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Antibodies Against *Chlamydia trachomatis* and Ovarian Cancer Risk in Two Independent Populations

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Abstract

Background: Pelvic inflammatory disease (PID) has been associated with ovarian cancer risk. To clarify the role of *Chlamydia trachomatis* and other infectious agents in the development of ovarian cancer, we evaluated the association of serologic markers with incident ovarian cancer using a staged approach in two independent populations.
Methods: Studies included: 1) a case–control study in Poland (244 ovarian cancers/556 control subjects) and 2) a prospective nested case–control study in the PLCO Cancer Screening Trial (160 ovarian cancers/159 control subjects). Associations of serologic marker levels with ovarian cancer risk at diagnostic as well as higher thresholds, identified in Poland and independently evaluated in PLCO, were estimated using multivariable adjusted logistic regression.
Results: In the Polish study, antibodies (based on laboratory cut-point) against the chlamydia plasmid-encoded Pgp3 protein (serological gold standard) were associated with increased ovarian cancer risk (adjusted odds ratio [OR] = 1.63, 95% confidence interval [CI] = 1.20 to 2.22); when a positive result was redefined at higher levels, ovarian cancer risk was increased (cut-point 2: OR = 2.00, 95% CI = 1.38 to 2.89; cut-point 3 [max OR]: OR = 2.19, 95% CI = 1.29 to 3.73). In the prospective PLCO study, Pgp3 antibodies were associated with elevated risk at the laboratory cut-point (OR = 1.43, 95% CI = 0.78 to 2.63) and more stringent cut-points (cut-point 2: OR = 2.25, 95% CI = 1.07 to 4.71); cut-point 3: OR = 2.53, 95% CI = 0.63 to 10.08). In both studies, antibodies against other infectious agents measured were not associated with risk.
Conclusions: In two independent populations, antibodies against prior/current *C. trachomatis* (Pgp3) were associated with a doubling in ovarian cancer risk, whereas markers of other infectious agents were unrelated. These findings lend support for an association between PID and ovarian cancer.

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Ovarian cancer is the most fatal gynecologic malignancy (1). Historically ovarian tumors were viewed as arising from ovarian surface epithelia; however, recent data suggest that many of these tumors may be initiated outside the ovary (eg, fallopian tubes, endometrium) (2–5). In the last decade, infectious agents (causing chronic inflammatory diseases) have become increasingly investigated as possible cancer initiators/promoters. Ovarian cancer has been linked to events and conditions related to inflammation and repair (eg, endometriosis, ovulation) (6–8). Primary infertility due to tubal disorders has been shown to predispose to ovarian cancer (9). The role of inflammation in the tube related to sexually transmitted infections, chronic salpingitis, and pelvic inflammatory disease (PID) in the pathogenesis of ovarian cancer has received little attention (10). Of interest, however, is that recurrent PID has been associated with increasing ovarian cancer risk in some studies (11–13). A major limitation in studying the role of chronic inflammation, specifically PID, and ovarian cancer is the lack of information about these

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conditions in epidemiologic studies. Most studies do not capture information regarding medical diagnoses of PID, and use of self-reported medical history is unreliable. Further, examination of risk factors by histologic subtype is important as the etiological pathways differ (14).

Chlamydia trachomatis (*C. trachomatis*) is a sexually transmitted bacterium and the most common cause of PID in high-income countries (15). A prior study found an association between antibodies against *C. trachomatis* infection and ovarian cancer (16), while other studies have reported null results (17,18). *Mycoplasma genitalium* (*M. genitalium*) is another sexually transmitted infection that can cause PID; however, its evaluation as a potential risk factor for ovarian cancer using serologic markers is limited to one prior study (17). We sought to comprehensively test for possible relationships between sexually transmitted infections and other known cancer-associated microorganisms and ovarian cancer using a recently developed and validated multiplex serology assay that allows simultaneous determination of antibodies to a large set of infectious agents. We hypothesized that increased seropositivity indicating previous/current chlamydia infection is associated with ovarian cancer, particularly at higher antibody levels likely indicative of chronic and/or persistent chlamydia infections that may be more strongly correlated with PID. To test this hypothesis, we evaluated associations of antibodies against *C. trachomatis* with ovarian cancer risk for different thresholds to define seropositivity using a two-stage strategy, identifying the cut-points in a population-based case–control study conducted in Poland and independently testing the cut-points in a prospective nested case–control study conducted in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. We also tested for associations with other potential causes/correlates of PID including *M. genitalium*, as well as markers of prior/current infections that are not known causes of PID (eg, human papillomavirus [HPV], hepatitis B virus, etc.) (10).

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Methods

Study Populations

Polish Ovarian Cancer Study

Details of the study have been previously reported (19). Briefly, eligible case subjects included women age 20 to 74 years living in Warsaw or Łódź, Poland, that were newly diagnosed with histologically confirmed epithelial ovarian cancer between June 2001 and December 2003. Cases were identified through a rapid identification system organized at participating hospitals, with periodic checks against cancer registries in both cities to ensure complete case identification. Population-based control subjects were randomly selected from the Polish Electronic System, a database with demographic information for all residents of Poland. Controls comprised women who did not report a previous diagnosis of ovarian cancer or bilateral oophorectomy at the time of enrollment and who were frequency matched to cases based on study site and five-year age category. Serum samples were collected from 278 borderline and invasive ovarian cancer cases (81.5% of 341 interviewed cases) and 1911 controls (95.8% of 1994 interviewed controls). We randomly selected a sample of 556 controls with available serum to approximate a 1:2 case:control ratio. We excluded borderline ovarian cancers (n = 34); thus our final analytic sample included 244 cases and 556 controls. Ethics boards in Poland and the United States approved the study protocol; all participants provided written informed consent.

