current or past oral contraceptive use reduces the incidence of endometrial cancer in otherwise similar women). Almost all of the hormonal contraceptive use in these studies is likely to involve combined oral contraceptives, which contain oestrogen plus progestin. These contraceptives might protect against endometrial cancer by minimising exposure to unopposed oestrogen during the follicular phase of the

menstrual cyde, thereby inhibiting oestrogen-induced cell proliferation;[56,57] moreover, the addition of a progestin to menopausal hormone therapy has been shown to reduce the adverse effects of oestrogen on the risk of endometrial cancer in postmenopausal women.[53,58-60] However, the exact mechanisms by which oral contraceptives cause substantial protection against endometrial cancer many years after cessation of use are still unclear.

Since the introduction of oral contraception in the early 1960s, about 400 million women have used it in high-income countries alone,[61] often for prolonged periods during early adulthood.[53] Medium-to-long-term use of oral contraceptives (eg, for 5 years or longer) results in a substantial proportional reduction in the incidence of endometrial cancer, the magnitude of which is similar to that seen for ovarian cancer.[53] Because this reduction in risk persists more than 30 years after use has ceased, and the incidence of endometrial cancer increases steeply with age, the public health effect of oral contraceptive use on endometrial cancer is most apparent many years after use has stopped. The present results, taken together with what what is known about past patterns of use, suggest that in high-income countries oral contraceptives have, over the past 50 years (1965-2014), already prevented a total of about 400 000 endometrial cancers before the age of 75 years, including 200 000 in the past decade (2005-14).

Contributors

NA, VB, SWK, RS, SS, and TOY identified studies, received and checked data, did analyses, and had full access to all materials and results. NA, VB, GR, SS, and RP drafted the report, and all writing committee members helped to revise it before and after circulation to the collaborators for comment.

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Declaration of interests

We declare no competing interests.

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APPENDIX R

Meta-Analysis of Intrauterine Device Use and Risk of Endometrial Cancer

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PURPOSE: We sought to study the association between intrauterine device (IUD) use and endometrial cancer.

METHODS: A comprehensive search of literature published through April 2007 was conducted, studies reviewed, and data abstracted. Data from ten studies were pooled and analyzed using both fixed- and random-effects models to examine the association of ever use of an IUD and endometrial cancer.

RESULTS: Based on the random effects model, a protective crude association between IUD use and endometrial cancer was observed (odds ratio [OR] = 039; 95% confidence interval [CI] = 0.29-0.51; heterogeneity $p < 0.001$) with a pooled adjusted risk of OR = 0.54 (95% CI, 0.47-0.63; heterogeneity $p = 0.40$). A decreased risk of endometrial cancer also was seen for increased years of IUD use (OR for 5 years of use 0.88; 95% CI = 0.84-0.92; n = 5; heterogeneity $p = 0.14$), increased years since last IUD use (OR for 5 years of use 0.91; 95% CI, 0.86-0.95; n = 4; heterogeneity $p = 0.02$), and increased years since first IUD use (OR for 5 years of use 0.89; 95% CI, 0.83-0.95; n = 4; heterogeneity $p = 0.04$).

CONCLUSIONS: Our results suggest that nonhormonal IUD use may be associated with a decreased risk for endometrial cancer; however, the exact mechanism for this association is unclear. Future investigations should address

the difference in the proposed association by specific type of IUDs.

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KEY WORDS: Endometrial Cancer, Intrauterine Device, Meta-analysis, Review.

INTRODUCTION

Endometrial cancer is the most prevalent female genital malignancy in the United States with an estimated 39,080 incident cases and 7,400 associated deaths expected in the United States during 2007 (1). Endometrial cancer primarily affects postmenopausal age women with a mean age at diagnosis of 61 years (2). Factors associated with an increased risk of endometrial cancer are exposure to unopposed estrogen increasing age, elevated body mass index, nulliparity, infertility, polycystic ovary syndrome, amenorrhea, early age at first menarche, delayed onset of menopause, unopposed estrogen therapy, and tamoxifen therapy (2, 3). Previous studies have indicated a protective association between use of combination oral contraceptives and risk of endome-trial cancer. Progesterone acts to limit endometrial proliferation, thereby decreasing the overall risk of endometrial cancer (4).

Intrauterine devices (IUDs) are a common method of reversible contraception in many countries, with an estimated 106 million women worldwide who have used an IUD (5). However, the rate of IUD use in North America ranks among the lowest in the world, with an estimated 1.5% of married women in the United States using an IUD, compared with the highest rate, 33.0% in China, and a global rate of 11.9% (5). IUDs were first marketed for use in 1964 (6). The first generation of IUDs was inert devices, followed by a second generation of copper IUDs, first approved by the U.S. Food and Drug Administration (FDA) in 1984 (7), and most recently

a third generation of progesterone IUDs, first introduced in 1990 in Finland (7, 8), and later approved by the FDA in December 2000 (7). Currently, two types of IUDs are marketed in the United States, the copper T380A (ParaGard) and the levonorgestrel-releasing intrauterine system (LNG-IUS) (Mirena) (5).

The overall aims of this study were to quantify the magnitude of the association between IUD use and risk of endometrial cancer, including potential contributing factors: duration of use, time since first use, time since last use, and type of device. A meta-analysis was conducted to evaluate these associations with endometrial cancer.

Selected Abbreviatinos and Acronyms

IUD = intrauterine device

LNG-IUS = levonorgestrel-releasing intrauterine system

SES = socioeconomic status

METHODS

Literature Review

For this meta-analysis, analytic studies that measured IUD use in relation to endometrial cancer were considered. First, a literature search from 1966 through the end of April 2007 was performed using PubMed. MeSH headings, key words, and text words searched included intrauterine devices, IUD, endometrial cancer, and endometrial neoplasms. The search of PubMed returned 42 articles, of which 11 (7, 9-18) were reviewed in detail. The 31 remaining articles were not relevant because they were commentaries, editorials, reviews, case-reports, diagnostic or treatment techniques, or other biological discussions. The references in the 42 articles were examined for additional relevant studies; however, no additional relevant studies were identified. In an attempt to locate possible unpublished studies, we searched the ProQuest database of dissertations and theses. This found three dissertations (by Castellsague, Hill, and Wemili) that have been published

elsewhere and are included in our analyses. Non—English-language articles were also reviewed but determined not to be relevant. Among 11 articles reviewed in detail, several reported on the same populations. Two articles were published using the same data from a 1989-1992 study in Israel; therefore these articles were considered to represent one study (9, 10). Several articles published data from subjects in Shanghai, China (16-18), but their diagnosis dates (1997-2003, 1991-1998, 1988-1990) were only minimally overlapping, so they were treated as separate studies.

The relationship between IUD use and endometrial cancer was examined from multiple perspectives. Specifically, total years of IUD exposure, years since first IUD exposure, years since last IUD exposure, and type of IUD used were assessed. Only two studies reported age at first IUD use; thus we did not pool such data.

Data Abstraction

Data were abstracted from all articles by one reviewer (R. B.). For each factor, raw data, adjusted factors, reported odds ratios (ORs), and 95% confidence intervals (CIs) were recorded. Information on study design, location, study dates, ethnic majority, case/control source populations, matching factors, and age ranges were also collected. Whenever possible, the most adjusted OR, having controlled for the greatest number of potential confounders, was obtained. Since we could not run the original data, we had to assume each article adjusted for appropriate confounders in the data obtained.

Statistical Methods

ORs were reported as an estimate of the relative risks. For studies in which no OR was reported, a crude OR was calculated from the tabulated raw data. For each study, the natural log of the OR was calculated and the variance was based on the corresponding 95% confidence intervals (CIs). Dichotomous factors (e.g., ever versus never IUD use) were

analyzed by using fixed-effects and random-effects models to compute pooled ORs (19). Assuming that there is a true overall quantity being estimated, inferences about the included studies can be obtained using the fixed-effects models. The amount of error in a fixed-effects model is assumed to be attributable to sampling error (19). Random-effects models apply inferences about hypothetical groups of studies, assumed to follow a probability distribution, rather than individual studies (19). To examine the consistency between associations, statistical tests of homogeneity (20) were performed. The estimated between-study variance was utilized to quantify the magnitude of heterogeneity among the studies (20).

To examine multiple ordinal categories of duration (total years of IUD use), latency (years since first IUD use), and recency (years since last IUD use) for possible linear associations, the categories were analyzed by using fixed-effects dose-response method (20). This method provides the ability to adjust within study correlation while combining levels of exposure in a linear regression of the natural log of the OR. To determine whether the linear model was appropriate for the data, a goodness-of-fit test for linear and quadratic models was performed. The analyses of linear association were performed using SAS software (SAS Software, Inc., Cary, NC).

RESULTS

Eleven articles reporting on 10 studies were reviewed (6, 10-18). Study characteristics, including diagnosis years of cases, study location, age range, and number of subjects are described (Table 1). We reported the ORs for the associations between ever versus never IUD use and risk of endometrial cancer, along with the adjustment factors described in each study (Table 1). Only three studies reported on specific types of IUD used; thus the data for types of IUDs used were too sparse to pool (Table 1). Duration of use, reported in years of IUD use, was examined for a linear, protective dose-effect for endometrial cancer. A summary of the duration of use along with time since first and last use of an IUD for each study is

provided (Table 1). Protective pooled effects were seen. Recency and latency effects were observed across studies; a protective association for endometrial cancer was observed with an increased period of time since first and last use of an IUD (Table 2). These data must only be interpreted within the range they cover.

The pooled ORs for the association between use of an IUD and endometrial cancer for both the fixed- and random-effects models were calculated (see Table 2). All studies, except one (16), reported protective effects, and the pooled analyses showed a significant protective effect for ever-use of IUDs and endometrial cancer. Based on the random effects model, a protective crude association between IUD use and endometrial cancer was observed (OR = 039; 95% CI = 0.29-0.51; heterogeneity $p < 0.001$) with a pooled adjusted risk of OR = 0.54 (95% CI = 0.47-0.63; hetero-geneity $p = 0.40$).

Table 1
Stud characteristics and ever-use of intrauterine device exposure and endometrial cancer among 10 studies, type of device for three studies, and total years of use for six studies

* * *

Table 2
Pooled odds ratios for intrauterine device used and endometrial cancer among 10 studies by study design along with duration of use reported for 5 year increments

* * *

This meta-analysis found a significant inverse association between IUD use and endometrial cancer. The overall pooled OR of 0.54 suggests a significant reduction in risk of endometrial cancer with ever-use of an IUD (see Table 2). The studies appear to be homogeneous with respect to ever-use of IUDs and endometrial cancer; therefore, the fixed-effects model estimates may be more appropriate for the meta-analyses. The linear duration analyses for a 5-year increase in

years of IUD use, latency, and recency effects are also reported (Table 2). An inverse association between IUD use and endometrial cancer was observed for duration of use (OR = 0.88 for 5 years), recency (OR = 0.91 for 5 years), and latency (OR = 0.89 for 5 years). The linear duration measures for years of IUD use, latency, and recency effects are reported as an increase for 5 years. The ORs pooled among studies reporting duration of use and duration since last use were not homogeneous.

The reported linear duration response ORs are the magnitude of association between IUD use and endometrial cancer that can be assumed for each 5 year increase in exposure within the range of the original studies (see Table 2). Among the four studies that examined recency, one had decreasing ORs, two decreased, then increased and the fourth study appeared to have no association. Thus the pooled risk estimates that show a 9% decrease over 5 years need to be interpreted within probably 5-10 years after last use based on the categories among the studies pooled (Table 2). Among the studies that examined first use, two showed a protective effect that was relatively flat, whereas the other two suggested more of a continued decrease, but showed no effect after 17 or 20 years since first use.

DISCUSSION

Hormonal (progesterone) IUDs have been marketed since. 1990 (7, 8). We assume all of the women included in these studies had used nonhormonal IUDs, since eight of the 10 studies had diagnosis dates of cancer prior to 1993 where participant exposure to IUDs would likely have occurred prior to the 1990s. When the two studies with diagnosis years in the 1990's were excluded, point estimates changed by 0.01 or less, suggesting that these two studies did not differ from the earlier studies. Considering the relative chronology of endometrial hyperplasia and subsequent cancer, it is unlikely that a significant portion of IUD users in the studies included in this analysis would have been exposed to hormonal IUDs at the time prior study data were collected.

The precise mechanism for the proposed protective association between IUD use and endometrial cancer is not clear. Cellular level changes in the normal endometrium include simple hyperplasia, complex hyperplasia, progressing to endometrial cancer. Two mechanisms through which IUD use may alter endometrial cancer risk have been enumerated: first, through influence on the production of estrogen and progesterone by inducing extrauterine effects on the ovary and the central hypothalamic-pituitary-ovarian axis; and second, through alteration of the endometrial response to hormones by exerting direct changes in the endometrial environment, resulting in chronic inflammation (11). Both mechanisms result in an overall reduction in endome-trial hyperplasia (11, 21, 22). An understanding of the magnitude and consistency of the association between IUD use and endometrial cancer may guide future recommendations in contraceptive health.

Mechanisms of different types of IUDs vary. Older, non-hormonal IUDs, including inert, copper, and stainless steel, 498 produce inflammation only. Studies have observed a significant reduction in both endometrial mitotic activity and estrogen receptor concentration, associated with copper IUD placement (23). Similarly, LNG-IUS acts by influencing the production of the hormone progesterone, which down-regulates estrogen receptors and results in a reduction of cellular-proliferation of the endometrial lining. Clinically, women with a LNG-releasing IUD in place tend to have a thinner endometrial lining than women without an IUD (24). This observation supports the theory that the patho-physiological response to an IUD is due to cellular level changes that decrease the rate of hyperplasia, thereby limiting dysplasia and subsequent progression to endometrial cancer. Therefore, if the use of nonhormonal IUDs had a protective effect on cancer development, then an association of the IUD that contains progesterone will likely have a similar or additive protective effect. To date, there are insufficient published data to address

the difference in association between types of IUDs and risk of endometrial cancer.

Identified risk factors for endometrial cancer have the potential to bias risk estimates if not adjusted for in the analyses. The four important risk factors for endometrial cancer (age, obesity, nulliparity, and type 2 diabetes mellitus) were not consistently adjusted for across the 10 studies included in this meta-analysis. Each of the 10 studies, except for one (14), adjusted for age. None of the 10 studies adjusted for all four risk factors and only two studies adjusted for at least three of the risk factors (6, 15). Protective factors for endometrial cancer include any previous use of combined oral contraceptives, tobacco use, and increased parity. Only one of the 10 studies adjusted for all three protective factors (6). Surprisingly, only two studies adjusted for combined oral contraceptive use (6, 14), which confers a lifelong protective effect (25). Failure to adjust for combined oral contraceptive use could potentially bias the protective association between IUD use and endometrial cancer toward a greater magnitude.

Socioeconomic status (SES) often influences healthcare behaviors and may have influenced the study populations included in this meta-analysis, based on differences in access to contraceptive methods (26). Conversely, other literature has suggested that IUD use rates do not differ by SES (27). In this review we were not able to discern how SES may have influenced the study population and therefore cannot assess potential bias.

It may not be possible at this point in time to discern the true magnitude of the proposed association between IUD use and endometrial cancer in reproductive-aged women because of the low incidence of endometrial cancer in premenopausal women and the limited IUD exposure in postmenopausal women included in this meta-analysis. The age range of women included in this meta-analysis was 20-74 years. The percentage of cases in each age stratum was not well enumerated in the 10 studies; however, it is likely that the

majority of endometrial cancer cases were skewed toward older age, consisting primarily of postmenopausal women. The majority of women who are diagnosed with endometrial cancer are not likely to be current IUD users. Correspondingly, the number of endometrial cancer cases in women of reproductive age is limited. In the study with the youngest age bracket of women, 75% of the endo-metrial cancer cases were diagnosed in women between the ages of 45 and 54 years, which would likely correspond to perimenopausal status (11). Considering the disparity in age between IUD use and diagnosis of endometrial cancer, there is a potential for exposure recall bias.

An overall magnitude of association can be estimated, through increased statistical power, without the collection of new data. The collective review of individual studies can lead to the identification of gaps in previous research or knowledge, thus potentially leading to the generation of new hypotheses.

Endometrial cancer can be confirmed through an endo-metrial biopsy. Of the 10 studies that were reviewed in this meta-analysis, all except three (12, 17, 18) clearly stated that each of their cases had a histologically confirmed diagnosis of endometrial cancer. The other three studies were unclear. Since most registries and hospitals require confirmation of cancer diagnoses, we assume that these three studies had confirmed cases but did not report this detail in their publications. However, we examined this further by conducting sensitivity analyses that excluded these three studies. This showed similar point estimates (OR = 0.53 for ever-use of IUDs) with wider confidence intervals that remained significant. Similar results were seen for measures of duration.

There were several inherent limitations to this meta-analysis. It is difficult to assess the overall level of bias in a meta-analysis. When analyses were stratified by study design, no differences were seen. Therefore we can assume

that the studies were comparable. In interpreting the associations, it must be considered that the individual studies adjusted for a variety of potential confounders, potentially influencing the level of bias in individual studies. In this meta-analysis, not every study controlled for each potential confounder. Thus the data for the individual studies may be biased in either a protective or an increased association depending on what factors were adjusted for. Since not all of the studies reported all duration measures of IUD use, the statistical power may be limited for several subanalyses. Additionally, it is unclear whether studies included in this meta-analysis that did not report on years of use or type of device originally collected such information. If the information was in fact collected, but not reported, then this would constitute a form of publication bias.

There have been a limited number of published studies addressing the association between IUD use and endome-trial cancer. It is more common for studies finding a positive association to be published than those concluding null associations (28). With only three or four studies reporting duration of use, latency, and recency, publication bias among measure of duration may exist, resulting in a bias of the overall magnitude and direction of the proposed association.

In conclusion, this meta-analysis found a protective association among women who reported ever-use of an IUD and risk of endometrial cancer. Future investigations should address the difference between exposure for the three types of IUDs-inert, copper, and hormonal. However, this study population may not be feasible because of exposure to multiple types of IUDs or exposure to nonhormonal IUDs and combined oral contraceptives. As time increases since IUDs were first marketed, it will be more feasible to study cohorts of women to assess the association between latency, recency, and duration of IUD use and risk of endometrial cancer, primarily in relation to the hormonal component. A large cohort study

would provide the ability to consistently control for potential confounders.

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APPENDIX S

CME REVIEW ARTICLE

VOLUME 57, NUMBER 2

OBSTETRICAL AND GYNECOLOGICAL SURVEY

CHIEF EDITOR'S NOTE: THIS ARTICLE IS THE SIXTH OF 36 THAT WILL BE PUBLISHED IN 2002 FOR WHICH A TOTAL OF UP TO 36 CATEGORY 1 CME CREDITS CAN BE EARNED. INSTRUCTIONS FOR HOW CREDITS CAN BE EARNED APPEAR ON THE LAST PAGE OF THE TABLE OF CONTENTS

NONCONTRACEPTIVE HEALTH BENEFITS OF INTRAUTERINE DEVICES: A SYSTEMATIC REVIEW

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Most women and their clinicians are unaware that IUDs confer important noncontraceptive health benefits. This

review summarizes the evidence from published articles on this topic. We conducted a series of systematic literature searches to identify articles on the noncontraceptive health benefits of IUD use. We reviewed the potentially pertinent ones for content, grouped them according to type of IUD, and evaluated them using the U.S. Preventive Services Task Force rating system. Over 500 titles were identified and several hundred abstracts were reviewed. Use of nonhormonal IUDs (plastic and copper) was associated with a decrease in endometrial cancer. The levonorgestrel intrauterine system can treat a variety of gynecological disorders, including men-orrhagia and anemia. The levonorgestrel system has also been used successfully as part of hormone replacement therapy, as adjuvant therapy with tamoxifen, and as an alternative to hysterectomy for women with bleeding problems. Like oral contraceptives, intrauterine contracep¬tives confer important noncontraceptive health benefits.

Target Audience: Obstetricians & Gynecologists, Family Physicians

Learning Objectives: After completion of this article, the reader will be able to describe the currently marketed IUDs in the U.S., to summarize the current literature about the noncontraceptive benefits of IUD use, and to list the noncontraceptive benefits of IUD use.

Intrauterine devices (IUDs) are known worldwide as contraceptives, but they also provide a variety of

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Dr. Hubacher has disclosed that he has no significant financial or other relationship with any commercial entity. Dr. Grimes has disclosed that he is a consultant for Alza,

Gynetics, GynoPharma, Mead-Johnson, Organon, Ortho-McNeil, Schering, Schmid, and Searle; is on the speakers bureau of Berlex Laboratories, Gyno-Pharma, Ortho-McNeil, Parke-Davis, Pharmacia-Upjohn, and Wyeth-Ayerst; and has received research grant support from Ber-lex Laboratories, Ortho-McNeil, and Wyeth-Ayerst.

The noncontraceptive uses of intrauterine devices discussed in this article have not been approved by the U.S. Food and Drug Administration. noncontraceptive health benefits. Although many ar¬ticles have chronicled the noncontraceptive benefits of oral contraceptives (1–4), the health benefits of intrauterine contraception are less well known and appreciated. Because IUDs are the most commonly used reversible contraceptive in the world today (5), the public health impact of disease protection and general health benefits that IUDs may provide may be substantial.

Two IUDs are currently marketed in the U.S. The copper T380A device (ParaGard, Ortho-McNeil Pharmaceutical Corp, Raritan, NJ) was approved by the FDA in 1984 and became available in the U.S. in 1988. Copper was first placed on plastic T-shaped devices in the 1960s, after researchers discovered the element's contraceptive properties. A dose-response relationship was established, culminating in the prod¬uct that now contains 380 mm2 of copper surface. The device is approved for 10 years of contraceptive protection, although data support high efficacy as long as 12 years (6). The T380A is the most used IUD worldwide.

In December 2000, the FDA approved the levonorgestrel-intrauterine system (LNG-IUS); it is marketed under the name of Mirena (Berlex Labora¬tories, Wayne, NJ). This plastic device is also T-shaped and the vertical stem is a reservoir containing 52 mg of levonorgestrel. It releases 20 pg of levonorgestrel per day over a period of 5 years, although data support effectiveness for as long as 7 years (7).

In recent years, several review articles on the ther-apeutic uses of hormone-releasing IUDs have been written (8–10),

but none has summarized all the possible therapeutic uses in a systematic fashion. In the U.S., the LNG-IUS was approved as a contracep-tive only; we discuss research of other indications that represent unlabeled use. Older types of IUDs (nonmedicated and copper-bearing) seem to be asso-ciated with protection from numerous gynecological maladies, however, this information has not been assembled in one article. This article summarizes the noncontraceptive benefits of IUD use and expands on past reviews (11) by evaluating the strength of the evidence.

METHODS

We conducted several online searches to collect pertinent English-language articles for this review. In Popline (June 2000 database), we located 103 articles with the following strategy: IUD [Beneficial Effects] or IUD [Therapeutic Use] or IUD, UNMEDICATED [Beneficial Effects] or IUD, UNMEDICATED [Therapeutic Use]. We also conducted separate PubMed (National Library of Medicine) searches: “intrauterine devices AND fibroids” (50 articles); “intrauterine devices AND (cancer OR neoplasm)” (379 articles); “intrauterine devices AND endometri-osis” (51 articles). To make sure key research on the levonorgestrel-IUS was not missed, we searched PubMed on that phrase and located 335 articles. Finally, we searched the Cochrane Library for arti¬cles on hormone replacement therapy and heavy menstrual blood loss, and added any original re¬search articles cited in the Cochrane topics to our review. For all the articles found in the initial searches, we removed duplicates and selected a subset for additional examination if they were original reports.

We divided the papers into two groups, depending on which type of IUD was investigated in the article. Nonmedicated and copper IUDs formed one group and hormone-releasing IUDs formed another group. The articles we found on nonmedicated and copper IUDs were epidemiologic studies focusing on cervi-cal cancer

(published reports on cervical intraepithe¬lial neoplasia were excluded from this review), en-dometrial cancer, and endometriosis. In these studies, retrospective data were collected on previous IUD use and most articles reported odds ratios for the association between previous IUD use and the end point of interest. In some instances, as noted in the tables, the published papers did not report odds ratios in the form we required for this review; we used data from the published reports to compute the crude odds ratios and 95% confidence intervals. We distin¬guished between crude and adjusted confidence in¬tervals in our tables. Many of the papers provided subanalyses examining type of IUD, duration of use, and timing of use; we reported many of these find¬ings from subanalyses in our tables. The literature on hormonal IUDs was generally derived from prospec¬tive trials focusing on gynecological problems; all the articles involved the levonorgestrel system only. Treatment effects were compared with an alternative therapy, baseline measurements, or in some cases both. Where available, we reported whether such comparisons were statistically significant using a P value of ~.05. After collecting and reviewing the reports on both types of IUDs, we used the U.S. Preventive Services Task Force rating system (12) to grade the quality of evidence and the strength of recommendation that can be based on that evidence.

RESULTS

Copper-bearing and nonmedicated (plastic only) IUDs have several noncontraceptive benefits, includ¬ing probable protection against endometrial cancer (Table 1). Seven studies reported the relationship between previous copper or nonmedicated IUD use and endometrial cancer (13–19). In all but one study, previous IUD use was associated with a decreased risk of endometrial cancer. The studies by Salazar-Martinez et al. (13), Hill et al. (15), and Castellsague et al. (18) all reported statistically significant associ¬ations between IUD use and a decrease in the risk of endometrial

cancer. Of note, the landmark Cancer and Steroid Hormone Study of the Centers for Dis-ease Control and Prevention was one of the studies to report significant protection against endometrial can¬cer (18). Three articles (14, 16, 17) suggested a protective effect of IUDs, but the measures of effect were not statistically significant. The final article (19) was based on research in China, where the steel ring IUD was used; the findings suggest that this type of IUD does not protect against endometrial cancer. The majority of articles on endometrial cancer also reported subgroup analyzes focusing on factors such as type of IUD and duration/timing of use. In general, no consistent pattern emerged from the articles to suggest that length or timing of use, or type of IUD was associated with an increase or decrease in the risk of endometrial cancer.

Cervical cancer was addressed in three articles (20–22) (Table 1). Although all three suggested a possible protective effect from previous IUD use, none was statistically significant. Each article re-ported subanalyses involving IUD use variables, and only the work by Li and colleagues (20) showed a statistically significant decrease in risk of cervical cancer in one subgroup: women who began intrauter¬ine contraception before age 33 years. Because the research by Li and colleagues (20) was done in China when the steel ring IUD was dominant, their findings apply to only this type of device.

