Could epithelial ovarian cancer be associated with chlamydial infection?

A. Wong[1], *MBBS, MRCOG, FAMS*; **A.B. Maclean**[1], *M.D., FRCOG, FRCPEdin*; **S.J. Furrows**[2], *MBBS, MSc, MRCP, MRCPath.*; **G.L. Ridgway**[2], *M.D., FRCP, FRCPath.*; **P.J. Hardiman**[1], *M.D., FRCOG*; **C.W. Perrett**[1], *Ph.D., MRSC*

[1]*Department of Obstetrics and Gynaecology, Royal Free and University College Medical School (Hampstead Campus), London;*
[2]*Department of Clinical Microbiology, University College London Hospitals, London (UK)*

Summary

Purpose of investigation: Epithelial ovarian cancer (EOC) is the leading cause of death from gynaecological malignancy in the UK. The pathogenesis of this disease is poorly understood. Our hypothesis was that chlamydial infection might play a role in the pathogenesis of EOC. *Methods:* 122 serum samples of patients undergoing surgery for benign or malignant gynaecological conditions were analysed. There was a total of 41 patients with EOC (33.6%), 27 with benign cystadenomas (22.1%) and 54 with normal ovaries (44.3%). *Results:* There was a higher incidence of IgA seropositivity and lower incidence of IgG seropositivity in the EOC group compared with the other groups; however, this was not statistically significant. There was no statistical difference in the serum IgM antibodies to chlamydia in the three different groups. *Conclusion:* Although chronic infection and persistent inflammation may contribute to the pathogenesis of EOC, and chlamydia is a common genital tract pathogen, our study did not find an association between chlamydia and EOC.

Key words: Cancer; Chlamydia; Epithelial ovarian cancer; Infection.

Introduction

Epithelial ovarian cancer (EOC) accounts for more than 4,000 deaths per annum in the UK. It is thus the leading cause of death from gynaecological malignancy. The pathogenesis of EOC generally remains unknown.

It has recently been recognised that certain chronic infectious agents are associated with human cancer. The best documented are human papillomavirus (HPV) and cervical cancer [1, 2], hepatitis B and liver cancer [3] and *Helicobacter pylori* and gastric cancer [4]. Since the female genital tract is a frequent site of chronic infection, it is possible that an infective agent may also play a role in the pathogenesis of ovarian cancer.

Chlamydia trachomatis is a frequent sexually transmitted pathogen among women. There have been several studies looking at *C. trachomatis* as a cofactor in the aetiology of invasive cervical cancer. In a recent study examining women in Brazil and the Philippines, there was a suggestion that *C. trachomatis* increased the risk of squamous cervical cancer among HPV-positive women [5].

We have recently published on how EOC and infertility are associated, and explored potential underlying mechanisms, including those involving infection [6]. In this current study we examine the role of chlamydial infection in EOC.

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Patients and Methods

Clinical samples

The serum samples were from a serum bank, accumulated over 12 years. Preoperative serum was obtained from 122 women undergoing surgery for ovarian or other gynaecological conditions. The serum was aliquotted out and stored at -80°C. The women fell into three distinct groups: women with histologically normal ovaries, women with benign ovarian cystadenomas and women with EOC. There was a total of 41 patients with EOC (33.6%), 27 with benign cystadenomas (22.1%) and 54 with normal ovaries (44.3%).

The mean age of the patients with EOC was 56.49 years (range 25-86, SD 15.84), while the mean age of patients with cystadenomas was approximately ten years younger at 45.81 years (range 17-89, SD 15.23). The mean age of the patients with normal ovaries was 48.37 years (range 27-81, SD 10.88). A cut-off of 50 years of age was used to sub-analyse the groups in order to estimate the number of women who were either pre- or postmenopausal. Approximately two-thirds of the EOC patients were more than 50 years of age, whereas two-thirds of the patients with normal ovaries or benign cystadenomas were less than 50 years of age.

Chlamydia serology

Serum antibodies to *Chlamydia spp.* were assayed using enzyme-linked immunosorbent assay (ELISA) kits from MEDAC Diagnostika (Hamburg, Germany). These kits were used to detect IgM, IgG and IgA antibodies to the genus *Chlamydia*.

The microplates for the ELISAs were pre-coated with chlamydia-specific lipopolysaccharide fragments as the basis for the reaction. Serum samples were incubated in the pre-coated microplate wells, where any chlamydia-specific antibodies from the sample would bind to the antigen. Following a

wash step, conjugate solution containing peroxidase-conjugated anti-human antibodies (either IgM, IgG or IgA) was added. These antibodies would bind to the appropriate class of human antibody in the well. The wells were washed as before, then the chromogenic substrate tetramethylbenzidine (TMB) was added to each well. A colorimetric reaction occurs between the peroxidase and the substrate. The reaction was stopped by adding stop solution containing sulphuric acid. The plates were read colorimetrically at 450 nm.

