

Histopathologic Features of Ovaries at Increased Risk for Carcinoma

A Case-Control Analysis

Mark E. Sherman, M.D., M.P.H., Jennifer S. Lee, M.A., R. Tucker Burks, M.D.,
Jeffery P. Struewing, M.D., Robert J. Kurman, M.D., and Patricia Hartge, Sc.D.

Summary: In this study, the pathogenesis of ovarian carcinoma was investigated by performing a masked histopathologic comparison of benign ovaries removed from 61 women predicted to be at increased risk for developing carcinoma (cases) with ovaries removed from 121 women without known predisposing conditions (controls). The cases included 26 women who had a unilateral invasive carcinoma and 35 women undergoing prophylactic oophorectomy for a family history of ovarian cancer. As predicted by previously developed models, epithelial inclusion cysts were identified more frequently with advancing age in both cases and controls. However, the mean and maximum number of cysts per slide in a woman were not increased among cases. Surface epithelial "atypia," a designation based on a composite impression of multiple features, was found in 13% of cases compared with 3% of controls (relative risk 7.1; 95% confidence interval, 1.9 to 26.1), but this result was based on small numbers. None of the other histologic features examined was found more often in cases following age-adjustment. Reexamination of sections with well-preserved surface epithelium or inclusion cysts under oil immersion demonstrated several differences in the detection of specific features between cases and controls and increased detection of "atypia" among cases, but none of these findings reached statistical significance. It is concluded that there may be subtle differences in the surface epithelium of ovaries predisposed to developing cancer as compared with controls, but these changes are difficult to identify reliably with light microscopy. Future etiologic studies should attempt to optimize specific handling and include molecular studies and epidemiologic analyses. **Key Words:** Ovary—Carcinoma—Pathogenesis—Epidemiology—Precursor—Carcinogenesis

Ovarian cancer is the most lethal neoplasm of the female genital system, accounting for approximately 14,800 deaths in the United States in 1996. Although 90% of women with well-documented stage I tumors are

cured, overall survival rates are poor because 80% of patients present with disseminated disease (1). To date, screening strategies using serum CA125 levels and transvaginal ultrasound do not appear sensitive or cost-effective, and efforts to develop new screening approaches have been limited by our lack of knowledge regarding the pathogenesis of these tumors.

Proposed precursors of ovarian cancer include serous inclusion cysts, "dysplasia" of the ovarian surface epithelium or the lining of cysts, borderline tumors (atypical proliferative tumors or tumors of low malignant potential), and endometriosis. It has been postulated that inclusion cysts are predisposed to malignant transformation because cyst lining cells are exposed to high levels of estrogen and other substances in cyst fluid. Although

From the National Cancer Institute, Division of Cancer Epidemiology and Genetics (M.E.S., J.S.L., J.P.S., P.H.), Rockville, Maryland, Johns Hopkins Medical Institutions, Departments of Pathology and Gynecology and Obstetrics (M.E.S., R.J.K.), Baltimore, Maryland, Howard Hughes Medical Institute (J.S.L.), Bethesda, Maryland, and Medical College of Virginia, Virginia Commonwealth University (R.T.B.), Richmond, Virginia, U.S.A.

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Address correspondence and reprint requests to Dr. Patricia Hartge, NCI/NIH, EPN 443, 6130 Executive Boulevard, Rockville, MD 20892.

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endometriosis and borderline tumors may be precursors of some ovarian carcinomas, the origin of most ovarian carcinomas is unknown (2).

To investigate the pathogenesis of ovarian cancer, we compared the histopathologic findings in benign ovaries judged to be at increased risk for developing carcinoma with those not known to be at increased risk. Ovaries were considered prone to tumor development if removed as part of staging for a contralateral carcinoma or prophylactically for a family history of ovarian cancer. Because these ovaries are thought to be predisposed to tumor development, it was hypothesized that differences between these ovaries and those removed for indications unrelated to ovarian pathology could reflect early changes related to cancer development.

MATERIALS AND METHODS

Case Selection

The study was designed as a case-control comparison. A total of 61 cases was selected, including 26 women whose benign ovaries were removed during staging for a contralateral invasive carcinoma and 35 women who underwent prophylactic oophorectomy for a family history of ovarian cancer. Ovaries removed from 121 women for reasons unrelated to ovarian disease were designated as controls.

