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Chlamydia trachomatis and Anti-MUC1 Serology and Subsequent Risk of High-Grade Serous Ovarian Cancer: A Population-Based Case–Control Study in Northern Sweden

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Abstract

BACKGROUND: Chlamydia trachomatis salpingitis causes inflammatory damage to the fallopian tube and could potentially cause initiation and progression of high-grade serous ovarian cancer (HGSC). Furthermore, C. trachomatis infection may stimulate mucin 1 (MUC1) protein production, possibly affecting anti-MUC1 antibody levels. The aim of this study was to examine if serology indicating past infection with C. trachomatis as well as anti-MUC1 production was associated with subsequent risk of HGSC.

MATERIALS AND METHODS: In a prospective nested case–control study within the Northern Sweden Health and Disease Study and the Northern Sweden Maternity Cohort, the prevalence of chlamydial and anti-MUC1 antibodies was analyzed in blood samples drawn more than one year before diagnosis from 92 women with HGSC and 359 matched controls. Matching factors were age, date at blood draw, and sampling cohort. Plasma C. trachomatis IgG was analyzed using commercial micro-immunofluorescence test; chlamydial Heat Shock Protein 60 IgG (cHSP60) and anti-MUC1 IgG were analyzed with ELISA technique.

RESULTS: The prevalence of C. trachomatis IgG and cHSP60 IgG antibodies, as well as the level of anti-MUC1 IgG was similar in women with HGSC and controls (16.3% vs. 17.0%, \( P = 0.87 \); 27.2% vs. 28.5%, \( P = 0.80 \); median 0.24 vs. 0.25, \( P = 0.70 \)). Anti-MUC1 IgG and cHSP60 IgG levels were correlated (\( r = 0.169; P < 0.001 \)).

CONCLUSIONS: The findings of this prospective nested case–control study did not support an association between C. trachomatis infection, as measured by chlamydial serology, or anti-MUC1 IgG antibodies, and subsequent risk of HGSC.

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Introduction

Epithelial ovarian cancer (EOC) can be divided into five subgroups: high-grade serous (HGSC), low-grade serous, endometrioid, clear-cell, and mucinous cancer [1]. HGSC is the most common and lethal subtype of EOC. Traditionally, the ovarian surface epithelium has been viewed as the site of tumor origin. However, accumulating evidence now suggests that the distal part of the fallopian tube, many times the junction between the ciliated epithelium of the endosalpinx and the peritoneal mesothelium, is the primary origin of HGSC [2,3]. Carcinogenesis and potential risk factors for HGSC is not fully elucidated [4].

Chlamydia trachomatis is a sexually transmitted, Gram-negative intracellular bacterium causing cervicitis and if not resolved can ascend to the upper female genital tract, causing acute and chronic inflammation of the fallopian tubes [5,6]. In experimental animal models, C. trachomatis bacteria have been shown to infect the
secretory cells of the distal fallopian tube [7,8], the same cell type
where serous tubal intraepithelial carcinoma, the suggested precursor
of HGSC, is found. There are several mechanisms described
explaining C. trachomatis potential carcinogenic properties. First,
C. trachomatis is suggested to induce DNA damage in the host cell,
inhibit DNA repair, and resist apoptotic stimuli [9,10]. Second, if left
untreated, C. trachomatis can enter a viable nonreplicative chronic
state [11–13] and the association of chronic inflammation with
cancer is well documented [14,15]. Third, during chronic inflamma-
tion C. trachomatis produces a 60-kDa protein called chlamydial heat
shock protein 60 (chsp60) [11]. The protein chsp60 is suggested to
stimulate tissue damage by triggering the immune response as well as
inducing resistance to apoptotic stimuli [6,12]. The ability to induce
chronic inflammation generating an environment favorable for
malignant transformation in combination with the ability to induce
DNA damage and avoid apoptosis increases the risk for cancer
initiation.