PLCO

We conducted a prospective nested case–control study within the screening arm of the PLCO Cancer Screening Trial. Details of the trial (20) and nested case–control study (21) have been previously reported. Briefly, between 1993 and 2001, approximately 155 000 subjects (78 216 women) age 55 to 74 years were recruited from 10 cities and randomly assigned to receive screening or no screening. Screening arm subjects provided blood samples at baseline and five subsequent annual medical examinations (22), and cancer diagnoses were pathologically confirmed via medical records. We identified 160 first primary ovarian cancer cases diagnosed between two and 14 years after blood collection from the eligible screening arm participants followed through December 31, 2010. Eligibility criteria included the availability of a serum sample, consent to biochemical studies, completion of the baseline questionnaire, and no history of cancer (other than nonmelanoma skin cancer) prior to ovarian cancer diagnosis. Controls were individually matched to cases on age at blood collection (five-year age category), race (white, black, other), study center, and time (AM, PM) and date (three-month categories) of blood collection. Controls were restricted to women alive without a history of oophorectomy at the time of diagnosis of their matched case. We were unable to identify a suitable matched control for one case; therefore our final analytic sample consisted of 160 cases and 159 matched controls. Institutional review boards at the National Cancer Institute and the study centers approved the trial; all participants provided written informed consent.

Laboratory Measurements

We measured antibodies against an extensive set of *C. trachomatis* antigens including the major outer membrane proteins (MOMP) from serovars A, D, and L2, translocated actin-recruiting phosphoprotein N and C terminal fragments (Tarp-F1 and Tarp-F2), heat shock protein 60 variant 1 (HSP60-1) (Hulstein SH, Matser A, Alberts CJ, et al., manuscript submitted for publication), and plasmid-encoded Pgp3 protein. The *C. trachomatis* Pgp3 antibodies are considered the gold standard for detecting current or past chlamydia infections (23,24) due to longer persistence of antibodies compared with other commonly used antigens (eg, MOMP peptide enzyme-linked immunosorbent assay). We tested for *M. genitalium*, a small pathogenic bacterium that infects the ciliated epithelial cells of the urinary and genital tracts in humans. *M. genitalium* is a relatively common, albeit recently identified, infection that has been associated with PID (25) and infertility (26). We also included HSV-2 as another potential cause of PID (27). To further evaluate the infection–ovarian cancer hypothesis, we measured serologic markers of HPV, which is not associated with PID but is a well-known cause of cervical cancer, as well as other cancer-associated infections that are not exclusively sexually transmitted.

Serum samples were tested for antibodies against a variety of infectious agents (listed in Supplementary Table 1, available online) using a multiplex, fluorescent bead-based assay (28). Antibodies bound to each bead were quantified as median fluorescence intensity (MFI). Continuous MFI values representing antibody levels were dichotomized as seropositive or seronegative based on previously defined cut-points (Hulstein SH,

Table 1. Demographic and health characteristics of cases and controls from the Polish Ovarian Cancer Case-Control Study and a nested case–control study within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial*

Characteristics	Poland		PLCO	
	Cases (n = 244) No. (%)	Controls (n = 556) No. (%)	Cases (n = 160) No. (%)	Controls (n = 159) No. (%)
Mean age at study enrollment (SD)	55.5 (11.4)	55.6 (11.2)	63.3 (5.4)	63.1 (5.4)
Race				
White	244 (100)	556 (100)	147 (92.5)	147 (91.9)
Nonwhite	–	–	13 (8.2)	12 (7.5)
Body mass index, kg/m^2				
<25	148 (60.7)	218 (39.2)	67 (42.1)	67 (41.9)
25–29.9	59 (24.2)	217 (39.0)	57 (35.8)	55 (34.4)
≥30	34 (13.9)	110 (19.8)	34 (21.4)	37 (23.1)
Parity				
Parous	196 (80.3)	486 (87.4)	148 (93.1)	151 (94.4)
Nulliparous	48 (19.7)	70 (12.6)	12 (7.5)	8 (5.0)
Duration of oral contraceptive use, y				
Never/<1	224 (91.8)	488 (87.8)	87 (54.4)	79 (49.7)
1–5	13 (5.3)	44 (7.9)	50 (31.3)	50 (31.5)
>5	3 (1.2)	15 (2.7)	23 (14.4)	30 (18.9)
Duration of menopausal hormone therapy use, y				
Never/<1	183 (75.0)	428 (77.0)	46 (28.8)	64 (40.3)
1–5	42 (17.2)	90 (16.2)	51 (31.9)	50 (31.5)
>5	15 (6.2)	30 (5.4)	63 (39.4)	45 (28.3)

*Columns may not sum to total due to missing data. PLCO = Prostate, Lung, Colorectal, and Ovarian trial.

Matser A, Alberts CJ, et al., manuscript submitted for publication, 28–31) or as described in the Supplementary Methods (including Supplementary Figures 1 and 2 and Supplementary Tables 2 and 3, available online) for *C. trachomatis* Pgp3 and *M. genitalium*. No values were below the detection limit; therefore, we used seronegative as the reference for the individual markers. Quality controls are described in detail in the Supplementary Methods (available online).

