Changes in pre-diagnostic serum C-reactive protein concentrations and ovarian cancer risk: a longitudinal study

A. T. Toriola¹,²*, K. Grankvist³, C. B. Agborsangaya², A. Lukanova⁴, M. Lehtinen² & H.-M. Surcel¹

¹National Institute for Health and Welfare, Oulu; ²Tampere School of Public Health, University of Tampere, Tampere, Finland; ³Department of Medical Biosciences, Umeå University, Umeå, Sweden; ⁴Department of Cancer Epidemiology, German Cancer Research Centre, Heidelberg, Germany

Received 29 September 2010; revised 26 October 2010; accepted 28 October 2010

Background: Evidence suggests that inflammation may be associated with increased risk of ovarian cancer but there is paucity of studies investigating this association, especially using over-time changes in inflammatory biomarkers.

Materials and methods: We conducted a prospective population-based case–control study nested within the Finnish Maternity Cohort (FMC). Within the FMC, 170 women with ovarian cancer who had donated serum samples to the cohort twice, 1 year apart, before cancer diagnoses were identified. One control per case was matched for age, parity and sampling date.

Results: Comparing the highest with lowest tertiles, the odds ratio (OR) of ovarian cancer using the first set of serum samples (mean lag time to cancer diagnosis 9.0 years) was 1.62 [95% confidence interval (CI) 0.93–2.83]. However, analysis conducted using the second set of serum samples donated closer to cancer diagnosis (mean lag time 6.4 years) revealed a significantly increased risk of ovarian cancer comparing extreme tertiles of C-reactive protein (CRP) concentrations; OR 1.96 (95% CI 1.11–3.4). Over time, increases in individuals’ CRP concentrations were also associated with increased risk; OR 1.90 (95% CI 1.12–3.23).

Conclusion: The results suggest that inflammation may precede ovarian cancer since increasing CRP concentrations, both across tertiles and longitudinally at the individual level, were associated with increased risk.

Key words: biobanks, C-reactive protein, inflammation, longitudinal study, ovarian cancer, prospective study

Introduction

Inflammation may play a role in the aetiology of ovarian cancer [1]. The hypothesis is based on the association of certain risk factors such as the use of perineal talc, endometriosis and pelvic inflammatory disease [2–6] that may induce ovarian inflammation with increased risk of ovarian cancer. Likewise, gynaecological surgeries such as tubal ligation and hysterectomy that prevent retrograde transport of pro-inflammatory factors from lower genital tracts to the ovaries have been found to offer protection against ovarian cancer [5, 7]. Further credence is lent to this hypothesis with the observation that use of non-steroidal anti-inflammatory drugs (NSAIDs) can reduce the risk of ovarian cancer [8, 9]. Nevertheless, some other studies have found neither any relationship between the above surrogates of inflammation [10, 11] nor any protective effect for the use of NSAIDs on the risk of ovarian cancer [12, 13].

C-reactive protein (CRP) is an acute-phase protein produced by the liver in response to tissue injury and inflammation and can be used as a marker for chronic systemic inflammation [14]. Two recent studies have investigated the risk of ovarian cancer with regard to circulating CRP concentrations [15, 16]. While the first study [15] observed an increased risk of ovarian cancer associated with circulating CRP concentrations, the second study showed no indication of an overall association [16]. The two studies, however, determined risks using only one-time measurement of CRP levels. It has been shown that CRP exhibits significant within-subject variation and measurements using more than one sample are better indications of long-term CRP concentrations [17].

In order to further explore the relationship between pre-diagnostic CRP concentrations and ovarian cancer risk, we conducted a longitudinal population-based case–control study nested within the Finnish Maternity Cohort (FMC) using cases and controls who had donated more than one serum sample each, at least 1 year apart.