Positive, negative and blank controls were included in the reaction and had to fall within an accepted range. A cut-off value was assigned based upon the mean OD value of the negative control, as per manufacturer's instructions. A "grey zone" around this cut-off was calculated as the cut-off ± 10% (for IgG and IgA) or ± 15% (for IgM). Results falling within the grey zone were interpreted as equivocal. Results above the grey zone were positive and those below the grey zone were negative.

For IgG and IgA, titres could be calculated from the OD value of the sample using a formula provided by the manufacturer. This formula is not valid for the IgM assay.

The MEDAC ELISA is not specific for *C. trachomatis*, as it detects antibody to all chlamydial species. It also includes an equivocal result usually clarified by follow-up testing with a second serum sample, which was not possible in this study. An equivocal result may represent low-level antibody, which could be found in a new, reactivated, subsiding or previous infection. Alternatively the result may be due to cross-reactivity in the assay with other, unrelated micro-organisms. Without a follow-up sample it is not possible to interpret such equivocal findings with any confidence. They were therefore excluded from the final analysis.

Statistics

The patient profiles and histological reports were reviewed and entered into an Excel database. Possible associations between diagnosis and presence of immunoglobulin types were analysed using Pearson chi-squared tests.

Results

When we analysed the patients for IgM antibodies to chlamydia, we found a total of 15 out of 111 patients (13.5%) were positive for IgM. There were 11 equivocal results that were excluded. Table 1 shows the IgM seropositivity against the patients' diagnoses. There was no statistically significant difference between the groups (p = 0.423). There was no significant difference in IgM seropositivity between women less than and more than 50 years of age.

Analysing the IgG antibody levels within the three groups of patients, there was a total of 41 out of 110 patients (37.3%) who were IgG positive (Table 2). There were 12 equivocal results. Twenty-five percent of the patients with EOC were IgG seropositive, whereas 43% and 44% of patients with normal ovaries or benign cystadenomas, respectively, were seropositive. Although there was a lower seropositivity among patients with EOC, this was not statistically significant (p = 0.178). When we analysed the groups of patients against the strength of the IgG titres or age, there were no significant associations.

With IgA antibody levels, a total of 42 out of 115 patients (36.5%) were IgA positive (Table 3). There were seven equivocal results. There was a higher seropositivity among EOC patients but again, this was not statistically different from the other patient groups (p = 0.698). There were no obvious trends with either age or titre.

Table 1. — *IgM seropositivity in ovarian patients by diagnosis.*

	IgM negative	IgM positive	Total
EOC	34 (89.5%)	4 (10.5%)	38
Benign cystadenomas	18 (78.3%)	5 (21.7%)	23
Normal ovaries	44 (88.0%)	6 (12.0%)	50
Total	96 (86.5%)	15 (13.5%)	111

Equivocal results - 11 (9.0%); p value = 0.423.

Table 2. — *IgG seropositivity in ovarian patients by diagnosis.*

	IgG negative	IgG positive	Total
EOC	27 (75.0%)	9 (25.0%)	36
Benign cystadenomas	14 (56.0%)	11 (44.0%)	25
Normal ovaries	28 (57.1%)	21 (42.9%)	49
Total	69 (62.7%)	41 (37.3%)	110

Equivocal results - 12 (9.8%); p value = 0.178.

Table 3. — *IgA seropositivity in ovarian patients by diagnosis.*

	IgA negative	IgA positive	Total
EOC	23 (59.0%)	16 (41.0%)	39
Benign cystadenomas	18 (69.2%)	8 (30.8%)	26
Normal ovaries	32 (64.0%)	18 (36.0%)	50
Total	73 (63.5%)	42 (36.5%)	115

Equivocal results - 7 (5.7%); p value = 0.698.

Discussion

An increasing number of infectious agents are thought to play a role in the development of various human cancers. Chronic infections contribute to about one-third of the world's cancer. Hepatitis B and C are involved in chronic hepatitis leading to liver cancer [3, 7]. *Helicobacter pylori* bacteria infect the stomachs of more than one-third of the world's population and are involved in stomach cancer, ulcers, and gastritis [4]. HPV is now recognised to be a major causative factor in cervical cancer [1, 2]. Other infections linked to cancer are *Schistosoma japonicum* and colon cancer, *Schistosoma haematobium* and bladder cancer, *Opisthorchis viverrini* (a liver fluke), and cholangiocarcinoma, and *Clonorchis sinensis* and biliary tract cancer [8].

One of the mechanisms by which some microorganisms may be involved in carcinogenesis is through persistent inflammation arising from chronic infection. As part of the host's response to infection, phagocytic cells release oxidants in an effort to eradicate invading pathogens. These oxidants kill and cause DNA damage to the host cells. It has been postulated that such DNA damage, together with compensatory cell division during the inflammatory response, increases the probability that a host's cells will acquire the mutations necessary for tumorigenesis [9, 10].