Statistical Analysis

Mean antibody levels were compared using generalized linear models adjusting for matching factors, and *P* values were calculated using a Wald test. Associations between continuous antibody levels were estimated using Pearson correlation coefficients. Conditional logistic regression was used to assess the odds ratios (ORs) and 95% confidence intervals (CIs) for the association between seropositivity (indicative of past/current infection) and ovarian cancer. Models were conditioned on matching factors and adjusted for the following a priori–defined ovarian cancer risk factors: nulliparity, duration of oral contraceptive use, and duration of menopausal hormone therapy (MHT) use (as defined in Table 1).

Associations of ovarian cancer with seropositivity were based on laboratory cut-points that were defined independently of the current studies (cut-point 1). We additionally evaluated higher antibody thresholds for chlamydia serology given that we were interested in evaluating risk associated with a marker of PID, which is an adverse sequala of severe, prolonged, and/or repeated *C. trachomatis* infection. We determined these cut-points by evaluating the trajectory of the odds ratio in Poland with increasing antibody level. We identified two additional cut-points in Poland: cut-point 2) where the odds ratio first increased substantially (at least 15%) from the laboratory cut-point-based odds ratio; and cut-point 3) the maximum odds ratio. We then tested these additional cut-points in PLCO. We applied this same approach to antibodies against *M. genitalium* and HSV-2, potential causes/correlates of PID, and HPV16, a common sexually transmitted infection not associated with PID.

In secondary analyses, we evaluated associations stratified by serous/nonserous ovarian cancer subtype in both studies as well as time between blood collection and diagnosis (2–<5 years and 5–14 years) in PLCO; we also evaluated associations excluding women age 70 years or older. $P_{heterogeneity}$ values were based on the likelihood ratio test from a cases-only model, with serous histotype (subtype analyses) or recent blood draw (time to diagnosis analyses) as the reference group. Statistical significance was defined as *P* value of less than .05; all statistical tests were two-sided. Data analyses were performed using SAS software (SAS Institute, version 9.3; Cary, NC).

Results

The Polish study population was younger (mean age = 55.5 years) than the PLCO study population (mean age = 63.2 years) (Table 1). Age-adjusted mean antibody levels tended to be higher in the Polish Study (eg, Pgp3 average antibody level among controls in Poland = 1878 MFI, 95% CI = 1569 to 2188; vs PLCO = 999, 95% CI = 561 to 1436) (Table 2). Mean chlamydia antibody levels were higher in cases than controls in both studies, and the differences in mean anti-pgp3, anti-MOMP-D, and anti-MOMP-L2 levels were statistically significant in the Polish study.

In the Polish case–control study, Pgp3 seropositivity (serological gold standard) was associated with a 63% increased ovarian cancer risk (OR = 1.63, 95% CI = 1.20 to 2.22), and MOMP-A and MOMP-L2 seropositivity were associated with 46% and 59% increased risk (OR = 1.46, 95% CI = 1.06 to 2.02; OR = 1.59, 95% CI = 1.16 to 2.17, respectively) (Table 3). Seropositivity for chlamydia (≥3 of 6 markers seropositive: MOMP-A, MOMP-D, MOMP-L2, Tarp-F1, Tarp-F2, HSP60-1) was also associated with increased

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Table 2. Adjusted arithmetic mean antibody levels (median fluorescence intensity) for antibodies against sexually transmitted and non–sexually transmitted infectious agents by case–control status and study