Materials and methods

Finnish Maternity Cohort

This is a prospective population-based case–control study nested within the FMC. The FMC was established by the National Institute for Health and Welfare, Oulu, Finland.
Institute for Health and Welfare, Finland.

control with paired samples was selected for every case. (ii) parity and (iii) date of index blood sampling were determined using the CRP distribution among the controls. The was skewed to the right. Spearman correlation coefficient was used to assess normally distributed data and median and percentiles for data whose statistical analysis. Descriptive statistics is presented as mean (range) for their controls were also excluded leaving 170 cases and controls for Biosciences, University of Umeå, Umeå, Sweden, using a high-sensitive laboratory analysis A control had to fulfil the case-matching criteria for both first and second had also donated at least two serum samples during different pregnancies. who were alive and free of cancer at the time of diagnosis of the case and available for final selection. Eligible controls were women from the FMC cases who donated their last serum sample within 1 year of ovarian cancer were identified. Two hundred and five cases (205) fulfilled these criteria and 33 in time, at least 1 year apart, ovarian cancer cases who had been pregnant screening for intrauterine infections. After the screening has been done, the remaining sample (1–3 ml of serum) is stored at +25°C in polypropylene cryovials in a well-protected biorepository at the National Institute for Health and Welfare in Oulu. More than 98% of pregnant women in Finland have donated blood samples to the cohort since 1983 and currently, >1.6 million samples are kept in storage. Each year, ~60 000 new serum samples are added to the repository.

identification of cases and controls

Incident ovarian cancer cases were identified by the population-based Finnish Cancer Registry (FCR). All cancer cases diagnosed in Finland since 1953 have been reported to the FCR (reporting mandatory since 1961). The coverage of the FCR is virtually complete with no losses to follow-up [19]. Every resident of Finland has a unique personal identity code, which is also used in official health registries like the FMC and FCR. Our study cohort was record-linked with the cancer registry data by using the personal identity code.

In order to determine serum CRP concentrations at more than one point in time, at least 1 year apart, ovarian cancer cases who had been pregnant on at least two occasions before cancer diagnosis and who had donated serum samples to the FMC during these different pregnancies were identified. Two hundred and five cases (205) fulfilled these criteria and 33 cases who donated their last serum sample within 1 year of ovarian cancer diagnosis further were excluded leaving 172 cases with paired samples available for final selection. Eligible controls were women from the FMC who were alive and free of cancer at the time of diagnosis of the case and had also donated at least two serum samples during different pregnancies. A control had to fulfil the case-matching criteria for both first and second samples. The controls were matched for (i) age at sample withdrawal ± 1 year, (ii) parity and (iii) date of index blood sampling ± 2 weeks. One control with paired samples was selected for every case.

The study was approved by the ethical committee of the National Institute for Health and Welfare, Finland.

laboratory analysis

CRP measurement was carried out at the Department of Medical Biosciences, University of Umeå, Umeå, Sweden, using a high-sensitive particle-enhanced immunoturbidimetric assay ‘Tina-quant CRP (Latex) HS’ on a Roche Cobas Modular P analyzer (Roche Diagnostics GmbH, D-68299, Mannheim, Germany). The method was calibrated against the IFCC/CRM 470 standard. The lower detection limit was 0.03 mg/l with an assay sensitivity ranging from 0.1 to 20 mg/l. During the analysis period, the total coefficients of variation estimated from CRP analyses of laboratory quality controls at 0.17 and 5.4 mg/l were 10.4% and 2.5%, respectively. Case and control samples were assayed together, ordered randomly and labelled to mask case-control status.

statistical analysis

Two cases had insufficient serum samples for laboratory analysis; hence, their controls were also excluded leaving 170 cases and controls for statistical analysis. Descriptive statistics is presented as mean (range) for normally distributed data and median and percentiles for data whose distribution departed from normality. Highly sensitive CRP data were log-transformed to reduce departure from normality because the distribution was skewed to the right. Spearman correlation coefficient was used to assess the correlation between the two CRP measurements.

Tertile cut-off points for CRP for both first samples and second samples were determined using the CRP distribution among the controls. The conditional logistic regression was used to estimate the relative risk [expressed as odds ratios (ORs)] of ovarian cancer across tertiles of CRP concentrations with the lowest tertile serving as the reference category. The model was adjusted for age at first full-term pregnancy, age at last full-term pregnancy and gestational age. Only age at first full-term pregnancy was included in the final model because it was the only variable that influenced the point estimates by >5%. Tests for trends were calculated using log-transformed CRP as a continuous variable in the model. Secondary analyses were conducted excluding subjects who had CRP values >10 mg/l and their matched cases/controls; thus, 14 and 20 case–control pairs were excluded from first and second sample secondary analyses, respectively. We examined the effects of individual changes in CRP concentration on the risk of ovarian cancer. Three groups were created based on the changes in CRP concentrations from sample 1 to sample 2. These groups had been determined a priori: (i) group 1—women with little changes in their CRP concentrations; these were women whose second sample CRP values did not deviate from the first sample value by >100% (either positive or negative); (ii) group 2—women with substantial decrease (negative change) in CRP concentrations; women whose second sample CRP values were less than first sample values by >100%; (iii) group 3—women with substantial increase (positive change) in CRP concentrations; women whose second sample CRP values increased from the first sample values by >100%. Group 1 was used as the reference group. Long-term stability of CRP according to pre-defined clinical cut-off points was evaluated. The defined clinical cut-off points are as follows: low (≤5 mg/l), moderate (1.1–3.0 mg/l), high (3.1–10 mg/l) and very high (≥10 mg/l) [20].