Benign ovaries contralateral to invasive ovarian carcinoma were identified by performing a computer search of the surgical pathology files of The Johns Hopkins Hospital for the years 1985 to 1993. The tumor types in these women included 9 clear cell carcinomas, 4 endometrioid, 3 serous, 9 other types, and 1 of unknown type. The majority of the "other" types were serous carcinoma admixed with minor components of other histopathologic patterns. The tumors were well differentiated in 3 cases, moderately differentiated in 3, poorly differentiated in 10, and of unknown grade in 10. The tumors were stage I in 10 patients, stage II in 4, and stage III in 12. The mean age of the women in this group was 57 ± 15 years.

Cases in the family history group were selected from among subjects participating in a National Cancer Institute-sponsored study of familial ovarian cancer. Initially, 47 patients who had undergone an elective bilateral prophylactic oophorectomy for a family history of at least two ovarian cancers (mean 2.6 per family) were identified. Genetic testing for BRCA mutations was unavailable at the time of oophorectomy (1958 to 1993), but 20 women were subsequently evaluated using linkage analysis and single-strand conformation polymorphism

assay. Germ-line BRCA mutations were identified in eight women, including 185delAG, 2798del14, 2800delAA, 4153delA, V1713A, and 5382insC. Seven of these women with interpretable ovarian slides were included. Twelve members of BRCA families tested negative for BRCA mutations after oophorectomy, and therefore were presumed not to be at increased risk for ovarian carcinoma and were excluded. BRCA status was unknown in the remaining 28 patients. The mean age of these 35 women was 41 ± 9 years.

The control group consisted of 121 women with a mean age of 53 ± 13 years whose ovaries had been removed at The Johns Hopkins Hospital between 1990 and 1994 during operations performed for diseases unrelated to the ovary. The final pathology in these women included leiomyoma uteri in 45, cervical dysplasia or neoplasia in 23, endometrial hyperplasia or neoplasia in 6, and a combination of benign findings in the remainder.

Histopathologic Review

The individual slides from the cases and controls were assembled in a single randomly ordered slide set, and the slide labels were masked with study identification numbers. The slides were evaluated by one of two pathologists (primarily R.T.B. and M.E.S.) for the following features: percentage of attached surface epithelium, number of inclusion cysts, other benign cysts, surface papillae, psammoma bodies, surface epithelial invaginations forming clefts, epithelial "atypia" of the surface epithelium, and other features.

After the initial review, slides from 37 cases that contained inclusion cysts or epithelium covering at least 10% of the ovarian surface and slides from 30 controls containing both inclusion cysts and at least 10% of the surface covered with epithelium were reexamined under oil immersion ($\times 1000$) to assess nuclear and architectural features in detail (M.E.S.). Nuclear features evaluated included anisonucleosis ($>50\%$ variation in diameter), membrane irregularity, chromatin clumping, and nucleoli. Architectural features analyzed included stratification and loss of polarity. A subjective classification of "atypia" was applied to cases displaying multiple features as noted above.

Analysis

The pathologic findings in individual slides were tabulated and then the results of all slides from a given patient were combined and analyzed by subject. Nine slides from six women were rated as unacceptable in quality and were excluded. If a histopathologic feature was identified on any slide from a woman, she was considered

positive for that feature. Because the histopathologic data were similar for benign ovaries removed prophylactically in women with BRCA mutations, ovaries removed prophylactically in women with unknown BRCA status, and those associated with a contralateral carcinoma, these data have been combined. Based on a preliminary analysis demonstrating a relationship between the detection of most histologic features and age, results were further analyzed using regression analysis, controlling for age, defined as a categorical variable. The number of inclusion cysts was also analyzed as an average per slide and maximum number of cysts per slide from each woman to control for the effect of differential sampling between groups. The features assessed under oil immersion were also analyzed, controlling for age as a categorical variable. Relative risks and 95% confidence intervals from univariate and logistic regression analyses were performed using SAS computer package (SAS Institute, Inc., Cary, NC) and the personal computer versions of EPITOME (3).