	Poland*			PLCO		
	Cases (n = 244)	Controls (n = 556)		Cases (n = 160)	Controls (n = 159)	
Infectious agent	Mean* (95% CI)	Mean* (95% CI)	P†	Mean‡ (95% CI)	Mean‡ (95% CI)	P†
Sexually transmitted infections						
Chlamydia trachomatis						
Pgp3	2670 (2233 to 3107)	1878 (1569 to 2188)	.004	1512 (1072 to 1953)	999 (561 to 1436)	.10
MOMP-A	476 (351 to 601)	320 (232 to 409)	.05	277 (184 to 370)	232 (140 to 324)	.50
MOMP-D	767 (601 to 933)	522 (405 to 640)	.02	406 (288 to 523)	270 (153 to 387)	.11
MOMP-L2	701 (549 to 852)	462 (354 to 569)	.01	383 (273 to 494)	259 (149 to 369)	.12
Tarp-F1	925 (720 to 1131)	730 (584 to 875)	.13	683 (484 to 883)	445 (247 to 644)	.10
Tarp-F2	824 (657 to 992)	628 (509 to 746)	.06	830 (550 to 1109)	789 (511 to 1066)	.84
HSP60-1	752 (593 to 911)	587 (475 to 670)	.10	602 (420 to 784)	493 (313 to 674)	.40
Mycoplasma genitalium						
MgPaN	759 (602 to 916)	631 (520 to 742)	.19	313 (198 to 429)	366 (250 to 481)	.53
rMgPa	789 (633 to 946)	703 (592 to 813)	.37	433 (297 to 570)	542 (407 to 678)	.27
Herpes simplex virus–2						
mgGunique	714 (495 to 932)	676 (521 to 831)	.78	651 (425 to 878)	480 (255 to 706)	.29
Human papillomavirus						
11 L1	138 (103 to 172)	138 (113 to 162)	.99	121 (95.8 to 145)	78.3 (53.7 to 103)	.02
16 L1	134 (94.7 to 173)	130 (102 to 158)	.88	71 (41.8 to 99.7)	85.4 (56.7 to 114)	.48
18 L1	154 (113 to 196)	153 (124 to 183)	.97	105 (78.7 to 131)	101 (75.1 to 127)	.84
31 L1	157 (112 to 201)	171 (139 to 202)	.60	145 (105 to 185)	152 (113 to 193)	.78
33 L1	95.0 (75.7 to 114)	90.4 (76.7 to 104)	.70	55.9 (46.7 to 64.9)	61.0 (52.0 to 70.0)	.43
45 L1	107 (78.4 to 136)	125 (105 to 145)	.31	90.1 (65.0 to 115)	78.7 (53.9 to 104)	.53
Other infections (predominantly non–sexually transmitted)						
Herpes simplex virus–1						
gD	5666 (5215 to 6116)	5926 (5608 to 6245)	.35	3184 (2657 to 3711)	4043 (3519 to 4567)	.02
Polyomavirus						
BK VP1	4321 (3877 to 4765)	4745 (4431 to 5059)	.13	4368 (3876 to 4860)	3952 (3464 to 4441)	.24
JC VP1	1586 (1386 to 1787)	1627 (1485 to 1769)	.74	1130 (937 to 1323)	1180 (988 to 1373)	.72
HPyV9 VP1	2395 (1894 to 2896)	2936 (2581 to 3290)	.08	578 (275 to 881)	1048 (747 to 1349)	.03
Hepatitis C virus						
Core	280 (151 to 410)	211 (120 to 303)	.39	126 (51.5 to 201)	96 (22.0 to 171)	.58
NS3	321 (196 to 447)	260 (171 to 349)	.43	121 (37.7 to 204)	72 (10.5 to 151)	.41
Hepatitis B virus						
HBc	1057 (686 to 1428)	1317 (1055 to 1580)	.26	55.3 (4.8 to 185)	170 (41.0 to 299)	.22
HBe	973 (643 to 1302)	1230 (997 to 1463)	.21	153 (23.8 to 331)	260 (83.8 to 435.9)	.40
Epstein-Barr virus						
Zebra	5416 (4976 to 5856)	5562 (5251 to 5873)	.60	4953 (4430 to 5476)	5002 (4483 to 5522)	.89
EA-D	3769 (3357 to 4181)	3505 (3214 to 3796)	.30	5222 (4612 to 5832)	4701 (4096 to 5307)	.23
EBNA-1	7199 (6684 to 7713)	7286 (6922 to 7650)	.79	5976 (5372 to 6580)	5927 (5327 to 6527)	.91
Cytomegalovirus						
pp150N	5717 (5271 to 6163)	5840 (5525 to 6156)	.66	3681 (3191 to 4172)	3481 (2994 to 3968)	.57
pp52	10 690 (10 109 to 11 270)	10 716 (10 306 to 11 127)	.94	7328 (6558 to 8097)	6966 (6201 to 7731)	.51
pp28	7065 (6538 to 7593)	7083 (6710 to 7456)	.96	4982 (4294 to 5672)	4751 (4066 to 5435)	.64

*Adjusted for age and study site. CI = confidence interval; HSP = heat shock protein; MOMP = major outer membrane protein; PLCO = Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; Tarp = translocated actin-recruiting phosphoprotein.
†P values calculated using a two-tailed Wald test with an F-distribution as the reference distribution, at alpha = .05.
‡Adjusted for age, race (white vs nonwhite), time of blood draw (AM vs PM), and month of blood draw.

ovarian cancer risk (OR = 1.45, 95% CI = 1.06 to 2.00). Odds ratios were elevated, albeit not statistically significantly so, for the other chlamydia markers MOMP-D2, Tarp-F1, Tarp-F2, and HSP60-1. When evaluating higher marker thresholds for seropositivity (Table 4), antibodies against Pgp3 were associated with a doubling in ovarian cancer risk in the Polish study (cut-point 2: OR = 2.00, 95% CI = 1.38 to 2.89; cut-point 3 [max OR]: OR = 2.19, 95% CI = 1.29 to 3.73). Higher antibody levels (cut-points 2 and 3) also resulted in statistically significantly increased ovarian cancer risk with all other chlamydia markers measured in Poland.

While the proportion of Pgp3 seropositive women was different by case (25.0%) and control (21.4%) status in PLCO, the increased risk of ovarian cancer was not statistically significant using the laboratory-determined seropositivity cut-point for Pgp3 (OR = 1.43, 95% CI = 0.78 to 2.63) (Table 3). Other chlamydia markers as well as overall chlamydia seropositivity were also not associated with increased risk in PLCO (overall seropositivity: OR = 1.18, 95% CI = 0.64 to 2.18). After applying the higher marker threshold identified in Poland to the PLCO population, Pgp3 positivity was associated with an increased ovarian cancer

Table 3. Serologic marker associations with ovarian cancer risk in a Polish case–control study and a nested case–control study conducted within the PLCO Cancer Screening Trial