All statistical analyses were carried out using SPSS 17 for windows (SPSS Inc., Chicago, IL). Two-sided \( P < 0.05 \) was considered statistically significant.

results

The mean age at serum sampling for both cases (28.6 years) and control (28.7 years) was similar. Likewise, no appreciable differences were observed between cases and controls with regards to ages at first and last full-term pregnancies and the average number of pregnancies. Mean time between first and second serum sample donation for cases was 2.6 years compared with 2.5 years for controls. The average times between first and second sampling and cancer diagnosis were 8.9 years (range 1.1–15 years) and 6.4 years (range 1.1–13 years), respectively. Median CRP concentrations were higher among cases compared with controls during both sample periods (2.1 versus 1.7 mg/l during first sampling and 3.7 versus 2.5 mg/l during second sampling). There were moderate correlations between the second and the first sample CRP concentrations for both cases (\( r_s = 0.58, P \leq 0.001 \)) and controls (\( r_s = 0.62, P \leq 0.001 \)) (Table 1). There was a weak correlation between CRP concentrations and gestational age during both sampling periods (\( r_s = 0.28, P \leq 0.001 \), and \( r_s = 0.11, P = 0.04 \), during first and second sampling periods, respectively) but no correlations were observed between CRP concentrations and parity.

Figure 1 shows how stable individuals’ CRP measurements are, over a long period of time according to defined clinical cut-off points [20]. Using these clinical cut-off points, CRP levels, especially among women with low levels, appear to be stable over many years. Almost 70% of the women who had clinically low CRP levels at the time of first sampling maintained their clinically low CRP levels at the time of second sampling indicating long-term CRP stability.
FMC, Finnish Maternity Cohort; CRP, C-reactive protein.

Table 1. Baseline characteristics of the study population who donated serial samples to the FMC over a 23-year period (from 1983 to 2006)

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 170)</th>
<th>Controls (n = 170)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at serum sampling</td>
<td>28.6 (17.9–40.7)</td>
<td>28.7 (18.6–39.9)</td>
</tr>
<tr>
<td>Age at first full-term pregnancy</td>
<td>27.0 (16.3–39.6)</td>
<td>27.4 (18.6–37.9)</td>
</tr>
<tr>
<td>Age at last full-term pregnancy</td>
<td>31.4 (22.1–42.7)</td>
<td>32.1 (21.6–43.7)</td>
</tr>
<tr>
<td>Time interval between first and second serum sampling</td>
<td>2.6 (1.0–7.6)</td>
<td>2.5 (1.0–7.5)</td>
</tr>
<tr>
<td>Lag time to cancer diagnosis, first sample</td>
<td>8.9 (2.1–14.9)</td>
<td></td>
</tr>
<tr>
<td>Lag time to cancer diagnosis, second sample</td>
<td>6.4 (1.1–13.2)</td>
<td></td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>2 (1–9)</td>
<td>2 (1–8)</td>
</tr>
<tr>
<td>Median CRP concentrations for first sample (25th to 75th percentile), mg/l</td>
<td>2.1 (1.1–4.2)</td>
<td>1.7 (0.8–3.5)</td>
</tr>
<tr>
<td>Median CRP concentrations for second sample (25th to 75th percentile), mg/l</td>
<td>3.7 (1.3–6.2)</td>
<td>2.5 (1.2–4.9)</td>
</tr>
</tbody>
</table>

Values presented are mean (range) unless otherwise stated. All ages and times are in years.

FMC, Finnish Maternity Cohort; CRP, C-reactive protein.

In order to evaluate how long-term changes in an individual’s CRP concentrations may impact on ovarian cancer risk, we calculated the risk of ovarian cancer among the cohort using women whose CRP concentrations did not change much over the years as reference. Women whose CRP concentrations increased over time by >100% of the initial value had an almost twofold significant increased risk of ovarian cancer, OR 1.90 (95% CI 1.12–3.23, P value 0.03), compared with those with minimal changes in CRP concentrations. Exclusion of subjects with extreme values did not affect the risk estimates (Table 3).

