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Immunological Markers of *Chlamydia trachomatis* Infection in Epithelial Ovarian Cancer

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Abstract. *Background/Aim: Pelvic inflammatory disease (PID) is a risk factor for epithelial ovarian cancer (EOC). Chlamydia trachomatis infection, a major cause of PID, may persist in some women. Serum IgG antibodies to chlamydial TroA and HtrA are more common in ascending or repeat chlamydial infection than in uncomplicated infection. The aim of this study was to explore the role of C. trachomatis infection in EOC by analyzing chlamydial TroA, HtrA and major outer membrane protein (MOMP) IgG serum antibody responses. Patients and Methods: The study is based on the review of Oulu University Hospital medical records of 162 women diagnosed with EOC between March 2008 and May 2018. Serum IgG antibody responses to recombinant C. trachomatis TroA, HtrA and MOMP were analyzed using enzyme-linked immunoassay. Complete response to the first line therapy and the three-year survival were the study endpoints. Results: Altogether, 16.7%, 11.1% and 12.3% women were C. trachomatis TroA, HtrA and MOMP IgG positive, respectively. Women with these antibodies were more likely to have a complete response to the first-line treatment, compared to women without these antibodies (63.0% vs. 34.1% for TroA IgG, 50.0% vs. 37.5% for HtrA IgG and 50% vs. 37.3% for MOMP IgG, respectively). The presence of these antibodies predicted better three-year survival. Conclusion: Women with EOC and positive markers of persistent C. trachomatis infection have better response to the first-line treatment and seem to have better three-year survival.*

Ovarian cancer is associated with high mortality, causing 140,000 deaths annually worldwide (1). Over 90% of ovarian cancers are of epithelial origin, of which 70% are high-grade serous cancers. The five-year relative survival of women with ovarian cancer varies between 40 and 50% in the Nordic countries (2). Current understanding points to the fallopian tube epithelium as the main origin of high-grade serous carcinomas (3). Many studies have suggested a role for pelvic inflammatory disease (PID) in the pathogenesis of ovarian cancer (4-7).

Chlamydia trachomatis infection is a major cause of PID typically characterized by silent inflammation of the fallopian tubes and ovaries (8). Although *C. trachomatis* infection usually clears after treatment, the inflammation can persist in some women, and viable atypical chlamydial forms remain in infected cells (9, 10). This ability of *C. trachomatis* to transform into a persistent form has been hypothesized as one of the key mechanisms leading to tissue damage and sequelae (11, 12). The persistence of *C. trachomatis* is associated with an altered bacterial gene transcription profile, including the expression of specific and highly immunogenic proteins, chlamydial TroA and HtrA (10). In our previous studies, serum immunoglobulin G (IgG) antibodies against TroA and HtrA were more common in women with ascending or repeat *C. trachomatis* infection than in those with first infection (11-

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13). Such antibody response was linked to pelvic adhesions (12) and tubal factor infertility (11).

In the present retrospective study, we explored *C. trachomatis* TroA, HtrA and MOMP IgG serum antibody responses in women with EOC, as well as the association of seropositivity with response to the first-line treatment and three-year survival.

Patients and Methods

Study design. The study cohort consisted of 378 women admitted to Oulu University Hospital because of undefined ovarian tumors between March 2008 and May 2018. In Finland, gynecologic cancer treatments are centralized to five university hospitals including Oulu University Hospital. Women with benign (n=130), borderline (n=32), sex cord-stromal (n=4) and germ cell tumors (n=2), as well as those with cancers other than ovarian tumors (n=48), were excluded. The final study cohort comprised 162 women diagnosed with EOC (Figure 1).

The medical records were obtained from Oulu University Hospital and reviewed. Information obtained from the records included age, parity, previous cancers or pre-malignant conditions and some other obvious confounding factors. Cancer-related information, such as the histology, cancer stage, residual tumor load, CA 125 values and the presence of adhesions, was also obtained from the hospital records. All ovarian cancer diagnoses were based on histology obtained from diagnostic laparoscopy or cytoreductive surgery. Serum samples for upcoming studies were collected before treatment after signed informed consent. At first, serum samples were stored at –20˚C in the hospital's laboratory and transferred monthly to research laboratory and stored at –80˚C until analyzed.