	Poland*			PLCO*		
Infectious agent	Cases No. + (% +)	Controls No. + (% +)	OR† (95% CI)	Cases No. + (% +)	Controls No. + (% +)	OR† (95% CI)
Chlamydia trachomatis						
Pgp3	111 (45.5)	192 (34.5)	1.63 (1.20 to 2.22)	40 (25.0)	34 (21.4)	1.43 (0.78 to 2.63)
MOMP-A	80 (32.8)	146 (26.3)	1.46 (1.06 to 2.02)	33 (20.6)	41 (25.8)	0.70 (0.40 to 1.22)
MOMP-D	89 (36.5)	186 (33.5)	1.25 (0.91 to 1.70)	40 (25.0)	38 (23.9)	1.20 (0.66 to 2.19)
MOMP-L2	99 (40.6)	160 (28.8)	1.59 (1.16 to 2.17)	41 (25.6)	39 (24.5)	1.14 (0.64 to 2.01)
Tarp-F1	94 (38.5)	189 (34.0)	1.21 (0.88 to 1.66)	50 (31.3)	41 (25.8)	1.44 (0.85 to 2.44)
Tarp-F2	131 (53.7)	275 (49.5)	1.21 (0.89 to 1.63)	67 (41.9)	64 (40.3)	1.09 (0.67 to 1.78)
HSP60-1	121 (49.6)	264 (47.5)	1.17 (0.86 to 1.60)	64 (40.0)	57 (35.8)	1.25 (0.77 to 2.03)
Seropositive‡	89 (36.5)	158 (28.4)	1.45 (1.06 to 2.00)	37 (23.1)	34 (21.4)	1.18 (0.64 to 2.18)
Mycoplasma genitalium						
MgPaN	84 (34.4)	166 (29.9)	1.24 (0.90 to 1.72)	26 (16.3)	36 (22.6)	0.68 (0.35 to 1.32)
rMgPa	94 (38.5)	183 (32.9)	1.32 (0.96 to 1.81)	41 (25.6)	50 (31.4)	0.71 (0.41 to 1.23)
Seropositive‡	58 (23.8)	103 (18.5)	1.47 (1.00 to 2.15)	13 (8.1)	19 (11.9)	0.53 (0.22 to 1.31)
Herpes simplex virus–2						
mgGunique	55 (22.5)	115 (20.7)	1.18 (0.83 to 1.69)	37 (23.1)	28 (17.6)	1.45 (0.79 to 2.68)
Human papillomavirus						
11 L1	11 (4.5)	25 (4.5)	0.97 (0.47 to 1.99)	11 (6.9)	3 (1.9)	4.57 (0.97 to 21.7)
16 L1	17 (7.0)	23 (4.1)	1.60 (0.82 to 3.12)	2 (1.3)	3 (1.9)	0.82 (0.13 to 5.19)
18 L1	17 (7.0)	34 (6.1)	1.04 (0.56 to 1.92)	5 (3.1)	6 (3.8)	1.04 (0.29 to 3.68)
31 L1	10 (4.1)	23 (4.1)	0.85 (0.40 to 1.82)	4 (2.5)	6 (3.8)	0.92 (0.23 to 3.68)
33 L1	10 (4.1)	13 (2.3)	1.30 (0.55 to 3.08)	0 (0.0)	1 (0.6)	–
45 L1	17 (7.0)	32 (5.8)	1.14 (0.61 to 2.11)	5 (3.1)	2 (1.3)	2.62 (0.49 to 14.1)
Seropositive‡	40 (16.4)	69 (12.4)	1.26 (0.83 to 1.93)	13 (8.1)	14 (8.8)	1.17 (0.49 to 2.80)
Herpes simplex virus–1						
gD	235 (96.3)	540 (97.1)	0.51 (0.21 to 1.24)	114 (71.3)	131 (82.4)	0.52 (0.29 to 0.96)
Polyomavirus						
BK VP1	217 (88.9)	520 (93.5)	0.60 (0.35 to 1.01)	147 (91.9)	145 (91.2)	1.34 (0.53 to 3.40)
JC VP1	149 (61.1)	377 (67.8)	0.81 (0.59 to 1.11)	95 (59.4)	94 (59.1)	1.16 (0.70 to 1.93)
HPyV9 VP1	114 (46.7)	314 (56.5)	0.75 (0.56 to 1.02)	55 (34.4)	53 (33.3)	1.16 (0.72 to 1.89)
Hepatitis C virus						
Core	14 (5.7)	22 (4.0)	1.61 (0.80 to 3.25)	4 (2.5)	3 (1.9)	1.76 (0.26 to 11.9)
NS3	15 (6.1)	49 (8.8)	0.78 (0.43 to 1.41)	1 (0.6)	1 (0.6)	0.53 (0.03 to 8.91)
Seropositive‡	10 (4.1)	18 (3.2)	1.61 (0.72 to 3.60)	1 (0.6)	0 (0.0)	–
Hepatitis B virus						
HBc	36 (14.8)	100 (18.0)	0.75 (0.50 to 1.14)	1 (0.6)	3 (1.9)	0.22 (0.02 to 2.33)
HBe	38 (15.6)	113 (20.3)	0.69 (0.46 to 1.03)	5 (3.1)	5 (3.1)	0.85 (0.23 to 3.12)
Seropositive‡	36 (14.8)	99 (17.8)	0.77 (0.51 to 1.16)	1 (0.6)	3 (1.9)	0.22 (0.02 to 2.33)
Epstein-Barr virus						
Zebra	231 (94.7)	531 (95.5)	0.83 (0.41 to 1.64)	146 (91.3)	149 (93.7)	1.00 (0.37 to 2.68)
EAD	187 (76.6)	403 (72.5)	1.34 (0.94 to 1.91)	128 (80.0)	130 (81.8)	1.01 (0.56 to 1.85)
EBNA1	228 (93.4)	534 (96.0)	0.55 (0.28 to 1.06)	141 (88.1)	141 (88.7)	1.10 (0.49 to 2.50)
Seropositive‡	232 (95.1)	531 (95.5)	0.86 (0.43 to 1.75)	145 (90.6)	148 (93.1)	0.90 (0.33 to 2.44)
Cytomegalovirus						
pp150N	226 (92.6)	526 (94.6)	0.79 (0.42 to 1.49)	119 (74.4)	116 (73.0)	1.23 (0.72 to 2.13)
pp52	233 (95.5)	537 (96.6)	1.00 (0.44 to 2.28)	130 (81.3)	129 (81.1)	1.09 (0.59 to 2.02)
pp28	230 (94.3)	536 (96.4)	0.67 (0.33 to 1.36)	127 (79.4)	127 (79.9)	1.09 (0.60 to 1.99)
Seropositive‡	230 (94.3)	533 (95.9)	0.95 (0.46 to 1.98)	123 (76.9)	122 (76.7)	1.15 (0.65 to 2.05)