**Discussion**

Our prospective population-based study supports the hypothesis that inflammation, of which CRP is a biomarker, may likely play a role in ovarian carcinogenesis.

We were able to demonstrate the association between inflammation and ovarian cancer by employing two methods. With the first method, the results revealed that increasing CRP concentrations were associated with increased risk of ovarian cancer across tertiles. In the second method, we employed changes in individuals’ CRP concentrations over a period of time before cancer diagnosis to determine the risk. We observed that women whose CRP concentrations increased substantially closer to cancer diagnosis were at higher risk of developing ovarian cancer. The principle behind this approach is that as inflammation becomes chronic, CRP concentrations will increase likewise. This may explain why significantly positive associations were observed only in analysis carried out using the second set of serum samples. Since the lag time between first sampling and cancer diagnosis extended to 15 years in some cases, it is possible that the inflammatory processes related to early stages of carcinogenesis may not have been initiated or at peak at this time. This is plausible considering the relatively young age of the cohort at the time of first serum sampling. As the years progress and the chronic inflammatory process persists, gradual increases in CRP concentrations will begin to ensue, which will most likely peak towards cancer diagnosis. This was corroborated in our observation that increase in CRP concentrations over the time period is an important denominator in the cancer process.

Another possible explanation for our result could be that the first set of samples were taken during the prodromal phase of the disease when the symptoms had not become manifest, since elevated risks, albeit insignificant, were also noted using the first
OR, odds ratio; CI, confidence interval; CRP, C-reactive protein.

Table 2. Relative risk (ORs with 95% CIs) of ovarian cancer by tertile of CRP distribution among Finnish women who donated serum samples at two separate occasions, at least 21 years apart before cancer diagnosis

<table>
<thead>
<tr>
<th>Tertile values, mg/l</th>
<th>At the time of first sample donation (n = 340)</th>
<th>At the time of second sample donation (n = 340)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 1</td>
<td>Tertile 2</td>
</tr>
<tr>
<td>n, case/control</td>
<td>47/57</td>
<td>56/57</td>
</tr>
<tr>
<td>ORb</td>
<td>1.0 (reference)</td>
<td>1.35 (0.77–2.35)</td>
</tr>
</tbody>
</table>

Excluding subjects with CRP concentrations >10 mg/l

<table>
<thead>
<tr>
<th>Tertile values, mg/l</th>
<th>At the time of first sample donation (n = 312)</th>
<th>At the time of second sample donation (n = 300)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 1</td>
<td>Tertile 2</td>
</tr>
<tr>
<td>n, case/control</td>
<td>41/55</td>
<td>59/52</td>
</tr>
<tr>
<td>ORb</td>
<td>1.0 (reference)</td>
<td>1.56 (0.87–2.79)</td>
</tr>
</tbody>
</table>

*Adjusted for age at serum sampling.

OR, odds ratio; CI, confidence interval; CRP, C-reactive protein.

Table 3. Relative risk (ORs with 95% CIs) of ovarian cancer associated with changes in CRP concentrations

<table>
<thead>
<tr>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>At the time of first sample donation (n = 340)</td>
<td>1.1 to ≤2.6</td>
<td>&gt;2.6</td>
<td>0.04</td>
</tr>
<tr>
<td>At the time of second sample donation (n = 340)</td>
<td>1.6 to ≤3.9</td>
<td>&gt;3.9</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Excluding subjects with CRP concentrations >10 mg/l

<table>
<thead>
<tr>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>At the time of first sample donation (n = 312)</td>
<td>1.0 to ≤2.5</td>
<td>&gt;2.5</td>
<td>0.20</td>
</tr>
<tr>
<td>At the time of second sample donation (n = 300)</td>
<td>1.4 to ≤3.2</td>
<td>&gt;3.2</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Adjusted for age at serum sampling.

OR, odds ratio; CI, confidence interval; CRP, C-reactive protein.

