Increased cyclooxygenase-2 (COX-2) expression is associated with chemotherapy resistance and outcome in ovarian cancer patients

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Background: Cyclooxygenase-2 (COX-2) expression is associated with aggressive clinicopathological parameters and unfavourable prognosis in several human malignancies. The aim of this study was to investigate the expression of COX-2 and its association with clinicopathological parameters, response to treatment, and clinical outcome in ovarian cancer patients.

Patients and methods: COX-2 expression was analysed by immunohistochemistry in 87 primary ovarian carcinomas from patients with measurable disease after primary laparotomy.

Results: COX-2 immunoreaction was observed in 39 (44.8%) cases, and did not differ in distribution according to age, FIGO stage, debulking at time of surgery, presence of ascites, histotype or tumour grade. Both in patients cytoreduced at first surgery and in those undergoing only explorative laparotomy, the percentage of COX-2 positivity was significantly higher in non-responding than in patients responding to treatment (P = 0.043 and P = 0.0018, respectively). In multivariate analysis, only COX-2 positivity and older age retained an independent role in predicting a poor chance of response to treatment. There was no significant difference of clinical outcome according to COX-2 status in patients undergoing primary debulking while, in the subgroup of patients who underwent explorative laparotomy, COX-2-positive cases showed a shorter time to progression (P = 0.025) and overall survival (P = 0.025).

Conclusions: The assessment of COX-2 status could provide additional information in order to identify ovarian cancer patients with a poor chance of response to chemotherapy and potentially candidates for more individualised treatments.

Key words: chemotherapy response, COX-2, ovarian cancer, prognosis

Introduction

Ovarian cancer is the most common gynaecological malignancy in western countries and represents the fifth leading cause of death due to cancer in women [1]. More than 70% of cases present with advanced stage of the disease at diagnosis and despite advances in cytoreductive surgery and chemotherapy regimens, chemotherapy failure and progression of disease occur [1]. Although several clinicopathological parameters have been reported to be of prognostic significance in ovarian cancer, including tumour FIGO (Federation Internationale de Gynecologie et d’Obstetrique) stage, volume of residual disease, presence of cytologically malignant ascites and grade of tumour differentiation [2], it is conceivable that the assessment of biochemical factors more strictly related to individual tumour cell biology and intrinsic aggressiveness could help in identifying high risk patients and facilitating management of this disease.

Recently, much attention has been focused on the involvement of cyclooxygenase (COX), the key enzyme in the conversion of arachidonic acid to prostaglandins, in critical steps of tumour onset and progression. Starting from epidemiological studies showing a 40–50% lower risk of colorectal cancer [3] and to a lesser extent of breast, prostate, and head and neck cancer [4–6] in people continuously taking non-steroidal anti-inflammatory drugs, which are well known COX inhibitors, much effort has been devoted to the understanding of the biological activities of COX.

Two COX isoforms have been characterised: COX-1, which is constitutively expressed in almost all tissues where it serves homeostatic functions, and COX-2, which is highly
inducible by growth factors, prostaglandins and tumour promoters, and has been mainly associated with the inflammatory response [7]. More recently, several in vitro and pre-clinical studies showed that COX-2 overexpression is associated in colorectal cancer cells with bcl-2 overexpression, apoptosis inhibition, increased adhesion to extracellular matrix, and increased metastatic potential and neoangiogenesis [8–11]. Moreover, it has also been hypothesised that overexpression of COX-2 could impair host immune responses, as suggested by the ability of COX-2 inhibitors or COX-2 antisense constructs to revert tumour-induced immunosuppression [12, 13].

COX-2 has been found to be overexpressed in the vast majority of colorectal cancers, as well as in other solid tumours, and has been associated with clinicopathological parameters of aggressiveness and unfavourable prognosis [14–20]. In particular, we found that COX-2 overexpression is a strong predictor of chemotherapy response and poor outcome in locally advanced cervical cancer patients [21]. As far as ovarian cancer is concerned, only preliminary data have reported that COX-2 is constitutionally expressed in ovarian cancer cell lines and that it is inducible by prostaglandins, resulting in protection from apoptosis [22].