Based on the abovementioned, C. trachomatis has been implicated
in ovarian cancer development [16–18]. Antibodies to C. trachomatis
and chsp60 is associated with pelvic inflammatory disease (PID)
[19,20]. Recent studies have shown an increased risk of HGSC after
PID [21,22]. During an inflammatory process, the glycoprotein
mucin 1 (MUC1), normally expressed by epithelial cells, are
overexpressed to provide a barrier between the epithelium and the
extra cellular milieu against infections [23]. This results in a humoral
immune response and anti-MUC1 antibody production. Not only
inflammation but also epithelial adenocarcinomas including EOC
induces overexpression of MUC1 protein [24] and anti-MUC1
antibody production [25]. On the contrary, higher anti-MUC1
antibody levels in prospective blood samples have been associated
with events known to reduce the risk for ovarian cancer (e.g. using
oral contraceptives, parity, tubal ligation, hysterectomy, and
salpingectomy) [25–28], suggesting that natural immunity against
MUC1 might have a long-term protective effect [26]. Low-grade
chronic events such as increasing number of ovulatory cycles and use
of talc have been shown to reduce the antibody level suggestively
because of immune tolerance [27,28]. Accordingly, infection of the
female genital tract with C. trachomatis could stimulate an immune
response to MUC1 protein and potentially both increase or decrease
anti-MUC1 antibody levels depending on the chronicity of the
infection.

Based on experimental and epidemiologic data, we hypothesized
that C. trachomatis may play a role in the development of HGSC, and
C. trachomatis antibodies to be associated with increased risk of
HGSC. The aim of this study was to assess the association of
C. trachomatis and anti-MUC1 antibodies with HGSC, in a
prospective population-based case–control study.

Materials and Methods

Study Population

This is a nested case–control study within the Northern Sweden
Health and Disease Study (NSHDS) and the Northern Sweden
Maternity Cohort (NSMC) to compare the prevalence of C. trachomatis
and the levels of anti-MUC1 antibodies in prospective
blood samples from women with HGSC and matched controls.
Blood samples, drawn more than one year before ovarian cancer
diagnosis were identified in NSHDS and NSMC, both previously
reported in detail [29–31]. In brief, the NSHDS cohort contains
three subcohorts. Plasma samples used in the present study were
collected from participants in the Västerbotten Intervention Program
(VIP) and the Mammography Screening Project (MSP). VIP invites
all residents of Västerbotten County to a general health examination
at 10-year intervals at ages 40, 50, and 60 years. The MSP collects
blood samples at mammography screening visits and the cohort
consists of women aged 18–82 years at blood draw, of whom 95%
are between 48 and 70 years. The NSMC collects serum samples for
research purpose from pregnant women attending the maternity
health care during the end of the first trimester of pregnancy. All
participants provided informed consent before donating their blood
samples for research purposes.

Ethical approval was given from The Human Ethics Committee of
the Medical Faculty, Umeå University (Dnr 06–053), Sweden.

Nested Case–Control Study Participant Selection

Women diagnosed with HGSC between 1993 through 2011 with
at least one prospective blood sample in NSHDS or NSMC were
included in the study. Cases were identified through the national
Cancer Register having one of the following malignant neoplasms;
peritoneum, ovary, fallopian tube, abdomen, or pelvis (International
Classification of Diseases 9th revision codes 158 and 183; 10th
revision code C48.1–2, C56.9, C57.0–8, C76.2–3). A pathology
report review was conducted. Only serous histotypes were included.
Serous adenocarcinomas Silverberg grade III [32] were defined as
HGSC. All cases with serous histotype Silverberg grade II or
unknown differentiation were reevaluated blindly by a senior
consultant in gynecologic pathology to confirm or reject HGSC
diagnosis as defined by the World Health Organization classification
(2014).

Women with HGSC were matched to four controls each with
respect to age (±1 year), date at blood draw (±3 months), and
sampling cohort (VIP, MSP, NSMC). Except for nonmelanoma skin
cancer, cases and controls had no history of cancer before blood
collection. Women having had bilateral oophorectomy before
diagnosis of the index case were excluded.

Plasma Antibody Analysis

C. trachomatis IgG. C. trachomatis IgG antibodies, as well as
Chlamydia pneumoniae IgG antibodies, were determined by the
serovar D–K specific micro-immunofluorescence (MIF) test (Focus
Diagnostics, USA). C. pneumoniae IgG antibodies were included in
the analysis to determine possible covariance/cross-reaction with
C. trachomatis. All procedures were performed according to the
manufacturer’s protocols. In short, serum dilutions 1/16 were used.
Serum was added to the well, incubated for 30 min, in 37 °C. Plates
were washed in PBS to remove unbound serum antibodies.
Fluorescein-labeled antibody IgG was added and samples were
incubated for 30 min at 37 °C. Then again washed, dried, and
mounted. All samples were examined by the same observer, blinded
to case–control status, using fluorescence microscopy. Positive and
negative controls from the kit were included.