Complete response and the three-year survival were selected as the two main endpoints. The definition of complete response was based on radiological imaging (computer tomography) according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria as a routine practice (14). The first-line treatment consisted of either primary surgery followed by adjuvant chemotherapy, or neoadjuvant chemotherapy followed by secondary debulking surgery and adjuvant chemotherapy. In addition, the response to the first-line treatment in women not eligible for surgery after neoadjuvant chemotherapy was estimated at the end of the first full-course chemotherapy. The most used chemotherapy regimens in Finland at that time were carboplatin and paclitaxel. Three-year survival was calculated from the date of first hospital visit to the date of death or end of data collection (36 months or November 10, 2020, whichever came first).

Serological methods. IgG antibody responses to recombinant *C. trachomatis* TroA and HtrA were analyzed using an in-house enzyme-linked immunosorbent assay (ELISA), as described in detail earlier (11-13). The intra-assay coefficients of variation (CV) of the TroA and HtrA assays was 4.6% and 4.3%, respectively. The inter-assay CV of the TroA assay was 12.3-12.9%, and that of the HtrA assay 7.6-13.8%. The serum samples were also analyzed for *C. trachomatis* MOMP IgG antibody using a commercial *C. trachomatis* IgG ELISA kit (Labsystems Diagnostics, Vantaa, Finland). Samples with S/CO of <1 were interpreted as negative, ≥1.4 as positive, and 1.0 ≤ S/CO <1.4 as equivocal, according to the manufacturer's instructions (15).

Statistical methods. Statistical analysis was performed using IBM SPSS Statistics, version 25 (IBM Corporation, Armonk, NY, USA) software. Parity was categorized as nulliparous and primi/multiparous (≥ one delivery). The tumors were classified into early and advanced stages according to the International Federation of Gynecology and Obstetrics (FIGO) staging system. The early-stage category included FIGO stages I and II, and the advanced-stage category included stages III, IV and X (X=surgically unstaged, advanced ovarian cancer). *C. trachomatis* TroA, HtrA and MOMP IgG antibodies were analyzed as categorical (positive/negative) variables.

When analyzing complete response, logistic regression was performed to adjust for EOC stage. The Cox proportional hazards model was used to obtain hazard ratios (HR) and 95% confidence intervals (CI) to investigate the association of three-year survival and antichlamydial seropositivity and EOC stage. The assumptions of the Cox regression model were checked graphically by drawing Kaplan–Meier curves.

Ethics statement. The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Helsinki University Central Hospital, Laboratory Division (permit number: HUS/211/2020, date April 29, 2020). Serum sample collection and data collection from the hospital's records were approved by the regional ethics committee of the Northern Ostrobothnia Hospital District (53/2010, date September 15, 2010) and the National Supervisory Authority for Welfare and Health (1339/05.01.00.06/2009, date March 12, 2009). Informed consent was obtained from research participants.

Results

The study cohort consisted of 162 women with EOC, of whom 101 (62.3%) had high-grade serous ovarian cancer, and 21 (13.0%) had endometroid ovarian cancer. The median age at the time of cancer diagnosis was 64 years (range=28-85 years). Most EOC cases (n=116, 71.6%) were diagnosed in advanced stage (stages III-IV and X). Forty-seven (29.0%) women died during the three-year follow-up. The mean follow-up time was 30.5 months (range=0-36 months).

The majority of women were treated with primary cytoreductive surgery (69.8%), followed by six cycles of adjuvant chemotherapy (81.5%). A minority of women were treated with 3-4 cycles of neoadjuvant chemotherapy (11.1%), followed by secondary debulking surgery (17.3%). Of the women who underwent surgery, 56.7% had no residual tumor. Sixty-three women (38.9%) had complete response after the first-line treatment.