We found seven articles (23–29) addressing the relationship between past use of an IUD and endo-metriosis (Table 2). Three of these articles (23, 24, 27) suggested an increased risk, but none was statis-tically significant. Of the two articles suggesting a possible protective effect (25, 26), only the results of Mahmood and Templeton (26) were statistically sig-nificant. As noted in Table 2, in all but one article, the odds ratios provided in this review were calcu-lated from data presented in the published article.

The last two articles (28, 29) did not provide data from which overall odds ratios could be calculated, although Kirshon and Poindexter (28) suggested IUD use is positively associated with endometriosis.

The LNG-IUS has two distinct categories of benefits that will be described separately; the first concerns the ancillary health benefit or disease pro-tection that this device confers, relative to the copper IUD (Table 3). Two large, randomized-controlled trials, subsequently referred to as the European trial and the multicontinent trial, compared the LNG-IUS with the Nova-T (copper) IUD and the copper T380 device, respectively. In the European trial, pelvic inflammatory disease (PID) rates were significantly lower among LNG-IUS users at 5-year (30) and 3-year follow-up (31). In the multicontinent trial (7), PID rates did not differ significantly between LNG-IUS and copper-T users at 2, 5, and 7 years after insertion. In a retrospective cohort study, Merki-Feld and colleagues (32) compared the incidenceof acti-nomyces-like organisms (ALO) in users of the LNG-IUS and users of copper IUDs; they found that ALO-positive PAP smears of the cervix were signif-icantly more common in users of copper IUDs com-pared with LNG-IUS users (20% vs. 3%).

In all four articles (7, 30, 33, 34) addressing hemoglobin changes, the LNG-IUS was shown to increase the concentration over measurements taken before insertion of the device (Table 3). The net gain in hemoglobin concentrations varied depending on the length of follow-up, ranging from as little as 0.5 gm/dl after 2 years (34) to as much as 1.6 gm/dl after 5 years (30). Both the European and multicontinent trials showed decreases in hemoglobin concentra¬tions among users of copper IUDs.

TABLE 1

Estimates from case-control studies on cancer and previous use of nonmedicated and/or copper IUDs

* * *

TABLE 2

Studies on endometriosis and previous IUD use

* * *

TABLE 3

Selected health benefits/disease protection from using the levonorgestrel (LNG) intrauterine system

* * *

The second category of papers on the LNG-IUS addresses the numerous therapeutic uses of this de-vice (Table 4). Idiopathic menorrhagia responds favorably to the levonorgestrel system; all nine stud¬ies (35–43) using a variety of designs and measures, showed positive results. The seven articles that mea¬sured menstrual blood loss estimated reductions of 74% to 97%. Four (37, 41–43) of the six studies used the alkaline hematin method (44, 45) for mea¬suring the amount of menstrual blood, and three studies (35, 39, 40) used menstrual diaries (46) to estimate the amount of blood loss. Lahteenmaki and colleagues (38) used menstrual diaries to record the number of days of bleeding, not amount of bleeding; after 12 months, women using the levonorgestrel device reduced their number of days of bleeding by about 50%.

Many hysterectomies are performed because of heavy menstrual blood loss that has become intoler-able; two studies reported the LNG-IUS as a possible alternative to surgery. Both studies were random¬ized trials, assigning either continued conservative (medical) treatments or the LNG-IUS for women who were contemplating hysterectomy. The proportion of women canceling their planned hysterectomy in the LNG-IUS arms of the two trials was 80% (47) and 64% (38); this compared with 9% and 14%, respectively, of women assigned to the medical treatments.

TABLE 4
Therapeutic uses of the levonorgestrel intrauterine system (LNG-IUS)

* * *

Two articles addressed uterine fibroids; the be-fore-after study by Starczewski and Iwanicki (48) involved 12 participants and concluded that the LNG-IUS reduced bleeding from uterine fibroids but did not reduce the size of the fibroids, based on ultrasound measurements. The other publication, a case report (49), noted an increase in hemoglobin from 5 gm/dl to 11 gm/dl and a decrease in fibroid volume. The LNG-IUS has also been tested in a population of 25 women with adenomyosis-associ-ated menorrhagia (50); the therapy reduced bleeding and reduced uterine volume, as measured by ultra¬sound. Only one study (51) was located that exam¬ined anemia; the researchers found that the LNG-IUS reduces the prevalence compared with nonusers or users of other IUDs.

Because oral progestins used in hormone replace¬ment therapy can cause frequent and irregular bleeding in some women (52), several groups of researchers sought to determine whether the LNG-IUS could avoid the effects of systemic progestin and mitigate bleeding. In all published articles on this topic (53–60), the LNG-IUS was found to reduce bleeding, as measured by the number of menstrual days, spotting days, or induced amenorrhea. In the subset of research that involved randomized trials comparing the LNG-IUS with other means of deliv¬ering progestins (54–56, 58), the LNG-IUS was su¬perior (in reducing bleeding) to the comparison methods in all but one study (55). Finally, as an adjuvant to tamoxifen therapy in women with breast cancer, the LNG-IUS caused a decidual response in the endometrium of all treated women (61); this in turn protected women from the uterine effects of tamoxifen.

CONCLUSIONS

The collected evidence supports several conclu-sions about noncontraceptive benefits of contempo-rary IUDs (Table 5). Case-control studies (level II-2 evidence) provide fair evidence that use of nonmedicated or copper IUDs protect against endometrial cancer (class B recommendation). Because random¬ized, controlled trials cannot be done, level II-2 stud¬ies will be the most rigorous evidence available. Case-control studies of cervical cancer and endometriosis are inadequate to reach a conclusion (class C recommendation).

TABLE 5

U.S. Preventive Services Task Force ratings (12) as applied to research on the noncontraceptive benefits of IUD

* * *

Concerning the LNG-IUS, randomized, controlled trials have produced conflicting conclusions regard-ing pelvic inflammatory disease; the European trial which compared the LNG-IUS with the Nova T copper IUD found a significant reduction in risk, whereas the multicontinent trial (using the copper T380 as a comparison) found no differences in risk. Compelling level I evidence indicates important im-provements in hemoglobin concentration (class A recommendation), and level II-3 evidence supports a role in preventing anemia (class B recommendation). Strong evidence from randomized controlled trials shows the LNG-IUS to be an effective treatment for menorrhagia (class A recommendation). Small case-series reports (level III evidence) provide some evi¬dence for a beneficial effect in treating heavy bleed¬ing related to fibroids, although the evidence is too limited to make a recommendation.

The studies on the LNG-IUS as an alternative to hysterectomy were well conducted (level I evidence) and showed conclusively that when offered this method, women will cancel their procedure in pro-portions far exceeding that of women assigned to continue their current therapy. Level I evidence also strongly supports the usefulness of the

levonorgestrel system as an adjunct to hormone replacement therapy (class A recommendation). One randomized, con¬trolled trial has also found benefit in preventing endometrial hyperplasia in women receiving tamoxifen (class B recommendation).

DISCUSSION

Like combined oral contraceptives (62) and inject-able depotmedroxyprogesterone acetate (63), non-medicated and copper IUDs seem to help prevent endometrial cancer. However, because of the diffi-culties in assessing causal relationships in case-con-trol studies, this protective effect must be viewed cautiously. The mechanism involved is unknown. The IUD might protect the endometrium against can¬cer by interfering with localized response to hor-mones and or by altering the production of hormones that are often associated with cancer development. Alternatively, the sterile inflammatory reaction may be hostile to atypical histology that might otherwise lead to cancer.

On a global scale, the public health impact of IUD use may be large. For example, in the U.S., endome-trial cancer is the most common gynecological ma-lignancy. Because over 100 million women world-wide currently use IUDs, even modest protection against endometrial cancer may avert thousands of deaths due to this cause. Although women are un-likely to choose an IUD expressly to prevent endo-metrial cancer, this information should probably be discussed as part of routine counseling. Given the powerful suppressive effect on the endometrium, the levonorgestrel system should also protect against en-dometrial cancer, although studies to date have been limited to women receiving tamoxifen (61).

Despite the seemingly strong evidence that the LNG-IUS is an alternative to hysterectomy (provided by level I evidence), we decided on a class B rec¬ommendation because of concerns that perhaps the women using the LNG-IUS were merely giving the method an honest chance to improve their condition; women who were not randomized to the LNG-IUS

had nothing to compel them to cancel their hyster¬ectomy procedure. Longer follow-up periods are needed to document the incidence of hysterectomy among the women assigned to the LNG-IUS.

In contrast to the nonhormonal IUDs, the LNG-IUS will be used specifically for many noncontra-ceptive purposes. Current off-label uses in the U.S. include treatment of menorrhagia, treatment of dys-menorrhea, and use as hormone replacement therapy. The list of potential therapeutic applications will likely grow as its use expands around the world.

We broached the topic of IUDs and pelvic inflam-matory disease because some research has shown that the LNG-IUS confers protection compared with other IUDs. This possible protective effect has bio-logic plausibility in two major ways. First, because levonorgestrel thickens the cervical mucus, bacteria may have a more difficult time ascending into the upper genital tract. Second, because of reduced menstrual blood loss with a LNG-IUS, there is less opportunity for retrograde menstruation to occur. More research is needed to determine whether the LNG-IUS, indeed, provides clinically significant protection.

Our review has both strengths and weaknesses. The methods we used were comprehensive and standard¬ized; they included an explicit search strategy, a thorough search for relevant articles (64, 65), and a quantitative assessment of the strength of evidence using widely accepted criteria (12). However, several limitations may have biased our assessment. For ex¬ample, some relevant articles may have escaped our attention because the authors did not report outcomes of interest in their abstract. Although our review may have missed some articles, we do not believe this would introduce systematic bias. Publication bias (66) is another concern that must be raised; our conclusions may be biased if favorable findings on these topics were more likely to be published than unfavorable results. This might exaggerate the

poten¬tial benefits. Publication bias is probably more of an issue in research involving the levonorgestrel device.

The IUD today poses a global paradox. Although the most common reversible contraceptive method in the world, it has the worst reputation of all contra¬ceptives. . .except among those using IUDs (67). Mass media clearly influences women's decisions about contraceptive choices (68); over the past 20 years, the media have focused on adverse effects of IUDs. In recent years, many gynecologists have pointed out that today's IUDs deserve a fresh look (69–72). Clinicians and, importantly, the media now have an ethical obligation to inform women that IUDs are not only safe and effective contraception, but they also have important health benefits. Without this information, women cannot make truly informed choices about contraception.

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APPENDIX T

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IUD USE AND THE RISK OF ENDOMETRIAL CANCER

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Abstract

Objective: Although the intrauterine device (IUD) is one of the most widely used forms of contraception throughout the world, its potential long-term effects on the uterus have not been thoroughly evaluated. This paper reports the long-term results of IUD use on the incidence of endometrial cancer. *Study design*: The data is part of a nationwide case-control, pilot study that was undertaken in order to evaluate the possible influence of ovulation induction drugs on the risk of endometrial cancer. The study included 128 living women 35–64 years old, with a histologically confirmed

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diagnosis of endometrial carcinoma. The controls were 255 women from the same dialing areas selected by random digit dialing. A multivariate logistic model, controlling for age, was used to assess the independent effects of factors found to be significantly associated with endometrial cancer on univariate analysis. *Results*: The following parameters were found to be independently associated with endometrial cancer controlling for age: nulliparity OR = 2.7 (95% CI 1.1–6.5, $P = 0.03$); history of infertility OR = 1.8 (95% CI 1.0–3.3, $P = 0.05$); BMI > 27 OR = 2.3 (95% CI 1.4–3.8, $P = 0.001$).

The use of oral contraceptives and IUD were found to be protective; OR = 0.29 and 0.37, respectively, (95% CI 0.14–0.61, $P = 0.001$, 0.19–0.70, and 0.003, respectively). *Conclusions*: IUD use may have a protective effect on endometrial cancer risk. The protective effect of IUD may be either, through the intense inflammatory response that leads to other lisosomal and inflammatory actions, which may include cells responsible for early elimination of hyperplastic endometrial epithelial cells or, the more complete shedding of the endometrium associated with IUD use may decrease hyperplasia of the endometrium, a known risk factor for endometrial carcinoma.

Keywords: Contraception; Endometrial cancer; Epidemiology; Intrauterine device (IUD)

1. Introduction

Although IUD use is one of the oldest and the most widely used form of contraception throughout the world, its potential long-term effects on the uterus have been poorly evaluated.

Since early this century, sporadic attempts have been made to design an intrauterine device (IUD) that would prevent pregnancy without serious adverse effects. In the 1960’s, Lippes (1962) and Margulis (1964) described the flexible plastic devices, which are the basis for the present IUDs in use. The IUD is believed to induce an intense local inflammatory response, which leads to recruitment of phagocytic cells and mast cells, and to provoke

lysosomal activation, and proteolytic enzymes release from this cells into the uterine cavity [1–3]. Furthermore, scanning electron microscope studies of the endometrium in IUD-wearing women, show alterations in the surface morphology of cells, especially of the microvilli of ciliated cells [4] and reduction of ciliated cells with impairment of the secretory activity in the epithelium next to the device [5]. Other reports indicated alterations in the composition of proteins within the uterine cavity [6], and alterations in endometrial response to estrogen and progesterone [7,8].

The epidemiological data on the relationship between IUD use and endometrial cancer is scanty, and only few have examined the possibility of such a link. To expand the existing data we report a secondary analysis of a pilot case-control study from Israel.

2. Material and methods

The general design of this study is fully described in our previous report [9]. In brief, cases of endometrial cancer were identified from the Israel Cancer Registry. Cases were eligible for this study if they had a histologically confirmed diagnosis of endometrial carcinoma that was first diagnosed and reported between 1 January 1989 and 31 December 1992; if they were born between 1 January 1929 and 31 December 1957; and if they were alive at the time of interview. Only living cases were used such that ascertainment of exposure was based on personal interviews exclusively.

Controls were obtained by telephoning randomly selected numbers within the same area codes as those of the cases, a method closely resembling that reported by Hartge et al. [10], and were interviewed during the same period as the cases. Thus, cases and controls were matched for geographic area by the sampling procedure. Eligibility for the control group was based on date of birth in the identical range to that of the cases. Once a household was reached, the interviewer asked if a woman born between 1 January 1929 and 31 December 1957 resided there. Women who had undergone hysterectomy were excluded as controls.

3. Statistical analysis

The data were analyzed using SPSS for Windows (SPSS Inc., Chicago, IL). The association between case and control status and demographic and clinical parameters was assessed using w^2 for categorical variables and *t*-test for continuous variables. Variables found to have a statistically significant association with endometrial cancer on univariate analysis were entered into a stepwise logistic regression model, which controlled for age. The criterion for entry for the model was $P = 0.05$ and for removal from the model was $P = 0.10$. Ninety-five percent confidence interval for adjusted odds ratios for the logistic model were calculated using computer programs for epidemiological analysis PEPI version 2.06.[1]

The study protocol was submitted and approved by the institutional review board of Hadassah Medical Organization and the Ministry of Health. For legal reasons, women located via the Cancer Registry could not be contacted directly. Rather, their physicians were contacted and consent to interview the patient was obtained through them. Verbal consent was obtained from both cases and controls.

4. Results

Before and during the study period, 21.6% of women with endometrial cancer reported to the Cancer Registry between the above dates had died. Of the 325 living women who could be included, we interviewed 128 (39.1%). The others were not interviewed because of inability to locate the patient or physician (69.3%), illness (5.0%), refusal of the physician (4.0%) or refusal by the patient (21.6%). Cases alive at the time of interview that were not interviewed, were compared with cases who participated and cases who were interviewed. There were no significant differences in age, area of residence, or histology. The distribution of cases and controls according to demographic and clinical characteristics is presented in Table 1. Cases tended to have a history of hypertension (24.8% versus 13.7%), and to be more obese (BMI greater or equal to 27). The study group had a mean BMI of 29.01 whereas the mean BMI was 25.93 in controls ($P = 0.0001$). Family

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history of endometrial cancer, a history of diabetes, and smoking were not different between the two groups. Obstetric and gynecologic characteristics, which were significantly different between controls and cases, were: a history of infertility (25.8% versus 16.5, $P = 0.05$), nulliparity (14.8% versus 5.1% $P = 0.005$), with no significant difference found for months of breast-feeding.

In our study, 19/128 (14.8%) of the cases and 121/256 (47.5%) of the controls were ever users of IUD. We found a significant negative association between IUD use and endometrial carcinoma. A similar negative association was demonstrated for oral contraceptive use, $P = 0.00001$ for both, Table 2.

Variables found to have statistically significant association with endometrial cancer on univariate analysis were entered into a stepwise logistic regression model, which controlled for age. According to the model, factors found to be significantly more prevalent in cases were; Obesity (BMI $\geq$ 27) with an adjusted OR = 2.47; infertility OR = 1.82; and nulliparity OR = 2.58.

Use of IUD and oral contraceptives were found to be protective (adjusted OR = 0.37 and 0.29, respectively).

5. Discussion

The results of our study suggest that IUD use significantly reduces the risk of endometrial cancer. After controlling for confounding factors such as: age, nulliparity, family history of endometrial cancer and other factors, ever users of IUD had an OR of 0.37 (95% CI 0.19–0.70, $P = 0.003$) to develop endometrial cancer as compared to non-users of IUD.

Our results are in agreement with those of the few papers published on this subject.

Castellsague et al. [11], reported the data from a large, multicenter, population-based, case-control study of epithelial endometrial cancer. The study included 437 cases and 3200 randomly selected controls. The adjusted OR for the association of ever users of IUD and endometrial cancer was 0.51 (95% CI 0.3–0.8).

Table 1
Selected sociodemographic and clinical characteristics of cases and controls
* * *

Parazzini et al. [12], reported similar results from a case-control study conducted in Italy between 1983 and 1992. Their study included 453 cases with histologically confirmed endometrial cancer and 1451 controls. When compared to never users, ever users of IUD had a relative risk of 0.4 (95% CI 0.1–1.0).

Sturgeon et al. [13], examined the relation between use of IUD and endometrial cancer risk using data from a multicenter case-control study comprising 405 endometrial cancer cases and 297 controls. IUD use was associated with a decreased risk of endometrial cancer (OR = 0.56 for ever use; 95% CI 0.3–1.0).

Table 2
IUD and oral contraceptives use of cases and controls
* * *

Hill et al. [14], reported similar results from a population-based case-control study. The study included women aged 45–74 from three counties in Washington State. They have found a risk of 0.61 (95% CI 0.41–0.89) of endometrial cancer in ever users of IUD as compared to a control group. The reduction in cancer risk was not found to be dependent on duration of IUD use. The relative risk among a small number of current users was 0.49 (95% CI 0.12–2.80).

However, data collected from seven countries [15], for a multinational case-control study, with 226 cases of endometrial cancer compared with 1529 matched controls, found no significant association between use of an IUD and risk of endometrial cancer (OR = 0.74, 95% CI 0.4–1.33). There were no trends in risk with respect to duration of use, time since first use, or ages at first or last use.

Theoretically IUD use may decrease endometrial cancer risk through at least two mechanisms: first the protective effect of IUD may be through the intense inflammatory response that leads to

other lisosomal and inflammatory actions which may include recruitment of cells responsible for early elimination of abnormal, precancerous, hyperplasia endometrial epithelial cells. Another theoretical mode of action may be that the more complete shedding of the endometrium, and the changes in endometrial environment and endometrial response to hormones associated with IUD use may decrease hyperplasia of the endometrium, a known risk factor for endometrial carcinoma.

Our study had a number of limitations. One of the main limitations was that the study was not primarily designed to examine such an association. Furthermore, mainly for technical reasons, we were not able to interview the majority of cases who were still alive. This may have introduced considerable bias since non-interviewed cases, as well as those who had died prior to interview may have differed substantially from interviewed cases. We had no access to the medical records of subjects, thus we could not verify the information about IUD use that was obtained from the study participants, and under-reporting cannot be excluded. The potential for non-response bias is present due to the low response, which raises doubts for whether the study group is representative. However, a comparison between cases and those who did not participate in the study shows that the age, area of residency and histology in the two groups were not different.

Cases were at least 3 years and up to 7 years older than controls at time of interview, which might explain difference in contraceptive history, as well as recall of other exposures.

In conclusion, the scanty available epidemiological data, including ours, is reassuring and points toward a protective effect of IUD use on endometrial cancer risk. However, the existing data is based on case-control studies, which were not designed to address such an association. Thus, the results should be interpreted carefully. Larger especially designed studies are warranted, as the use of IUD is increasing.

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APPENDIX U

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ENDOMETRIAL CANCER IN RELATION TO INTRA-UTERINE DEVICE USE

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Data from a population-based case-control study were used to evaluate the risk of endometrial cancer among women who have used an intra-uterine device (IUD). Incident cases were identified between 1985 and 1991 among women aged 45-74 years who were residents of one of 3 counties in Washington State. Controls were selected by random digit dialing, and both groups of subjects received an in-person detailed interview. In this study population, women who had ever used an IUD were

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estimated to have a risk of endome-trial cancer that was 0.61 times that of other women (95% CI 0.41-0.89). The reduction in cancer risk was not found to be dependent on duration of IUD use. There was a suggestion that women who had used intra-uterine contraception relatively late in reproductive life experienced a greater reduction in risk than those whose use was more distant or at a younger age. The relative risk among the small number of women who were currently using an IUD was 0.49 (95% CI 0.12-2.80). These results apply to the use of inert and copper IUDs as there was no use of progestin-releasing IUDs among women in the study population. The data from this and several other studies of the question support the hypothesis that use of an IUD has a favorable effect on the subsequent risk of endometrial cancer. The reason(s) for such a reduced risk is unclear. *Int. J. Cancer,* 70:278-281, 1997.

To date, 4 studies have examined the possibility that use of an intra-uterine device (IUD) alters a woman's subsequent risk of endometrial cancer. Two have observed that women who had ever used an IUD had half or less the risk of endometrial cancer as never-users (Castellsagué *et al.,* 1993; Parazzini *et al.,* 1994), while in a third, risk among users was 0.7 that of non-users (Rosenblatt *et al.,* 1994). However, the 4th study found that IUD users and non-users had a similar incidence (Shu *et al.,* 1991).

The presence of an IUD is known to alter the intra-uterine environment. The IUD evokes a number of immunological and biochemical changes, including localized acute and chronic inflammation (Moyer and Mishell, 1971) and increases in cytokine expression (Ammala *et al.,* 1995). IUDs also have been associated with elevations in uterine prostaglandins (Toppozada, 1985) and fibrinolytic activity (Liedholm *et al.,* 1983). IUDs induce morphological changes such as ulceration and erosion of surface epithelium and

exposure of the underlying basement membrane (Shaw and Macaulay, 1979). It is not clear whether these or other responses to the presence of an IUD ought to alter the risk of endometrial cancer development after the device has been removed.

Although use has declined in the United States in recent years, an IUD is used by over 93 million women internationally (Shah, 1994), so the question of a relation to endometrial cancer is an important one. Furthermore, understanding the relationship between IUD use and development of endometrial cancer, if one exists, may suggest mechanisms by which endometrial cancer may be prevented.

Previous studies of endometrial cancer have included relatively few women who had used IUDs and, therefore, have not been able to examine specific patterns of use. As part of several case-control studies investigating endometrial cancer risk in western Washington, we were able to evaluate the association further.

SUBJECTS AND METHODS

The Cancer Surveillance System, a population-based cancer registry, identified women newly diagnosed with epithelial endometrial cancer in western Washington during the study period. Cases diagnosed during 1985 and 1986 were included in the study if they were residents of King County and 45–64 years of age. Women residing in King, Pierce and Snohomish counties were eligible if they were diagnosed during 1987–1990 and were 45–74 years of age or diagnosed during 1991 and 45–69 years of age.

Of the 1,254 identified cases, 100 were deemed ineligible: 72 women had a non-epithelial or *in situ* tumor, and the other 28 were excluded because they either were unable to communicate in English, were not residing in a household in the 3 county region or did not have a telephone when they were diagnosed. Of the remaining 1,154 eligible cases, 100

died before interview, 222 were not interviewed because of physician or subject refusal and one interview was lost. A total of 832 cases (72%) are included in this analysis.

Controls were identified using random digit dialing (Waksberg, 1978) and were broadly matched to cases by county of residence and by 5 year age group. Random digit dialing calls to identify controls were initiated to 52,045 numbers, of which 26,405 were found to be non-residential. Residential status could not be determined for an additional 2,113. Among the 23,527 identified households, in all but 877 it could be ascertained whether an eligible woman was a household member. Of the 2,619 women found to be eligible for the study, interviews were conducted with 1,975 (75.4%). Included in this analysis are the 1,114 controls who did not have a prior hysterectomy or prior endometrial cancer. Each control was assigned a reference date, analogous to the date of diagnosis of the cases, and all interviews collected data on the experiences of cases and controls prior to the reference date. Control reference dates were approximately matched to those of cases on year of diagnosis, and within that year reference months were assigned randomly.

All study subjects were interviewed in person by trained interviewers, except that 3% of cases and 5% of controls were interviewed over the telephone. Reproductive and medical histories were collected as well as routine demographic data. A detailed contraceptive history was obtained using calendars to aid recall and photographs of common IUDs, as well as contraceptive and non-contraceptive hormones. Subjects interviewed by telephone received photographs by mail prior to interview.

Information on IUD use available for analysis included type of device used, duration of use, age at first and last use and years since first and last use. Variables evaluated for potential confounding included demographic variables, such as age, ethnicity, county of residence, income and education, as well as factors known or suspected to be related to

endometrial cancer, such as oral contraceptive use; use of estrogen alone or combined with a progestin; smoking; number of births; incomplete pregnancies; age at menarche; weight, height and body mass index; history of diabetes, hypertension or infertility and treatment for any of these conditions; and family history of endometrial or breast cancer. We also evaluated potential confounding by factors known or suspected to be related to IUD use: amenorrhea, endometriosis, fibroids, ectopic pregnancy, age at last full-term pregnancy, history of pelvic inflammatory disease or of other sexually transmitted diseases, number of sexual partners and use of other methods of birth control. Unconditional logistic regression was used to compute odds ratios (ORs) and associated 95% confidence intervals (CIs) for the relationship between IUD use and endometrial cancer and to evaluate possible confounding or modification of this relationship by other factors.