The female lower genital tract is a common site of infection. *Chlamydia trachomatis* has been implicated as

a cofactor with HPV in the causation of cervical cancer. Koskela *et al.* found that serum antibodies to *C. trachomatis* were associated with an approximate doubled increased risk for cervical squamous cell carcinoma after adjusting for smoking and HPV [11]. In a more recent study, *C. trachomatis* serotype G was found to be most strongly associated with the subsequent development of cervical cancer. Increasing numbers of exposures to different *C. trachomatis* serotypes also increased the risk [12]. As part of an International Agency for Research on Cancer multicentre case-control study, Smith *et al.* found that *C. trachomatis* increased the risk of squamous cervical cancer among HPV-positive women by an odds ratio of 2.1 in women in Brazil and the Philippines [5]. There was also a suggestion of increasing squamous cervical cancer risk with increasing *C. trachomatis* antibody titres. The group therefore concluded that *C. trachomatis* infection was a possible cofactor with HPV in the aetiology of squamous cervical cancer, and its effect may be mediated by chronic inflammation.

The upper genital tract, i.e., the endometrium, fallopian tubes and ovaries, although much less commonly infected than the lower genital tract, is also a site of infection. The infections commonly occur by ascending through the vagina. The more common pathogens of pelvic inflammatory disease (PID) infecting the tubes and ovaries include *C. trachomatis*, *Neisseria gonorrheae* and genital mycoplasmas. *C. trachomatis* can cause chronic persistent infection of the upper genital tract as this organism is especially adept at maintaining a long-term relationship with its host. *C. trachomatis* is an obligate intracellular parasite and can cause chronic, clinically inapparent infections of the upper genital tract that may result in significant damage to the reproductive organs [13].

Ovarian cancer seems to be related to factors that increase the division of surface epithelial cells [14]. Inflammation may underlie ovulatory events because an inflammatory reaction is induced during the process of ovulation. Ness and Cottreau proposed that various initiators of epithelial inflammation, such as PID, may also contribute to ovarian carcinogenesis [15].

To date, the role of chronic infection and persistent inflammation in the pathogenesis of EOC has received relatively little scientific attention. Two epidemiological case-control studies of ovarian cancer conducted in China and Canada reported an increased risk for EOC in women who self-reported a history of pelvic infection/PID [16, 17]. A third study from the United States, however, found only a modest association and a fourth from Italy failed to find an association [18, 19].

In a recent small study examining *C. trachomatis* IgG titres to investigate the possibility of their association with EOC, serum from 19 patients with EOC was analysed, along with that from ten patients with non-malignant disease and 21 women with pain and infertility [20]. The group found *C. trachomatis* IgG antibodies were present in 79% of women with ovarian cancer, 90% of age-matched controls and 67% of patients with infertility and pain. There was however a significant cross-reaction with *Chlamydophila pneumoniae* titres so that the authors were not able to draw conclusions regarding *C. trachomatis* and ovarian cancer. Ness *et al.* have now reported a much larger case-control study, with 117 women with ovarian cancer and 171 controls, which showed a greater probability of having ovarian cancer in those with the highest levels of chlamydia-elementary body and chlamydia heat shock protein 60-1 antibodies [21].

Although our study found no significant association between chlamydial antibodies and EOC, there may be several reasons for this. Firstly, we looked for total chlamydial antibodies rather than specific antibodies to *C. trachomatis*, which is the common genital tract pathogen. Most laboratories would initially test for total chlamydial antibodies and only in the presence of a high titre would the laboratory go on to look for the specific sub-type. Another limitation of our study is that we looked for chlamydial antibodies in the serum. Perhaps a more accurate assessment of an association may be obtained by looking for chlamydia in the ovarian tissue itself. We currently have a large tissue bank of ovarian samples, matching our serum bank, and this is one of the aims of future work.

Our study has an advantage in that we compared women with malignant and benign ovarian tumours with women with normal ovaries, all based on histological confirmation. It is important that any future study into infectious agents and ovarian cancer should document the prevalence of these agents in non-neoplastic ovarian specimens, including the contralateral ovaries of women with unilateral ovarian carcinomas undergoing bilateral oophorectomy.

In conclusion, our study found no association between EOC and chlamydia. Further studies are needed to clarify the pathogenesis of ovarian cancer, so that in future we may be able to prevent and reduce ovarian cancer mortality.

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Address reprint requests to:
C.W. PERRETT, Ph.D., M.R.S.C.
Department of Obstetrics and Gynaecology
Royal Free and University College Medical School (Hampstead Campus)
Rowland Hill Street
London
NW3 2PF (UK)