In total, 27 (16.7%) women were *C. trachomatis* TroA IgG positive, 18 (11.1%) women were HtrA IgG positive, and 20 (12.3%) women were MOMP IgG positive (Table I, Figure 2). Forty-one women tested positive in any antichlamydial IgG assay, 20 women had antibodies against at least two chlamydial antigens, and four women tested positive for all three antibodies (Figure 2). The women with TroA, HtrA or MOMP IgG antibody were younger than the seronegative women (Table I). HtrA IgG positivity rate was higher with more advanced stage of EOC, and in the presence of residual tumor after operation (Table I).

The presence of TroA IgG antibody was associated with complete response to the first line platinum-taxane

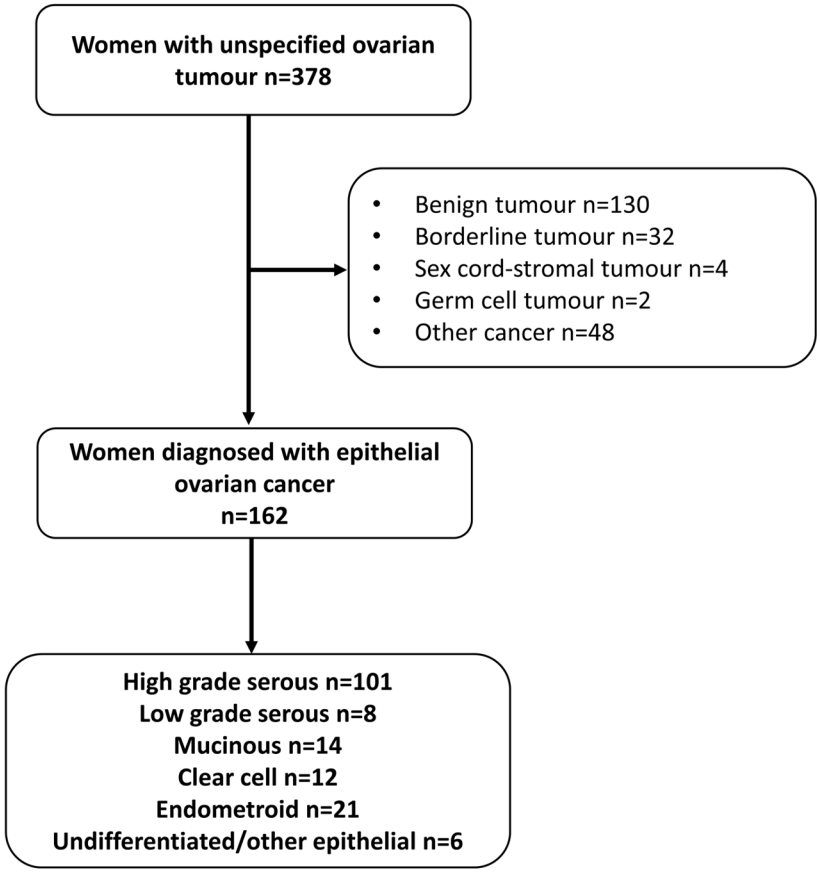

Figure 1. *Flowchart of the study population.*

chemotherapy, as 17 (63.0%) of women with TroA IgG showed complete response, compared to 43 (34.1%) of those without TroA IgG. Similarly, complete response was associated with HtrA IgG positivity, since nine (50%) of women with HtrA IgG had complete response, compared to 54 (37.5%) of those without HtrA IgG. Ten (50.0%) women with MOMP IgG, and 53 (37.3%) without MOMP IgG showed complete response. Complete response was associated with TroA IgG (OR=0.18, 95%CI=0.07-0.49) and HtrA IgG seropositivity (OR=0.25, 95%CI=0.08-0.72) when adjusted for stage (Table II).

After three years, 115 (71%) women were alive. Among TroA IgG positive women, 23 (85.2%) were alive, compared to 92 (68.1%) TroA IgG seronegative women. Similarly, 16 (88.9%) of HtrA IgG, and 18 (90%) of MOMP IgG positive women were alive compared to 99 (68.8%) of HtrA IgG negative and 97 (68.3%) of MOMP IgG negative women.