All analyses were adjusted only for variables that were found to alter the OR estimate: age (45–54, 55–64, 65–74), number of births (0, 1 *vs.* 2+) and use of unopposed estrogen for 3 or more years (yes, no).

RESULTS

Cases were somewhat older than controls and were more likely to be nulliparous or to have had only one birth, to have a higher body mass index and to have used unopposed estrogen for 3 or more years (Table I). A higher proportion of controls than cases reported having taken oral contraceptives. Cases and controls were broadly similar according to ethnicity, income and education.

A history of use of an IUD was reported by 5.2% of cases (n = 43) and 10.6% of controls (n = 118). Compared with nonusers, women who had ever used an IUD had a reduced risk of endometrial cancer (OR 0.61, 95% CI 0.41–0.89) (Table II). Few women (2 cases, 7 controls) reported that they currently used an IUD (*i.e.*, within 1 year of reference date), and their cancer risk was 0.49 (95% CI 0.12–2.80) that

of never-users. Although the relative risk did not vary substantially when evaluated among separate groups of women with and without other risk factors for endometrial cancer, estimates were imprecise because of small stratum sizes.

The length and timing of exposure to IUDs and age at first and last use were examined to ascertain any differential association with endometrial cancer risk. The duration of IUD exposure, once a woman became a user, was not associated with risk (Table II). There was a suggestion that use relatively late in reproductive life might be related to a reduced incidence of endometrial cancer.

Table I
Characteristics of Endometrial Cancer Cases and Control Women

* * *

We examined whether the type of intra-uterine contraception was related to development of endometrial cancer. The most frequently reported IUD was the Lippes loop (57.8%) (Table III). The reduction in risk seen among ever-users was not limited to a particular IUD. However, the total number of women who used some types was very small, and no women reported use of a progestin-releasing device. Among women who had used a Lippes loop, risk did not vary by duration of use.

DISCUSSION

Compared with women who had never used an IUD, the risk of endometrial cancer was more than one-third lower among those who had ever done so. The reduction in cancer risk was not dependent on duration of exposure and was only slightly influenced by use that was more recent or ended at a later age. Most women had ceased IUD use over 10 years prior to the reference date. Thus, the reduced risk was unlikely to be due to screening for precursor lesions (such as endometrial hyperplasia) that might take place among

women being considered for an IUD, the presence of which might prevent women from becoming IUD users (Weiss and Rossing, 1996).

Table II
Use of An Intrauterine Device (IUD) Among Endometrial Cancer Cases and Control Women

* * *

Table III
Intrauterine Device (IUD) Use Among Endometrial Cancer Cases and Control Women According to Type of Device

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Our observations that neither duration of use, time since first or last use or age at first or last use was appreciably related to risk among IUD users are consistent with those of previous studies (Castellsagué *et al.*, 1993; Rosenblatt *et al.*, 1994). The lower risk associated with IUD use was most apparent among women who were current users in one investigation (OR 0.10, 95% CI 0.01–0.78) (Rosenblatt *et al.*, 1994), but there were too few current users in our study to determine whether they had a notably decreased risk.

A number of potential biases should be considered in the interpretation of the results. The study included about 72% of eligible cases and 75% of eligible controls, and any differences in IUD use between participants and non-participants could result in biased estimates of risk. Differential recall between cases and controls is unlikely to have influenced our data. It is unlikely that cases or controls were aware of a potential association between IUD use and endometrial cancer at the time of the interview. We believe that women are likely to remember IUD use when interviewed as a physician visit is required for insertion and removal. Of potential concern is the fact that 16% of IUD users (n 5 26) did not recall the type of device. However, the

reduction in risk associated with use was not confined to a particular device.

Women who have practiced intra-uterine contraception may differ from women who have chosen other methods by their medical history or other factors that are related to endometrial cancer risk. Currently, IUDs are contra-indicated for women who have a history of pelvic inflammatory disease (PID) or ectopic pregnancy or who have recent unresolved conditions such as bleeding, infection or an abnormal Pap smear (Tatum and Connell, 1989). However, most women in our study used IUDs prior to the wide application of these restrictions. Inclusion in the analysis of variables indicating a history of PID or ectopic pregnancy did not alter the OR estimate. Data were not available on the remaining conditions. Compared to other women, those who have experienced conditions such as amenorrhea, uterine fibroids or infertility might have been less often given an IUD or might have less often tolerated it. Adjustment in the analysis for a history of fibroids further reduced the OR estimate slightly, while inclusion of amenorrhea or a history of infertility did not alter the risk estimate. Some residual confounding could be present due to lack of data on conditions, such as oligomenorrhea or anovulatory bleeding, that are possibly related to IUD use and to imprecise measurement of others, such as infertility, which required a physician visit to be included in the analysis. However, these conditions are at most only weakly related to endometrial cancer and could not completely account for our results. In summary, the characteristics of women who use IUDs do not appear to explain the lowered risk of endometrial cancer associated with IUD use in our data.

The presence of an IUD induces numerous physiologic changes that could alter risk of endometrial cancer. The IUD evokes a “foreign body” immune response in the endometrium, characterized by localized inflammation and increased concentrations of neutrophils, macrophages and

plasma cells (Moyer and Mishell, 1971) and increased expression of the cytokines interleukin 1 and tumor necrosis factor a (Ammala *et al.,* 1995). Although the acute inflammation generally subsides, tissue concentrations of lymphocytes and macrophages remain elevated 2 years or more after beginning use (Moyer and Mishell, 1971). The sustained contact of an IUD with the endometrium is associated with minor tissue trauma, including ulceration, erosion and necrosis of the superficial layer of the epithelium and exposure of the underlying basement membrane or stroma (Shaw and Macaulay, 1979). Women using IUDs have been reported to experience heavier menses than they did prior to IUD insertion (Guillebaud *et al.,* 1976). Among IUD users, increased fibrinolytic activity in endometrial biopsy tissue (Liedholm *et al.,* 1983) or increased uterine fluid prostaglandin levels (Toppozada, 1985) have been found in comparison with control or baseline values and may be associated with the increased bleeding. Conceivably, some or all of the above could contribute to a reduction in endometrial cancer risk. However, the persistence of most of these changes after IUD removal has not been investigated.

Hormonal changes occur in the endometrial environment after IUD insertion, though their relevance to endometrial cancer incidence is uncertain. In animal studies, uterine concentrations of estrogen or progesterone receptors were lower among rats provided with suture-type IUDs than in control animals (Myatt *et al.,* 1980*a,b*). Estrogen uptake in the uterus was found to be increased but progesterone uptake unchanged among rats provided with copper IUDs, while no variation was found in association with inert devices (Aedo and Zipper, 1973). Few studies have examined hormonal changes in women in relation to IUD use. Among women using high-load copper IUDs, endometrial progesterone receptor concentrations were lower after 1 year than baseline levels, but there was no difference among other copper IUD users (De Castro and Gonzalez-Gancedo, 1986). Hormone

receptor concentrations were found to be similar among users and non-users of copper devices in another study (Punnonen *et al.*, 1984). After 1 year of IUD use, endometrial biopsy did not reveal changes in estradiol or progesterone concentrations among users of inert IUDs compared with pre-insertion levels, but progesterone was decreased and estradiol increased in women provided with a copper IUD (Hagen-feldt and Landgren, 1975). Serum hormone levels have not been observed to differ between IUD users and non-users (Nygren and Johansson, 1973), suggesting that the IUD does not exert an influence on ovarian hormone production. The persistence of hormonal changes, if any, after IUD removal has not been determined.

Our results provide evidence consistent with those of others that there is a reduced risk of endometrial cancer among women who have used an IUD. The reduced risk persists for many years after use is discontinued and is not restricted to one or a few types of IUDs, though the biologic basis for it remains unclear.

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APPENDIX V

INTERNATIONAL JOURNAL OF EPIDEMIOLOGY

INTRAUTERINE DEVICE USE AND ENDOMETRIAL CANCER RISK

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Background. Because intrauterine devices (IUD) invoke acute and chronic inflammatory responses in the endometrium, it is possible that prolonged insertion of an IUD could induce endometrial cancer.

Methods. We examined the relation between use of an IUD and endometrial cancer risk using data from a multicentre case-control study involving 405 endometrial cancer cases and 297 population controls.

Results. A total of 20 (4.9%) cases and 34 (11.4%) controls reported any use of an IUD. After adjustment for potential confounders, IUD use was not associated with an increased risk of endometrial cancer (RR = 0.56 for ever use; 95% CI : 0.3-1.0). Little reduction in risk was observed among women who last used an IUD within 10 years of the index date (RR = 0.84; 95% CI : 0.3-2.4) but risk was decreased among women who used an IUD in the more

distant past (RR = 0.45; 95% CI : 0.2-1.0). Risk did not vary consistently with number of years of IUD use or with years since first use. Risk was not increased among women who used inert devices (RR = 0.46; 95% CI : 0.3-3.6) or those who used devices containing copper (RR = 1.08; 95% CI : 0.1-3.6).

Conclusion. These data are reassuring in that they do not provide any evidence of an increased risk of endometrial cancer among women who have used IUD.

Keywords: intrauterine device (IUD), endometrial cancer, contraception, epidemiology

Because intrauterine devices (IUD) invoke acute and chronic inflammatory responses in the endometrium, it is possible that prolonged insertion of an IUD could induce endometrial cancer.[1] IUD containing copper may be particularly suspect because they tend to produce more serious endometrial irritation than inert devices.[2] IUD could also theoretically increase endometrial cancer risk because they alter uterine sensitivity to oestrogen and progesterone.[3]

Although IUD are used by an estimated 85 million women worldwide,[4] only four small studies have examined the relation between their use and the occurrence of endometrial cancer [5-8] and none were able to examine risks associated with specific types of IUD. Thus, we used data from a large multicentre case-control study in the US to evaluate further the relation between IUD use and endometrial cancer.

METHODS

This case-control study was a collaborative effort with seven participating hospitals in five areas of the US—Chicago, Illinois; Hershey, Pennsylvania; Irvine and Long Beach, California; Minneapolis, Minnesota; and Winston-Salem, North Carolina. A total of 498 women between the ages of 20 and 74 years with newly diagnosed endometrial cancer were identified between 1 June 1987 and 15 May 1990. Detailed information on the selection of cases and

controls and other study methods are presented elsewhere.[9] Briefly, random digit dialling techniques were used to select controls for cases younger than age 65 whereas older controls were selected using information provided by the Health Care Financing Administration. We attempted to select one control for each case, matched for age (5-year age groups), race, and location of residence at diagnosis (telephone exchange or zip code).

Random digit dialling controls were selected by identifying a residential cluster matched for the telephone exchange for each eligible case. Telephone numbers were called, and an enumeration of female members aged 20-64 in each household was attempted. Of 15 820 telephone numbers sampled, 10 184 were assessed to be residential working numbers, and an enumeration of female members was obtained for 85%. Older controls were derived from Health Care Financing Administration computer records a subject of the same age, race and zip code as each eligible case. After the initial selection of subjects, a short telephone questionnaire was administered to determine whether the subjects had intact uteri. A total of 125 of the initially selected random digit dialling controls and 88 of the Health Care Financing Administration controls were eliminated because of their not being at risk of developing endometrial cancer. These subjects were replaced with other eligible controls so that there was an eventual accrual of 304 controls through random digit dialling techniques and 173 through Health Care Financing Administration records.

Trained interviewers completed home interviews on 434 (87%) of the eligible cases and 313 (66%) of the eligible controls. Eligible subjects who could not be interviewed were not replaced. Reasons for non response included refusal (5% of the cases and 22% of the controls), communication problems (4% versus 3%) and other problems (2.2% versus 9%). In addition, physician consent was not obtained for 2.0% of the cases. The response rate

was considerably higher for the random digit dialling than the Health Care Financing Administration controls (76% vs 47%).

Pathology reports were obtained and reviewed for all cases, with 93% of the interviewed cases having a classification of epithelial cancer. Because of the distinct epidemiological characteristics of sarcomas,[10] this analysis focused on data from interviews with 405 epithelial cancer cases and their 297 matched controls. The mean ages of the cases and controls were 59.2 (standard deviation [SD] = 9.96) and 58.0 years (SD = 10.4), respectively.

A structured interview, on average 76 minutes in length, was administered to obtain information on hypothesized risk factors, including demographics, pregnancy history, menstrual history, contraceptive behaviour, use of exogenous hormones, changes in body weight, diet and alcohol intake, family history of cancer, medical events and physical activity. The dietary section consisted of 60 food items and provided an estimate of usual adult caloric intake and intake of specific nutrients.[11] Anthropometric measurements, including waist-to-thigh circumference ratio as a measure of intra-abdominal fat,[12] were also taken at the time of interview. Information on birth control usage was obtained using lifetime calendars to record usage of specific methods on a monthly basis. For each mention of IUD use, information on brand was elicited. No subjects reported using progestagen containing IUD.

Because of the large number of cases without an interviewed matched control, adjusted maximum likelihood relative risk estimates (RR) and 95% confidence intervals (CI) are presented using unconditional logistic regression techniques.[13] The main results of the study were confirmed using conditional logistic regression on the smaller subset of 274 matched pairs of cases and controls.

Risk factors identified in this study, adjusted for each other, included education (RR = 2.0 for $\geq$16 versus <12 years), age

at menarche (RR = 2.8 for <12 versus ≥15 years), menopausal oestrogen use (RR = 15.3 for ≥10 versus 0 years), diabetes (RR = 1.6), saturated fat intake (RR = 2.0 for highest versus lowest quartile), current body mass index (weight in kg/height in m^2) (RR = 3.2 for ≥32 versus <25) and waist to thigh circumference (RR = 2.7 for highest versus lowest quartile). Factors associated with reductions in risk included multiple livebirths (RR = 0.2 for ≥5 versus 0 births), cigarette smoking (RR = 0.3 for current versus never smokers), and oral contraceptive use (RR = 0.4 for versus 0 years). Menopausal status and age at natural menopause were unrelated to risk.[9]

RESULTS

Table 1 presents the prevalence of risk factors among controls who never used any method of birth control, those who ever used an IUD and those who only used other forms of birth control. Compared to women who had never used any method of birth control, women who had used an IUD were younger, better educated and had a higher intake of saturated fat. Women who had used an IUD also had a lower waist to thigh circumference ratio, and were less likely to smoke and to be nulliparous. Differences tended to be less striking between women who had ever used an IUD and those who had only used other forms of birth control. Compared to those who only used other forms of birth control, women who had used an IUD were younger, better educated, had a later age at menarche and a lower waist to thigh circumference ratio. A total of 27 (79.4%) of the 34 controls who had ever used an IUD also had taken oral contraceptives (data not shown).

A total of 20 cases (4.9%) and 34 controls (11.4%) reported any use of an IUD, resulting in an age-adjusted relative risk of 0.43 (95% CI : 0.2-0.8). Further adjustment for oral contraceptive use attenuated this reduction in risk (RR = 0.53, 95% CI : 0.3-1.0). After further controlling for the other potential confounders identified in Table 1 (education,

intake of saturated fat, waist to thigh circumference ratio, number of livebirths, cigarette smoking, and age at menarche), risk remained modestly lowered among women who used an IUD (RR = 0.56; 95% CI : 0.3-1.0) (Table 2). In this fully-adjusted model, risk did not vary with increasing years of use and years since first IUD use was unrelated to risk of endometrial cancer. Risk did, however, appear to vary by years since last IUD use. Little reduction in risk was observed among women who last used an IUD within 10 years of the index date (RR = 0.84; 95% CI : 0.3-2.4) but risk was reduced among those who last used an IUD more than 10 years before (RR = 0.45; 95% CI : 0.2-1.0).

Table 1
Characteristics of controls by their birth control practices

* * *

Table 2
Risk of endometrial cancer associated with use of an intrauterine device

* * *

IUD were also categorized into two groups for analysis based on the presence or absence of copper. Inert device use was associated with a reduction in risk (RR = 0.46; 95% CI : 0.1-3.6) whereas copper device use was unrelated to risk (RR = 1.08; 95% CI : 0.3-3.6). The small number of IUD users precluded further stratification to investigate the separate effects of years since last use and type of IUD device on risk.

Additional adjustment for diabetes, current body mass index, cigarette smoking, menopausal oestrogen use, use of barrier methods of contraception, spermicides, female sterilization, and vasectomy of a partner did not materially change the risk estimates presented in Table 2. Excluding 86 women who had never used any form of birth control from the referent category also did not alter the results. Women who had used an IUD remained at modestly reduced risk of endometrial cancer (RR = 0.67; 95% CI : 0.3-1.6) in a

separate analysis that excluded 188 women who bad ever used oral contraceptives.

Because IUD were first commercially available in the US in 1964, few of the women 65 years and older in this study would have had an opportunity to use IUD. Results were similar when we restricted the above analyses to women younger than 65 years.

DISCUSSION

Three of four previous studies have observed a modest overall reduction in endometrial cancer risk among women who bad ever used an IUD." No evidence of a positive relation between IUD use and risk was found among women under age 55 in an analysis of data from the Cancer and Steroid Hormones (CASH) study (RR = 0.5 for ever use versus none; 95% CI : 0.3-0.8).[5] In the analysis of data from a case-control study in Italy,[6] the relative risk associated with ever use of an IUD was 0.4 (95% CI : 0.1-1.0). A study carried out in developing countries also reported no increased risk associated with use of an IUD (RR = 0.7 for ever use versus none; 95% CI : 0.4-1.3).[7] One conducted in Shanghai, China found no relationship between IUD use and endometrial cancer risk (RR = 1.1 for ever use; 95% CI : 0.5-2.5).[8]

With respect to type of IUD device, we did not find any evidence of an increased risk of endometrial cancer among women who used either inert devices or those who used devices containing copper.

Studies have been inconsistent with respect to their findings on the effects of years of IUD use and years since last IUD use on risk. In the present investigation, the reduction in risk associated with IUD use was apparent only among women whose use had ceased more than 10 years ago. In the CASH study conducted in the early 1980s,[5] however, risk did not vary by time elapsed since last IUD use. By contrast, Rosenblatt *et al.*[7] found that risk was

lowest among current users (RR = 0.1; 95% CI : 0.01-0.8). In accord with the study by Rosenblatt *et al.*[7] we found no evidence that risk decreased with increasing years of IUD use. Castellsague *et al.*[5] however, observed that risk decreased from 0.62 among women who used IUD for less than 4 years to 0.41 for those who used an IUD for more than 8 years. No details were available on the relation between risk and various exposure measures from the other two studies.[6-8]

It is unclear why relationships with years since last IUD use and years of IUD use have differed across studies. This inconsistency may reflect the difficulty in obtaining stable risk estimates from studies involving small numbers of IUD users. Another possible explanation relates to the fact that the materials and shapes of IUD devices have varied across populations and calendar time.[5] If certain IUD have more of an effect on endometrial cancer risk, studies conducted in different populations could observe disparate findings. Alternatively, the lack of consistency across studies may indicate that the modest reduction in risk associated with IUD use is the result of indication bias. Such bias could result if women at increased risk of developing endometrial cancer were less likely to be prescribed IUD (e.g. those with uterine bleeding from endometrial hyperplasia).

The major limitation of the present study is that the response rate was low among the population-based controls. If the controls who were IUD users were disproportionately more likely to be interviewed than cases, this could result in a spurious reduction in risk associated with IUD use. It is somewhat reassuring, however, that findings from this study with respect to generally accepted endometrial cancer risk factors, are similar to those presented in previous studies.[14]

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APPENDIX W

INTRAUTERINE DEVICE USE AND RISK OF ENDOMETRIAL CANCER

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Summary The relationship between intrauterine device (IUD) use and risk of endometrial cancer has been analysed in a case-control study conducted in Italy between 1983 and 1992, including 453 patients with histologically confirmed endometrial cancer and 1.451 controls admitted for acute, non-gynaecological, non-hormonal, non-neoplastic conditions to the same network of hospitals where cases had been identified. Two (0.4%) cases versus 36 (2.3%) controls reported ever using a❑ IUD. The corresponding multivariate relative risk was 0.4 (95% CI 0.1-1.0). The results of this study and the few published available epidemiological data suggest a protective role of IUD use on endometrial carcinogenesis, but potential selective mechanisms for IUD utilisation (indication bias) should be carefully considered in the interpretation.

Intrauterine device (IUD) use may induce endometrial alterations, such as inflammatory changes (Sheppard, 1987), loss of epithelial surface (El-Badrawi *et al.*, 1981) and reduction in ciliated cells (Gonzalez-Angulo *et al.*. 1973), which may affect the risk of neoplastic changes of the endometrium. In terms of biological inference, the risk of

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endometrial cancer might be either increased or decreased by such changes.

Epidemiological data on the relation between IUD use and risk of endometrial cancer are, however, scanty. A recent analysis of data from the Cancer and Steroid Hormones (CASH) Study suggested that the risk of endometrial cancer is approximately halved in women reporting ever IUD use, and the protective effect tended to increase with duration of use (Castellsague *et al.*. 1993). To offer further data on the issue. we report the results from a case—control study conducted in Northern Italy (Parazzini *et al.*. 1991a).

Patients and methods

The general design of this study has been previously described (Parazzini *et al.*. 1991a). Cases included in the study were 453 patients with histologically confirmed endometrial cancer aged <65 years (median age 56 years. range 28-64). They were admitted to the Ospedale Maggiore (including the four largest teaching and general hospitals in the greater Milan area). to the University Obstetrics and Gynecology Clinics and to the National Cancer Institute of Milan between 1983 and 1992. They were interviewed during their stay in hospital for surgery. medical treatment, radiotherapy: their diagnosis of endometrial cancer dated back no more than 1 year (median time from diagnosis to interview 2 months, range 0-12 months).

Controls were patients younger than 65 years admitted for acute, non-gynaecological, non-hormone-related, non-neoplastic conditions to the same network of hospitals where cases had been identified. Women who had undergone hysterectomy were not eligible as controls. A total of 1,541 controls (median age 53 years, range 27-64) was included in the present analyses. Of these, 32% were admitted for traumatic conditions (mostly fractures and sprains). 35% had non-traumatic orthopaedic disorders (mostly low back pain and disc disorders), 15% had surgical conditions (mostly abdominal, such as acute appendicitis or strangulated hernia)

and 18% had other illnesses, such as ear, nose and throat or dental disorders. Less than 3% of identified cases and controls refused to be interviewed.

Trained interviewers identified and questioned cases and controls using a standard questionnaire. Information was collected on general characteristics and habits. gynaecological and obstetric data, related medical history and use of oral contraceptives, intrauterine devices (IUD) and female hormones for other indications.

Odds ratios, as estimators of relative risks **(RR)** of endometrial cancer, together with their 95% confidence intervals (CI). according to use of IUD were computed from data stratified for quinquennia of age by the Mantel—Haenszel procedure (Mantel & Haenszel. 1959). In order to allow simultaneously for the effects of several potential confounding factors, unconditional multiple logistic regression. with maximum likelihood fitting. was used (Breslow & Day. 1980). Included in the regression equations were terms for age and selected factors significantly associated in this data set with the risk of endometrial cancer (parity. Quetelet's index and oestrogen replacement therapy use).

Results

The distribution of cases and controls according to age and selected covariates is presented in Table I. Cases were more frequently null-parae **(RR** age adjusted. parae versus null-parae. 0.6: 95% CI 0.4-0.9). of higher body mass index (age adjusted **RR.** kg m^{-2} $\geq$25 is <25. 2.0: 95% CI 1.7-2.4) and more often oestrogen replacement therapy users **(RR** ever versus never 2.0. 95% CI 1.3-3.1).

The relation between IUD use and endometrial cancer risk is considered in Table **II.** Out of the 453 endometrial cancer cases, two (0.4%) reported ever having used an IUD: the figures for controls were 36 ever users (2.3%) out of the 1.541 controls. The corresponding **RR** of endometrial cancer

was, in comparison with never users, 0.4 (95% CI 0.1-1.0) for ever IUD users. The data were insufficient for analysis of duration of use or other time-related factors.

Discussion

The results of this analysis further suggest that IUD use reduces the risk of endometrial cancer, but the interpretation deserves caution. In fact, indication bias may. at least partially. explain this inverse association. IUD may be less frequently prescribed in women with long, heavy menstrual flows or reporting pre-, post- or inter-menstrual blood spotting, conditions that may be associated with unopposed oestrogen endometrial stimulation and consequently increased endometrial cancer risk. Another potential limitation of this study is the low number of IUD users in Italy, which did not provide the opportunity to analyse the role of duration and any other time-related factors. In relation to other potential biases, cases and controls were identified in institutions covering broadly comparable catchment areas, and participation was almost complete. Likewise, recall bias is unlikely, since the interviewed cases and controls and the interviewers were unaware of the potential association between IUD use and endometrial cancer risk.

Table l
Distribution of 453 endoetrial cancer cases and 1.541 controls according to selected characteristics. Milan, Italy, 1983-1992

* * *

We did not have information on type of IUD used, thus we cannot evaluate the role of different types of IUD, particularly progestin-releasing ones. Despite these considerations. some biological evidence, the consistency of our results with data from the CASH study (Castellsague *et al.*, 1993) and the magnitude of the association offer some support to the hypothesis that IUD use reduces the risk of endometrial cancer. The CASH study showed a decreased risk of endometrial cancer in IUD users of about 50%; in that study the risk tended to decrease with duration of use,

offering some support to the hypothesis of a causal relationship, although the trend in risk with duration was not significant (Castellsague *et al.,* 1993).

In biological terms, laboratory and animal studies have suggested that IUD use may alter the response to steroids of the endometrium. These changes are mediated by the device itself as well as by the copper ions present in some devices. These alterations inhibit binding of oestrogen and progesterone to the endometrial cell receptors (Tamaya *et al.,* 1976) and decrease the steroid nuclear receptor concentration in the endometrial cells (Myatt *et al.,* 1980). These changes, however, may influence both oestrogen and progesterone activity, which have opposing effects on endometrial carcinogenesis (Paramini *et al.,* 1991b).

In conclusion, the few available epidemiological data suggest a protective effect of IUD use on endometrial cancer risk, but potential indication or selection bias is difficult to overcome in any epidemiological study on the issue, and should therefore be carefully considered in the interpretation.