cHSP60 IgG. Analysis of the levels of chlamydial Heat Shock
Protein 60 (cHSP60) IgG antibodies was performed using a
commercial ELISA technique (Medac, Germany) and optical density
(OD) values were measured at 450 nm; reference wavelength at
620 nm). Protocol and validation criteria of the assay were followed
according to the manufacturer’s instructions. The OD values were
analyzed continuously and dichotomized; cutoff was defined as the
Microtiter plates were coated overnight at 4 °C with 1 μg of synthetic MUC1 100-mer peptide (vaccine antigen) dissolved in 0.9% Dulbecco’s PBS. Corresponding control plates received PBS but no antigen. The plates were washed three times with PBS and 1-hour incubation in room temperature with 2.5% bovine serum albumin (BSA) in PBS (PBS-BSA) to fully coat the microtiter plate wells with protein and block nonspecific binding. PBS-BSA was removed and plasma diluted in PBS-BSA was added to the wells. After 1-hour incubation at room temperature, the plates were washed 5 times with PBS with 0.1% Tween-20 (Sigma–Aldrich), and alkaline phosphatase-conjugated anti-human IgG, IgM, or IgA secondary antibody (Sigma–Aldrich) in PBS-BSA was added. After a 1-hour incubation, the plates were washed 5 times and the substrate, p-nitrophenyl phosphate (Sigma–Aldrich), was added to each well. The reaction was terminated after 1-hour by adding 0.5 mol/L NaOH. The results were read at OD 405 nm on a spectrophotometer. The OD values from the control wells containing no antigen were subtracted from the OD values in test wells coated with peptide. Every sample was assayed multiple times at multiple dilutions, in at least triplicate wells. The OD values were analyzed continuously and dichotomized. Based on a previous study, a cutoff for high anti-MUC1 IgG level was set at an OD value ≥ 1.0 [26].

### Statistical Analysis

Statistical analysis was performed using the SPSS software (version 25.0). The differences in proportions of the categorical variables were evaluated by chi-square or Fisher’s exact test, whichever appropriate. Nonparametric Mann–Whitney U test was applied to analyze continuous data not normally distributed. Spearman rank test was used analyzing correlations. Association of antibodies with HGSC was also evaluated by lag time between blood draw and diagnosis, using the median time as a cutoff (1–7.3 years or > 7.3 years) as well as age group using mean age at diagnosis as cutoff. A two-sided P-value less than 0.05 was considered significant.

### Results

#### Cohort

Ninety-two women diagnosed with HGSC were included. Most cases were low-differentiated serous adenocarcinomas (n = 75, 81.5%) followed by serous surface papillary carcinoma (n = 12, 13.0%) and serous cystadenocarcinoma "not otherwise specified" (n = 5, 5.4%). Cases were diagnosed at a mean age of 62 years (range 38–83 years) and matched to four controls each. However, for nine cases, only three controls fulfilling the criteria were identified. Forty-two cases (46%) had more than one prospective blood sample. Most blood samples were collected within the NSHDS cohort (n = 317, 70.3% whereof MSP: n = 188, 59.3%; VIP: n = 129, 40.7%) and the rest from NSMC (n = 134, 29.7%). The median time between blood collection and diagnosis was 7.3 years (range 1.1–34.0 years).

### Chlamydial Antibodies and HGSC

Women having _C. trachomatis_ IgG antibodies were younger at blood collection than women who did not have _C. trachomatis_ IgG antibodies (median 47.1 vs. 58.7 years, respectively). There was no correlation between age and the level of _C. pneumoniae_ IgG or chHS60 IgG (data not shown). The prevalence of _C. trachomatis_ IgG and chHS60 IgG was similar in the different sample cohorts among cases as well as controls (data not shown). The prevalence of _C. trachomatis_ IgG was similar in women with HGSC and controls (16.3% vs. 17.0%, P = 0.87) (Table 1). There was no difference in chHS60 IgG antibodies between cases and controls neither as continuous parameter (P = 0.85) (Table 2) nor dichotomized (27.2% vs. 28.5%, P = 0.80) (Table 1). There was no difference in the proportion of chHS60 IgG antibodies in Q4 vs. Q1 (quartile cutoff based on control population) between cases and controls (P = 0.79). Analysis on lag time between blood draw and diagnosis (1–7.3 years or > 7.3 years) did not result in any significant differences in