According to the Cox regression analysis, the presence of TroA IgG (HR=2.55, 95%CI=0.92-7.11), HtrA IgG (HR=4.37, 95%CI=1.06-18.03) and MOMP IgG (HR=4.27, 95%CI=1.04-17.62) were associated with better three-year survival (Table III, Figure 3). However, when restricting analysis for high grade serous histology, the association of TroA IgG positivity (HR=2.65, 95%CI=0.82-8.61), HtrA IgG positivity (HR=3.46, 95%CI=0.83-14.37) and MOMP IgG positivity (HR=2.96, 95%CI=0.72-12.30) with better three-year survival was not statistically significant.

Discussion

The role of *C. trachomatis* infection in the development of EOC has been suggested in many studies (16-19), but chlamydial infection as a prognostic factor has not been addressed. We studied immune responses to *C. trachomatis* among women with EOC by measuring serum IgG antibodies to *C. trachomatis* TroA and HtrA proteins, *i.e.*, markers of persistent chlamydial infection (11, 13), and *C. trachomatis* MOMP, which represents the most commonly used antichlamydial antibody test. Overall, 16.7% had antibodies to *C. trachomatis* TroA, 11.1% to HtrA and 12.3% to MOMP. Surprisingly, women with immunological markers of persistent *C. trachomatis* infection had better response to the first-line platinum-taxane treatment. Our data also suggest better three-year survival among *C. trachomatis* TroA, HtrA and MOMP IgG seropositives.

Table I. *Selected characteristics of women with epithelial ovarian cancer by C. trachomatis serology.*

	C. trachomatis TroA IgG		*C. trachomatis* HtrA IgG		*C. trachomatis* MOMP IgG	
	pos (n=27)	neg (n=135)	pos (n=18)	neg (n=144)	pos (n=20)	neg (n=142)
Age (years)						
Mean	60.33	63.69	59.83	60.54	59.60	63.63
Median	61.0	65.0	59.5	64.5	58.0	64.5
STD	08:19	11:39	07:22	11:31	09:29	11:13
Parity (n, %)*						
Nullipara	6 (22.2)	20 (14.8)	3 (16.6)	23 (16.0)	3 (15.0)	23 (16.2)
Primi/Multipara	21 (77.8)	109 (80.7)	15 (83.3)	115 (79.9)	16 (80.0)	114 (80.3)
Previous (pre)malignant condition (n,%)						
Yes	3 (11.1)	18 (13.3)	2 (11.1)	19 (13.2)	4 (20.0)	17 (12.0)
No	24 (88.9)	117 (86.7)	16 (88.9)	125 (86.8)	16 (80.0)	125 (88.0)
Adhesions in surgery (n,%)**						
Yes	2 (7.4)	14 (10.4)	3 (16.7)	13 (9.0)	2 (10.0)	14 (9.9)
No	25 (92.6)	120 (88.9)	15 (83.3)	130 (90.3)	18 (90.0)	127 (89.4)
Missing	0	1 (0.7)	0	1 (0.7)	0	1 (0.7)
Operation type (n, %)						
Primary cytoreductive surgery	20 (74.1)	93 (68.9)	13 (72.2)	100 (69.4)	15 (75.0)	98 (69.0)
Secondary debulking surgery	4 (14.8)	24 (17.8)	1 (5.6)	27 (18.8)	3 (15.0)	25 (17.6)
No operation	3 (11.1)	18 (13.3)	4 (22.2)	17 (11.8)	2 (10.0)	19 (13.4)
Residual tumor after operation (n, %)						
No	17 (63.0)	63 (46.7)	11 (61.1)	69 (47.9)	9 (45.0)	71 (50.0)
R1	2 (7.4)	9 (6.7)	1 (5.6)	10 (6.9)	1 (5.0)	10 (7.0)
R2	5 (18.5)	45 (33.3)	2 (11.1)	48 (33.3)	8 (40.0)	42 (29.6)
No operation	3 (11.1)	18 (13.3)	4 (22.2)	17 (11.8)	2 (10.0)	19 (13.4)
CA 125 (kU/l)						
Mean	651	1.565	1.015	1.465	1.014	1.472
Median	286	391	390	369	402	364
Min–Max	17-3121	8-30,016	17-9,669	8-30,016	27-5,876	8-30,016
Stage (n, %)						
Early	7 (25.9)	39 (28.9)	1 (5.6)	45 (31.3)	4 (20.0)	42 (29.6)
Advanced	20 (74.1)	96 (71.1)	17 (94.4)	99 (68.8)	16 (80.8)	100 (70.4)
Histology (n, %)						
Low grade serous	2 (7.4)	6 (4.4)	2 (11.1)	6 (4.2)	1 (5.0)	7 (4.9)
High grade serous ovary	8 (29.6)	60 (44.4)	8 (44.4)	60 (41.7)	9 (45.0)	59 (41.5)
High grade serous (fallopian tube/peritoneum)	8 (29.6)	25 (18.5)	6 (33.3)	27 (18.8)	3 (15.0)	30 (21.1)
Mucinous	3 (11.1)	11 (8.1)	0	14 (9.7)	2 (10.0)	12 (8.5)
Clear cell	3 (11.1)	9 (6.7)	2 (11.1)	10 (6.9)	2 (10.0)	10 (7.0)
Endometroid	3 (11.1)	18 (13.3)	0	21 (14.6)	2 (10.0)	19 (13.4)
Undifferentiated/other epithelial	0	6 (4.4)	0	6 (4.2)	1 (5.0)	5 (3.5)