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APPENDIX X

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Despite the increasing world-wide popularity of contraceptive intra-uterine devices (IUDs), their potential long-term effects on the risk of developing endometrial carcinoma have been poorly studied. This paper reports on the relationship between intra-uterine contraception and endometrial cancer by analyzing epidemiological data from a large, multicenter, population-based, case-control study of epithelial endometrial cancer. Cases were 437 women, 20 to 54 years of age, with histologically confirmed epithelial endometrial cancer ascertained through 6 population-based cancer registries in the United States. Controls were 3200 women selected at random from the populations of these areas. The age- and parity-adjusted odds ratio (OR) for the association between ever having used intra-uterine contraception and endometrial cancer

was 0.51 (95% confidence interval (CI) 0.3-0.8). Although the protective effect increased with duration of use, a dose-response relationship among users was not statistically demonstrable. The association did not vary significantly with age at first or last IUD use or with time elapsed since first or last IUD use. Years of education significantly modified the effect of intra-uterine contraception. Thus, intra-uterine contraception appeared to be strongly protective for women with at least 13 years of education (OR = 0.29, 95% CI, 0.15-0.6). It is proposed that intra-uterine contraception exerts its protective effect through local structural and biochemical changes in the endome-trium that may alter endometrial sensitivity and response to circulating estrogen and progesterone.

In this century, 3 major events in the field of contraception have occurred: the introduction of intra-uterine contraception, the formulation of oral contraceptives, and the development of laparoscopic tubal sterilization. In contrast to oral contraceptives, the potential effects of intra-uterine contraception and tubal sterilization on the risk of endometrial carcinoma have been poorly studied. This paper focuses on the epidemiological relationship between intra-uterine contraception and endo-metrial cancer.

A contraceptive intra-uterine device (IUD) is not just an inert device seated inactively in the uterus. IUDs have been reported to induce profound endometrial changes, including sterile inflammatory changes (Sheppard, 1987; Sagiroglu and Sagiroglu, 1970), an increased number of mast cells (Tursi *et al.,* 1984; Kobayashi *et al.,* 1983), superficial loss of surface epithelium (Sheppard and Bonnar, 1980; El-Badrawi *et al.,* 1981), reduction of ciliated cells with impairment of the secretory activity in the epithelium contiguous to the device (Gonzalez-Angulo *et al.,* 1973), and alterations in endometrial response to estrogen and progesterone (Tamaya *et al.,* 1976; Ghosh *et al.,* 1975; Ghosh and Roy, 1976; Kontula *et al.,* 1974).

IUD use could theoretically alter endometrial cancer risk through at least 2 mechanisms: first, by inducing extra-uterine effects on the ovary and the central hypothalamic-pituitary-ovarian axis that could affect the production of ovarian estrogens and progesterone; and second, by exerting direct changes in the endometrial environment that could induce a chronic inflammatory process or an alteration of the endome-trial response to hormones.

To explore the relationship between IUD use and endometrial carcinoma, we analyzed data from the Cancer and Steroid Hormones (CASH) Study (CDC CASH Study, 1983), a large, multicenter, population-based, case-control study.

METHODS

Data for the CASH Study were collected in 8 areas of the USA that are part of the Surveillance, Epidemiology and End Results (SEER) program of the US National Cancer Institute. The areas included: the metropolitan areas of Atlanta, Detroit, San Francisco and Seattle; the states of Connecticut, Iowa and New Mexico; and the 4 urban counties of Utah.

The design and methods used in the CASH Study, which included breast- and ovarian-cancer patients as well as the endometrial-cancer patients reported here, have been detailed elsewhere (CDC CASH Study, 1983; Wingo *et al.*, 1988). Here we summarize those features of the CASH Study that are relevant to the association investigated.

Cases

Eligible cases were 905 women, 20 to 54 years of age, who resided in one of the 8 participating areas and who were newly diagnosed with a primary epithelial endometrial cancer between December 1, 1980, and December 31, 1982. Of those, 673 (74%) were interviewed. Cases from Utah and New Mexico were excluded because histologic reports and slides of endometrial cancer specimens were not retrieved. Of the 599 women identified

and interviewed in the 6 remaining areas, the SEER centers were able to retrieve the histologic information and slides from 575 women (96%). These were independently reviewed by a panel of 3 pathologists, each an expert in endometrial cancer. The panel agreed that 437 women (76%) met the criteria for a primary epithelial malignant neoplasm of the endometrium.

Controls

The pool of eligible controls consisted of women 20 to 54 years of age selected through the Waksberg (1978) method of random digit dialing of households with telephones in the same geographic locations and during the same time interval as when the cases were diagnosed. A stratified sample, frequency-matched by geographic location and by the 5-year age distribution of breast-cancer cases, was selected from the pool (5698 women). Of these women, 4755 (83%) were interviewed; of that sub-group, 1271 were excluded because they had either had a previous hysterectomy or had reported having had a dilation and curettage procedure of unknown or questionable outcome prior to interview. All Utah and New Mexico controls (284) were further excluded, leaving a control group of 3200 women available for analysis.

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Data collection

Each study participant was interviewed in person in her home according to a pre-tested, standardized questionnaire. Details of the questionnaire and the information collected have been presented elsewhere (Wingo *et al.,* 1988).

Each woman was asked whether she had ever used an IUD, loop or coil as a form of birth control. If she had,

dates of use were recorded. Of the 3637 study participants, 520 (14%) reported having used some form of intra-uterine contraception, 3114 (86%) reported never having used intra-uterine contraception, and 3 (2 cases and 1 control) did not know if they had used IUDs. Therefore, 3634 women (435 cases and 3199 controls) were available for analysis.

Analyses

The measure of association used to compare the risk of developing endometrial cancer among exposed women with that in unexposed women was the odds ratio (OR) as an approximation to the rate ratio. Logistic-regression models with maximum-likelihood estimation of parameter values were used to estimate unadjusted and adjusted odds ratios and to test for linear trends. An alpha value of 0.05 was used as the criterion for statistical significance and, accordingly, 95% confidence intervals (CI) around the OR are reported.

A 3-step process for screening for confounding variables was performed: first, the Mantel-Haenszel procedure was used, treating IUD use dichotomously and the confounders categorically (one confounder at a time); second, logistic regression was used, treating IUD use and confounding variables as continuous (one confounder at a time); and finally, multivariate logistic regression was used to assess the joint confounding effects of those confounding variables selected individually in either of the 2 previous steps. Potential confounders assessed included, among others: age, parity, age at menarche, menopausal status, age at menopause, race, years of education, use of other non-hormonal contraceptive methods (tubal ligation, vasectomy, diaphragm, contraceptive foam/ cream suppositories, condom, rhythm and withdrawal), frequency of Pap smears, frequency of pelvic examinations, infertility, smoking, history of selected diseases (diabetes, hypertension, arthritis and

pelvic inflammatory disease), Quetelet's index, use of oral contraceptives, use of estrogens, data collection center, and family history of cancer.

To assess the specificity of effects of IUD use, adjusted odds ratios were estimated for 3 histologic sub-types of epithelial endometrial cancer: adenocarcinoma, adenoacanthoma and adenosquamous carcinoma. We also assessed the effects of duration of IUD use, age at first and last IUD use, and time since first and last IUD use.

To identify effect modifiers (factors that may alter the association between exposure and disease) a 2-step process was carried out. First, potential effect modifiers were examined one at a time in logistic-regression models that included IUD use (dichotomous), age and parity (confounders), the potential effect modifier, and an interaction term between IUD use and the potential effect modifier. Second, those variables that were statistically significant *(p* < 0.1) effect modifiers individually were included, along with their interaction terms with IUD use, in a single multiple logistic-regression model. Through a backward elimination process, significant *(p* < 0.05) effect modifiers were retained. Potential effect modifiers assessed included: age, parity, age at menarche, menopausal status, age at menopause, race, years of education, frequency of Pap smears, infertility, smoking, history of selected diseases (diabetes, hypertension, arthritis and pelvic inflammatory disease), Quetelet's index, use of oral contraceptives, use of estrogens, data collection center, and family history of cancer.

The chi-square statistic proposed by Lemeshow and Hosmer (1982) was used to assess the goodness of fit of the final

adjusted and interaction logistic regression models. None of the models showed a statistically significant lack of fit.

RESULTS

In this population, the observed differences between cases and controls were consistent with those of other studies of risk factors for endometrial cancer. These results will not be presented here in detail, since they have been published elsewhere (CASH Study, 1987). In brief, as shown in Table I, endometrial cancer cases were more likely than controls to be of white race, obese, and nulliparous or of low parity. They tended to have completed fewer years of education and to have a slightly younger age at menarche. Cases were more likely to be peri-menopausal or to have had an early menopause. Cases were also more likely to have received treatment with estrogens and less likely to have used hormonal and non-hormonal contraceptive methods (tubal sterilization, diaphragm, condom). Cases reported more frequently than controls having received treatment for hypertension and diabetes and having a positive family history of cancer in a first-degree relative. Cases reported slightly less frequently than controls a history of cigarette smoking (Table I). Because controls were frequency matched by the 5-year age distribution of breast-cancer cases, differences in age between cases and controls are not interpretable.

Women who used intra-uterine contraception were less likely to develop endometrial cancer than women who did not use this contraceptive method (unadjusted OR, 0.32; 95% CI, 0.21 to 0.49). Only 6% (24/435) of the cases reported intrauterine contraception use, as compared with 16% (496/3199) in the control group (Table II). After the effects of 68 potential confounders had been assessed, only age and parity were found to appreciably reduce the estimated magnitude of the protective effect, but the association after adjustment remained statistically significant (adjusted OR, 0.51; 95% CI, 0.33 to 0.79; $p = 0.003$). All subsequent models

were adjusted for age and parity. In the adjusted analysis, the protective effect of intra-uterine contraception increased with duration of use, but the dose-response relationship among IUD users did not reach statistical significance (Table II).

Table III summarizes the stratum-specific ORs by age group. IUD use was consistently protective in all age categories.

The protective effect of intra-uterine contraception use on the risk of endometrial cancer increased with younger ages at first IUD use, although this effect did not reach statistical significance. Women who first used IUDs before age 35 had an adjusted OR of 0.47 (95% CI, 0.27 to 0.81), whereas women who first used IUDs at later ages had an adjusted OR of 0.64 (95% CI, 0.33 to 1.25). The test for linear trend with age at first IUD use was not statistically significant (p = 0.41). Age at last IUD use did not substantially modify the association between endometrial cancer and IUD use (data not shown).

The association between endometrial cancer and IUD use varied with time since first IUD use, although not significantly (Table IV). Women who first used intra-uterine contraception more recently had greater protection against endometrial cancer than women who first used intrauterine contraception in the more distant past. The OR for women who first used an IUD within 10 years before the index date was 0.35 (95% CI, 0.15 to 0.80), whereas the OR for women who first used an IUD at an earlier time was 0.63 (95% CI, 0.38 to 1.04). Intra-uterine contraception appeared to be most protective among women who first used an IUD within the past 10 years and used it for at least 96 months (OR, 0.21; 95% CI, 0.06 to 0.77).

Recency of IUD use, on the other hand, did not substantially change the association between endometrial cancer and intrauterine contraception. Women who

stopped IUD use more recently (less than 72 months before the index date) had an adjusted OR of 0.49 (95% CI, 0.27 to 0.90) and women who stopped IUD use less recently (72 months or more) had an adjusted OR of 0.56 (95% CI, 0.31 to 1.02).

Table I
Characteristics of Women with Epitherlial Endometrial Cancer and Controls

* * *

Table II
Crude and Adjusted Odds Ratios for the Associatin Between Epithelial Endoetrial Cancer and Intra-Uterine Contraception Use

* * *

Table V shows the age- and parity-adjusted OR for the 3 histologic sub-types studied. A protective effect was consistently found for each of the 3 histologic sub-types, although the risk estimates did not reach statistical significance.

Of the 21 potential effect modifiers assessed, only years of education was found to be a statistically significant effect modifier. Per one-year differential in education, the OR for the association between endometrial cancer and intra-uterine contraception decreased by about 20% (OR, 0.80, 95% CI, 0.68 to 0.93). To better summarize the modifying effects of years of education, we fitted another logistic regression model in which years of education were divided into 2 categories, less than 12 years of education and more than 12 years of education. As shown in Table VI, women who had completed less than 13 years of education were not significantly protected by intra-uterine contraception (OR, 1.02, 95% CI, 0.58 to 1.79), whereas women who had completed more than 12 years of education were strikingly protected by IUD use (OR,

0.29, 95% CI, 0.15 to 0.58). The ratio of these ORs is 0.29 (95% CI, 0.12 to 0.70), indicating that the OR among more educated women was about one third the magnitude of the corresponding OR among less educated women.

Table III
Odds Ratios for the Association Between Endometrial Cancer and Intra-Uterine Contraception Use by Age Group

* * *

Table IV
Odds Ratios for the Association Between Endometrial Cancer and Intra-Uterine Contraceptin Use by Time Since First IUD Use

* * *

Table V
Odds Ratios for the Association Between Endometrial Cancer and Intra-Uterine Contraception Use by Histologic Sub-Type

* * *

Table VI
Odds Ratios for the Association Between Endometrial Cancer and Intra-Uterine Contraception Use by Years of Education

* * *

DISCUSSION

This analysis of the data from the CASH Study shows an overall significant decrease in the risk of developing endome-trial cancer among women who used intra-uterine contraception, as compared with women who never used it. After taking into account the combined confounding effects of age and parity, women who had used intra-uterine contraception were about half as likely to develop endometrial cancer as were women who had

not used that method of contraception. Although the magnitude of the protective effect increased with duration of IUD exposure, a dose-response relationship was not statistically demonstrable among exposed women. However, due to low power, a fairly strong dose-response relationship could not be ruled out with statistical confidence.

The population-based design of the CASH Study reduced, but did not completely eliminate, the possibility of various types of bias that could distort the true relationship between intra-uterine contraception and endometrial cancer.

One of the main limitations was that the CASH Study was not primarily designed to investigate this association. As a consequence, procedures to specifically assist in the recall of IUD use were not incorporated in the study, thereby, at least theoretically, leading to mis-classification of exposure status.

However, poor recall of exposure to IUDs is unlikely to have played a role in the observed association. Intra-uterine contraception should be readily remembered by women, since insertion of the device involves not only a procedure but also a number of visits to the gynecologist before and after insertion. Moreover, since equally poor recall of exposure status by both cases and controls would tend to bias the magnitude of the association toward the null value, the observed magnitude of the protective effect would be an underestimate of the real effect.

Another issue, however, is the role of reporting bias. A case may have been more likely than a control to over-report IUD use, since it is reasonable for a woman with cancer to focus on possible exposures, such as IUDs, that may be related to her disease. However, since the observed effect was protective, over-reporting of IUD use by cases would lead to underestimation of the

observed protective effect. For differential reporting to account for the observed protective effect, either cases would have had to under-report IUD exposure more frequently than controls; or, alternatively, controls would have had to over-report IUD use more frequently than cases. Neither situation seems likely.

A second important limitation of this study was that information on the type of device used was not collected and thus it was not possible to assess whether the protective effect of intra-uterine contraception differed by type. It is likely that the nature and degree of the changes observed in the IUD-

exposed endometrium vary among inert, copper-releasing and progesterone-releasing devices. The shapes and materials of inert devices have changed over time, and in copper IUDs the amount of copper incorporated into the device, and consequently that released into the endometrial cavity, has also varied. Other epidemiologic, experimental and animal studies in which the effect of different types of IUDs on endometrial cancer risk can be evaluated are warranted to further investigate this protective relationship.

The finding that years of education significantly modified the association between IUD use and endometrial cancer is difficult to interpret. It can be speculated that more highly educated IUD users would be more likely than less educated IUD users to be involved in regular medical surveillance. However, this would more probably result in a positive relationship between IUD use and endometrial cancer among more highly educated women, rather than the negative relationship observed in this study. Failure to observe similar interactions between IUD use and actual screening behavior, such as frequency of Pap smears, further weakens an explanation based on detection bias. It should be borne in mind that the assessment of effect

modification in this analysis was merely exploratory, and that education was one of many effect modifiers considered.

A number of animal and clinical studies suggest mechanisms by which IUDs may protect against endometrial cancer. Several lines of evidence suggest that IUDs may alter endome-trial sensitivity and response to the circulating steroid hormones estrogen and progesterone. Hormonal studies in animal uteri and clinical studies in women suggest that changes in the endometrial sensitivity to ovarian hormones caused by an IUD could be mediated through the effects of the copper ions released into the endometrial cavity and through the inherent structural and biochemical endometrial changes triggered by the device itself. More specifically, the effects of the copper ions and the changes in the endometrium may (a) inhibit binding of estrogen and progesterone to their endometrial cell receptors, (b) lower the concentration or synthesis of hormonal nuclear receptors and (c) alter the physical properties of estrogen and progesterone receptors. Tamaya *et aL* (1976) have observed in rabbits that copper IUDs inhibit both estrogen- and progesterone-receptor binding, suggesting that copper ions aggregated or dissociated hormone-receptor macromolecules, making the receptors biologically inactive. Other animal studies have shown that in an IUD-exposed endome-trium the response to progesterone is inhibited (Brown-Grant, 1969) or blocked (Nutting and Mueller, 1974), that estradiol and/or progesterone uptake is significantly decreased (Ghosh *et al.,* 1975; Ghosh and Roy, 1976), and that hormonal nuclear-receptor concentrations are lower than in a non-IUD exposed endometrium (Myatt *et al.,* 1978, 1980a,b).

Kontula *et al.* (1974) demonstrated that the presence of copper ions in concentrations similar to those

prevailing in an human endometrium exposed to a copper-bearing IUD was capable of locally inhibiting progesterone binding in the human endometrium and that the inhibition was caused by decreased affinity of the receptors for progesterone.

The unopposed estrogen hypothesis of endometrial cancer causation maintains that exposure to estrogen that is not sufficiently opposed by progesterone increases endometrial

mitotic activity, and consequently, endometrial-cancer risk (Henderson *et al.*, 1982). According to this hypothesis, decreased endometrial sensitivity/response to estrogen would be protective, while decreased sensitivity/response to progesterone would increase risk. Thus, the animal and clinical observations made appear only partially consistent with the protective effect observed in the present study. More directed studies, including those specifically focussed on the effect of IUDs on endometrial mitotic activity, arc needed to clarify the mechanism by which IUDs may protect against endometrial cancer.

From a public-health view point, the significance of these findings is not the protective association itself, since it is unlikely that women will change contraception practices because of these results. What is informative in this study is that even a small positive association has been ruled out with a high degree of confidence *(p* = 0.003). This is important, because, although the literature does not provide any scientific evidence for a positive association between IUD use and endometrial cancer, neither does it rule out such a possibility. We should keep in mind that the etiology of various human cancers is thought to be associated with chronic inflammatory processes, which the IUD could well induce in the endometrium.

The finding of a 50% reduction in the risk of endometrial cancer among IUD users in this study is reassuring, but requires replication. Given the increasing worldwide popularity of IUDs, further research designed to address this association is warranted.

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APPENDIX Y

CONTRACEPTION METHODS, BEYOND ORAL CONTRACEPTIVES AND TUBAL LIGATION, AND RISK OF OVARIAN CANCER

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PURPOSE: Few studies have examined methods of contraception, beyond oral contraceptives (OCs) and tubal ligation, in relation to ovarian cancer risk.

METHODS: Nine hundred two cases with incident ovarian/peritoneal/tubal cancer were compared with1800 population-based control subjects. Women self-reported all methods of contraception by using life calendars.

RESULTS: Each of the contraceptive methods examined reduced the risk of ovarian cancer as compared with use of

no artificial contraception. Comparing ever versus never use, after adjustment for potentially confounding factors and all other methods of contraception, the methods of contraception that emerged as protective were OCs (adjusted odds ratio [adj OR] 0.75, 95% confidence interval [CI] 0.61-0.93); tubal ligation (adj OR 0.63, 95% CI 0.51-0.77); intrauterine devices (IUDs) (adj OR 0.75, 95% CI 0.59-0.95); and vasectomy (adj OR 0.77, 95% CI 0.61-0.99). Although for OCs and tubal ligation we found that the longer the duration of use, the greater the effect, for IUDs the pattern was reversed: significant protection occurred with short duration and progressively greater risk (albeit nonsignificant) was seen with longer duration of use.

CONCLUSIONS: In the largest case-control study to date, a range of effective methods of contraception reduced the risk for ovarian cancer. OCs and tubal ligation reduced ovarian cancer risk with lower odds ratios with longer duration of use, whereas IUDs reduced risk overall, having the greatest impact with short duration of use.

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KEY WORDS: Contraception, Contraceptive Methods, IUDs, Oral Contraceptives, Ovarian Cancer, Tubal Ligation.

INTRODUCTION

Several forms of contraception have been shown to reduce the risk of developing ovarian cancer. Oral contraceptives (OCs) reduce risk in a duration-dependent fashion, and the effects of oral contraceptives last for at least 20 years after cessation of use (1–4). Tubal ligation has also been shown to consistently reduce risk (5–8). Increasingly, OCs are considered for chemoprophylaxis against ovarian cancer, particularly in high-risk women (9–11).

Few studies have examined use of other methods of contraception in relation to ovarian cancer risk. Small case-control studies demonstrated some risk reduction with

nonhormonal contraceptive methods; however, only small numbers of women used each method, the findings were not entirely consistent by method, and risk reductions were not significant (1, 12–14). Two larger studies reached somewhat-conflicting conclusions. In a large, case-control study by our group, all methods of contraception (intrauterine devices or intrauterine devices [IUDs], barrier methods, and vasectomy, as well as OCs and tubal ligation) reduced ovarian cancer risk as compared with no contraception use; ever use versus never use also reduced risk in multiparous but not nulliparous women (15). An analysis from the prospective Nurses' Health Study cohort reported an increased risk associated with IUD use and no association for other contraceptive methods (16).

The finding that multiple contraceptive methods reduce ovary cancer risk must be scrutinized as possibly representing a bias by fertility status. Ovarian cancer rates are greater among infertile women (17, 18). In turn, many infertile women spend long periods of time practicing unprotected intercourse. Contraceptive users may thus appear to be protected from ovarian cancer only because they are less likely to be infertile. Alternatively, the finding that methods of contraception beyond OCs and IUDs are protective may provide insights into ovarian carcinogenesis.

Here we attempt to re-examine the results from our earlier case-control study in a newly conducted population-based case-control design. Our earlier study was conducted in the Delaware Valley in and around Philadelphia, and our current study was conducted in Western Pennsylvania and surrounding regions (15). As we did previously, we attempt to separate parity from contraceptive use and to examine the specificity of contraceptive effects on risk reduction for ovarian cancer. Here we examined OCs, IUDs, any barrier methods, tubal ligation, and vasectomy (in a partner) in relation to ovarian cancer risk.

Selected Abbreviations and Acronyms

OC = oral contraceptive

IUD = intrauterine device

OR = odds ratio

CI = confidence inerval

SUBJECTS AND METHODS

Subjects were enrolled in a population-based case-control study conducted in a contiguous region comprising Western Pennsylvania, Eastern Ohio, and Western New York State. Cases were residents of this geographic region with histologically confirmed primary epithelial ovarian, fallopian tube, or peritoneal cancer diagnosed between February 2003 and November 2008. Both invasive and borderline tumors were included. Women were referred from hospital tumor registries, clinical practices, or pathology databases and contacted with the permission of their gynecologists. Eligible women were at least 25 years of age and within 9 months of initial diagnosis. Controls consisted of women at least age 25 who lived in telephone exchanges wherein cases resided. Random digit dialing was used to identify age-eligible women, and these were further screened by the study team to ensure that they had not had a previous oophorec-tomy or diagnosis of ovarian cancer. Eligible women were then invited to participate. Potential controls were frequency matched by 5-year age group and telephone exchange to cases in a ratio of approximately 2:1. Women were interviewed in their homes by trained interviewers. The questionnaire included a reproductive and gynecological history, a contraceptive history, a medical history, a family history, and information on lifestyle practices. All study subjects gave informed consent for participation.

From Pennsylvania and Ohio, we identified 2458 potential cases with histologically confirmed borderline or invasive epithelial ovarian cancer or tubal/peritoneal cancer. After excluding women who were ineligible on the basis of age and

time since diagnosis; deceased; residence outside of the counties in which referral hospitals were located; previous diagnosis of ovarian cancer; or inability to speak English, there were 997 who had incident cancer and were eligible for the study. Two hundred thirty one women were untraceable and 115 women refused to participate or their physicians refused on their behalf. Thus, 651 women completed case interviews. From New York, we identified 420 potential cases. After excluding women who were ineligible based on the aforementioned criteria, there were 273 who had incident cancer and were eligible for the study. Fourteen women were untraceable, and eight women refused to participate or their physicians refused on their behalf, resulting in a sample of 251 women who completed interviews.

Overall, 902 women with ovarian, tubal/peritoneal cancer completed an interview and are included in these analyses. For brevity, we subsequently use the term ovarian cancer to describe all cases.

Controls were identified from 90,540 random digit dialing calls. Of these, 46,752 reached nonworking numbers; 26,237 were unresolved (never reached a person); 14,899 reached an ineligible or indeterminate household (no woman within the age range or no information given); and 808 refused to participate. Of the 1844 eligible women willing to be interviewed in the initial screening, 1802 controls completed an interview. Two controls had an oophorectomy before the interview and were further excluded from our study, and 1800 controls completed an interview.

Cases included 677 women with invasive epithelial ovarian tumors, 97 with borderline epithelial ovarian tumors, 75 with peritoneal tumors, 32 with fallopian tumors, and 21 women with “other” or a missing type. The diagnosis of ovarian cancer was confirmed by local pathology in all cases.

Contraceptive Use

Standardized 2-hour-long interviews were conducted by trained interviewers in the homes of participating women. A "life" calendar marked with important events that each participant recalled during her life was used to enhance memory of distant information. Using the calendar, the interviewer led each woman through a recall of her sexual activity, use of various contraceptive methods, pregnancy attempts, and reproductive events for every month from sexual debut until a reference date. The reference date was calculated as 9 months before the interview (for both cases and controls) to ensure that exposures occurred before ovarian cancer diagnoses in cases and within a similar time frame for cases and controls. All contraceptive use was recorded, including the type of contraception, frequency of use, duration of use, and reason for use. Finally, we asked women about any medical consultation for fertility problems.

Covariates

Detailed information on demographic factors, physical characteristics, medical history, lifestyle, and family history was obtained by interview. These included factors that have been previously associated with ovarian cancer: race, education, family history of ovarian cancer, number of live births and pregnancies, and breast-feeding.