To our knowledge, no data have been reported until now about the expression of COX-2 and its possible clinical significance in ovarian cancer. The aim of the study therefore was to investigate by immunohistochemistry the expression of COX-2 and its association with clinicopathological parameters, response to treatment and clinical outcome in a single institutional series of primary untreated ovarian cancer patients.

Patients and methods

Patients

The study included 87 ovarian cancer patients admitted to the Department of Obstetrics and Gynecology (Division of Gynecologic Oncology, Catholic University of Rome) between February 1995 and April 2000. Staging was performed according to the FIGO classification. In order to make the analysis of the association with response to chemotherapy and outcome as reliable as possible, a highly homogenous series of stage IIIC–IV ovarian cancer patients with measurable disease at time of first surgery were included in the study. Median age was 57 years (range 27–81 years). Sixty-eight (78.2%) patients had stage IIIC, and 19 (21.8%) patients had stage IV disease. Most of the tumours (74.7%) were serous adenocarcinomas and showed a poor grade of differentiation (81.5%). Other clinicopathological characteristics are listed in Table 1.

As summarised in Figure 1, showing the flow chart of the overall population, patients were divided into two groups according to the possibility of performing primary debulking: 52 (59.8%) cases underwent primary debulking with <2 cm or ≥2 cm residual disease (n = 40 and n = 12, respectively), while 35 (40.2%) cases were considered unresectable and submitted only to multiple biopsies. Most of these patients had upper abdominal or peritoneal tumours with infiltration of the upper gastrointestinal tract and/or major vessels. All patients, independent of age, underwent four to six cycles of cisplatin-based chemotherapy (total dose at least 400 mg/m²) 2–3 weeks after primary surgery. In particular, patients cytoreduced at first surgery received six cycles of chemotherapy unless they showed clinical progression during treatment. As far as patients undergoing explorative laparotomy are concerned, they received three or four cycles of chemotherapy before attempting a second cytoreductive surgery, unless they showed clinical progression during treatment.

In patients cytoreduced at first surgery, response to chemotherapy was assessed by clinical (gynaecological) and ultrasound examination and analysis of CA125 levels, and was recorded according to World Health Organisation (WHO) criteria [23].

In the subgroup of patients who had tumour secondary debulking, response to chemotherapy was assessed by ultrasound examination, computed tomography (CT) scan and CA125 analysis performed before and after chemotherapy. Moreover, a direct assessment of the extent of response to chemotherapy was carried out at the time of the second laparotomy, taking into account the findings of the primary explorative laparotomy.

Figure 1. Flow chart of the patient population.
Immunohistochemistry

Tumour tissues biopsies from primary tumours were obtained at first surgery in all cases. Tissue specimens were fixed in formalin and paraffin-embedded according to standard procedures. Four micrometres of representative blocks from each case were deparaffinised in xylene, rehydrated, treated with 0.3% H2O2 in methanol for 10 min to block endogenous peroxidase activity, and subjected to heat-induced epitope retrieval in a microwave oven using the Dako ChemMate detection kit (Dako, Glostrup, Denmark) according to the manufacturer’s instructions. Slides from all cases studied were then processed simultaneously for immunohistochemistry on the TechMate Horizon automated staining system (Dako) using the Vectastain ABC peroxidase kit (Vector Laboratories, Burlingame, CA, USA). Endogenous biotin was saturated by a biotin blocking kit (Vector Laboratories). Sections were incubated with normal rabbit serum for 15 min, then with rabbit polyclonal antiserum against COX-2 (Cayman, Ann Arbor, MI, USA) diluted 1:300 for 1 h. Negative controls were performed using non-immunised rabbit serum or by omitting the primary antiserum.