RESULTS

Histopathologic Comparisons

A total of 442 slides obtained from 182 women were reviewed, including 120 slides from prophylactic oophorectomy specimens (mean 3.4 ± 2.8), 45 from ovaries removed for ovarian cancer staging (mean 1.7 ± 1.3), and 277 from ovaries removed from controls without recognized cancer risk (mean 2.3 ± 1.1). Overall, the mean number of slides examined per case (prophylactic oophorectomy or contralateral cancer) was 2.7. At least partial denudation of the ovarian surface epithelium was found in nearly all sections obtained from both cases and controls. Most notably, <10% of the surface epithelium was present in 72% of cases and 74% of controls. The frequency of the histopathologic features evaluated in the cases and controls is displayed in Table 1 by subject. Inclusion cysts were identified in at least one slide in 51% of cases compared with 66% of controls. Surface papillae, nodular surface contour, and cortical clefts were more common among controls, whereas follicular cysts were more frequent among cases. "Atypia" of the surface epithelium was identified among 13% of cases compared with 3% of controls. Because the frequency of detecting these features generally increased with age, except for corpus luteum and follicular cysts, which decreased with age, these results were analyzed after age-adjustment (Table 2). Increased detection of "atypia" in cases compared with controls reached significance (relative risk 7.1; 95% confidence interval, 1.9 to 26.1), but this result was based on small numbers and the confidence interval

TABLE 1. Comparison of features in 61 cases (benign ovaries at increased risk for carcinoma) and 121 controls

Ovary feature by subject	No. of subjects by reason for oophorectomy	
	Cases (prophylaxis or contralateral cancer) N = 61 no. (%)	Controls (no ovarian pathology) N = 121 no. (%)
Inclusion cyst		
≥ 1 cyst on any slide	31 (51)	80 (66)
Papillae in inclusion cyst	3 (5)	7 (6)
Psammoma bodies	6 (10)	7 (6)
Surface papillae	13 (21)	37 (31)
Lobulated or nodular contour	7 (11)	28 (23)
Cortical clefts	19 (31)	50 (41)
Corpus luteum cyst	21 (34)	31 (26)
Follicular cyst	34 (56)	43 (36)
Epithelial "atypia"	8 (13)	4 (3)

was wide. None of the relative risks for any of the other features examined was statistically significant in this analysis.

Inclusion Cysts

The relationship between number of inclusion cysts and ovarian cancer risk was further analyzed in Table 3 and Fig. 1. The average number of inclusion cysts per slide in a subject increased with advancing age in all groups. The age-adjusted average number of inclusion cysts per slide in a woman was higher among controls than in cases when assessed categorically (Table 3), or as the mean of the average per woman (Fig. 1). However, logistic regression models, including age as a categorical variable, demonstrated that the average number of cysts per woman was not statistically different between the groups. In addition, the total number of cysts across all slides per woman and the maximum number of cysts noted on any slide for a woman were not statistically different (data not shown).

Oil Immersion Microscopy of Surface Epithelium and Cysts

After observing a possible increase in surface epithelial "atypia" in cases compared with controls with routine microscopy, we decided to reevaluate the best preserved sections under oil immersion in order to perform a more detailed microscopic analysis. Examination of the ovarian surface epithelium and the lining of the inclusion cysts under oil immersion demonstrated that anisonucleosis, nuclear irregularity, chromatin clumping, prominent or multiple nucleoli, cellular stratification, and a composite impression of "atypia" were notably more common among cases than controls (Table 4). Age-

TABLE 2. Comparison of features in 61 cases (benign ovaries at increased risk for carcinoma) and 121 controls, stratified by age at oophorectomy

Ovary feature by subject	Subjects by age at oophorectomy in years						Crude relative risk (95% CI)	Relative risk ^{a,b} (95% CI)
	Cases (prophylaxis or contralateral cancer) N = 61			Controls (no ovarian pathology) N = 121				
	<45 (n = 31) no. (%)	45-54 (n = 12) no. (%)	55+ (n = 18) no. (%)	<45 (n = 29) no. (%)	45-54 (n = 50) no. (%)	55+ (n = 42) no. (%)		
Inclusion cyst								
≥1 cyst on any slide	9 (29)	8 (67)	14 (78)	13 (45)	33 (66)	34 (81)	0.5 (0.3-1.0)	0.7 (0.3-1.5)
Papillae in cyst	1 (3)	0 (0)	2 (11)	0 (0)	3 (6)	4 (10)	0.8 (0.2-3.8) ^c	1.2 (0.3-4.8) ^c
Psammoma bodies	3 (10)	1 (8)	2 (11)	0 (0)	2 (4)	5 (12)	1.8 (0.5-6.3)	2.0 (0.6-6.5)
Surface papillae	4 (13)	6 (50)	3 (17)	1 (3)	21 (42)	15 (36)	0.6 (0.3-1.3)	1.0 (0.4-2.3)
Lobulated/nodular contour	2 (6)	2 (17)	3 (17)	1 (3)	15 (30)	12 (29)	0.4 (0.2-1.1)	0.6 (0.2-1.7)
Cortical clefts	7 (23)	5 (42)	7 (39)	5 (17)	27 (54)	18 (43)	0.6 (0.3-1.3)	0.9 (0.4-1.9)
Corpus luteum cyst	17 (55)	3 (25)	1 (6)	16 (55)	15 (30)	0 (0)	1.5 (0.7-3.1)	1.1 (0.5-2.5)
Follicular cyst	28 (90)	5 (42)	1 (6)	19 (66)	23 (46)	1 (2)	2.3 (1.2-4.5)	2.0 (0.8-5.1)
Epithelial "atypia"	1 (3)	3 (25)	4 (22)	0 (0)	2 (4)	2 (5)	4.4 (1.3-15.3) ^d	7.1 (1.9-26.1) ^d