Table 1
Demographic and reproductive characteristics of ovarian cancer cases and controls in the HOPE study

* * *

Statistical Analysis

All analyses were restricted to women who had ever had sexual intercourse with a man. Thirty-three cases and 23 controls that had never had intercourse were excluded on the basis that they would not have had the opportunity for exposure to contraceptive methods for contraception.

Because we did not engage in individual matching of cases and controls, we used unconditional logistic regression analyses. We adjusted the odds ratios (ORs) for any residual effect of age and for gravidity (each as continuous variables), race (white/black/other), self-reported infertility (yes/no for diagnosis or use of infertility medications), and history of ovarian cancer in any first-degree relative (yes/no). We included these covariates because they were the strongest covariates related to ovarian cancer in our data. The inclusion of education and breast-feeding did not affect our results. We subsequently adjusted for all other forms of contraception other than the one of interest (e.g., for OCs, this analysis included the covariates listed above plus ever use of IUD, tubal ligation, and vasectomy). Oral contraceptive use was categorized as use for contraception, for noncontraceptive uses such as to control abnormal bleeding or menstrual pain, or for both contraception and other uses. Barrier methods included condoms, diaphragms, foam, sponges, or cervical caps. The reference group of no contraception included women who reported never using OCs, birth control implants, IUDs, any barrier methods, tubal ligation, or vasectomy (in a partner). These women may have used natural family planning (that is, having intercourse during times when the woman believed she was not ovulating), withdrawal, or nothing. We do not report as a separate category of contraception birth control implants, because the number of women using these methods in our study was small (16 cases and 46 controls).

Table 2
Odds ratios for ovarian cancer comparison of ever-use of contraceptive methods with never-use

* * *

Table 3
Odds ratios for ovarian cancer: comparison of ever use of contraceptive methods with no artificial contraception

* * *

RESULTS

Study subjects were predominantly white, post-high school graduates, 60 or older, and postmenopausal (Table 1). The commonly-found protections with increasing education, numbers of pregnancies/live births and breast-feeding were observed. Cases were more likely to be African American than controls, suggesting a selection bias among this small segment of subjects.

We found a reduction in the risk of ovarian cancer for ever versus never use of each of the methods for contraception analyzed (Table 2). However, after adjustment for age, race, family history of ovarian cancer, infertility, and gravidity, significant protection was seen only with IUDs as well as OCs for contraception and tubal ligation. After further adjustment for all other forms of effective contraception, IUDs, OCs for contraception, and tubal ligation remained significantly protective; now vasectomy also reached a level of significant protection.

Because ever use of contraceptive methods is complicated by admixing users of other methods, mixed methods, and none of these methods over a lifetime, we also compared users of each method with women who used no artificial contraception, defined as use of only natural family planning, withdrawal, rhythm, or no contraception (Table 3). Each of the methods significantly reduced the risk of ovarian cancer as compared to no artificial contraception.

Next, we examined the association between contraception and ovarian cancer among women with zero, one, two, or three or more pregnancies (Table 4). Both OCs for contraception and tubal ligation significantly reduced risk in some but not all gravidity categories, without a clear pattern of greater or lesser effects as gravidity increased. IUD use, despite generating protective odds ratios similar to those for OCs, did not produce significant results in any gravidity category, possibly because of small sample sizes. Vasectomy

also did not produce significant reductions in risk in any gravidity category.

By duration, OCs had a progressively greater impact with 4 or fewer years, 5 to 9 years, and 10 or more years of use (Table 5). Similarly, longer duration of tubal ligation was associated with lower risk. For IUD use, the pattern was reversed: significant protection occurred with short duration (< 4 years) use and progressively greater risk was seen with longer duration of use (adjusted ORs 0.53 for <4 years; 1.11 for 5-9 years; 1.40 for >10 years). We further explored whether time since last IUD use might drive these observations. Although there was a trend toward reduced risk with increasing time since last use, this was eliminated with adjustment for (i.e., not independent of) IUD duration. We did not have data on duration of vasectomy.

Additional analyses showed our observations to be robust. Contraception use before the first pregnancy or episode of trying was protective (OCs for contraception, OR = 0.87, 95% confidence interval [CI] 0.69-1.11); IUD, OR = 0.81, 0.41-1.60). Adjustment for breast-feeding in the multivariate analyses had no substantial effect on our results for ever-never use. Analyses including only epithelial ovarian cancer (excluding fallopian and peritoneal cancers) essentially replicated those shown here with the result for vasectomy slightly enhanced in these analyses when adjusted for confounders plus all other contraceptive methods (OR 0.73, 95% CI 0.56-0.94).

Finally, we examined use of concomitant methods of birth control over a lifetime (Table 6). The majority of women used more than one contraceptive method over a lifetime and of these, the most common combination was OC use plus another method. For instance, of the 424 women whose contraceptive use included vasectomy, only 89 (21%) did not also use OCs.

Table 4
Adjusted odds ratios for ovarian cancer: comparison of ever-use of contraceptive methods with never-use by gravidity group

* * *

DISCUSSION

We report here the largest case-control study to examine whether effective methods of contraception, beyond OCs and tubal ligation, reduce the risk of ovarian cancer. Consistent with the results of our previous case-control analysis (15), we found that use of a variety of different contraceptive methods generally reduced risk of ovarian cancer as compared to use of no artificial contraception. Such an analysis is almost certainly confounded by fertility in that women who are infertile or subfertile would be less likely to use effective methods of contraception and more likely to develop ovarian cancer. In our current, more discriminating analyses of ever versus never use, comparisons to OCs, and use within parity categories, the methods of contraception that emerged as protective were OCs, tubal ligation, IUDs, and vasectomy. Vasectomy is intriguing but we were less informed about this relationship with ovarian cancer since we had no data on duration of use. IUDs were particularly interesting because (i) they are not traditionally thought to reduce the risk of ovarian cancer; (ii) duration-response analyses showed a counter-intuitive pattern wherein shorter use reduced risk and longer use (albeit nonsignificantly) increased risk.

Table 5
Adjusted ORs and 95%CIs for ovarian cancer comparison of duration of use of contraceptive methods with never use

* * *

Table 6
Contraception methods use by cases and controls

* * *

Our results replicated a plethora of earlier studies linking OCs and tubal ligation to reduced ovarian cancer risk (5–7, 19, 20). In particular, our results mirror adjusted ORs for ever-use of OCs reported from a meta-analysis (0.7) and a pooled analysis (0.66) (2, 21). We partially, but did not fully, replicate a much more limited literature that has addressed the relation between other forms of contraception (barrier, IUD, or vasectomy) and the risk of ovarian cancer. In these studies, the reported ORs were generally less than 1.0; however, none of those studies had enough women in any contraception category, other than OCs, to show strong effects or to explore more fully comparisons between categories (1, 12–15, 22). In the only prospective study to examine contraception methods beyond OCs and tubal ligation, Tworoger et al. (16) found a significant relative risk of 1.76 associated with IUD use. Unfortunately, only 18 IUD users informed the analysis and thus duration and time since last use of IUDs was not reported. Here, we did not show significant risk reductions for barrier methods and vasectomy but we did find that shorter-duration IUD use reduced risk while longer duration IUD use increased risk.

In previous analyses stratifying by parity or gravidity groups, results have been mixed. In our previous study, we found risk reductions to be limited to multigravid women (15). In our current study, we found a patchy set of associations that did not clearly demonstrate a limitation by gravidity category but was not fully consistent between gravidity categories, perhaps on the basis of the sizes of individual stratification cells. All methods were protective before the first pregnancy, a time during which women might not yet know their fertility potential and thus not yet adjust their contraceptive strategy. All of this suggests that confounding by fertility status is an unlikely explanation for our observations.

A variety of biological explanations have been offered to explain the protective effect of OCs on ovarian cancer risk.

These include: (i) excessive ovulation promotes risk; (ii) elevated steroid hormone levels increaser risk; (iii) unopposed estrogen increases risk; and (iv) pelvic inflammation increases risk (23–27). Tubal ligation has been posited to have an effect via a reduction in utero-ovarian blood flow resulting in altered local hormonal and growth factor levels, or via its protection against the ascension of inflammants (26–28). Some IUDs contain progestin, which has been proposed to reduce the risk of ovarian cancer (25). However, only a tiny fraction of IUD users in the current analysis (n = 14) reported using progestin-containing IUDs. IUDs, particularly older formulations, such as the Dalkon Shield, increased the risk for pelvic inflammatory disease. The hazard likely occurred because of the particular construction of the multifilament string attached to the Dalkon Shield. But it also may have related to insertion through a cervix infected with the bacterial sexually transmitted infections that cause pelvic inflammatory disease, as suggested by the close temporal relationship between insertion and pelvic inflammatory disease and the relative safety of modern-day use, which is confined to monogamous women without cervical infections (29). These facts may explain our counterintuitive finding of a reversed duration-response relationship (longer use associated with increasing risk). IUDs must be replaced every 5 to 10 years depending on the product; longer use would imply more insertions and thus greater risk of infection and inflammation. Shorter use might actually reduce upper genital tract inflammation by virtue of killing sperm. Indeed, the marginal reduction in risk from vasectomy might suggest some protection from reduced exposure to sperm.

Strengths of this study include the population-based ascertainment of cases and controls, the large number of women interviewed, the use of life calendars and emphasis on recall of contraceptive use and reproductive experiences, and the structured interviews to enhance recall. All of these

methodological features reduce the potential for selection and information bias. The greatest study limitation was the challenge of separating the effects of various contraceptive methods because the use of more than one method over a lifetime was the norm. We attempted to separately delineate methods by adjusting for all methods in logistic regression analyses and by comparing each method to no effective method. Nonetheless, residual confounding remains a real concern. Other study limitations included: (i) selection against women with short life expectancies postdiagnosis who may have become debilitated or died before interview and (ii) inaccurate recall of past contraceptive experiences. Women may have incorrectly recalled past events, such as the duration of use of contraceptive methods. It is less likely that women would misremember ever versus never-use of contraceptive methods (30, 31). Previous investigators (30–35) have found that among ever-users of OCs identified by medical records, 80% or more reported OC use; an even larger proportion of IUD users identified by medical records reported IUD use.

In summary, from this large study of contraceptive methods and ovarian cancer, we confirmed that OCs and tubal ligation reduced risk for ovarian cancer. Short-term IUD use reduced risk but long term IUD use tended toward elevating risk. Because contraceptive methods are modifiable and because ovarian cancer is highly lethal, these findings should be added to other considerations when selecting contraceptive methods.

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APPENDIX Z

AMERICAN ASSOCIATION FOR CANCER

CONDITIONS ASSOCIATED WITH ANTIBODIES AGAINST THE TUMOR-ASSOCIATED ANTIGEN MUC1 AND THEIR RELATIONSHIP TO RISK FOR OVARIAN CANCER

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Abstract

Many cancers, including ovarian, overexpress epithelial mucin (MUC1) and promote anti-MUC1 antibodies that may correlate with more favorable prognosis. By extension, risk for ovarian cancer might be reduced by preexisting MUC1-specific immunity. We measured anti-MUC1 antibodies in 705 control women, identified events predicting antibodies, and estimated ovarian cancer risk by comparing profiles of events generating antibodies in controls with those in 668 ovarian cancer cases. Factors predicting antibodies included oral contraceptive use, breast mastitis, bone fracture or osteoporosis, pelvic surgeries, nonuse of talc in genital hygiene, and to a lesser extent intrauterine device use and current smoking. There was a significant increase in the

likelihood of having anti-MUC1 antibodies from 24.2% in women with 0 or 1 condition, to 51.4% in those with five or more conditions. By the same index of events, the risk for ovarian cancer was inversely associated with number of conditions predisposing to anti-MUC1 antibodies. Compared with having experienced 0 or 1 event, the adjusted risk for ovarian cancer decreased progressively with relative risks (and 95% confidence limits) of 0.69 (0.52-0.92), 0.64 (0.47-0.88), 0.49 (0.34-0.72), and 0.31 (0.16-0.61), respectively for women with two, three, four, and five or more events related to the presence of antibodies ($P_{trend} < 0.0001$.) We conclude that several traditional and new risk factors for ovarian cancer may be explained by their ability to induce MUC1 immunity through exposure of MUC1 to immune recognition in the context of inflammatory or hormonal processes in various MUC1-positive tissues. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1125–31)

Introduction

Human mucin (MUC) family member, MUC1, is a high molecular weight protein expressed in a highly glycosylated form and low levels by many types of normal epithelial cells and in a hypoglycosylated form and high levels by most epithelial adenocarcinomas, including breast and ovarian cancer (1). Anti-MUC1 antibodies have been described and correlated with a more favorable prognosis (2-5) showing that patients generate immunity against MUC1 produced by their tumors and defining MUC1 as a tumor-associated antigen and candidate for cancer vaccines (6). Anti-MUC1 antibodies are also found in healthy individuals, especially in women during pregnancy and lactation. It has been hypothesized that a natural immunity against tumor MUC1 might develop and account for the long-term protective effect of pregnancy or breast-feeding on breast cancer risk (7), an elaboration on the so called “fetal antigen theory”(8). Indeed it has been shown that sera from multiparous women, but not from nulliparous women or from men, are able to mediate

killing of breast cancer cells (9). Supporting a key role for MUC1 in these reactions, core peptide sequences from MUC1 can induce proliferation of T cells and cytotoxic T-cell responses in multiparous women (10). Recently, the "fetal antigen" hypothesis was extended to ovarian cancer after it was shown that sera from multiparous women also reacted with multiple antigens from ovarian cancer cells more strongly than sera from nulliparous women or men (11), although MUC1 was not specifically examined in these experiments.

In this study, we used an ELISA to determine the presence and relative amounts of MUC1-specific antibody in women from the general population who served as controls in a study of ovarian cancer. In analyses confined to these controls, we identified the predictors of anti-MUC1 antibodies and used case-control comparisons to evaluate these predictors in relation to ovarian cancer risk. We hypothesized that events predicting anti-MUC1 antibodies would be inversely associated with ovarian cancer risk and that there would be a cumulative effect of such events.

Materials and Methods

Subject Recruitment and Study. This report is based on the second phase of a population-based case-control study of ovarian cancer conducted between 7/98 and 7/03 and involving eastern Massachusetts and all of New Hampshire, approved by the Brigham and Women's Hospital and Dartmouth Medical Center's Institutional Review Boards. We identified 1,267 cases from tumor boards and Statewide Registries and excluded 119 cases who died, 110 who moved from the study area, one who had no telephone, 23 who did not speak English, and 46 found to have a nonovarian primary upon review. Of the remaining 968, physicians denied permission to contact 106 and 171 declined to participate, leaving 691 cases interviewed. Of these, 668 had an epithelial ovarian cancer (including borderline

malignancies) and are included in this report. A small number of cases (n = 48) were enrolled before surgery.

Controls were identified through town books in Massachusetts and Drivers' License lists in New Hampshire and sampled to match the age and residence of previously accumulated cases. Invitations to participate were sent to 1843 potential controls. Of these 318 had moved and could not be located or had died, 197 (in Massachusetts) could not be recontacted because subjects returned an "opt out" postcard required by the hospital's Institutional Review Boards, and 47 no longer had a working telephone. Of the remaining 1,281 who were contacted, 152 were ineligible because they had no ovaries or were not the correct age, 59 were incapacitated or did not speak English, and 349 declined, leaving 721 who were interviewed and included in this report.

After written informed consent, an in-person interview dealing with demographic, medical, and family history was conducted. Subjects also completed a self-administered dietary questionnaire. Heparinized blood specimens were collected from subjects agreeing to provide one; separated into red cell, buffy coat, and plasma components; and stored at -80°C.

ELISA Assay for Anti-MUC1 Antibodies. Plasma specimens were available for measuring anti-MUC1 antibodies in 48 cases with preoperative bloods and 705 controls. Antibodies were measured against a synthetic 100-mer MUC1 peptide corresponding to five tandem repeats of the MUC1 polypep-tide core tandem repeat region, according to our previously published protocol (2). Briefly, 0.5 Ag of MUC1 peptide in 100 AL of PBS was added to each well of Immulon 4 plates (Dynax, Chantilly, VA) and incubated overnight at 4°C. Control plates without the MUC1 peptide were also prepared. The plates were washed thrice with PBS and 100 AL of 2.5% bovine serum albumin in PBS added for 1 hour at room temperature to coat remaining sites in the well

(blocking step). Fifty microliters of serially diluted plasma (1:20 to 1:80 in PBS) were added to the MUC1 peptide-coated and control plates and incubated for 1 hour at room temperature. The plates were washed 5x with 100 AL PBS and 0.1% Tween 20 detergent. Fifty microliters of secondary antibody, alkaline phosphatase– labeled goat anti-human polyvalent IgM, IgG, IgA (Sigma-Aldrich, St. Louis, MO), diluted 1:1,000, was added for 1 hour at room temperature, and plates again washed 5x with PBS-Tween. One hundred microliters of alkaline phosphatase substrate pNPP (Sigma-Aldrich) were added at 3 mg/mL in 0.05 mol/L NaCO3 and 0.5 mmol/L MgCl2 and the plates incubated in the dark for exactly 1 hour before adding 50 AL of the stop solution (0.5 mol/L NaOH). The absorbance at 405 to 410 nm was measured using the plate reader MRX Revelation (Thermo Labsystems, Chantilly, VA). Absorbance values for each sample in the MUC1-coated plate were compared with values in the antigen-negative plates to subtract nonspecific binding. Based upon the previous responses in over 500 cancer cases and controls, absorbance reactions at the 1:20 dilution at <0.6 are scored as negative, reactions in the 0.6 to 0.79 range as low, reactions in the 0.8 to 0.99 range as intermediate, and reactions z1.0 as high. In the current study, 20 blinded replicate specimens were included and the Spearman correlation coefficient between the paired absorbances was 0.93 ($P < 0.0001$).

Statistical Methods. Logistic regression analysis was used to compare those with an antibody reaction at any level against those considered negative for MUC1 antibody ($A < 0.6$), while adjusting for potential confounding variables. Spearman rank correlations or generalized linear modeling was used to assess differences in absorbance levels (using log-transformed values of absorbance) for a more quantitative assessment of factors affecting anti-MUC1 antibody production. Combinations of factors were examined to identify the best cumulative index of experiences

associated with likelihood of having antibodies. Ovarian cancer cases and controls were then categorized by the presence or absence of events found to affect the likelihood of antibodies and risk for ovarian cancer calculated using unconditional multivariate logistic regression to adjust for potential confounders. In our models, we adjusted for the matching variables, age (continuous), and study site (Massachusetts, New Hampshire), as well as ethnicity (White, non-White), religious background (Jewish, non-Jewish), and parity as a continuous variable except where noted in the text or footnotes.

Results

The distributions of absorbance readings (corresponding to the amount of anti-MUC1 antibodies measured in the ELISA assay) seemed bimodal in cases with preoperative bloods and skewed right in controls prompting log transformation for statistical testing (Fig. 1). By a cutoff of *A* z 0.6, 33.8% of controls and 45.8% of cases were positive for antibodies. By a cutoff of *A* z1.0, 12.3% of controls and 25% of cases had a high level of antibodies, a significant difference that likely reflects an ongoing immune response to tumor in the cases.

Events Predicting Occurrence of Anti-MUC1 Antibodies. A number of demographic, reproductive, and medical conditions were examined as they affected the likelihood of controls having a low, intermediate, high level, or any anti-MUC1 antibody (Table 1). The last two columns show the (geometric) mean absorbance value, its SE, and the *P* from the linear regression model. Age was a strong predictor with 50% having antibodies at ages <35, declining to 29.3% at ages 55 to 64 years, and increasing back up to 32.6% in those ages z65 years, prompting age adjustment when testing for the significance of further variables. The proportion of women who were positive for anti-MUC1 antibody was similar for women who had never been pregnant (33.3%), had at least one live birth (34.1%), or had breast-fed without experiencing a mastitis (33.0%) but

elevated for women who had experienced mastitis while breast-feeding (46.1%). Notably, 25.0% of women reporting mastitis had high antibody levels compared with 10% to 14% of parous women who either never breast-fed or breast-fed and reported no mastitis ($P = 0.05$). Women who had used oral contraceptives (OC), compared with those who had not, were more likely to have antibodies; and this was most apparent among premenopausal women in whom 40.7% of OC users had antibodies compared with 26.7% of nonusers ($P = 0.05$). The proportion of women with antibodies was also higher for those who reported a bone fracture or osteoporosis after age 40 or within 20 years of their age at interview (36.0%) than in those who had not (33.0%) and 17.1% of women with fracture/osteoporosis had high antibody levels compared with 10.8% of women who had not ($P = 0.03$). Several types of pelvic/gynecologic surgery, including tubal sterilization, cervical conization, and cesarean section increased the likelihood of a positive antibody reaction and 47.2% of women who had more than one surgery had antibodies compared with 30.9% of women who never had pelvic surgery ($P = 0.01$). A surprising finding was that 38.1% of women who reported no use of cosmetic talc in hygiene had antibody compared with 28.6% of women who regularly used talc in genital hygiene ($P = 0.04$). The final entry shows the trend for elevated anti-MUC1 antibody levels by increasing number of antibody-promoting conditions. These included all variables significant in univariate analyses, such as OC use, bone fracture, mastitis, pelvic surgery, and genital talc use (where no use was considered the “condition”) as well as variables of marginal significance in the univariate analysis, which nevertheless improved the overall model including current smoking and use of an intrauterine device (IUD). A significant trend ($P = 0.0005$) in the likelihood of having antibodies was observed such that 24.2% of women who had zero or one of condition had antibodies compared with 51.4% of women who had experienced five or more of these conditions.

Figure 1
Distribution of absorbances from anti-MUC1 antibody assay in cases with preoperative bloods and all controls

* * *

Table 1
Occurrence of anti-MUC1 antibodies in control women by epidemiologic variables

* * *

Spearman (rank) correlations were calculated between the absorbance reading and several variables quantifiable on a numerical scale. No significant correlations were noted with number of live births, months of breast-feeding, or pack-years of smoking (data not shown). Weak but significant positive correlations were noted between absorbance values and months of OC use (r = 0.09, P = 0.02) and number of cesarean sections (r = 0.10, P = 0.02). A nonsignificant inverse correlation was noted between absorbance and estimated total applications of talc. When genital talc users were characterized by <weekly, weekly, and daily use, there was a trend of borderline significance (P = 0.11) for women using talc more frequently to have the lower antibody levels after adjustment for age, smoking, bone fractures, and OC or IUD use.

Risk for Ovarian Cancer Associated with Antibody-Promoting Events. The variables examined in relation to anti-MUC1 antibodies were then examined in relation to ovarian cancer risk, based upon case-control comparisons (Table 2). Odds ratios for ovarian cancer with each of these variables (except for age which was a matching variable) were calculated and adjusted for age, study site, exact parity, non-White race, and Jewish religion. Our study confirmed the influence of known ovarian cancer risk factors including parity, breast-feeding, and OC use. In addition, we identified previously unreported risk factors, including mastitis, relative risk (and 95% confidence limits) of 0.35 (0.16-0.77); IUD use, relative risk of 0.68 (0.50-0.91); and bone fracture,

relative risk of 0.70 (0.530.91). The final entry shows the association between number of antibody-promoting conditions and ovarian cancer risk. Compared with women with zero or one condition, the risk for ovarian cancer decreased progressively with relative risks (and 95% confidence limits) of 0.69 (0.52-0.92), 0.64 (0.47-0.88), 0.49 (0.34-0.72), and 0.31 (0.16-0.61), respectively, for women with two, three, four, and five or more conditions (*P*trend < 0.0001). This pattern mirrored the effect of these same conditions on the likelihood that control women had anti-MUC1 antibody (Fig. 2). Finally, risk by number of antibody-promoting conditions was examined separately for major histologic subtypes of ovarian cancer (Table 3). The inverse association was most evident for endometrioid cancers followed by undifferentiated and then invasive serous cancers. Numbers were too limited to make any definitive comments about predictors of antibodies among the 48 cases with preoperative bloods in whom anti-MUC1 antibodies were measured.

Discussion

To date, this is the largest study to examine determinants of anti-MUC1 antibodies and the first to show that conditions that generally increase the likelihood of having antibodies decrease the risk for ovarian cancer. MUC1 is normally present in a glycosylated, membrane-bound form on the apical surface of most polarized epithelial cells of the respiratory, genitourinary, and digestive tracts as well as breast ducts (12). With malignant transformation, epithelial cells lose polarity and overexpress MUC1 on their entire cell surface. A soluble, underglycosylated form circulates in cancer patients, thus becoming available for recognition by the immune system (6, 13). Some healthy women and men also have detectable MUC1 (albeit much lower levels) as well as anti-MUC1 antibodies. In women mostly ages 50 to 70 years, McGuckin et al. assessed the presence of circulating MUC1 using the cancer-associated serum antigen

assay. Cancer-associated serum antigen concentrations were elevated in smokers and increased progressively with age (14). In a sample of women from the same study, Richards et al. then measured anti-MUC1 antibodies and found that virtually all women less than age 40 had antibodies and this percentage declined with age (4), somewhat similar to the pattern we observed. It is well established that women have MUC1 and anti-MUC1 antibodies during pregnancy and breast-feeding, presumably due to changes within the breast or uterus that alter MUC1 expression, glycosylation, or shedding (4, 15, 16). In addition, Hinoda et al. observed antibodies specific for the peptide backbone of MUC1 in patients with ulcerative colitis and speculated that inflammation may change MUC1 glycosylation and enhance its immunogenicity (17). One difficulty in evaluating these studies is that assays both for MUC1 and anti-MUC1 antibodies may differ. In measuring antibodies, assays will vary by the specific epitope of MUC1 and the secondary immunoglobulin antibody used. The assay in our study is based on the peptide backbone of MUC1 that we believe is closer to tumor MUC1 and we assessed total immunoglobulin levels including all isotypes, IgG, IgM, and IgA.

In our data, anti-MUC1 antibodies were associated with events affecting the reproductive tract, whose epithelia heavily express MUC1 (18). Injury and/or inflammation of these tissues, surgery, and other events might allow enhancement of MUC1 expression, spillage into circulation, and presentation to the immune system. Thus, the mechanism by which tubal sterilization reduces ovarian cancer risk, previously attributed to preventing substances like talc or endometrial cells from reaching the ovaries (19, 20), may include production of protective antibodies. In our data, cervical conization involving injury and repair of endocervical tissue was also associated with a nonsignificant increase in antibody formation and decrease in risk for

ovarian cancer. Antibody formation was also directly correlated with number of cesarean sections, which involve incision and repair of the uterine wall and endometrium. Endometrial expression of MUC1 might also be affected by IUD use, as suggested by biopsies showing a low-grade, chronic inflammation with enhanced mucin staining (21). We found that IUD use increased the likelihood of antibodies in the “low”range and significantly decreased the risk for ovarian cancer. This is the first study to identify an inverse association between ovarian cancer and IUD use, whereas there is considerably more evidence that IUD use reduces risk for endometrial cancer (22), another tumor with high MUC1 expression (23).