*Frequency (No.) and percentage (%) reported based on seropositive (+) for individual markers or the combined marker ("Seropositive"). The reference category is seronegative for an individual marker or negative for the combined marker. CI = confidence interval; HSP = heat shock protein; MOMP = major outer membrane protein; OR = odds ratio; PLCO = Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; Tarp = translocated actin-recruiting phosphoprotein.

†Odds ratios and 95% confidence intervals are from logistic regression models conditioned on matching factors (Poland: age and study site; PLCO: age, race, time of blood draw, month of blood draw) and adjusted for nulliparity, duration of oral contraceptive use, and duration of menopausal hormone use.

‡The definition for seropositive for a specific antigen was based on the following: *C. trachomatis*, three or more markers positive (MOMP-A, MOMP-D, MOMP-L2, Tarp-F1, Tarp-F2, HSP60-1); *M. genitalium*, hepatitis B, hepatitis C, both markers positive; human papillomavirus, if any of high-risk types are positive (HPV-16, -18, -31, -33, -34); Epstein-Barr virus and cytomegalovirus, at least two of three positive.

risk (cut-point 2: OR = 2.25, 95% CI = 1.07 to 4.71; cut-point 3: OR = 2.53, 95% CI = 0.63 to 10.08) (Table 4). Odds ratios in PLCO were also elevated for MOMP-D and MOMP-L2 when applying the seropositive marker cut-point for the maximum odds ratio in Poland (MOMP-D: OR = 1.57, 95% CI = 0.65 to 3.80; MOMP-L2: OR = 1.72, 95% CI = 0.77 to 3.82).

Ovarian cancer was not associated with prior/current infection of the other agents studied, either with individual antibody

Table 4. Chlamydia marker associations with ovarian cancer risk with increasing cut-points for marker positivity selected from a Polish case–control study and applied in a nested case–control study conducted within the PLCO Cancer Screening Trial

		Poland*			PLCO*		
Chlamydia trachomatis	Marker cut-point	Cases No. + (% +)	Controls No. + (% +)	OR† (95% CI)	Cases No. + (% +)	Controls No. + (% +)	OR† (95% CI)
Pgp3							
Lab cut-point for positivity (cut-point 1)	300	111 (45.5)	192 (34.5)	1.63 (1.20 to 2.22)	40 (25.0)	34 (21.4)	1.43 (0.78 to 2.63)
Increased OR from Poland (cut-point 2)	3878	70 (28.7)	97 (17.4)	2.00 (1.38 to 2.89)	28 (17.5)	17 (10.7)	2.25 (1.07 to 4.71)
Max OR from Poland (cut-point 3)	8839	28 (11.5)	36 (6.5)	2.19 (1.29 to 3.73)	7 (4.4)	3 (1.9)	2.53 (0.63 to 10.1)
MOMP-A							
Lab cut-point for positivity	165	80 (32.8)	146 (26.3)	1.46 (1.06 to 2.02)	33 (20.6)	41 (25.8)	0.70 (0.40 to 1.22)
Increased OR from Poland	386	52 (21.3)	78 (14.0)	1.72 (1.17 to 2.52)	24 (15.0)	25 (15.7)	0.94 (0.49 to 1.80)
Max OR from Poland	1727	22 (9.0)	22 (4.0)	2.33 (1.26 to 4.31)	6 (3.8)	6 (3.8)	0.98 (0.28 to 3.42)
MOMP-D							
Lab cut-point for positivity	165	89 (36.5)	186 (33.5)	1.25 (0.91 to 1.70)	40 (25.0)	38 (23.9)	1.20 (0.66 to 2.19)
Increased OR from Poland	638	56 (23.0)	81 (14.6)	1.93 (1.31 to 2.85)	22 (13.8)	21 (13.2)	1.19 (0.52 to 2.73)
Max OR from Poland	1042	47 (19.3)	59 (10.6)	2.12 (1.38 to 3.24)	16 (10.0)	13 (8.2)	1.57 (0.65 to 3.80)
MOMP-L2							
Lab cut-point for positivity	250	82 (33.6)	160 (28.8)	1.59 (1.16 to 2.17)	41 (25.6)	39 (24.5)	1.14 (0.64 to 2.01)
Increased OR from Poland	602	59 (24.2)	77 (13.8)	2.03 (1.39 to 2.96)	22 (13.8)	21 (13.2)	1.27 (0.58 to 2.77)
Max OR from Poland	834	55 (22.5)	65 (11.7)	2.27 (1.51 to 3.40)	20 (12.5)	16 (10.1)	1.72 (0.77 to 3.82)
Tarp-F1							
Lab cut-point for positivity	265	94 (38.5)	189 (34.0)	1.21 (0.88 to 1.66)	50 (31.3)	41 (25.8)	1.44 (0.85 to 2.44)
Increased OR from Poland	1326	48 (19.7)	78 (14.0)	1.51 (1.00 to 2.27)	21 (13.1)	19 (11.9)	1.25 (0.61 to 2.57)
Max OR from Poland	1689	41 (16.8)	62 (11.2)	1.76 (1.12 to 2.76)	18 (11.3)	17 (10.7)	1.19 (0.55 to 2.61)
Tarp-F2							
Lab cut-point	165	126 (51.6)	275 (49.5)	1.21 (0.89 to 1.63)	67 (41.9)	64 (40.3)	1.09 (0.67 to 1.78)
Increased OR from Poland	1667	37 (15.2)	50 (9.0)	1.90 (1.18 to 3.03)	22 (13.8)	23 (14.5)	0.95 (0.48 to 1.87)
Max OR from Poland	2106	32 (13.1)	38 (6.8)	2.24 (1.33 to 3.78)	18 (11.3)	21 (13.2)	0.82 (0.40 to 1.67)
HSP60-1							
Lab cut-point for positivity	200	121 (49.6)	264 (47.5)	1.17 (0.86 to 1.60)	64 (40.0)	57 (35.8)	1.25 (0.77 to 2.03)
Increased OR from Poland	1308	42 (17.2)	55 (9.9)	2.07 (1.34 to 3.19)	18 (11.3)	15 (9.4)	1.09 (0.49 to 2.43)
Max OR from Poland	3407	12 (4.9)	16 (2.9)	2.25 (1.08 to 4.70)	5 (3.1)	6 (3.8)	0.93 (0.23 to 3.70)