CI, confidence interval.

^a Adjusted for age at oophorectomy in years (categorical: <45, 45-54, ≥55).

^b Added 0.5 to each cell with zero counts.

^c Relative risks similar for the subgroup of subjects with ovaries having ≥1 inclusion cyst on any slide.

^d $p < 0.05$; confidence interval excludes unity.

adjustment reduced the magnitude of the observed differences, and none of the relative risks associated with these features was statistically significant. Even under oil immersion, the morphologic changes identified were subtle (Fig. 2).

DISCUSSION

We performed a masked histopathologic comparison of features in ovaries at increased risk for carcinoma with controls in an attempt to define early morphologic changes related to ovarian carcinogenesis. In agreement with the model proposed by Pike (4), our data demonstrate that inclusion cysts are identified more frequently

with advancing age in all groups of women. However, our age-adjusted analysis of the average number and maximum number of cysts on a slide per woman failed to demonstrate excess cyst formation in ovaries predicted to be at increased risk for developing carcinoma. Similarly, Westhoff et al. (5) previously reported no association between number of inclusion cysts and ovarian cancer risk in a study comparing 37 ovaries contralateral to a primary ovarian tumor with controls matched for age, laterality, and time of diagnosis. Although surface epithelial "atypia" was identified more frequently among cases than controls, these data are difficult to evaluate due to small numbers and a wide confidence interval. None of the other specific features that we assessed was definitely identified more frequently in ovaries at in-

TABLE 3. Crude and age-adjusted relative risk associated with inclusion cysts

Average no. of inclusion cysts/slide per subject	Subjects by age at oophorectomy in years						Crude relative risk (95% CI)	Relative risk ^{a,b} (95% CI)
	Cases (prophylaxis or contralateral cancer) N = 61			Controls (no ovarian pathology) N = 121				
	<45 (n = 31) no. (%)	45-54 (n = 12) no. (%)	55+ (n = 18) no. (%)	<45 (n = 29) no. (%)	45-54 (n = 50) no. (%)	55+ (n = 42) no. (%)		
No cyst on any slide	22 (71)	4 (33)	4 (22)	16 (55)	17 (34)	8 (19)	1.0 ^c	1.0 ^c
>0 and ≤1	7 (23)	5 (42)	2 (11)	9 (31)	15 (30)	9 (21)	0.6 (0.3-1.4)	0.7 (0.3-1.8)
>1 and ≤4	2 (6)	3 (25)	5 (28)	4 (14)	12 (24)	13 (31)	0.5 (0.2-1.2)	0.7 (0.2-2.0)
>4	0 (0)	0 (0)	7 (39)	0 (0)	6 (12)	12 (29)	0.5 (0.2-1.6)	0.8 (0.2-3.4)

^a Adjusted for age at oophorectomy in years (categorical: <45, 45-54, ≥55).

^b Added 0.5 to each cell with zero counts.

^c Referent group.