An increased likelihood of MUC1-specific antibodies in the “high” range was found in women reporting bone fracture or a diagnosis of osteoporosis. Both conditions are known to be associated with high interleukin 6 levels (24, 25), an important regulatory cytokine for MUC1 expression (26). Furthermore, a bone fracture might be associated with release of hemato-poetic precursors into the circulation, some of which may express MUC1 and be immunogenic (27). We also found an inverse association between bone fracture/osteoporosis and ovarian cancer risk, which to our knowledge has not been shown previously. Interestingly, bone fracture is associated with reduced endometrial and breast cancer risk (28). Whereas this may simply reflect low estrogen, an influence of anti-MUC1 antibodies should also be considered. Besides bone fracture and IUD use, a third factor, which may link the etiology of ovarian and endometrial cancer, is smoking. A decreased risk for endometrial cancer is found in smokers, especially current smokers (29, 30). The data are less clear for ovarian cancer with two recent studies suggesting that smoking may increase the risk only for mucinous histologic subtypes (31, 32). Although current smoking was not clearly related to either anti-MUC1 antibody development or ovarian cancer

risk in our univariate analyses, it did improve the cumulative index models in Tables 1 and 2. Furthermore, McGuckin's observation that smokers have higher serum MUC1 levels (presumably from damaged lung epithelium) provides a basis for linking current smoking to anti-MUC1 antibody production (14).

Table 2
Adjusted risk of ovarian cancer by epidemiologic variables in ovarian cancer cases and controls

* * *

Figure 2
Likelihood of anit-MUC1 antibodies by index of number of conditions and risk for ovarian cancer by same index

* * *

OC use is a strong protective factor for ovarian (and endometrial) cancer and also seemed to generate anti-MUC1 antibodies, particularly among premenopausal women. CA15-3 (MUC1) levels in saliva were found to be 75% higher in OC users compared with nonusers, a nonsignificant difference in that small study (33). Other studies suggest that MUC1 expression in the endometrium is progesterone dependent (34) and up-regulated by exogenous progesterone (35). Considered together, these observations support the speculation that OC users may have higher MUC1 levels that could translate into higher antibody production.

History of mastitis was associated with both increased anti-MUC1 antibodies and decreased ovarian cancer risk in our study. We believe this is an important finding in light of our previous report of a long-term breast cancer survivor in whom MUC1-specific antibody production and mucin-specific T lymphocytes became elevated following mastitis in pregnancy (36). The lactating breast secretes a form of MUC1 that is similar to the underglycosylated form of MUC1 produced by tumors. Thus, mastitis may lead to a potent anti-MUC1 and antitumor immune response, which

could explain the substantial decreased risk for ovarian cancer associated with mastitis found in our current study.

Curiously, we found that use of talc in the genital area was associated with significantly decreased levels of anti-MUC1 antibodies. Use of talc in the genital area would expose at least lower genital tract epithelia to talc and conceivably affect MUC1 expression in these tissues. In serial assays of pleural fluid in patients who received talc pleurodesis, inflammatory mediators eventually became depressed (37). Use of talc in the genital area has been consistently found to increase the risk for ovarian cancer in several meta-analyses (38-40). However, some investigators have challenged the association because of uncertainty about its biological basis and the absence of a dose-response relationship (38, 40). Although our present finding may also meet with skepticism, a testable hypothesis is now suggested by the possible link between genital talc exposure and systemic diminution of anti-MUC1 antibodies.

Existing theories of ovarian cancer pathogenesis have invoked incessant ovulation, gonadotropin excess, androgen excess, progesterone deficiency, or deleterious effects of inflammation to explain risk factors for ovarian cancer (41-44). Our findings offer an additional perspective on how OC use, tubal sterilization, and even talc use might exert their effects on ovarian cancer risk and suggests an entirely new set of protective factors such as mastitis, IUD use, and bone fracture that might be explained by the same immune-mediated mechanism. Interestingly, this mechanism may also explain the decreased risk for ovarian cancer associated with mumps parotitis noted in older studies conducted before the widespread use of vaccination (45, 46). Analogous to mastitis, infection of MUC1-rich salivary glands might also lead to an anti-MUC1 immune response and antibody production. Clearly, we have not explained all features of ovarian cancer including the “dose-related” decrease in risk associated with multiple pregnancies and length of breast-

feeding. Based on the studies reporting anti-MUC1 antibodies in women currently pregnant or breast-feeding, we had expected, but did not observe, that antibodies would increase with the more pregnancies a woman had or the longer she breast-fed. However, it should also be appreciated that anti-MUC1 antibodies are just one of several immune-effecter mechanisms that may also include helper and cytotoxic MUC1-specific T cells that are generated by MUC1 presentation to the immune system. Indeed, the reactions described in sera and T cells from multiparous women suggest that a complete picture of the link between ovarian cancer risk and MUC1 immunity will require assessment of cell-mediated reactions. In addition, immunity to other human mucins, including MUC16 (CA 125), may also need to be examined.

The principal limitation of our study comes from its case-control design. Exposure information was collected by self-report after the diagnosis in cases, introducing the possibility of misclassification. More importantly, we were unable to directly compare anti-MUC1 antibody levels in cases and controls and directly calculate odds ratios based on antibody levels because the cancer itself leads to production of antibodies. Consequently, assessing antibodies in cases after the diagnosis is not useful for identifying earlier events that influenced antibody generation or the predictive value of such antibodies. Prospective studies, in which blood samples are obtained decades or years before the development of ovarian cancer, will be necessary to assess directly the predictive value of anti-MUC1 antibodies on ovarian cancer risk. In addition, prospective studies before and after events like tubal sterilization, IUD use, mastitis, etc. that document the precise changes in the status of anti-MUC1 antibodies will refine our “cumulative index model” with its crude assumption that all events might be of equal potency in ability to generate antibodies. Thus, we make no claim this model is final but rather represents a simple foundation for a

paradigm shift that will incorporate MUC1 immunity as a key mechanism through which many risk factors for ovarian cancer may exert their influence.

Table 3
Adjusted risk, 95% confidence intervals, and trends for ovarian cancer of different histologic types associated with number of conditions predisposing to MUC1 antibodies

* * *

In summary, evidence from this case-control study of ovarian cancer suggests that events predicting the presence of anti-MUC1 antibodies are inversely associated with ovarian cancer risk and that the more conditions a woman experienced to raise antibodies the lower is her risk for ovarian cancer. We believe these data support the immune response as one mechanism of action of “traditional” ovarian cancer risk factors such as OC use and tubal sterilization, as well as novel ones observed in this study including mastitis, bone fracture, and IUD use. If, as we would like to propose, the immune response is a major mechanism, the implications are profound because it may eventually offer new avenues for ovarian cancer prevention through vaccines to stimulate immunity against MUC1 and perhaps other antigens expressed in ovarian cancer. Much work would need to be done, including prospective documentation of the precise changes in cell-mediated and humoral responses to MUC1 associated with pregnancy, breast-feeding, mastitis, and other events. Such studies may have implications beyond ovarian cancer and apply to other cancers with high MUC1 expression including endometrial and breast cancer.

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APPENDIX AA

INTRAUTERINE DEVICE USE, CERVICAL INFECTION WITH HUMAN PAPILLOMAVIRUS, AND RISK OF CERVICAL CANCER: A POOLED ANALYSIS OF 26 EPIDEMIOLOGICAL STUDIES

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Summary

Background Intrauterine device (IUD) use has been shown to reduce the risk of endometrial cancer, but little is known about its association with cervical cancer risk. We assessed whether IUD use affects cervical human papillomavirus (HPV) infection and the risk of developing cervical cancer.

Methods We did a pooled analysis of individual data from two large studies by the International Agency for Research on Cancer and Institut Catala d'Oncologia research programme on HPV and cervical cancer; one study induded data from ten case—control studies of cervical cancer done in eight countries, and the other induded data from 16 HPV prevalence surveys of women from the general population in 14 countries. 2205 women with cervical cancer and 2214 matched control women without cervical cancer were induded from the case—control studies, and 15 272 healthy women from the HPV surveys. Information on IUD use was obtained by personal interview. HPV DNA was tested by PCR-based assays. Odds ratios and 95% CIs were estimated using multivariate unconditional logistic regression for the associations between IUD use, cervical HPV DNA, and cervical cancer.

Findings After adjusting for relevant covariates, induding cervical HPV DNA and number of previous Papanicolaou smears, a strong inverse association was found between ever use of IUDs and cervical cancer (odds ratio 0.55, 95% CI 0.42-0.70; $p<0.0001$). A protective association was noted for squamous-cell carcinoma (0.56, 0.43-0.72; $p<0.0001$), adenocarcinoma and adenosquamous carcinoma (0.46, 0.22-0.97; $p=0.035$), but not among HPV-positive women (0.68,0.44-1.06; $p=0.11$). No association was found between IUD use and detection of cervical HPV DNA among women without cervical cancer.

Interpretation Our data suggest that IUD use might act as a protective cofactor in cervical carcinogenesis. Cellular

immunity triggered by the device might be one of several mechanisms that could explain our findings.

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Introduction

Epidemiological studies have consistently shown that intrauterine device (IUD) use reduces the risk of endometrial cancer.[1-4] However, the question of whether IUDs might also affect the risk of cervical cancer remains unanswered. Clinical and epidemiological studies done in several countries have reported inconsistent results,[3,5,6] and none of these studies accounted for human papillomavirus (HPV) status in their analyses. Since HPV is now firmly established as the cause of cervical cancer, HPV should be considered when exploring the potential effects of IUD use on cervical cancer risk, and the association between IU exposure and cervical HPV infection should be assessed.

During the past 20 years, the International Agency for Research on Cancer (IARC; Lyon, France), in collaboration with the Institut Català d'Oncologia (ICO; Barcelona, Spain), has done several large epidemiological studies on HPV and cervical cancer in different countries. We analysed pooled individual data from the IAR programme to explore the potential effects of IUD use on the risk of cervical HPV infection in healthy women, and on the risk of developing cervical cancer.

Methods

Patients

Women included in these analyses were recruited from two large series by the IARC and ICO programmes on HPV and cervical cancer: a series of HPV prevalence surveys, and a series of case-control studies of HPV and cervical cancer.

Procedures

A series of population-based HPV prevalence surveys was done by IARC in 15 areas in four continents between 1993 and 2007. Methods of population sampling have been described previously for the individual areas: Hanoi and Ho Chi Minh City, Vietnam; Lampang and Songkla, Thailand; South Korea; Shanxi, Shenzhen, and Shenyang, China; Mongolia; Mexico; Argentina; Colombia; Chile; Nigeria; Spain; and Poland.[7-2°] Briefly, in each area an attempt was made to obtain a random age-stratified sample of the population that induded at least 100 women in each 5-year age group, from 15-19 years to 65 years and older. Participation ranged from 48% in Songkla, Thailand, to 96% in Colombia. Trained interviewers questioned study participants face-to-face with a standardised questionnaire that induded information on IUD use and duration. Study participants had a pelvic examination during which samples of exfoliated cells from the cervix were obtained for cytology and HPV testing. All participants gave written informed consent according to the recommendations of IARC and the local ethical review committees.

Table 1
Characteristics of Participants Included in IARC HPV Surveys, by HPV Status

* * *

From 1985 to 1997, 13 case-control studies of cervical cancer were done in 11 countries with a broad range in the incidence of cervical cancer. Regions covered induded Africa (Algeria, Morocco, and Mali), South America (Brazil,

Paraguay, Peru, and Colombia), southeast Asia (India, Thailand, and the Philippines), and Europe (Spain)2[1-28] Studies from Brazil, Paraguay, and Mali were exduded from the pooled analyses because they did not contribute information on IUD use. Case patients were women with incident, histologically confirmed, invasive squamous-cell carcinoma, adenocarcinoma, or adenosquamous carcinoma of the cervix. Control patients were hospital-based or clinic-based and were frequency matched to case patients by 5-year age groups, in all studies except in Colombia and Spain, where random population-based controls were used for the invasive cervical cancer cases. All study participants were interviewed using a standardised questionnaire to elicit information on potential risk factors for cervical cancer, including IUD use and duration. All women had a pelvic examination and two cervical scrapes were obtained for cytology and HPV-DNA detection. A tumour biopsy was also taken from case patients and kept frozen. All protocols were approved by IARC and local ethics committees. All participants gave written informed consent.

The detailed protocol for detection of HPV DNA by PCR in cervical specimens obtained in the case-control studies has already been published.[21-28] Briefly, L1 consensus primers MY09-MY11, as modified by Hildesheim and colleagues,[29] were used in the Colombia and Spain studies, and GPS+/6+ general primers in the remaining studies. PCR products were assessed for HPV positivity using a cocktail of HPV-specific probes and were further genotyped by hybridisation of the PCR products with type-specific probes for 33 HPV types.[30,31]

For all HPV surveys, apart from the one in Mexico, cervical cells were tested with general GPS+/6+ primer-mediated PCR.[30] PCR products were tested using low-stringency Southern blot analysis of PCR products with a cocktail probe of HPV-specific DNA fragments. Typing of samples positive for HPV was done by enzyme

immunoassay or reverse line-blot analysis of GPS+/6+ PCR products using HPV type-specific oligoprobes for 36 HPV types.[30,32] The oligoprobe cocktail was extended to indude HPV types 30, 32, 64, 67, 69, cand85, 86, and JC9710 in the most recent HPV surveys done in Chile, Poland, Mongolia, and China (Shanxi, Shenzhen, and Shenyang). HPV testing and genotyping of samples collected in the Mexican HPV survey was done as previously described,[14] using biotinylated MY09/11 consensus primers and a single-hybridisation, reverse line-blot detection method.[33]

Figure 1
Adjusted odds ratios* for the association between IUD use and cervical HPV-DNA detection in IARC HPV prevalence surveys.

* * *

Statistical analysis

Unconditional logistic regression models were used to estimate odds ratios (ORs) and 95% CIs for associations between IUD use and both cervical HPV and cervical cancer. We did three analyses. The main analysis explored the association between IUD use and cervical cancer risk overall, by country, histology, years of use, and categories of reproductive and behavioural covariates possibly related to cervical cancer risk. We also estimated the association between IUD use and cervical HPV DNA among control women enrolled in the case-control study. Finally, we explored the association between IUD use and cervical HPV DNA among women enrolled in the HPV prevalence surveys.

Unless otherwise specified, all logistic regression models using the case-control data were adjusted by study area, age in tertiles (18-42, 43-53, ≥54 years), years of schooling in quartiles (0, 1-4, 5-9, ≥10), age at first sexual intercourse in quartiles (≥23, 20-22, 18-19, ≥17 years), number of previous screening Pap smears the woman had until 12 months before

enrolment in the study (0, 2-5, ≥6), and cervical HPV-DNA status. Logistic regression models using data from the HPV prevalence surveys were adjusted for study area, age group (≤24, 25-34, 35-44, 45-54, ≥55 years), years of schooling (0, 1-5, 6-10, ≥11), lifetime number of sexual partners (0-1, 2, ≥3) and Pap history (number of Paps unless otherwise specified: 0, 1, 2-4, .5). Heterogeneity in OR between study areas was tested using the likelihood ratio test for interaction between the study area and exposure of interest.

Statistical analyses were done with SAS version 9.2 and the computing environment R, version 2.12.0. Graphs were created using the plot.meta function of the R software.

Figure 2
Adjusted odds ratios* for the association between IUD use and cervical cancer in IARC case-control studies.

* * *

Role of the funding source

The funding institutions of the studies included in this pooled analysis had no role in the design and conduct of the study; in the collection, management, analysis, and interpretation of the data; in the writing, review, and approval of the manuscript; or in the decision to submit the report for publication. The corresponding author had full access to all the data and the final responsibility to submit for publication.

Results

Table 1 summarises the main characteristics of participants recruited from the IARC HPV prevalence surveys, according to HPV status, and table 2 summarises characteristics of participants recruited from the IARC case-control studies, according to cervical cancer status.

The original series of HPV prevalence surveys induded 13 924 HPV-negative and 2556 HPV-positive women from 16 studies, of whom 745 (5.4%) and 463 (18.1%), respectively,

were exduded because of missing information on IUD use. A total of 13 179 HPV-negative and 2093 HPV-positive women were induded in the final pooled analysis. Compared with HPV-negative women, women who tested positive for HPV were younger, had a lower educational level, fewer pregnancies, fewer screening Pap smears, more sexual partners, more exposure to cigarette smoking, and an earlier age at sexual debut. Overall, 4.7% (721 of 15 272) of the recruited women had an abnormal result in the cytological sample obtained for the study, ranging from 0.7% (seven of 994) in Hanoi, Vietnam, to 13.1% (127 of 969) in Mongolia (data not shown).

Figure 3
Adjusted odds ratios* for the association between IUD use and cervical cancer, by years of use in IARC case-control studies.

* * *

The original series of case-control studies included 2905 cases and 2906 controls from 11 studies. Women from Brazil, Mali, and Paraguay were excluded because they did not contribute information on IUD use, leaving 2508 cases and 2483 controls. A total of 2205 cases with cervical cancer and 2214 control women with information on IUD use were included in the final pooled analysis.

***Figure* 4**
Adjusted odds ratios* for the association between IUD use and cervical cancer, by strata of selected variables in IARC case-control studies

* * *

The percentage of women with unknown IUD use status was similar between cases and controls (12.1% [303 of 2508] *vs* 10.8% [269 of 2483]). By contrast, the percentage of women with unknown IUD use was somewhat higher among HPV-positive than among HPV-negative women (14.4% [301 of 2094] vs 9.7% [182 of 1882]), although the

corresponding 95% CIs greatly overlapped (webappendix p 2). Women with cervical cancer were more likely than control women to be single, divorced, or widowed, to have a lower educational level, more pregnancies, higher number of lifetime sexual partners, fewer screening Pap smears, and a younger age of sexual debut.

The potential effect of IUD use on cervical HPV infection was assessed in two groups: among control women recruited in the case-control studies and among women recruited in the HPV prevalence surveys. No association was found between IUD use and cervical HPV-DNA detection among control women in the case-control studies (OR 0.95, 95% CI 0.64-1.39); webappendix p 4 shows the ORs by country and years of IUD use. Further analyses stratified by potential risk factors and cofactors did not show any relevant associations across subgroups of age, education, menopausal status, number of sexual partners, number of pregnancies, use of hormonal oral contraception, and number of previous screening Pap smears within 12 months before study enrolment (data not shown).

Figure 1 shows the ORs for the association between IUD use and cervical HPV-DNA detection in the IARC HPV prevalence surveys, overall, and by study area and years of use. Although there is some significant heterogeneity between studies, none of the 16 surveys yielded a significant association between IUD use and cervical HPV. The overall combined adjusted OR was very dose to unity and not significant (OR 0.96, 95% CI 0.85-1.08; p=0.47). As shown in figure 1, years of IUD use was not associated with risk of cervical HPV. Further analyses stratified by selected characteristics did not show any significant associations in any of the subgroups explored (data not shown).

The potential effect of IUD use on cervical cancer risk was assessed in women enrolled in the case-control studies. Figure 2 shows data on IUD prevalence and ORs for cervical cancer overall, by country, and by cancer histology. The

combined prevalence of IUD use was 13.0% among women with cervical cancer and 22.5% among control women. Inverse associations between IUD use and cervical cancer risk were found for all study areas except Morocco. Inverse associations were dearly or borderline significant, apart from in Thailand, the Philippines, and India. After adjusting for relevant covariates, a strong and significant inverse association was found between ever use of an IUD and cervical cancer risk for all cervical cancers combined (OR 0.55, 95% CI 0.42-0.70; p<0.0001), and for each of the two histological groups: squamous-cell carcinoma (OR 0.56, 0.43-0.72; p<0.0001) and combined adenocarcinoma and adenosquamous carcinomas (OR 0.46, 0.22-0.97; p=0.035; figure 2). These estimates were not substantially altered when adjusting for finer age categories (ie, 18-24,35-42, 43-53, z54 years) instead of tertiles (data not sown).

Figure 3 shows the relationship between years of IUD use and cervical cancer. Compared with never users, the risk was reduced nearly by half in the first year of use (OR 0.53, 95% CI 0.27-1.02) and was maintained with longer durations of use. The formal test for linear trend with years of use was not significant (p=0.69).

To address further the potential effect of residual confounding we did a stratified analysis to assess the association between IUD use and cervical cancer risk within subcategories of selected covariates known to be potential confounders or cofactors in cervical carcinogenesis. These stratified analyses showed a consistent inverse association between cervical cancer and IUD use within each category of age, education, marital status, number of screening Paps, number of sexual partners, parity (except in nulliparous women), among premenopausal (but not postmenopausal) women, and in HPV-positive women (figure 4). The ORs in the younger age categories were 0.16 (95% CI 0.02-1.12) and 0.51 (0.51-0.37) for the 18-24 and 25-42 years age groups, respectively. The OR among HPV-negative women (0.44;

0.26-0.74) was similar to that among HPV-positive women (0.68; 0.44-1.06) and to that among women with unknown HPV status (0.46; 0.30-0.69). An inverse association was also seen among ever users (0.62, 0.39-0.98) and never users of oral contraceptives (0.50; 0.29-0.84), and among short-term (<2 years) users (0.79, 0.29-2.16) and long-term (<10 years) users of oral contraceptives (0.23, 0.09-0.62; webappendix table 2). The percentage of oral contraceptive users was somewhat higher among IUD users than in non-users, in cases (71.0% [191 of 269] *vs* 50.6% [736 of 1455], respectively) and in controls (66.4% [303 of 456] vs 49.8% [629 of 1262], respectively; webappendix p 3). Finally, condom use did not modify the inverse association found between IUD use and cervical cancer risk, among women who never or rarely used condoms (0.59, 0.44-0.79), and among women who regularly or always used condoms (0.55, 0.29-1.05; p for interaction 0.76).

Discussion

Several studies show that contraceptive methods such as oral contraceptives and condom use can affect the risk of cervical cancer[34,35] and cervical HPV infection,[36] respectively. Use of contraceptive IUDs has consistently been shown to reduce the risk of endometrial cancer;" however, little is known about the potential effects of IUD use on the risk of developing cervical cancer or cervical HPV infection. To our knowledge, this is the first large epidemiological study, with almost 20000 women induded, to explore such potential associations taking into account cervical HPV status and Pap screening history.

We found a strong and consistent inverse association between IUD use and cervical cancer risk; women who reported previous IUD use had half the risk of developing cervical cancer compared with women with no history of IUD use. An inverse association was detected for the two major cervical cancer histological types, squamous-cell carcinoma and adenocarcinoma or adenosquamous

carcinoma, as well as in most of the subgroups explored, although many were not significant. The lack of association among postmenopausal women is puzzling, but might be due to the fact that IUD exposure history was low: less than 10% of postmenopausal women reported having ever used an IUD. They were also substantially older and with a lower parity than the premenopausal women (data not shown).

The inverse association between IUD use and cervical cancer risk was not significantly affected by duration of use: an association was found within 1 year of use and it remained significant even after 10 years of use, but did not significantly increase or wane with increasing years of use. By contrast, neither the analysis among the 2214 control women from the case—control studies nor among the 15 272 women recruited in the international HPV surveys identified an association between IUD status or years of use and cervical HPV infection, as assessed by PCR methods. The lack of association between IUD use and cervical HPV was generally consistent across studies and among the covariates explored (data not shown).

Although the hypothesis that IUD use might promote cervical cancer has been considered since the introduction of these devices in 1930s, studies are inconclusive. A large multicentre case—control study in the USA found a non-significant reduced risk of cervical cancer associated with copper IUD use (adjusted OR 0.6, 95% CI 0.3-1.2), but almost no effect was found for the inert IUD (1.1; 0.9-1.7). Decreased risk with increased duration of copper IUD use supported a possible protective effect for copper IUDs on development of invasive cervical cancer.' By contrast, a 2007 review that included four case—control studies did not find an association between IUD use and cervical cancer risk.[3]

Overall, the associations found in our study strongly suggest that IUD use does not modify the likelihood of prevalent HPV infection, but might affect the likelihood of

HPV progression to cervical cancer. Thus, IUD use could possibly be regarded as a protective cofactor in cervical carcinogenesis. One of the mechanisms by which IUDs might exert this protective effect is through the induction of a reactive, chronic, low-grade, sterile inflammatory response in the endometrium, endocervical canal, and cervix that could modify, via changes in the local mucosal immune status, the course of HPV infections. Microscopic observation of typical cellular changes in the cervices of IUD users support this theory?' It is possible that these IUD-related subjacent mechanisms induce an immune deviation with a Th1 type of biased immune response, which might affect IUD users' risk of HPV persistence, progression to cervical cancer, or both. Also, for hormonal IUDs, release of progestins or progesterone into the uterus might affect the natural history of HPV infection. Unfortunately, information on IUD type was not obtained in any of the studies, preduding our assessment of the effect of copper IUDs and hormone-releasing IUDs on cervical cancer risk or cervical HPV DNA.

Alternatively, it can be postulated that the local trauma to the cervical tissue associated with insertion or removal of the device induces local small foci of chronic inflammation and a long lasting immune response similar to that noted in patients after colposcopically guided punch biopsies. This alternative hypothesis would explain better the immediate protective effect found for short-term users, and the observation that there was no difference in the protective effect by years of IUD use.

Another possible explanation for the protective effect of IUDs against cervical cancer is elimination of preinvasive cervical lesions when the device is inserted or removed. This hypothesis would help explain the lack of effect with duration of IUD use. More importantly, removal of preinvasive cervical lesions is compatible with some of our subgroup findings—ie, the strongest protective effect was in

women 37-45 years, among whom preinvasive cervical lesions might have already accumulated in inadequately screened populations but not yet progressed to invasive cancer. These possible mechanisms are speculative and provocative, but emphasise our limited knowledge and the need for other study designs to explore the underlying mechanisms by which IUDs might exert a protective effect on cervical cancer risk.