*Frequency (No.) and percentage (%) reported based on seropositive (+) for individual markers. The reference category is seronegative for an individual marker. CI = confidence interval; HSP = heat shock protein; MOMP = major outer membrane protein; OR = odds ratio; PLCO = Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; Tarp = translocated actin-recruiting phosphoprotein.

†Odds ratios and 95% confidence intervals are from logistic regression models conditioned on matching factors (Poland: age and study site; PLCO: age, race, time of blood draw, month of blood draw) and adjusted for nulliparity, duration of oral contraceptive use, and duration of menopausal hormone use.

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positivity or overall seropositivity (Table 3). Specifically, antibodies against the other possible correlates of PID, *M. genitalium* and HSV-2, had elevated odds ratios in the Polish Study (*M.genitalium* [MgPaN and rMgPa]: OR = 1.24 and 1.32; antibodies against HSV-2 [mgGunique]: OR = 1.18), and HSV-2 also had an elevated odds ratio in PLCO (OR = 1.45), but none reached statistical significance (Table 3). Ovarian cancer risk was elevated with the combined seropositive measure of *M. genitalium* in Poland (OR = 1.47, 95% CI = 1.00 to 2.15) but not in PLCO (OR = 0.53, 95% CI = 0.22 to 1.31). Ovarian cancer risk was not associated with antibodies against HPV (seropositive, Poland: OR = 1.26, 95% CI = 0.83 to 1.93; PLCO: OR = 1.17, 95% CI = 0.49 to 2.80), nor was risk associated with markers of prior/current non–sexually transmitted infections (ie, antibodies against HSV-1, human polyomaviruses, hepatitis C, hepatitis B, Epstein-Barr virus, and cytomegalovirus).

When evaluating higher marker thresholds for seropositivity, antibodies against *M. genitalium* (rMgPa) were associated with ovarian cancer risk at higher thresholds in the Polish study (cut-point 2: OR = 1.62, 95% CI = 1.00 to 2.64; cut-point 3: OR = 1.69, 95% CI = 1.02 to 2.79), but no association was observed in PLCO (cut-point 2: OR = 0.74, 95% CI = 0.28 to 1.94; cut-point 3: OR = 0.69, 95% CI = 0.25 to 1.95) (data not shown). We did not find any statistically significant associations between antibodies against *M. genitalium* (MgPaN), HSV-2, or HPV-16 L1 and ovarian cancer in Poland (results not shown).

Ovarian cancer risks were not substantially different by serous/nonserous subtype for either study (Supplementary Table 4, available online). In PLCO, no substantial differences in ovarian cancer risk were observed for diagnostic or higher marker thresholds of prior/current infection when evaluating time between blood draw and diagnosis, supporting that case status did not influence antibody levels (Supplementary Table 5, available online). Results were not substantially changed when excluding individuals age 70 years or older at blood draw from either study (eg, antibodies against Pgp3 at laboratory cut-point in Poland: OR = 1.67, 95% CI = 1.19 to 2.33; PLCO: OR = 1.51, 95% CI = 0.73 to 3.09) (data not shown).