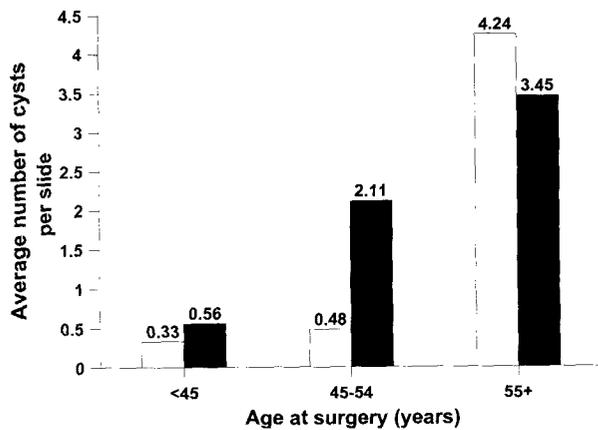


FIG. 1. Average number of inclusion cysts per slide per subject by age at oophorectomy. □, cases; ■, controls.

creased risk for cancer development. Finally, the appearance of ovaries removed from women with BRCA mutations did not appear distinctive compared to ovaries removed prophylactically for a family history of cancer with unknown BRCA status and ovaries removed for a contralateral carcinoma.

Our results contrast with those of two recent comparisons of prophylactic oophorectomy specimens and controls. In an unmasked review, Salazaar et al. (6) reported that inclusion cysts were identified more frequently in 20 prophylactic oophorectomy specimens than in 20 controls removed from women of similar age. The indication for operation in the controls was "leiomyomatosis" in 17, adenomyosis in 2, and endometriosis in 1. Salazaar et al. identified inclusion cysts in only 25% of their control

specimens compared with 66% of controls in our masked review. In addition, Salazaar et al. identified two microscopic cancers in the prophylactic group and reported that numerous histologic features were more common in prophylactic oophorectomy specimens. Recently, Afify et al. (7) also reported an increased frequency of inclusion cysts in 65 prophylactic oophorectomy specimens removed from women with a strong family history of ovarian cancer compared with 30 controls in a masked, age-stratified analysis.

Resta et al. (8) previously reported that inclusion cysts and epithelial hyperplasia or metaplasia (squamous, mucinous, endometrioid, or tubal) were more common in benign ovaries removed for endometrial cancer staging, polycystic ovary disease, or a contralateral benign or malignant ovarian neoplasm than in ovaries removed from women without hyperplastic or neoplastic disease. However, these data were not age-adjusted and ovaries that were subjectively viewed to have been damaged in removal or inadequately fixed were excluded from the analysis. Mittal et al. (9) reported increased numbers of inclusion cysts and an increased frequency of serous metaplasia in benign ovaries contralateral to tumors (cases) compared with controls matched on age and laterality. In contrast to our study, these authors observed a relationship between increasing age and number of inclusion cysts only among cases, but not controls.

In our masked review, epithelial atypia was identified in 13% of cases compared with 3% of controls under routine microscopy, based on small numbers. Accordingly, we reexamined our cases under oil immersion in an attempt to extend these findings. Using oil immersion microscopy, several subtle cytologic features were ap-

TABLE 4. Relative risk for features observed under oil immersion microscopy in benign ovaries at increased risk for carcinoma versus controls

Ovary feature by subject	Reason for oophorectomy among subjects		Crude relative risk (95% CI)	Relative risk ^{a,b} (95% CI)
	Prophylactic or contralateral to cancer n = 37	No ovarian pathology n = 30		
Anisonucleosis	9 (24)	2 (7)	4.5 (0.8-33)	2.8 (0.6-14)
Irregularity	13 (35)	6 (20)	2.2 (0.6-7.7)	2.0 (0.6-7.3)
Chromatin clumping	14 (38)	9 (30)	1.5 (0.5-4.7)	1.4 (0.5-4.5)
Hyperchromasia	1 (3)	5 (17)	0.1 (0.0-1.4)	0.3 (0.0-1.6)
Thickened nuclear membrane	8 (22)	6 (20)	1.1 (0.3-4.2)	1.2 (0.3-4.7)
Prominent nucleoli	26 (70)	14 (47)	2.7 (0.9-8.4)	2.6 (0.8-8.3)
Multiple nucleoli	4 (11)	1 (3)	3.5 (0.3-88)	1.9 (0.3-12)
Stratification	11 (30)	2 (7)	5.9 (1.1-43)	3.6 (0.9-17)
Loss of polarity	9 (24)	6 (20)	1.3 (0.3-4.8)	1.1 (0.3-4.8)
"Atypia"	11 (30)	3 (10)	3.8 (0.8-20)	2.9 (0.8-12)

^a Adjusted for age at oophorectomy in years (categorical: <45, 45-54, ≥55).

^b Added 0.5 to each cell with zero counts.