We also attempted to assess whether the protective effect on cervical cancer risk was driven by reduced persistent infection, as opposed to reduced progression to cervical intraepithelial neoplasia. We assumed that, by contrast with younger women, a substantial proportion of HPV infections detected in older women were more likely to be persistent rather than transient. If IUDs reduce the persistence of HPV, we should find a larger inverse association in older than in younger women. However, our analysis showed that the OR for association between IUD use and cervical HPV infection was exactly the same in women younger (OR 0.99, 95% CI 0 83-1.19) and older (OR 0.95, 0.81-1.11) than 35 years.

An important challenge in interpreting these results is to assess the possible effect of screening bias, induced by IUD use, on explaining the inverse association with cervical cancer risk. Insertion, follow-up, and removal of IUDs are often done in adult, parous women. In developed countries, these procedures involve several visits to the gynaecologist, providing many opportunities for these women to be directly diagnosed or screened for cervical cancer, through visual identification or repeated cervical cytology. Therefore, the reduced risk of cervical cancer seen in IUD users might not be due to the biological effect of the device, but rather to the higher likelihood of more intensive cervical screening or diagnosis in these women compared with non-users. To address whether IUD-induced screening bias had a confounding effect on the observed results, we estimated associations by specific strata of number of previous Pap

smears women had until 12 months before diagnosis or study entry. As shown in figure 4, an inverse association was consistent among women who never had a screening Pap smear (OR 0.62), and those who had one (OR 0.64), two to five (OR 0.45), and six or more Pap smears (OR 0.48). Thus, history of previous Pap smears did not significantly affect the observed inverse association between IUD use and risk of cervical cancer. Furthermore, since most of the populations included in these analyses are from developing areas of the world, where screening is opportunistic and has little effect in preventing cervical cancer, it is unlikely that screening bias would explain the observed inverse association.

Finally, information bias regarding self-reporting of IUD use and other covariates might also have had a confounding role in the observed associations. This bias is inherent to all epidemiological studies that rely on data collected through a questionnaire or interview. However, since the hypothesis that IUD use might affect HPV infection or cervical cancer risk was unknown to all study participants, it is unlikely that IUD-use misclassification was differential with regard to case—control status or HPV status, the latter being impossible because HPV status was unknown to the participants and interviewers. It is well established that non-differential misclassification of the exposure of interest (ie, IUD use) can attenuate the real OR, but it can never artificially increase it. Thus, the most likely effect of this potential bias on our study would be an underestimation of the true underlying effect.

In conclusion, our data suggest that use of IUDs substantially reduces the risk of cervical cancer and that this effect does not seem to be due to differences in screening histories between users and non-users. By contrast, IUD use is not associated with risk of cervical HPV infection, suggesting that the presence of the device does not affect HPV acquisition and detection in the exfoliated cells of the

cervix. We postulated that repeated microtrauma and subsequent chronic mucosal inflammation processes induced by the device might be the underlying mechanism through which IUDs can reduce the risk of cervical HPV progression, consequently reducing the risk of cervical cancer. Alternatively, even though our stratified analyses do not support this possibility, we cannot totally rule out the potential effects of residual confounding, and screening and diagnosis bias. In view of the wide use of IUDs worldwide, women, gynaecologists, and reproductive-health professionals can be reassured that IUDs do not seem to increase the risk of cervical HPV infection; and our study contributes solid evidence that IUD use might even reduce the risk of developing cervical cancer.

Contributors

XC, NM, SdS, RH, SF, CJLMM, and FXB were responsible for the conception, design, and supervision of the study. NM, SS, RH, SF, CJLMM, and FXB were responsible for data acquisition and obtaining funding. XC drafted the report. XC, MD, and SV did the statistical analysis. All authors analysed and interpreted data, revised the report, and provided administrative, technical, and material support.

Conflicts of interest

We declare that we have no conflicts of interest.

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APPENDIX BB

ORIGINAL RESEARCH ARTICLE
IMPACT OF THE FEDERAL CONTRACEPTIVE COVERAGE GUARANTEE ON OUT-OF-POCKET PAYMENTS FOR CONTRACEPTIVES: 2014 UPDATE[◊,◊◊]

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Abstract

Background: The Affordable Care Act requires most private health plans to cover contraceptive methods, services and counseling, without any out-of-pocket costs to patients; that requirement took effect for millions of Americans in January 2013.

Study design: Data for this study come from a subset of the 1842 women aged 18–39 years who responded to all four waves of a national longitudinal survey. This analysis focuses on the 892 women who had private health insurance

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and who used a prescription contraceptive method during any of the four study periods. Women were asked about the amount they paid out of pocket in an average month for their method of choice.

Results: Between fall 2012 and spring 2014, the proportion of privately insured women paying zero dollars out of pocket for oral contraceptives increased substantially, from 15% to 67%. Similar changes occurred among privately insured women using injectable contraception, the vaginal ring and the intrauterine device.

Conclusions: The implementation of the federal contraceptive coverage requirement appears to have had a notable impact on the out-of-pocket costs paid by privately insured women, and that impact has increased over time.

Implications: This study measures the out-of-pocket costs for women with private insurance prior to the federal contraceptive coverage requirement and after it took effect; in doing so, it highlights areas of progress in eliminating these costs.

Keywords: Contraception; Oral contraceptive pills; Insurance; Health reform; Out-of-pocket costs

1. Introduction

One high-profile provision of the Affordable Care Act is a requirement that private health plans cover contraceptive methods, services and counseling for women, without any copayments, deductibles or other patient out-of-pocket costs [1]. This federal contraceptive coverage guarantee part of a broader provision requiring coverage without cost sharing for dozens of recommended preventive care services was phased in starting in August 2012 and began affecting health plans widely in January 2013.

Even before that requirement took effect, coverage of a wide range of contraceptive methods was standard in U.S. private health plans [2]. Where the federal requirement broke new ground, at least for private health plans, was in its

prohibition on patient cost sharing. That change brought with it the potential to eliminate cost as a reason for choosing one method of contraception over another, a change that could be particularly important for low-income women and women considering methods with substantial upfront costs.

This report provides new, national-level data about the reach and impact of the contraceptive coverage requirement. It utilizes information collected from a longitudinal survey of women, comparing women's responses in fall 2012, before the contraceptive coverage requirement would have taken effect for most women, with their responses to three subsequent rounds of the survey (at 6-month intervals) that were fielded after the requirement was implemented for millions.

An earlier analysis, using just the first two waves of this survey (fall 2012 and spring 2013), was published in December 2013 and found substantial increases in the proportions of privately insured women paying zero dollars out of pocket for oral contraceptives and the vaginal ring over just the first few months of the federal guarantee [3]. An April 2014 report from the IMS Institute for Healthcare Informatics found similar trends and estimated that women saved nearly half a billion dollars in out-of-pocket costs for contraception in 2013 in the wake of the guarantee [4]. Our report provides more up-to-date information to bolster this body of knowledge.

2. Materials and Methods

Data for this analysis come from all four waves of the Guttmacher Institute's Continuity and Change in Contraceptive Use Study, which surveyed women about their contraceptive use repeatedly over an 18-month time period. This analysis is based on the methodology used for the Guttmacher Institute's first analysis described above [3]. More details on the methodology can be found in that article, but we provide a brief description below.

The survey was administered online to a national sample of women aged 18–39 years. It was administered by the market research firm GfK using their KnowledgePanel, a national household panel recruited using a probability-based methodology.

The survey was conducted over 3-week periods in fall 2012, spring 2013, fall 2013 and spring 2014. Of the 4634 women who participated in the baseline study, 3207 participated at Wave 2, 2398 participated at Wave 3 and 1842 participated at Wave 4, resulting in between-survey response rates of 69%, 75% and 77%, respectively. The sample for the current analysis was limited to women who participated in all four waves of the study or 40% of the baseline sample. The sample used for this analysis was further limited to women who had private health insurance and used a prescription contraceptive method during any of the four study periods (892 women).

In this analysis, we focused on survey questions about out-of-pocket payments for contraception among women who used hormonal methods in the last 30 days or obtained an intrauterine device (IUD) between surveys. We examined the percentage of women who reported paying nothing, as well as the mean and median amounts that women paid for the pill; the number of women paying for methods other than the pill was too small for an analysis of means and medians.

Women who reported that they used the pill, injectable or vaginal ring during the last 30 days were asked how much they paid for the method out of pocket each month. We assessed change over time in cross-tabulations using Rao-Scott–corrected χ^2 tests in order to include as many women as possible in all analyses while also taking into account the clustering of data within individuals. Our focus is change over time, and χ^2 statistics allow us to assess differences across all waves at once rather than whether specific waves are statistically different from each other. Our analysis is based on a total of 1916 observations of pill use, 107

observations of injectable use and 151 observations of ring use as reported by 892 women; some women contributed up to four observations per method, while others only contributed one.

IUD users were only asked about cost the first time they reported use of the method. Because we captured relatively few new IUD users covered by private health insurance in waves two through four (***n***=45), we used ***t*** tests to assess for differences between the proportions who paid nothing for the method at Wave 1 compared to the users at Waves 2, 3 and 4 grouped together. Our analysis is based on 165 IUD users. We did not ask about type of IUD — copper vs. hormonal — and both are grouped together.

The number of users of the patch and implant were too small to be reliable; thus, those methods were excluded from this analysis. Analyses were performed using Stata 13. All findings presented were statistically significant at the $p<.05$ level.

3. Results

Among women who reported using the pill and having private health insurance, the proportion who did not pay anything out of pocket increased from 15% to 67% between Waves 1 and 4 (Fig. 1). The most substantial increase occurred between Wave 1 and Wave 2 (from 15% to 44%[1]), but there was a continuing upward trend over the 18-month time period.

[1] The previously published article in Contraception reported that 40% of pill users paid nothing out of pocket during Wave 2. The difference is because the prior study restricted analyses to women who were privately insured and using the pill at both points in time, while the current study incorporated women who may have experienced changes in insurance coverage or method use. Moreover, respondents included in the earlier analyses who failed to participate in subsequent waves are excluded from the current study.

We conducted a sensitivity analysis that examined changes in out-of-pocket costs when the sample was restricted to women who were privately insured and using the pill during all four waves (n=308, obs=1227). The proportions paying US$0 were virtually the same, 15%, 45%, 57% and 69% (p<.001), respectively (data not shown). In addition, we also examined these changes when the sample was restricted to women who were privately insured and using the pill at both Waves 1 and 4 (***n***=350). The proportions paying US$0 were 16% and 69%, and a paired ***t*** test indicated that the difference was significant at p<.001 (data not shown). Both analyses confirmed the patterns found in analyses using all available observations.

Similar increases in the proportion paying zero dollars out of pocket were observed for injectable contraception users and vaginal ring users with private insurance. For injectable users, the proportion increased from 27% to 59% between Wave 1 and Wave 4. For ring users, it increased from 20% to 74% over the same time period.

Among IUD users with private health insurance at Wave 1, 45% indicated that they paid nothing for the method. This increased to 62% among new users in all three subsequent waves combined (data not shown).

Fig. 1
Percent of privately insured women who paid US$0 out of pocket for their method

* * *

Fig. 2
Mean and median out-of-pocket costs for privately insured women using the pill

* * *

Among privately insured women using the pill, the Wave 1 mean out-of-pocket payment was US$14.35 and the median was US$10; by Wave 4, this had declined to US $6.48 and US$0, respectively (Fig. 2).

3.1. Limitations

This study is subject to some limitations. Although our response rates were comparable to those of other studies using online administration, only 40% of the baseline sample participated in all four waves of the study, which compromises the representativeness of the data. The findings might be further biased if our respondents differed from the national population in ways that correlate with contraceptive use. Nonetheless, the data are still useful because they serve as one of the only sources of information about trends in contraceptive copays among the same group of women over time.

Despite the abovementioned concerns, it is reassuring that the findings here are similar to prior published research: The mean (US$14.35) and median (US$10) out-of-pocket payments for the pill in Wave 1 of our study are almost identical with the mean (US$15.13) and median (US$10) out-of-pocket payments from another nationally representative study carried out before the new federal policy took effect [5].

Some 45% of baseline IUD users reported that they had paid US$0 for the method, a higher proportion than reported paying US$0 for the pill, the ring or the injectable at Wave 1. Prior to the contraceptive coverage guarantee, many women had to pay several hundred dollars out of pocket for the IUD. One potential interpretation of the pattern in our data is that many women unable to obtain the method at no cost were unable to afford it at all. That is, prior to coverage guarantee, women may have opted to pay a relatively modest copayment each month for the pill rather than come up with several hundred dollars to cover out-of-pocket costs for the IUD.

4. Discussion

The findings of this study suggest that the federal contraceptive coverage guarantee has had a substantial

impact in eliminating out-of-pocket costs among privately insured women using some methods of contraception including oral contraceptives, the most popular reversible method in the United States. Between fall 2012 and spring 2014, the proportion of pill users paying zero dollars out of pocket increased from 15% to 67%, with similar trends for injectable, ring and IUD users.

Further progress may still be expected as more private health plans become subject to the requirement. Notably, existing plans are grandfathered exempt from the requirement so long as they make no significant negative changes, such as benefit reductions or cost sharing increases. That status is designed to be temporary to allow for a smoother transition to new federal rules, and the number of people enrolled in grandfathered plans has been declining rapidly, from 48% of covered workers in 2012 to 36% in 2013 and 26% in 2014 [6].

However, the proportion of women paying zero dollars will never reach 100%, for several reasons:

- Federal guidance allows insurers to charge copayments in limited situations, such as when a woman chooses a brand-name drug with a generic equivalent or when a woman receives services from an out-of-network provider [7].
- Federal regulations exempt some employer-sponsored health plans sponsored by houses of worship from the contraceptive coverage requirement on religious grounds, [8] and the U.S. Supreme Court's June 2014 decision in *Burwell v. Hobby Lobby* has extended that to certain closely held for-profit employers.

In addition, several other problems may result in women paying out of pocket for contraceptive methods despite the federal guarantee:

- There is evidence that some private health plans are not adequately complying with what the law clearly requires

coverage of "the full range" of contraceptive methods approved by the Food and Drug Administration when prescribed for a woman and are instead denying coverage, requiring cost sharing or otherwise restricting access to specific methods [9].

- Other religiously affiliated nonprofits have been offered an accommodation under which they are supposed to be absolved from involvement in covering contraception, but their employees and family members must still receive that coverage through the insurance company [8]. However, there are serious questions, and a complete dearth of information, about whether and how plans are complying.

Despite these gaps in the reach of the federal guarantee, the findings of this study bode well for the health and well-being of women, couples and families. Government bodies and private-sector experts have long recognized contraceptive services as a vital and effective component of preventive health care, and an extensive body of research shows that contraceptive use helps women avoid unintended pregnancy and improve birth spacing, resulting in substantial health, social and economic benefits [10–12]. By guaranteeing that women have coverage for a wide range of contraceptive choices without cost sharing, the federal requirement may help them overcome financial barriers to choosing a contraceptive method they will be able to use consistently and effectively, thus increasing their likelihood of avoiding unplanned pregnancies.

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APPENDIX CC

ORIGINAL RESEARCH ARTICLE

CHANGES IN OUT-OF-POCKET COSTS FOR HORMONAL IUDS AFTER IMPLEMENTATION OF THE AFFORDABLE CARE ACT: AN ANALYSIS OF INSURANCE BENEFIT INQUIRIES

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Abstract

Background: The Affordable Care Act (ACA) requires that privately insured women can obtain contraceptive services and supplies without cost sharing. This may substantially affect women who prefer an intrauterine device (IUD), a long-acting reversible contraceptive, because of high upfront costs that they would otherwise face. However, imperfect enforcement of and exceptions to this provision could limit its effect. Study design: We analyzed administrative data for 417,221 women whose physicians queried their insurance plans from January 2012 to March 2014 to determine whether each woman had insurance coverage for a hormonal IUD and the extent of that coverage.

Results: In January 2012, 58% of women would have incurred out-of-pocket costs for an IUD, compared to only 13% of women in March 2014. Differentials by age and region virtually dissolved over the period studied, which suggests that the ACA reduced inequality among insured women.

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Conclusions: Our findings suggest that the cost of hormonal IUDs fell to US$0 for most insured women following the implementation of the ACA.

Implications: Financial barriers to one of the most effective methods of contraception fell substantially following the ACA. If more women interested in this method can access it, this may contribute to a decline in unintended pregnancies in the United States.

Keywords: Contraception; Healthcare reform; Out-of-pocket costs; IUD; LARC; Insurance

1. Introduction

In the United States, 43 million women are at risk of unintended pregnancy, and 39 million of them (90%) use contraception [1,2]. Some 30 million (78% of contraceptors) use a method more effective than condoms, and 4 million (10%) use an intrauterine device (IUD), while fewer than a half million use another long-acting reversible method [1,3]. Fewer than 1% of women who use IUDs will become pregnant within a year, in contrast to 18% of women who use condoms to prevent pregnancy, and 9% of women who use the pill [4].

Women who would otherwise prefer the IUD face barriers that can lead them to use less effective contraceptives; these include high upfront costs that can exceed a thousand dollars [5–11]. Greater uptake of the IUD and the implant preceded fewer births in Colorado and fewer abortions in Iowa, and in St. Louis, teenagers provided these methods at no-cost exhibited rates of pregnancy, birth and abortion far lower than the national average [8,12,13].

Insurance mandates may help women to access the contraceptive of their choice. In 1993, 32% of insurers covered the IUD [14]. By 2002, in part because insurance mandates came into effect in many states, this increased to

94% [14]. However, when an insurance company covers a contraceptive, a woman may still incur costs for example, women may incur copayments for the prescription and visits to a doctor's office or clinic.

A provision of the Affordable Care Act (ACA) requires that patients do not face out-of-pocket costs for contraceptive services and supplies at in-network providers. This provision matters particularly in the context of the high upfront costs of an IUD. This ACA mandate phased in starting in August 2012, and it took effect for many health insurance plans in January 2013. This may improve the ability of millions of women to afford safe and effective contraception [15].

The ACA can affect insurers exempt from state mandates, whereas states lacked authority over self-funded employer plans. However, other exceptions may limit the effect of the ACA's contraceptive coverage mandate. These include grandfathered insurance plans and the contraceptive exclusion. Grandfathered plans are those that came into being no later than March 2010 and have not seen substantial benefit changes since then [16]. The contraceptive exclusion exempts certain religious employers from the ACA's contraceptive coverage provision. As such, even if insurance companies adhere perfectly to the law, some women covered by private insurance may still have to pay the full cost of the IUD and other contraceptives.

Women interested in an IUD may face a higher financial burden if their insurance plan requires out-of-pocket costs. In addition to the cost of the device itself and the initial doctor's visit, women may also face costs to insert and remove their IUD [5–7,10,17]. In 2002, a year after the hormonal IUD came on the market (complementing the nonhormonal copper IUD, which had been available in the United States since 1988), 94% of insurers covered IUDs, but cost sharing continued to make the IUD unaffordable for many women interested in it [8,9,14].

To understand the impact of the ACA's contraceptive coverage provision on IUD cost sharing, we would need to know what costs women faced before and after the ACA. Unfortunately, the extant literature on IUD cost sharing after the ACA went into effect is limited. One analysis estimated that full coverage increased from 45% to 62% after the ACA, based on data from 165 privately insured women [18]. Data from the National Survey of Family Growth (NSFG), the best available representative survey of women's contraceptive behavior, do not indicate when women obtained their IUDs or how much they paid. Even if the NSFG asked women how much they paid, this information would not provide us the percentage of women seeking an IUD who faced out-of-pocket costs: if cost inhibits IUD uptake, the extant data will under-represent women with higher costs [8–10]. All surveys that measure cost based on women who obtained IUDs share this limitation, as do claims data. Finally, none of these surveys address the effect of the contraceptive exclusion, which exempts certain religious employers from providing full coverage.

To help address these limitations, we analyzed data on insurance inquiries; these show what an insured woman would have paid if she had chosen to obtain an IUD, between January 2012 and March 2014, a period covering the introduction of the ACA's contraceptive coverage provision and its initial implementation for many plans.

2. Materials and methods

2.1. Data

Bayer HealthCare, the manufacturer of the Mirena® and Skyla® IUDs, used by some 3 of 4 million American IUD users [3] offers a voluntary "benefit inquiry" service to healthcare providers to determine the type and extent of a patient's insurance coverage for an IUD and whether the patient's insurance company requires cost sharing. Bayer utilizes an outside benefits-verification contractor and does not obtain the data directly. Within a few days after a

healthcare provider's inquiry, typewritten reports with a narrative summary of coverage are faxed by the contractor to healthcare providers, and details of each benefit inquiry are recorded in the contractor's database[1]. Though healthcare providers can pursue this information independently, they may elect to use this free service to reduce their administrative caseload.

The dataset we obtained contained 444,316 women whose physicians inquired about a Mirena or Skyla IUD between January 2012 and March 2014. Of these, we excluded 27,095 women from the analysis because they were minors (4,577, in order to focus on adults who were likely to have their own insurance), they had no insurance (11,363)[2], a woman's insurer would not reveal benefit information to a third party (10,382), women or their healthcare providers did not completely fill out the form (763), or the healthcare provider canceled the inquiry (10). The resulting number of cases we analyzed was 417,221.

The analysis period includes time both before and after the ACA's key provision regarding contraceptive coverage took effect, which allowed us to study its impact. We hypothesized that there would be a sharp decline in the percentage of women subject to cost sharing in the first quarter of 2013, since patients with existing coverage typically sign up for new plans or renew their insurance at the beginning of a calendar year, and January 2013 was the first new year after the implementation of the ACA's contraceptive coverage provision.

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[1] The data record whether patients were subject to cost sharing, and if so, what the copayment or coinsurance rate was and not what providers charge.

[2] This could arise if, for example, a woman's coverage is not yet active or is no longer active, but the data do not record this. Because our goal was an analysis of insured women's IUD benefits, we excluded these women.

2.2. Methods

We analyzed changes between January 2012 and March 2014 in the percentage of women who would have had out-of-pocket costs for a hormonal IUD. The ACA's contraceptive coverage provision came into effect in August 2012, but did not affect most women until January 2013, as most employer-based insurance plans are typically renewed on January 1.

For 2013 onward (n=231,086), we assessed how these results were affected when taking into account two additional factors that affect cost sharing: copayment for insertion and cost sharing owing to a deductible (data not available in 2012). This may affect our results as, for example, women whose insurers covered the cost of the device might not have interpreted the ACA mandate to apply to services as well as supplies.

We estimated trends for all women by month in whether a woman's insurance coverage required cost sharing. We also estimated trends by quarter for age and region subgroups to examine inequality in coverage before, and after, the ACA came into effect.

In an analysis of a very large dataset, trivial fluctuations can reach statistical significance. It is therefore inappropriate to compare p-values, as, for example, a trivial decline of 0.01%, which might only reflect random fluctuations, may be described as "statistically" significant [19]. Therefore, we highlight the substantive size of change over time[3].

In order to understand how much women who still have costs would be required to pay, we also computed cost estimates at the median and 90th percentiles. A woman's out-of-pocket cost is the sum of a fixed copayment and the product of the IUD's price and her coinsurance rate.

[3] Results of logistic regressions, which compare each month to January 2013 or each quarter to the first quarter of 2013, are available from the authors upon request.

Unfortunately, we do not know the price that a healthcare provider would charge a patient for the IUD. Therefore, for the 13% of women subject to coinsurance, we multiplied their coinsurance rate by the most recent published estimates for Mirena's wholesale price, US$844 [7]. This strategy understates the actual cost because patients may also be required to pay for an initial visit to their healthcare provider and for the device's insertion.

Finally, the dataset indicates whether a woman's coverage was subject to the contraceptive exclusion for religious employers, and we use this to estimate the percentage of women without coverage who would have had coverage if not for this exclusion.

2.3. Sensitivity analyses

Of the women in our data, 50,804 have multiple insurers. We do not know their insurers' names or why they have duplicative coverage. We suspect, for example, that some may have private insurance from their employer, as well as secondary insurance from Medicaid or their spouse's employer. In our main analysis, we assumed that women with multiple insurers can choose which insurer to use. They may not have this choice, however[4]. Therefore, we performed a sensitivity analysis in which we assume that a woman with duplicative coverage must use whichever insurer offers the worst coverage.

Fig. 1.
Percentage of women who would have had out-of-pocket costs for a hormonal IUD, by month. Note: The lighter line begins in

[4] We speculate, for example, that a woman's employer's insurance may be her *primary* insurer in some cases, and she may also have insurance from her spouse's employer; she may have to use her employer's insurer even if her spouse's insurer offers a lower copay. Alternatively, a woman's primary insurer may cover the IUD but may require a copayment; if she has Medicaid, then, Medicaid should cover the copayment.

January 2013 because the 2012 data do not contain insertion copayments and deductible applicability.

* * *

3. Results

The black line in Fig. 1 shows the decreasing percentage of women who faced out-of-pocket costs for a hormonal IUD (and at least some cost for its insertion) over the 27 months between January 2012 and March 2014. In January 2012, out-of-pocket costs were required of 58% of insured patients; by March 2014, this number dropped to 13%. The percentage of women who faced out-of-pocket costs did not decrease during the first half of 2012; we first observe decreases toward the end of 2012, as the ACA's contraceptive coverage requirement first took effect for patients signing up for new health plans. Coverage increased substantially at the end of 2012, when many patients' annual plans were renewed and the ACA took effect for those without grandfathered plans; the percent with out-of-pocket costs declined 3 percentage points in December 2012, from 52% to 49%, and 21 points in January 2013, from 49% to 28%. Over the next 15 months, from February 2013 through March 2014, the percentage of women who faced out-of-pocket costs fell to 13%, or by 1 percentage point per month.

We analyzed whether a woman's insurer required a copayment for the device's insertion or otherwise required cost sharing due to a deductible from 2013 onwards (as these data were not available for 2012). The results did not substantively differ from the trend described above for full coverage. The gray line in Fig. 1 shows that 16% rather than 13% of women faced out-of-pocket costs for both the device and its insertion. These estimates of change over time may be conservative, however, as the percentage of women with insurers who required them to share in the cost of the device's insertion might have been higher in 2012 than in 2013.

Figs. 2 and 3 show trends in IUD coverage by age and region, respectively. Before the implementation of the ACA provision, young and Northeastern women experienced higher levels of coverage than other women; after implementation, differences by age and region narrowed sharply.

Fig. 2
In each age group: percentage of women who would have had out-of-pocket costs for a hormonal IUD, by quarter.