Antibodies against *C. trachomatis* were positively correlated with one another; correlations with Pgp3 antibodies ranged from 0.43 to 0.72 (Pearson correlation coefficients) across both populations (Supplementary Table 1, available online). As anticipated, antibody responses to *M. genitalium* and HSV-2 were modestly correlated with chlamydia marker Pgp3, while antibody responses to other infections measured were not correlated with Pgp3.

Discussion

Our results suggest that antibodies against *C. trachomatis* infection are associated with an increased risk of ovarian cancer in two independent populations. The findings are specific to the serologic gold standard of prior chlamydia infection (Pgp3) as well as other markers of chlamydia infection (ie, MOMP-A, MOMP-L2, overall chlamydia seropositivity). Antibodies against other sexually transmitted infections evaluated (eg, HPV) or non–sexually transmitted infections that are not implicated in PID were not associated with ovarian cancer risk in either study. This supports the hypothesis that chlamydia infection, possibly through the associated adverse sequala PID, is associated with ovarian cancer risk. The finding that higher antibody thresholds showed a stronger association with ovarian cancer risk likely suggests that only a subset of the most severe chlamydia infections are associated with ovarian cancer. Higher antibody titers have been associated with severe infection previously, particularly for clinically manifest salpingitis (32,34).

Prior studies evaluating serologic markers of *C. trachomatis* or *M. genitalium* infection are few, limited in sample size, and inconsistent (16–18). IgG antibodies to chlamydia extracellular elementary bodies (EB) were associated with ovarian cancer risk in a study of 117 cases and 171 healthy controls (16). However, an inverse association among younger women was reported with serovar D IgG EB in a larger study, and associations with other chlamydia serology markers, including HSP60-1, have been null (17,18,35). None of the prior studies have evaluated the association between chlamydia Pgp3 and ovarian cancer risk. *M. genitalium* serology was not associated with ovarian cancer risk in one study (17), and *M. genitalium* DNA was detected in one study of ovarian carcinoma but not in another (36,37).

The evaluation of HPV serology and ovarian cancer so far has been limited to one prospective study that reported no association between ovarian cancer and HPV16 L1 antibodies (38). Numerous studies evaluating HPV in tissue have also been primarily null (36,39–42), with only one study suggesting a higher prevalence of HPV16 E6 gene expression in ovarian tumor tissues compared with nonmalignant tissue (41). A study evaluating viral DNA and ovarian cancer from sequencing data from The Cancer Genome Atlas (TCGA) did not identify transcribed viral elements to HPV, Epstein-Barr virus, or hepatitis B virus in 419 serous cystadenocarcinomas (43). Our results, from two independent populations, further support that there is no association between HPV infection and ovarian cancer risk.

An association between *C. trachomatis* infection and ovarian cancer is supported by molecular data demonstrating an antiapoptotic effect of prolonged chlamydia infection resulting in survival of chlamydia bacteria within the host cell. This in turn facilitates survival of DNA damaged cells, which may lead to increased risk of cancer initiation (33,44,45). Additionally, upper genital tract chlamydial infections can cause adhesions between tube and ovary, leading to juxtaposition of these organs and transfer of tubal-initiated cells to the growth-promoting microenvironment in the ovary.

Study strengths included the comprehensive evaluation of infection-related antibodies measured using a validated technology as well as the availability of extensive risk factor data in both studies with careful control of confounding. Additional strengths included the population-based design and recruitment of incident ovarian cancer cases in the Polish study and the prospective design of the PLCO study. Limitations included the assessment of serologic antibodies at the time of diagnosis in Poland; however, when evaluating the timing of blood draw and cancer diagnosis in PLCO, we noted no appreciable difference. Information on prior chlamydia infections or PID were not available in either study; however, this is a limitation of most epidemiologic studies. We were not able to test for antibodies to *Neisseria gonorrhoeae*, a known cause of PID. Further, we were not able to adjust for treatment of chlamydia infections (eg, antibiotic use), which may have affected our findings. Although we included all ovarian cancer cases from the PLCO screening arm, the study was limited in power, especially for evaluating relations with specific subtypes. Another potential limitation included the persistence of antibody response, which has been demonstrated over a 12-year period. We cannot rule out that the waning of marker positivity observed in the PLCO study (older mean age at blood draw) is due to reduced persistence of the antibody response over long periods (23); however, results were not substantially changed in analyses excluding women age 70 years or older. Increasing the seropositivity cut-point in PLCO did demonstrate increased risk of ovarian cancer; additional research establishing a cutoff for both persistent chlamydia infections and PID would further enable unbiased testing of the PID-ovarian cancer hypothesis.

In conclusion, the current data support the hypothesis that tubal inflammation and damage are likely etiologically relevant phenomena for ovarian cancer, arguing for additional research to confirm and extend these findings. While *C. trachomatis* is the most prevalent cause of PID in high-income countries, other causes include *N. gonorrhoeae*, *M. genitalium*, bacterial vaginosis pathogens (eg, *Peptostreptococcus* and *Bacteroides* species), and enteric pathogens (eg, *E. coli*); respiratory pathogens (eg, *Haemophilus influenzae*), which may also increase risk of ovarian cancer. Chronic PID (lasting longer than 30 days) has been attributed to *Mycobacterium tuberculosis* and *Actinomyces* (46). Identifying serologic markers specific to subclinical or chronic PID is warranted. If corroborated, these findings support evaluation of potential ovarian cancer risk reduction through treatment of *C. trachomatis* infections.

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