*Data missing in six women; **data missing in one woman; pos: positive; neg: negative.

The presence of antibodies against MOMP or MOMP-derived peptides can persist for years after chlamydial PID (20). A positive IgG MOMP titer can indicate infection but cannot discriminate cleared infection from persistent infection. Persistence of *C. trachomatis* infection has, in turn, been considered one of the key factors in the process leading to pelvic adhesions and reproductive sequelae (10). This process is caused by immune mediators (21) and is associated with chlamydial antigens, including *C. trachomatis* TroA and HtrA (9), as well as chlamydial heat shock protein (chsp60) (10). In line with this, we have earlier found increased seropositivity rates and levels of TroA and HtrA antibodies with ascending or repeat (13) chlamydial infection, as well as tubal factor infertility (11). This suggests that these circulating markers are associated with persistent chlamydial infection and hyperinflammation, which leads to scarring of the fallopian tubes. A subgroup of EOC cases is immunoreactive with tumor-infiltrating lymphocytes, and have better clinical outcome compared to non-immunoreactive EOC (22). Our finding may reflect such immunoreactivity representing a favorable prognostic sign in EOC.

The host immune response to intracellular *C. trachomatis* is complex, extending from complete clearance of infection to prolonged persistence and excessive inflammation (23). In persistent chlamydial infection, T lymphocytes infiltrate the site

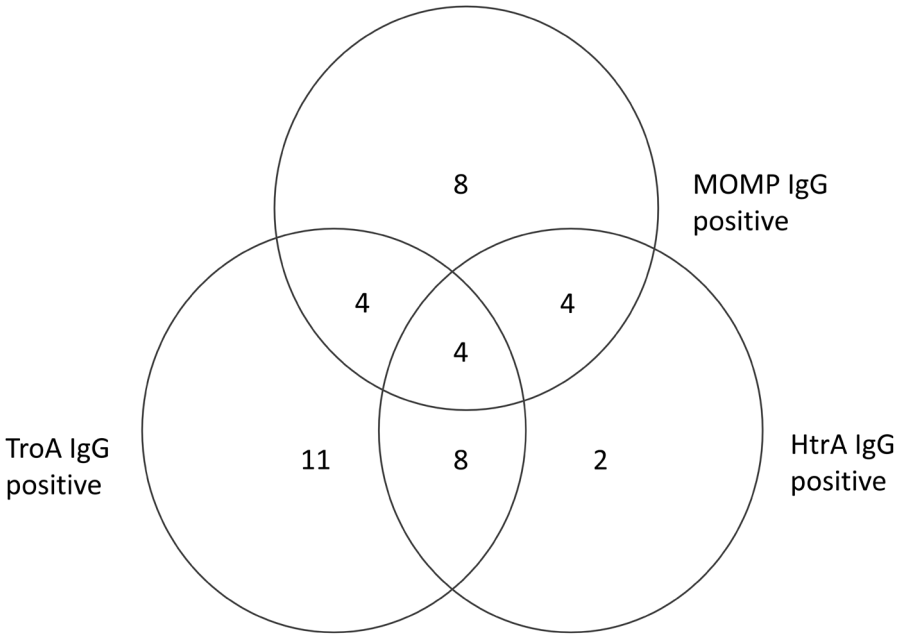

Figure 2. *Overlap between C. trachomatis TroA, HtrA, and major outer membrane protein (MOMP) IgG seropositivity.*