Tumour sections showing immunoreaction were scored as positive. The analysis of all tissue sections was performed without any prior knowledge of the clinical parameters by two different pathologists (L.L. and F.O.R.) by means of light microscopy. The proportion of immunostained cells was scored at low magnification (×5 objective lens) by evaluating the entire tumour area. When tumour area with positive immunostaining was >10% of the total tumour area, the case was scored as positive. Intensity of staining was also evaluated subjectively using a range from 0 (none) to 1 (feint) to 2 (strong). Cases in which the intensity of staining was scored <2 were considered negative. In case of disagreement (n = 7, 8%), the sections were subjected to a joint re-evaluation.

Statistical analysis

Fisher’s exact test or χ2 test were used to analyse the distribution of COX-2-positive cases according to several clinicopathological features.

Table 1. COX-2 expression according to the clinicopathological characteristics of the ovarian cancer population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of patients</th>
<th>No. of COX-2-positive cases (%)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>87</td>
<td>39 (44.8)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>54</td>
<td>23 (42.6)</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>33</td>
<td>16 (48.4)</td>
<td>NS</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>68</td>
<td>29 (42.6)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>19</td>
<td>10 (52.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Cytoreduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual tumour volume &lt;2 cm</td>
<td>40</td>
<td>17 (42.5)</td>
<td></td>
</tr>
<tr>
<td>Residual tumour volume ≥2 cm</td>
<td>12</td>
<td>6 (50.0)</td>
<td>NS</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Explorative laparotomy</td>
<td>35</td>
<td>16 (45.7)</td>
<td></td>
</tr>
<tr>
<td>Ascites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>24</td>
<td>8 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>63</td>
<td>29 (46.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Histotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>65</td>
<td>31 (47.7)</td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
<td>2</td>
<td>2 (100)</td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>8</td>
<td>3 (37.5)</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>9</td>
<td>2 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>1 (33.3)</td>
<td>NS^</td>
</tr>
<tr>
<td>Grade of differentiation</td>
<td>1–2</td>
<td>15</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>29 (43.9)</td>
<td></td>
</tr>
<tr>
<td>Not available</td>
<td>6</td>
<td>–</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Calculated by Fisher’s exact or χ² test for proportion.
^Serous histotype compared with other histotypes.
^According to the FIGO system.
NS, not significant.
Overall survival (OS) and time to progression (TTP) were calculated from the date of diagnosis to the date of death/progression or date last seen. Medians and life tables were computed using the product-limit estimate by the Kaplan–Meier method [24], and the log-rank test was employed to assess statistical significance [25]. Multiple logistic analysis [26] was used to analyse the role of clinicopathological parameters and COX-2 staining as a predictor of response to treatment. Statistical analysis was carried out using SOLO (BMDP Statistical Software, Los Angeles, CA, USA).

Results

COX-2 immunostaining

COX-2 immunostaining was observed mainly in the cytoplasm of tumour cells. Figure 2 shows a representative example of an ovarian tumour with intense COX-2 immunostaining (A), compared with a tumour with a very low percentage of COX-2-stained tumour cells (B). Stromal lymphoid cells sometimes showed different intensities of COX-2 immunoreaction. Thirty-nine cases (44.8%) were scored as COX-2 positive.

Correlation with clinicopathological parameters and response to chemotherapy

No significant association between the extent of residual tumour at surgery and stage of disease was observed, while patients with greater tumour volume showed ascites more frequently than patients with lesser residual disease ($P = 0.04$). The extent of residual disease was also shown to be associated with poor grade of differentiation ($P = 0.04$) and older age ($P = 0.013$). A direct association between older age and poor grade of differentiation was found ($P = 0.016$). Finally, the presence of ascites was found more frequently in the case of serous adenocarcinomas than in other histotypes ($P = 0.013$).