* * *

In Q1 2012, 49% and 63%, respectively, of women aged 18– 24 and 40–49 years would have had to pay out of pocket, a 14-point difference (Fig. 2). In Q1 2013, less than a third of this gap remained (4 points, 24% versus 28%); differences by age nearly dissolved by the end of the analysis period. Similarly, in Q1 2012, 53% and 61–64%, respectively, of women in the Northeast and elsewhere would have had to pay something out of pocket (Fig. 3). In Q1 2013, four fifths of this gap remained, and after another year, differences by region nearly dissolved (to 0–3 points). Differences by region dissolved as much as differences by age but less rapidly.

Table 1 reports the percentage of women with full coverage for a hormonal IUD (and at least partial coverage for its insertion), with partial coverage for the IUD or without coverage, by quarter, between Q1 2012 and Q1 2014. The table indicates that very few women in these data had no coverage at all. Thus, most of the increase in full coverage appears to be driven by insurance companies moving from partial to full coverage.

Fig. 3
In each region: percentage of women would have had out-of-pocket costs for a hormonal IUD, by quarter.

* * *

Table 1 also reports that the percentage of women in these data affected by the contraceptive exclusion for religious

employers varies from 0.4% to 2.2% in the five quarters between January 2013 and January 2014. Dividing the percentage without coverage due to the contraceptive exclusion by the percentage of women with no coverage shows, however, that these 0.4–2.2% of women who sought an IUD amount to 8.8–37.9% of women who sought an IUD and had no coverage; this may suggest that a nontrivial portion of women with interest in an IUD but without any coverage worked for a religious employer that denies contraceptive coverage. Considering the wide variation in these numbers, however, they should be interpreted with caution.

Table 2 reports cost estimates for the IUD itself at the median and 90th percentiles. The 90th percentile declines to $169 in the first quarter of 2013 and to $15 in the first quarter of 2014, from $844 in the first three quarters of 2012. Median estimates are much smaller, at $20 in the first half of 2012, and fall to $0 in Q4 2012, as by then fewer than half of women (49.9%) faced out-of-pocket costs for the IUD itself.

In a sensitivity analysis, we examined the percentage of women who faced out-of-pocket costs for obtaining an IUD under the assumption that women with multiple insurers for example, backed up by Medicaid — could not rely on the insurance with the lowest out-of-pocket cost available to them. In this scenario, 20% of women would have had out-of-pocket costs for the IUD and insertion in March 2014, compared to 16% as shown in Fig. 1. In both coverage scenarios, 58–59% faced out-of-pocket costs in January 2012, so this sensitivity analysis corroborates the overall analysis.

4. Discussion

Following implementation of the ACA, we observed a substantial decline in the percentage of women having to pay out of pocket for a hormonal IUD and the elimination of cost disparities by age and region. Potential for further decline remains, as 13% of women still did not have complete coverage as of March 2014.

Some of the decrease in women who face costs could follow from other causes aside from the ACA. However, we note the complete absence of any trend prior to the point in time at which the ACA's provisions came into effect.

Either the ACA reduces differences between the North-east and other regions or the characteristics of the healthcare providers who use the benefit inquiry service differ in the Northeast. If so, then these findings may reflect a convergence in coverage not by region but by unobserved socioeconomic characteristics. We cannot identify effects by individual characteristics such as income or race, but trends by region suggest that IUD coverage increased substantially under ACA throughout the United States.

To address the representativeness of the benefit inquiry data, we compared the available demographics — age and geographic region — to U.S. Census data and the NSFG. With regard to age, the women in the benefit inquiry data do not differ significantly from all women of reproductive age. With regard to geography, the comparisons indicate that the benefit inquiry data overrepresent women in the Northeast and underrepresent women in the West, although women in the West are more likely to have an IUD in the NSFG and in a recent Centers for Disease Control and Prevention analysis of services provided to teenagers in Title X clinics [3,20]; this may reflect differences by region in the use of the benefit inquiry service.

Table 1
Percentage of women with different levels of coverage for a hormonal IUD and percentage affected by the contraceptive exclusion for religious employers, by quarter

* * *

We note several limitations of our approach. A key limitation is that we rely upon both the manufacturer of the hormonal IUDs and the manufacturer's benefits-verification

contractor for the data's authenticity and accuracy. We also cannot determine how many of the 13% of women who remain without complete coverage in March 2014 do so because of imperfect adherence to the ACA requirement or because they have a grandfathered insurance plan. Evidence of imperfect adherence leads advocates like the National Women's Law Center to publish advice to women faced with costs in spite of the federal mandate [21–23]. Also, as previously noted, these data do not represent all women seeking Mirena or Skyla, nor do we know the percentage of these women who actually went on to obtain an IUD or the number of IUDs sold. Finally, we expect but cannot confirm that these data predominantly represent women with private insurance, as a doctor familiar with the public insurance plans within his or her state would likely know a publicly insured woman's coverage. While we note these limitations, our findings corroborate similar results from other studies that analyze other contraceptives [17,18].

Table 2
Median and 90th percentile cost estimates for a hormonal IUD, by quarter

* * *

Earlier studies reported that most women with private insurance had at least partial coverage [10,14,17,18,24], but these studies could have underestimated the number of women with no coverage because they analyzed women who obtained an IUD, and women who discovered that their insurance did not cover an IUD might not obtain one. In contrast to these earlier studies, our results are not biased by this limitation.

Noticeable gaps in the percentage of women who are covered and not subject to cost sharing, between women by region and women by age, dissolved after the ACA took effect. This convergence suggests that the ACA reduced inequality among insured women. Were race or income available in these data, it would have been interesting to test

whether race or income inequality in coverage declined over time. We believe that this is worth further study.

Our study also contributes the first nonanecdotal estimates of the extent to which the contraceptive exclusion for religious employers inhibits women's access to the contraceptive of their choice. We interpret these results with caution, however, given the between-quarter fluctuations in the percentage of women denied IUD coverage due to the exclusion. We might expect that as the share of women without coverage declines, the proportion of uncovered women subject to the religious exclusion would increase, but we observe the opposite, with a higher proportion of women without coverage affected by the religious exclusion in the first quarter of 2013 than in the first quarter of 2014.

Between 2006 and 2010, unintended pregnancy rates declined in all but 2 of the 41 states for which data are available [25]. This decline corresponded with a national increase in long-acting reversible contraceptive (LARC) use, predominantly of the IUD, from 3.7% in 2007 to 8.5% in 2009 [26]. As noted earlier, IUD use has since risen further, reaching 10% in 2011–2013 [3], and prior research shows that eliminating costs can lead to increased LARC use, which in turn can contribute to lower pregnancy, abortion and birth rates [8,9,12]. Other factors may also contribute to the decline in unintended pregnancy. However, if the ACA leads to additional uptake, this may contribute to continued declines in unintended pregnancy.

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authors made the final determination as to the content of the paper, and the conclusions and opinions expressed are theirs alone.

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APPENDIX DD

WOMEN'S HEALTH

By Nora V. Becker and Daniel Polsky

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WOMEN SAW LARGE DECREASE IN OUT-OF-POCKET SPENDING FOR CONTRACEPTIVES AFTER ACA MANDATE REMOVED COST SHARING

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ABSTRACT The Affordable Care Act mandates that private health insurance plans cover prescription contraceptives with no consumer cost sharing. The positive financial impact of this new provision on consumers who purchase contraceptives could be substantial, but it has not yet been estimated. Using a large administrative claims data set from a national insurer, we estimated out-of-pocket spending before and after the mandate. We found that mean and median per prescription out-of-pocket expenses have

decreased for almost all reversible contraceptive methods on the market. The average percentages of out-of-pocket spending for oral contraceptive pill prescriptions and intrauterine device insertions by women using those methods both dropped by 20 percentage points after implementation of the ACA mandate. We estimated average out-of-pocket savings per contraceptive user to be $248 for the intrauterine device and $255 annually for the oral contraceptive pill. Our results suggest that the mandate has led to large reductions in total out-of-pocket spending on contraceptives and that these price changes are likely to be salient for women with private health insurance.

Contraceptives are among the most widely used medical services in the United States, and 99 percent of sexually active women have used at least one type of contraceptive in their lifetime.[1] Contraceptives are much less costly than maternal deliveries for insurers and patients, and their use has been shown to result in net savings to insurers.[2]

Contraceptive use also has important effects on families and the economy. Studies of the effects of legalization of the contraceptive pill in the 1960s and 1970s found that increased access to contraception was associated with lower rates of subsequent entry into poverty, higher rates of labor-force participation and entry into professional school, and higher wages for women.[3-6] These economic gains also affect subsequent generations: The children of women with increased access to contraception have higher rates of college completion and higher incomes, compared to children whose mothers did not have access to family planning.[7]

A variety of contraceptive products are currently available to women in the United States. Some—like the oral contraceptive pill—are relatively inexpensive but must be purchased monthly. Others can be very expensive but require only a one-time purchase for months or years of contraceptive coverage. These methods of long-act-ing

reversible contraceptives (sometimes called LARCs) are the intrauterine device (IUD) and the subdermal implant. Both are much more effective than oral contraceptives, but before the ACA they could require a one-time out-of-pocket payment of several hundred dollars.

This high up-front cost may have deterred some women from using long-acting reversible contraception methods. A recent study of women enrolled in private health insurance who ex-pressed interest in an IUD found that women with a lower out-of-pocket spending requirement for the device and insertion procedure were significantly more likely to receive an IUD than women who faced higher out-of-pocket expenses.[8]

The Affordable Care Act (ACA) includes a mandate that "preventive services"—a category of services that includes both prescription contraceptives and their related medical services—be covered with no consumer cost sharing. This mandate went into effect August 1, 2012. It required that insurance plans come into compliance at the beginning of the subsequent plan year, which for many women was January 1, 2013. The mandate includes all contraceptive methods approved by the Food and Drug Administration (FDA), including female sterilization and prescription emergency contraception, but it excludes over-the-counter emergency contraception and abortifacients.[9] The mandate does not require that insurance companies cover every brand of prescription contraceptive on the market.

The ACA mandate applies nationally to all private health insurance plans, including those offered in the health insurance Marketplaces and by employers. The only exceptions are grand-fathered plans and those offered by employers that receive an exemption for religious reasons. Grandfathered plans are health plans that have not substantially changed their cost-sharing requirements since March 2010, the month when the ACA became law. These plans are gradually being phased out of the employer-

sponsored health insurance marketplace but still covered 36 percent of insured workers as of 2013.[10] This means that a significant subset of women are still enrolled in plans that are not yet subject to the ACA's mandate of zero cost sharing for contraception.

The inclusion of prescription contraceptive coverage in the ACA's mandate has drawn a large amount of political attention. Much of the debate surrounding the mandate has focused on either the effect of the mandate on employers' religious freedom or the potential impact of the mandate on women's health.[11-12] Its financial impacts on women as consumers have attracted far less attention. However, one recent survey of several hundred privately insured women found that the average out-of-pocket price for the pill had dropped from $14.35 per month in 2012 to $6.48 in 2014.[13]

Our aim was to systematically quantify declines in out-of-pocket spending between 2012 and 2013 for all available reversible prescription contraceptive methods. This will allow an understanding of relative changes in price across methods, particularly between the pill and long-acting reversible contraception methods. We also put these spending changes into their financial context for women as consumers by examining how these price declines affect both their total out-of-pocket spending on health care and the proportion of that spending that is spent on prescription contraceptives.

Study Data And Methods

We used a 10 percent sample of the Clinfor-matics™ Data Mart from Optum Insight, a claims database from a large national insurer, to calculate monthly out-of-pocket spending between January 2008 and June 2013 for the eight categories of prescription contraceptives listed in Exhibit 1. Our sample consisted of 17.6 million month-level observations for 790,895 women ages 13-45 who were enrolled in private health insurance for at least one month during this period. The mean and median lengths of insurance enrollment were

22.3 and 17.0 months, respectively. The data set included women in all fifty states and the District of Columbia.

EXHIBIT 1

Characteristics Of Prescription Contraceptives And Consumers' Out-Of-Pocket Expenses

* * *

THE INCLUSION OF PRESCRIPTION CONTRACEPTIVE COVERAGE IN THE ACA'S MANDATE HAS DRAWN A LARGE AMOUNT OF POLITICAL ATTENTION.

ESTIMATING AVERAGE OUT-OF-POCKET SPENDING Per claim out-of-pocket spending was calculated using pharmacy claims for contraceptive methods delivered in a pharmacy, such as oral contraceptives, the contraceptive patch and ring, and diaphragms and cervical caps. Contraceptive methods delivered in a physician office (IUDs, implants, and injections) were identified in the medical claims data using *Current Procedural Terminology*, Fourth Edition (CPT-4); level 2 Healthcare Common Procedure Coding System (HCPCS); and *International Classification of Diseases*, Ninth Revision (ICD-9), procedural and diagnostic codes. We estimated out-of-pocket spending for these three methods by aggregating all patient cost sharing for the encounter during which the method or device was delivered, because procedural costs associated with these methods are billed separately from the cost of the device itself.

For all contraceptive methods, we report the six-month mean or median per claim out-of-pocket expense. For short-term products such as the pill, the patch, and the ring, this calculation is not equivalent to the per month out-of-pocket expense because many women receive two to three months

of contraceptive supplies when they fill their prescriptions. Our cost estimates are therefore not comparable with monthly estimates reported previously in the survey literature.

Before the ACA mandate, contraceptives were subject to yearly deductibles and out-of-pocket limits. The average costs per method therefore declined predictably over the course of a given year as some women used up their deductibles or hit their out-of-pocket spending limits and incurred lower out-of-pocket expenses for their method of contraception. To remove the influence of deductibles and out-of-pocket limits from our estimates, in some of our analyses we regressed pre-August 2012 out-of-pocket expenses on a set of monthly dummies and then plotted the residual variation in out-of-pocket spending.[14]

ESTIMATING CHANGES IN TOTAL OUT-OF-POCKT SPENDING To estimate the share of out-of-pocket spending for prescription contraceptives, we focused on users of the pill and women who had new IUD insertions, since the pill and the IUD are the two most commonly used reversible prescription contraceptive methods in the United States.[15] To minimize selection bias, we limited our spending analysis to women who were continuously enrolled in insurance from January 2012 to June 2013. We then compared spending patterns among pill users and women who received IUD insertions in the pre period (January-June 2012) to patterns in the post period (January-June 2013).

We defined *pill users* as women who had at least one claim for an oral contraceptive pill in both the pre and post periods. We included spending in both periods for pill users. We defined *IUD users* as women who had an IUD inserted in either the pre or the post period. We included spending for IUD users only in the period in which they received their IUD.

For each woman, we summed her out-of-pocket spending on either pills or IUD insertion and divided that value by her

total out-of-pocket spending during that period. Using these percentages and the mean and median total out-of-pocket spending values for these users, we then estimated the mean and median implied savings on pills and IUD insertions per woman attributable to the ACA mandate.

Implied savings were calculated by multiplying the mean (or median) total spending by the mean (or median) percentage of spending spent on that method for each period and then subtracting the 2013 estimate from the 2012 estimate. This calculation took into account the possibility that total average out-of-pocket spending might have changed during this time period. For pill users, this value was then multiplied by two to estimate total yearly spending.

All costs are presented in inflation-adjusted 2013 dollars. Analyses were performed using Stata/MP, version 13.

LIMITATIONS There were a number of important limitations to our study. Claims for emergency contraception and diaphragms or cervical caps were infrequent in our data, so we recommend caution when interpreting estimates for these methods. Additionally, we did not include cost sharing for physician appointments or costs of IUD or implant removals in our estimates, which resulted in a conservative estimate of out-of-pocket spending.

For contraceptive methods obtained in a physician office and reported in medical claims (the IUD, implant, and injection), we calculated expenses per encounter. If a woman received another expensive service at the same encounter—for instance, if an IUD or implant was inserted immediately after maternal delivery—it is possible that we erroneously included the costs of those procedures in some of our totals. We therefore report both means and medians in our results. We also conducted a sensitivity analysis in which we excluded the top 1 percent of expenses for each of these methods. This lowered the estimated mean expenses slightly but had almost no effect on the estimated median expenses.

Finally, our implied savings estimates assumed that in the absence of the mandate, out-of-pocket expenses for consumers would have stayed the same as they were in the period January-June 2012. This could be an unrealistic assumption in particular for IUDs, which demonstrated a dynamic average monthly out-of-pocket price prior to the mandate's implementation. Because of this limitation, the savings estimates should be interpreted as short-term changes in out-of-pocket spending only and should not be used for long-term estimates of out-of-pocket spending reductions.

Study Results

Adjusted mean per claim out-of-pocket spending declined for both the pill and the IUD after implementation of the ACA mandate (Exhibit 2). The average adjusted out-of-pocket expense for a pill prescription fell from $33.58 in June 2012 to $19.84 in June 2013, and the out-of-pocket expense for an IUD insertion fell from $293.28 to $145.24.

$255

Per year

The average user of the pill saved $254.91 per year after the ACA mandate took effect.

To better examine the change in costs for all contraceptive methods, we report the unadjusted six-month mean and median per claim out-of-pocket spending for each prescription contraceptive method in the pre and post periods (Exhibit 3). At baseline in 2012, the method that was most expensive up front was the implant, with a mean expense of $320.31, followed by the IUD, at $262.38. The methods with the lowest per claim expense were the pill ($32.74), emergency contraceptives ($26.16), and diaphragms or cervical caps ($34.48).

However, out-of-pocket spending for short-term methods compared to that of long-term methods must be considered in the context of the length of time the methods are used. Short-term methods such as the pill must be purchased

repeatedly over time, while the out-of-pocket expense for long-term methods such as IUDs is a one-time expense. In the long run, long-acting reversible contraception methods such as the IUD or implant have been shown to be less costly than repeatedly purchasing a short-term method such as the pill for an equivalent length of time.[16]

We observed large decreases in the mean out-of-pocket expenses of most methods following implementation of the mandate (Exhibit 3).

EXHIBIT 2

Trend In Mean Adjusted Per Claim Out-Of-Pocket Expenses For Oral Contraceptive Pill Prescription Fills And Intrauterine Device (IUD) Insertions, 2008-13

* * *

EXHIBIT 3

Mean And Median Per Prescription Out-Of-Pocket Expenses For Prescription Contraceptive Methods Before And After Implementation Of The Affordable Care Act Mandate, 2012 And 2013

* * *

From June 2012 to June 2013 the mean out-of-pocket expense for the pill declined by 38 percent, and the mean out-of-pocket expense for an IUD declined by 68 percent. We also found decreases in spending for emergency contraception (93 percent), diaphragms or cervical caps (84 percent), the implant (72 percent), and the injection (68 percent). In contrast, spending for the ring and the patch declined only 2 percent and 3 percent, respectively, over this period.

Median out-of-pocket per prescription spending fell to zero for almost all prescription contraceptive methods following implementation of the ACA mandate. This suggests that while some women were still paying large amounts out of pocket for their contraception, the majority of women were

paying nothing by June 2013. The ring and the patch were the exceptions: Their mean and median out-of-pocket expenses remained similar during this time period.

To assess the relative magnitude of these out-of-pocket spending changes for contraceptive users, we examined total mean and median out-of-pocket spending and the percentage of that spending spent on contraceptives for pill users and women who received IUD insertions (Exhibit 4). Because the mandate was implemented mid-2012, we compared spending percentages in the first six months of 2012 with those in the first six months of 2013. For women who were enrolled in insurance continuously and had at least one claim for oral contraceptive pills in both periods, the mean and median percentages of out-of-pocket spending spent on the pill dropped from 44.0 percent and 36.0 percent to 22.4 percent and 0.0 percent, respectively. For women who received an IUD during the same periods, the mean and median out-of-pocket spending percentages in the period they received their IUD dropped from 30.3 percent and 13.2 percent to 11.3 percent and 0.0 percent, respectively.

We used these values to estimate the per woman savings on yearly oral contraceptive pill costs for pill users and on IUD insertions for women receiving IUDs. We estimated that the average pill user saved $254.91 per year, and the median pill user saved $204.65 per year (Exhibit 4). The mean and median savings on IUD insertions were estimated to be $248.30 and $107.95, respectively, per woman.

Discussion

Out-of-pocket expenses used in this study for the period before the implementation of the ACA mandate were roughly equivalent to those in other available data.[16-17] However, we found substantial drops in both the mean and the median out-of-pocket spending for most contraceptive methods after the mandate's implementation. Median spending for almost all contraceptive methods fell to zero within ten months of implementation, and mean spending dropped by large

percentages (38-93 percent, depending on the method). Mean out-of-pocket spending remained above zero for two reasons: Not all brands are required to be covered with zero cost sharing, and a subset of women in the data were enrolled in grandfathered plans that were not yet subject to the mandate.

Before the mandate's implementation, out-of-pocket expenses for contraceptives for women using them represented a significant portion (30-44 percent) of these women's total out-of-pocket health care spending. This is a finding that, to our knowledge, has not been previously reported. It is likely that contraceptives are a significant proportion of total health spending because contraceptive users tend to be young women with few serious health issues. For these women, obtaining contraceptives is likely their primary reason for visiting a health care provider and paying out-of-pocket amounts. Because contraceptives represented a large portion of their health care spending before the mandate, the price reductions caused by the ACA are likely to be salient for these women.

A recent industry report estimated that the ACA mandate saved women $483 million in out-of-pocket spending on the pill in 2013.[18] Our findings suggest that reductions in out-of-pocket expenditures on contraceptives in 2013 were in fact much higher, as demonstrated using a quick back-of-the-envelope calculation. The most recent estimates suggest that there are 6.88 million privately insured pill users in the United States.' Multiplying this by our conservative median estimate of $204.65 peryear yields an estimate of $1.4 billion per year in out-of-pocket savings on the pill alone.

EXHIBIT 4

Out-Of-Pocket Spending On Prescription Birth Control By Oral Contraceptive Pill Users And Women Receiving Intrauterine Devices (IUDs), 2012 And 2013

* * *

Policy Implications

Our findings suggest that the ACA mandate will likely significantly reduce the out-of-pocket expenditures of contraceptive users, in some cases to nothing. But it is still too early to predict the final impact of the mandate on health care use and spending, or the mandate's impact on other health and socioeconomic outcomes for women.

Economic theory and empirical evidence suggest that decreasing out-of-pocket contraception expenses to consumers will result in increased use.[19-20] An increase in the use of contraceptives could have long-ranging impacts upon women's health and the economy, potentially lowering fertility rates and increasing economic opportunities for women and their families.[4-6, 21]

The ACA mandate also changes the relative prices of different contraceptive methods. Because long-acting reversible contraceptive methods are more costly up front, it is possible that removing financial barriers to all methods might induce women to choose long-acting reversible contraceptive methods at higher rates.

The CHOICE Project, a recent prospective cohort study of 9,256 women ages 14-45, offered participants their choice of contraceptive at no cost after they received counseling and education about all available methods.22'23 With the barriers of cost, knowledge, and access removed, 75 percent of participants chose a long-acting reversible contraception method. Participants who chose such methods had higher rates of continuing to use their device and of satisfaction at twelve and twenty-four months of follow-up. In addition, their rates of pregnancies, births, and abortions in the twenty-

four-month follow-up period were much lower than national rates during the same period.

Some policy makers and media outlets have raised concerns that no-cost contraceptives, or increased use of more effective contraceptives, might increase risky sexual behavior. However, the CHOICE Project found no evidence of increased sexual risk taking among the study cohort.

The CHOICE Project enrolled only women who were interested in starting a new contraceptive method and specifically counseled participants about the relative effectiveness of long-acting reversible contraception methods compared to more short-term methods. In contrast, the ACA mandate lowered the out-of-pocket expense for contraceptives for all women in private health plans, many of whom might be uninterested in changing their current contraceptive method.

IT IS STILL TOO EARLY TO PREDICT THE FINAL IMPACT OF THE MANDATE ON HEALTH CARE USE AND SPENDING, OR ON OTHER HEALTH AND SOCIOECONOMIC OUTCOMES FOR WOMEN.

Furthermore, the ACA mandate does not directly change providers' behavior or affect consumers' knowledge about contraceptives, although some providers may take it upon themselves to educate their patients about the mandate. In some cases, women may not even be aware that their coverage has changed. A recent study of young adults' experiences in shopping for health insurance on HealthCare.gov found that many were unaware that well-women visits and contraception were included as preventive services with no cost sharing.[24]

The impact of the ACA mandate on contraceptive utilization will therefore depend on how sensitive consumers are to out-of-pocket expenses for contraceptives and how

many women were dissuaded from using contraceptive products by that expense before the mandate's implementation." Very few studies have estimated the responsiveness of consumers to the out-of-pocket expense of contraceptives in the United States, and no study has estimated it for the population of privately insured women affected by the ACA mandate. Future work will need to measure whether these spending changes result in increased use of contraceptives or changes in the choice of contraceptive methods.

Lastly, insurance companies are required to cover all contraceptive methods with no consumer cost sharing in plans that are not grandfathered, but they are not required to cover all brands. The large national insurer that provided our data appeared to be interpreting this broadly, as out-of-pocket spending for the patch and the vaginal ring did not follow the same pattern as spending for other methods. Mean and median out-of-pocket expenses for the patch and vaginal ring remained very similar to premandate levels.

These findings are consistent with results from several recent studies suggesting that not all insurers are fully complying with the mandate.[26, 27] In response to these reports, the Departments of Labor, Health and Human Services, and the Treasury jointly issued new guidelines May 11, 2015, clarifying the requirements of the mandate. These guidelines specify that insurers must cover with no cost sharing at least one of the eighteen FDA-approved contraceptive methods, including methods such as the patch and the ring.[28] Insurers can use cost sharing to direct consumers to lower-cost methods within a category, as long as at least one method within each category is covered with zero cost sharing.

With this new clarification from the administration of President Barack Obama, we expect that the pattern of out-of-pocket expenses for the patch and the ring among the

population we studied will soon resemble that of other methods.

Conclusion

We found the ACA-mandated removal of consumer cost sharing for prescription contraceptives in nongrandfathered insurance plans resulted in large reductions in out-of-pocket spending on contraceptives. A woman who uses oral contraceptive pills or the IUD, the two most commonly used reversible prescription contraceptive methods, has the potential to save several hundreds of dollars each year. This represents a significant portion of the average total out-of-pocket medical spending in this population. The impact of these reductions in out-of-pocket expenditures on the use of contraceptives, fertility, and women's health will depend on the price sensitivity of privately insured women for prescription contraceptives.

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