Table II. *The results of logistic regression analysis of complete response to first line treatment. Epithelial ovarian cancer stage as confounding factor was applied to the model together with chlamydial TroA immunoglobulin G (IgG), HtrA IgG or major outer membrane protein (MOMP) IgG data.*

	Complete response		
	Odds ratio	95% Confidence interval	*p*-Value
TroA IgG (pos *vs*. neg)	0.18	0.07-0.49	**<0.01**
Stage (early *vs*. advanced stage)	0.06	0.02-0.14	**<0.01**
HtrA IgG (pos *vs*. neg)	0.25	0.08-0.72	**0.01**
Stage (early *vs*. advanced stage)	0.06	0.02-0.13	**<0.01**
MOMP IgG (pos *vs*. neg)	0.37	0.13-1.08	0.07
Stage (early *vs*. advanced stage)	0.06	0.03-0.15	**<0.01**

Significant *p*-values are shown in bold.

Table III. *The results of Cox regression analysis of three-year survival by antichlamydial antibodies. Epithelial ovarian cancer stage was included as a confounding factor.*

	Three-year survival		
	Hazard ratio	95% Confidence interval	*p*-Value
TroA IgG (pos *vs*. neg)	2.55	0.92-7.11	0.07
Stage (early *vs*. advanced stage)	11.21	2.72-46.25	**<0.001**
HtrA IgG (pos *vs*. neg)	4.37	1.06-18.03	**0.04**
Stage (early *vs*. advanced stage)	12.36	2.99-51.03	**<0.001**
MOMP IgG (pos *vs*. neg)	4.27	1.04-17.62	0.05
Stage (early *vs*. advanced stage)	11.64	2.82-48.04	**<0.001**

Significant *p*-values are shown in bold.

of infection more rapidly and more extensively than in primary infection (24), resulting in cellular proliferation and chronic inflammation which may increase the risk of carcinogenesis. Also, gastric cancer after Helicobacter pylori infection and colon cancer related to inflammatory bowel disease, arise at sites of infection and chronic inflammation. We found that 11-17% of the women had TroA or HtrA IgG responses consistent with persistent chlamydial infection. *C. trachomatis* infection may predispose to transformation through impaired repair of DNA damage during persistent infection (25).

The major strength of our study was inclusion of women with unselected ovarian cancer, who underwent uniform management during the study period. In addition, this was a single-center study in which the data were obtained via a systematic review of our hospital database. Furthermore, we used validated assays to determine *C. trachomatis* infection-related antibodies (11-13). The limitations included the assessment of serological antibodies only at the time of the EOC diagnosis but not post-treatment. The small sample size limited the power of the outcome analyses and precluded adjustments for all potential confounding factors,