Table 1 shows the distribution of COX-2 positivity according to clinicopathological characteristics. COX-2 positivity was not distributed differently according to age, FIGO stage, debulking at time of surgery, presence or absence of ascites, or tumour grade. COX-2 positivity did not show any significant variation according to different histotypes, although it is noteworthy that the only two cases with a mucinous histotype were both COX-2 positive.

With respect to response to chemotherapy, in the group of 52 patients in which cytoreduction was performed there were 41 (78.8%) responses (29 complete and 12 partial responses), while 11 (21.1%) patients were classified as non-responders. In patients who underwent only explorative laparotomy, clinical response was documented in 23 (65.7%) cases, while 12 (34.3%) patients experienced disease progression.

In the group of 52 patients who were cytoreduced at first surgery, COX-2 positivity was found in a statistically significantly higher percentage of non-responding cases ($n = 8/11; 72.7\%$) than in patients responding to chemotherapy ($n = 15/41; 36.6\%$) ($P = 0.043$). Similar results were obtained in the subgroup of patients undergoing only exploratory laparotomy since the percentage of COX-2 positivity was higher in non-responders ($n = 10/12; 83.3\%$) than in patients responding to treatment ($n = 6/23; 26.1\%$) ($P = 0.0018$).

In Table 2 the univariate and multivariate analysis of clinicopathological parameters and COX-2 status as predictors of response to chemotherapy in the whole population are summarised.

When logistic regression was applied, only COX-2 positivity and older age retained an independent role in predicting a poor chance of response to treatment.

Similar results were obtained after subgrouping in patients undergoing cytoreduction or exploratory laparotomy (data not shown).

Survival analysis

Follow-up data were available for 87 patients. As of May 2001, the median follow-up period was 25 months (range 4–147 months). During the follow-up period, progression and death of disease were observed in 64 (73.6%) and 42 (48.3%) cases, respectively. Figure 3 shows the TTP and OS curves in our population according to COX-2 status.

There was no significant difference of clinical outcome in terms of both TTP and OS according to COX-2 status in

![Figure 2. COX-2 immunostaining in primary ovarian carcinoma. (A) COX-2-positive tumour showing intense cytoplasmatic immunoreaction in tumour cells. (B) COX-2-negative tumour showing only scattered COX-2-positive cells. Bar = 35 µm.](image_url)
patients undergoing primary debulking (Figure 3A and B), while in the subgroup of patients who underwent explorative laparotomy (Figure 3C and D), COX-2-positive cases demonstrated a shorter TTP (median 11 months compared with 17 months in COX-2-negative cases; \( P = 0.025 \)) and OS (median 22 months compared with >50% survival in COX-2-negative cases at last follow-up; \( P = 0.025 \)).

**Discussion**

This is the first study demonstrating the association between COX-2 and reduced susceptibility to chemotherapy and poor outcome in a large series of primary advanced ovarian cancer with measurable disease at first surgery.

Overexpression of COX-2 has been reported in tumour cells compared with normal cells, which showed low or no COX-2 expression in several tissue types [16, 17, 19]. Although the mechanism of COX-2 up-regulation is unknown, recent evidence suggests that it could result from the dysregulation of key steps in the epidermal growth factor receptor signalling pathway, namely of \( ras \) and mitogen-activated protein kinases [18, 27].

Although we failed to demonstrate an association between COX-2 status and any of the clinicopathological character-

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**Table 2.** Univariate and multivariate analysis of clinicopathological parameters and COX-2 status as predictors of chemotherapy response in ovarian cancer patients

<table>
<thead>
<tr>
<th>Variable(^a)</th>
<th>All cases</th>
<th>Univariate</th>
<th>Multivariate(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \chi^2 )</td>
<td>( P ) value</td>
</tr>
<tr>
<td>Age</td>
<td>8.4</td>
<td>0.004</td>
<td>7.3</td>
</tr>
<tr>
<td>Stage</td>
<td>0.1</td>
<td>0.8</td>
<td>–</td>
</tr>
<tr>
<td>Cytoreduction</td>
<td>1.2</td>
<td>0.26</td>
<td>0.2</td>
</tr>
<tr>
<td>Ascites</td>
<td>4.2</td>
<td>0.04</td>
<td>2.3</td>
</tr>
<tr>
<td>Histotype</td>
<td>0.1</td>
<td>0.8</td>
<td>–</td>
</tr>
<tr>
<td>Grade</td>
<td>0.2</td>
<td>0.6</td>
<td>–</td>
</tr>
<tr>
<td>COX-2 status</td>
<td>12.3</td>
<td>0.0005</td>
<td>10.7</td>
</tr>
</tbody>
</table>