Our study has some similarities and differences with the two other prospective studies that have investigated the relationship between inflammation and ovarian cancer using serum CRP concentrations. The three studies are relatively large (for ovarian cancer studies) prospective nested case–control studies but while our study was a population-based study of women from a monogenetic pool (only Finnish women), the others contained women from at least two differing nationalities. This may, however, not be an important factor as our results are similar to those obtained by McSorley et al. [15] using American and British subjects. It, however, differed from those obtained by Lundin et al. [16] who observed no overall association between serum CRP concentrations and ovarian cancer risk but a positive relationship for women with CRP concentrations ≥10 mg/l, using American, Swedish and Italian subjects. A particular novelty of our study lies in the longitudinal measurements of CRP and estimation of risk estimates using changes in CRP concentrations as this has not been done previously.

Over the years, the dominant hypotheses regarding ovarian carcinogenesis have been those of incessant ovulation and excessive stimulation by hormones and gonadotrophins [22] with relatively less attention paid to other potential causes such as inflammation. The role of inflammation in the etiology of ovarian cancer is supported by the observed increased risk in women with chronic inflammatory diseases such as endometriosis and pelvic inflammatory disease. Furthermore, the role of inflammation in the etiology of ovarian cancer is supported by the observed increased risk in women with chronic inflammatory diseases such as endometriosis and pelvic inflammatory disease. Furthermore, the increased risk in women with chronic inflammatory diseases such as endometriosis and pelvic inflammatory disease. Furthermore, the increased risk in women with chronic inflammatory diseases such as endometriosis and pelvic inflammatory disease. Furthermore, the increased risk in women with chronic inflammatory diseases such as endometriosis and pelvic inflammatory disease.

In conclusion, our study suggests that serum CRP concentrations may be a potential biomarker for ovarian cancer risk. Further studies are needed to confirm these findings and to explore the role of inflammation in the etiology of ovarian cancer.

References

as inflammation. However, recent data seem to suggest that these hypotheses especially incessant ovulation and inflammation may actually share a similar pathway and some aspects of their contribution to ovarian carcinogenesis may not be mutually exclusive. The ovulatory process may be akin to an inflammatory process because it causes disruption and remodelling of the ovarian surface epithelium with resultant invasion by leukocytes, infiltration of inflammatory mediators such as cytokines, nitric oxide release and DNA repair [1, 23, 24]. Repetitive DNA repairs can result in replication errors especially at key regulatory sites such as in the tumour suppressor DNA regions, thereby increasing risk for carcinogenesis [25]. Hence, the incessant ovulation hypothesis may inadvertently incorporate some aspects of the inflammation hypothesis. It has also been recently suggested that there may be interplay between hormones and inflammation in hormone-dependent cancers [26], of which ovarian cancer is one.

Other potential sources of inflammatory stimuli can be within the peritoneal cavity [6] and external causes. The ovarian surface epithelium is a constituent of the peritoneal lining, thus any insults causing inflammation within the peritoneal cavity can also affect the ovaries [6]. Likewise, many studies have observed increased risks of ovarian cancer associated with conditions such as endometriosis, pelvic inflammatory diseases and use of talc powder, all of which stimulate inflammation within the ovaries, and protective effects for surgical conditions (hysterectomy and tubal ligation), which prevent retrograde transfer of inflammatory products from the lower genital tract to the ovaries [1, 4, 5, 27, 28].

Our study has the following strengths: it is prospective, population-based and CRP concentrations were measured twice for each subject, allowing us to explore how variations in serum CRP concentrations may impact on ovarian cancer risk. Nevertheless, the following limitations in our study need to be taken into consideration. The study population is limited to women who have been pregnant more than once, hence we do not know if the same will hold true in nulliparous women, who are at more risk of developing ovarian cancer compared with multiparous women. We did not have information on confounding factors such as body mass index, metabolic syndrome and the use of non-steroidal anti-inflammatory drugs (NSAIDs) within this subset of women to determine their effect on the risk estimates.

In conclusion, by showing that increasing CRP concentrations and changes in CRP concentrations before cancer diagnosis are associated with increased risk of ovarian cancer, our study supports the hypothesis that inflammation may play a role in ovarian carcinogenesis. This is important as there needs to be more research elucidating the mechanisms involved, not just in isolation but probably in conjunction with other suspected risk factors for ovarian cancer. Studies exploiting the association of polymorphisms in inflammatory genes with risk of ovarian cancer will also be warranted.

disclosure

The authors declare no conflict of interest.

references

24. Bonello N, Mickie K, Jasper M et al. Inhibition of nitric oxide: effects on interleukin-1 beta-enhanced ovulation rate, steroid hormones, and ovarian