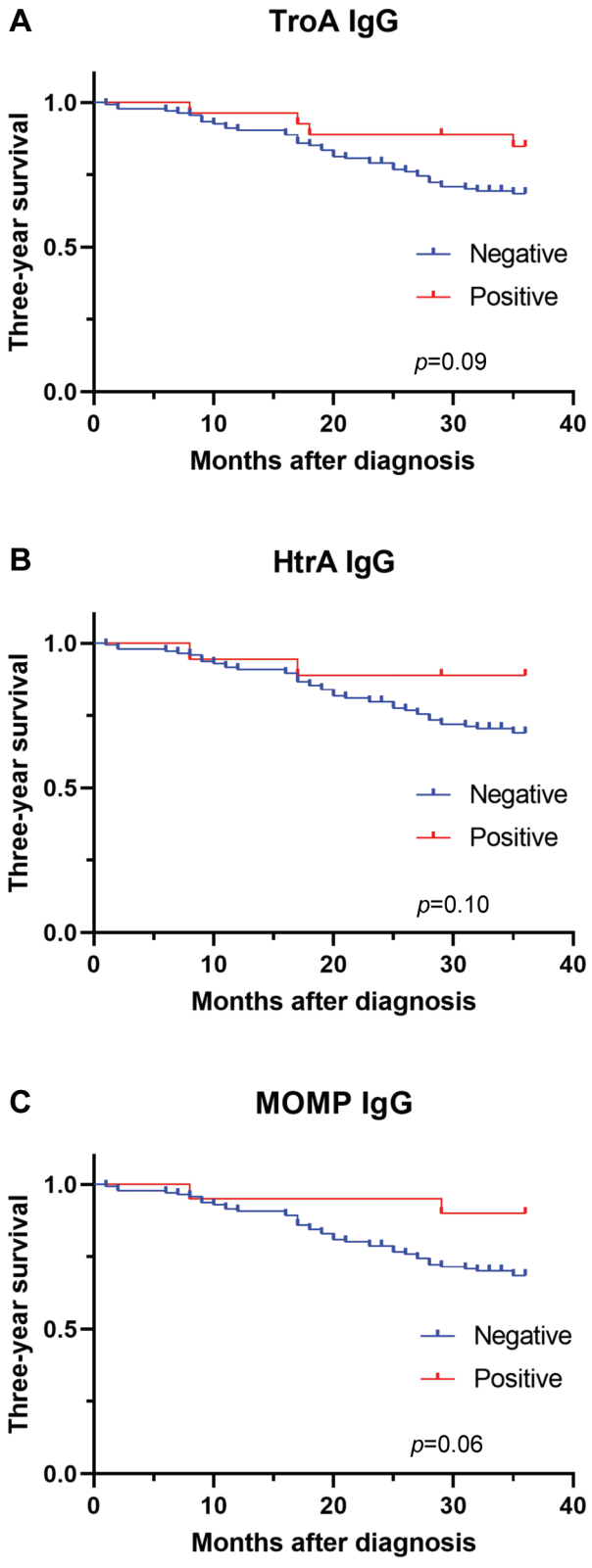

Figure 3. *Kaplan–Meier curves for three-year survival of women with epithelial ovarian cancer by serum C. trachomatis TroA (A), HtrA (B), or major outer membrane protein (MOMP) (C) IgG antibodies.*

such as age and the presence of residual disease after surgery hence increasing the risk for residual confounding. Also, genetic factors, socioeconomic status, and lifestyle factors could have affected the outcome, but were not measured in this study. Comorbidity, on the other hand, did not explain the differences between the groups. The best endpoint in cancer studies, progression-free survival (PFS) could not be used since the exact date of initiation of chemotherapy for relapse was not available. Therefore, we selected three-year survival as a surrogate marker for PFS. This is biologically feasible since most relapses take place during three years after completion of treatment. We acknowledge that historical studies usually have limitations due to potential biases. For instance, we could not exclude immortal time bias as we began the follow-up at the time of the first hospital visit. Other possibilities for follow-up starting point would have been the date of the surgery and the date of first chemotherapy. The exact end date of first line treatment was not available in the hospital records and could not be used as a starting point for the follow-up. Although the period of our study was long, *i.e.*, ten years, EOC treatment guidelines did not change during the study period, which certainly decreases confounding bias.

Conclusion

Women with EOC and TroA and HtrA antibodies as novel serological markers of persistent *C. trachomatis* infection had better response to the first-line platinum-taxane treatment than those without, and also had better three-year survival.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Conceptualization, U.P, J.P, T.H and M.P.; Methodology, M.P, T.H. and E.U.; Data curation, E.U. and U.P.; Funding acquisition, T.H., E.U., U.P. and M.P.; Visualization, T.H. and E.U; Writing – original draft preparation and review & editing, T.H., E.U., J.P., U.P. and M.P. All Authors have read and agreed to the published version of the manuscript.

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