\(^a\)Variables were subgrouped as follows: age, \( \leq 60 \) years compared with >60 years; stage IIIIC compared with stage IV; cytoreduced cases compared with explorative laparotomy; absence compared with presence of ascites; serous histotype compared with other histotype; grade 1–2 compared with grade 3; COX-2 negative compared with positive.

\(^b\)Only variables with \( P < 0.3 \) in the univariate analysis were included in the multivariate analysis.

\( \chi^2 \) of the model = 27.7 (\( P = 0.00001 \)); NS, not significant.

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**Figure 3.** TTP and OS curves according to COX-2 status in ovarian cancer in cytoreduced cases (A and B) and in cases undergoing explorative laparotomy (C and D).
istics, a very strong association between COX-2 positivity and a poor chance of response to treatment was observed. More importantly, the ability of COX-2 expression to predict ovarian tumour susceptibility to chemotherapy was retained in multivariate analysis. Furthermore, older age retained an independent role in predicting a poor chance of response to treatment. This could be explained by the correlation between older age and both poor tumour grade of differentiation and the reduced feasibility of cytoreduction.

We reported recently that high COX-2 expression is associated with a poor chance of response to neoadjuvant cisplatin-based chemotherapy in locally advanced cervical carcinoma [21]. Moreover, it is noteworthy that COX inhibitors are able to enhance the cytotoxicity of several chemotherapeutic agents in vitro [28] and to potentiate tumour cell radiosensitivity in vivo [29].

In this context, our current findings support the hypothesis that the association between COX-2 expression and chemoresistance could be a generalised phenomenon that is yet to be clarified on a biochemical level. It is conceivable that the involvement of COX-2 in the biochemical pathways influencing tumour cell susceptibility to cytotoxic agents could play a major role: in particular, COX-2 overexpression has been associated with the function of Her2/neu, which is renowned for inducing resistance to several cytotoxic agents. Moreover, COX-2 has been reported to induce the antiapoptotic bcl-2 protein [30] and to be associated with neoangiogenesis in tumour-bearing mice [31]. Since both inhibition of apoptosis and promotion of neoangiogenesis are strictly related to chemotherapv resistance [32, 33], it is conceivable that COX-2 expression could play a role as an indicator of chemoresistance in ovarian cancer.

The correlation between COX-2 overexpression and unfavourable clinical outcome has been demonstrated in several human malignancies and has been associated with clinicopathological characteristics of tumour aggressiveness such as advanced stage, node involvement or invasive properties [12, 14–16, 18–20].

It remains to be verified why, although COX-2 positivity seems to identify chemoresistant tumours in patients undergoing both cytoreduction and explorative laparotomy, COX-2 positivity behaves as a marker of poor TTP and OS only in the latter patient group.

It is conceivable that cytoreduction can modify the tumour–host interactions and then tumour biology, hindering the prognostic impact of COX-2 positivity.

An alternative hypothesis is that the selection of chemosensitive cases based on the feasibility of interval debulking surgery (IDS) could be more reliable than in patients optically debulked at first surgery.

In conclusion, these findings need to be confirmed in a larger series, mainly because the assessment of response rate is a difficult end point in this tumour. However, our study showed that the assessment of COX-2 status could provide additional information in order to identify ovarian cancer patients with a very poor chance of response to chemotherapy, and therefore who are potentially candidates for more individualised treatments.

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References