### Table 1: Prevalence of Chlamydial trachomatis IgG, chHS60 IgG, and Anti-MUC1 IgG in Prospective Blood Samples in Women With High-Grade Serous Ovarian Cancer and Matched Controls

| Antibodies | Cases | Controls | P* |
|------------|-------|----------|----|
|            | n     | n+      | (%)|            | n     | n+     | (%)|   |
| _C. trachomatis_ IgG | 92    | 15 (16.3%)|     | 358   | 61 (17.0%)|     | 0.87|
| 1–7.3 years | 46    | 8 (17.4%)  |     | 176   | 24 (13.6%)|     | 0.52|
| >7.3 years  | 46    | 14 (20.3%) |     | 270   | 45 (16.7%)|     | 0.48|
| chHS60 IgG | 92    | 25 (27.2%) |     | 358   | 102 (28.5%)|    | 0.80|
| 1–7.3 years | 46    | 16 (34.8%) |     | 176   | 54 (30.7%)|     | 0.59|
| >7.3 years  | 69    | 18 (26.1%) |     | 270   | 67 (24.8%)|     | 0.83|
| Anti-MUC1 IgG | 92    | 11 (12.0%) |     | 359   | 24 (6.7%) |     | 0.09|
| 1–7.3 years | 46    | 2 (4.3%)  |     | 177   | 13 (7.3%) |     | 0.74|
| >7.3 years  | 69    | 2 (3.0%)  |     | 270   | 17 (6.3%) |     | 0.06|

Abbreviations: _C. trachomatis_ IgG = _Chlamydia trachomatis_ IgG; chHS60 IgG = chlamydial Heat Shock Protein 60 IgG; MUC1 = mucin 1.

* Chi-square test
* One control was not evaluated because of technical problems.
* The same individual might have samples in both the 1–7.3 years or >7.3 years subgroups.
* Cutoff for chHS60 IgG was defined as the mean optical density value of the negative control plus 0.350 and results are presented as positive (+) or negative (−).
* Cutoff for high anti-MUC1 IgG level was considered as an optical density of ≥1.0.
* Fisher’s exact test.
prevalence of *C. trachomatis* IgG nor cHSP60 IgG between cases and controls (Table 1). There were no significant differences in the results in analysis by age group (< 62 or ≥ 62 years at diagnosis) (data not shown).

**Anti-MUC1 Antibodies and HGSC**

There was a negative correlation between age and the level of anti-MUC1 antibodies (r = −0.162, P < 0.001). The prevalence of anti-MUC1 IgG was similar in the different sample cohorts among cases as well as controls (data not shown). The median OD values between the cases and controls were equal (0.24 vs. 0.25, P = 0.70) (Table 2). The prevalence of higher anti-MUC1 IgG antibody levels was similar between cases and controls, based on anti-MUC1 IgG cutoff with OD ≥ 1.0 (12.0% vs. 6.7%, *P* = 0.09) (Table 1). Women with HGSC had a trend of higher anti-MUC1 IgG levels compared with controls, when analyzing blood samples collected more than 7.3 years before diagnosis and using anti-MUC1 IgG cutoff OD ≥ 1.0 (13.0% vs. 6.3%, *P* = 0.06) (Table 1). There were no significant differences in the results in analysis by age group (< 62 or ≥ 62 years at diagnosis) (data not shown). Sensitivity analysis excluding the cohort of pregnant women did not change the results in any of the analyses aforementioned (data not shown).

**Association and Correlation Between Antibodies**

No association was found between *C. trachomatis* IgG and *C. pneumoniae* IgG (χ² = 0.012; *P* = 0.91). There was a significant association between the presence of *C. trachomatis* IgG and cHSP60 IgG (χ² = 160.20; *P* < 0.001). In nonparametric test, the level of cHSP60 IgG was similar in *C. trachomatis* IgG positive and negative women (*P* = 0.81). Spearman rank test showed no correlation between cHSP60 IgG and hHSP60 IgG (*P* = 0.99). Women with *C. trachomatis* IgG did not have higher or lower levels of anti-MUC1 IgG (median 0.26 vs. 0.23; *P* = 0.49), nor did women with *C. pneumoniae* IgG (0.23 vs. 0.23; *P* = 0.75). Spearman rank test showed a significant positive correlation between cHSP60 IgG and anti-MUC1 IgG (*r* = 0.169; *P* < 0.001). No correlation was found between hHSP60 IgG and anti-MUC1 IgG (*r* = 0.045; *P* = 0.25).