FIG. 2. **A:** Surface cells showing minimal stratification, loss of polarity, and nuclear variability ($\times 1,000$, oil immersion). **B:** Epithelial lining of inclusion cyst displaying tubal metaplasia with focal loss of cell polarity ($\times 1,000$, oil immersion).

preciated more frequently among cases than controls, and a subjective interpretation of "atypia" was rendered three times more often in cases. However, these results were not statistically significant after age-adjustment, perhaps in part due to the small number of specimens examined. Gusberg et al. (10) previously reported that prophylactic oophorectomy specimens obtained from three women whose monozygotic twin sisters had been diagnosed with ovarian carcinoma showed "dysplastic" changes with routine microscopy, including stratification, loss of polarity, and nuclear atypia. In an unmasked retrospective comparison of surface epithelium adjacent to cancer and controls, Plaxe et al. (11) also reported that identification of nuclear pleomorphism or irregular chromatin in combination with either loss of cellular polarity or stratification permitted the distinction of malignant from benign ovaries with 98% sensitivity and 100% specificity using conventional microscopy.

In several studies, Deligdisch and colleagues (12–14) and Gil and Deligdisch (15) have reportedly identified

morphologic changes recognizable as "dysplasia," using interactive computer-assisted morphometry and, more recently, neural networks (16). Using computer-based image analysis, Afify et al. (7) have also suggested that the surface epithelial cells of prophylactically removed ovaries possess larger and more heterogeneously dense nuclei than controls. In summary, these data suggest that there may be subtle cytologic changes in ovaries at increased risk for carcinoma compared with normal ovaries; however, these features are difficult to appreciate microscopically.

Isolated reports have suggested that the epithelial lining of surface inclusion cysts elaborates different proteins compared with normal surface epithelium (17,18) and that similar proteins are identifiable in cysts and ovarian carcinoma (19). However, the significance of these findings with respect to ovarian carcinogenesis is unclear. Auersperg et al. (20) demonstrated that cultures of normal appearing surface epithelial cells derived from ovaries removed prophylactically for a family history of cancer show prolonged expression of CA125 and a sustained epithelial phenotype with multiple passages compared with controls. These findings provide additional preliminary evidence that ovaries at increased risk for carcinoma differ biologically from normal.

Retrospective comparisons of the histopathology of ovaries at increased risk for cancer compared with controls are limited by multiple factors. Surface epithelial denudation is frequently extensive without special handling, rendering assessment difficult. Variability in sampling and difficulties related to estimating the number of cysts in two-dimensional representations pose added challenges. We attempted to control statistically for differences in histologic sampling and age distributions between groups, but these adjustments may have been incomplete.

Accurately defining the level of cancer risk in benign ovaries is challenging. The risk of cancer in a woman undergoing prophylactic oophorectomy for a family history of cancer may vary widely (21). Women with many affected family members may be at greater risk for genetic reasons, but this does not account for other known factors such as obstetric history and oral contraceptive use. In addition, women who are related to carriers of BRCA mutations, but who have not been tested, may not be carriers, and therefore are not demonstrably at increased risk. Similarly, 12 women initially included in our prophylactic case group were ultimately excluded because testing for BRCA mutations was negative. Additional studies may identify genes other than BRCA that are related to heritable risk in women with family histories of multiple ovarian cancers. Similarly, the evidence

for a two- to threefold increased risk for the development of cancer in benign ovaries contralateral to carcinoma is based largely on reports of a small number of patients in whom a second ovarian cancer developed 5 or more years after the removal of an initial contralateral tumor (22). Finally, we identified some features such as surface inclusion cysts in a higher percentage of controls compared with other studies, suggesting that variation in histologic sampling or morphologic evaluation could account for differences between studies. Because prophylactic ovaries were removed in many different hospitals without using a standardized protocol, the extent of sampling in these cases varied. As a result of the recent report by Salazaar et al. (6) describing occult microscopic cancers in prophylactic oophorectomy specimens, pathologists should be encouraged to meticulously examine these ovaries and liberally submit tissue for histologic examination.

Progress in our understanding of the pathogenesis of ovarian cancer has been hampered by our inability to identify a common precursor of most of these tumors. Furthermore, prospective studies of ovarian cancer are expensive and time consuming because the relative incidence of these tumors is low. The results of this study, in combination with previous reports, suggest that without optimized specimen preparation and pathologic examination, use of molecular markers, and collection of epidemiologic information, advances in understanding the pathogenesis of this lethal tumor may be slow.

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