**Discussion**

We examined chlamydial and anti-MUC1 IgG antibodies in women with HGSC and matched controls using prospective blood samples collected one year or more before diagnosis. No significant association of *C. trachomatis* antibodies with subsequent risk for HGSC was detected. Neither were the levels of anti-MUC1 IgG antibodies significantly different between cases and controls. Women with higher cHSP60 IgG antibodies had significantly higher anti-MUC1 antibody levels, suggesting that chronic chlamydia infection might stimulate MUC1 production thereby inducing a humoral immune response.

The results of previous studies are inconsistent regarding *C. trachomatis* serology and ovarian cancer. Our findings are in line with studies where no associations were found [34,35]. Recent studies show an association of *C. trachomatis* serology with subsequent risk for ovarian cancer [36,37]. However, their findings were specific to the *Chlamydia* Pgp3 antibody, while in this study, we used a commercial MIF-test which may explain the differences. Similar to our result, Trabert et al. found no association between cHSP60 IgG and ovarian cancer [36].

Our study did not support any association between anti-MUC1 IgG and subsequent risk for HGSC. This was in line with Cramer et al. [38]; they concluded that the anti-MUC1 antibody levels may be informative in the pathogenesis of EOC mucinous subtypes, but less useful for informing risk for all EOC. The focus of this study was HGSC and the mucinous subtype was not included.

We found that women with higher cHSP60 IgG antibody levels had significantly higher anti-MUC1 antibody levels, whereas *C. trachomatis* IgG and *C. pneumoniae* IgG were not associated with higher levels of anti-MUC1 antibodies. One explanation could be that the chronicity of *C. trachomatis* infection is important for induction of anti-MUC1 antibody production, but the result has to be interpreted with caution because the strength of the correlation was low. On the contrary, previous studies have shown that low-grade chronic inflammations are associated with lower levels of anti-MUC1 antibodies, suggestively because of immune tolerance [27,28]. Our results did not support lower anti-MUC1 antibody levels in women with serology indicating previous chronic *C. trachomatis* infection.

**Strengths**

This study consists of a well-defined cohort regarding the histopathological diagnosis. Most cases were successfully matched to four controls each with respect to age, date at blood donation, and sample cohort. All tests were performed with laboratory personnel unaware of case–control status, using validated methods.

**Limitations**

Only HGSC cases were included while results in more recent studies have shown associations with other histotypes that were not part of our aim. Reproductive variables were not collected in most cases and controls and therefore not adjusted for. It is shown that pregnancy influences the anti-MUC1 antibody level [25] and 30% of

**Table 2.** Optical Density Values of cHSP60 and Anti-MUC1 Antibodies in Prospective Blood Samples in Women With High-Grade Serous Ovarian Cancer and Matched Controls

| Antibodies      | Cases                   | Controls                  | P* |
|-----------------|-------------------------|---------------------------|----|
|                 | n Median (min–max)      | n Median (min–max)        |    |
| cHSP60 IgG      | 92 0.53 (0.15–7.80)     | 358 0.58 (0.01–8.59)     | 0.85|
| 1–7.3 years     | 46 (0.58 (0.15–7.80)    | 176 0.62 (0.01–8.59)     | 0.59|
| ≥7.3 years      | 69 (0.53 (0.15–6.06)    | 270 0.50 (0.03–8.09)     | 0.51|
| Anti-MUC1 IgG   | 92 0.24 (0.00–2.45)     | 359 0.25 (0.01–3.67)     | 0.70|
| 1–7.3 years     | 46 (0.23 (0.00–2.05)    | 177 0.24 (0.02–3.67)     | 0.51|
| ≥7.3 years      | 69 (0.24 (0.05–3.29)    | 270 0.26 (0.03–3.63)     | 0.51|

Abbreviations: cHSP60 IgG = chlamydial Heat Shock Protein 60 IgG; MUC1 = mucin 1.

* Mann-Whitney U test.

† One control was not evaluated because of technical problems.

‡ The same individual might have samples in both the 1–7.3 years and >7.3 years subgroups.
the blood samples in our study were collected from pregnant women. However, sensitivity analysis of anti-MUC1 levels excluding the pregnant subcohort showed similar results.

Conclusions
In this prospective population-based case–control study, there was no significant association of *C. trachomatis*, cHSPE60, or anti-MUC1 IgG antibodies with HGSC. Hence, the hypothesis that past infection with *C. trachomatis* would confer increased risk of HGSC was not supported. Given the divergent results in the current literature, the hypothesis needs to be further elucidated.

Conflicts of interest
None.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.tranon.2019.09.007.

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