Increased cyclooxygenase-2 expression is associated with better clinical outcome in patients submitted to complete ablation for severe endometriosis

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BACKGROUND: Recent studies have demonstrated the overexpression of cyclooxygenase-2 (COX-2) in endometriosis. The aim of this study was to investigate the correlation between COX-2 expression and the clinical outcome rate in a homogeneous series of patients undergoing fertility-sparing complete laparoscopic ablation for severe endometriosis.

METHODS: COX-2 expression was analysed by immunohistohemistry in 103 samples, 71 endometriomas (group 1) and 32 peritoneal implants and or recto-vaginal nodules (group 2) of endometriotic tissue from 85 patients submitted to complete laparoscopic ablation of severe endometriosis. RESULTS: At median follow-up of 54 months, a recurrence rate of 24.7% (n = 21) was observed. Patients with COX-2-positive endometriotic cysts showed a lower relapse rate than COX-2-negative cases (16.7 versus 41.2%; P = 0.036). Patients with COX-2-positive peritoneal implant and or recto-vaginal nodule showed a similar trend. Taking the two groups of patients together, we found a significantly lower relapse rate in COX-2-positive patients in comparison to COX-2-negative patients (16.4 versus 40%; P = 0.015). Moreover, COX-2-positive patients showed a longer relapse-free survival in comparison to COX-2-negative patients (P = 0.016). CONCLUSIONS: In patients with severe endometriosis who underwent fertility-sparing complete ablation, COX-2 overexpression characterizes a subgroup of patients with lower risk of relapse and longer relapse-free survival.

Key words: COX-2/endometriosis/relapse

Introduction

Endometriosis is a common gynaecological disease which has a complex, multifactorial aetiology and is strongly associated with pelvic pain, severe dysmenorrhoea and deep dyspareunia.

Although retrograde menses has been indicated as the crucial step in the development of endometriosis (Sampson, 1927), factors allowing the implantation and propagation of endometriotic lesions are still unclear.

Ectopic endometrium has been reported to produce prostaglandins which, in turn, contribute to the pathophysiology of endometriosis (Vermon et al., 1986). In fact, some studies reported higher prostaglandin levels in peritoneal fluids of women affected by endometriosis in comparison to controls (Darke et al., 1981; Badawy et al., 1985).

Cyclooxygenase-2 (COX-2), the inducible isoform of the enzyme responsible for the conversion of arachidonic acid to prostaglandins, has been reported to be involved in apoptosis inhibition, angiogenesis induction and immunosuppression. COX-2 expression can be markedly increased by several growth factors, oncogenes and tumour-promoting factors, and has been associated with worse clinical outcome in several solid tumours including endometrial cancer (Tucker et al., 1999; Tomozawa et al., 2000; Ferrandina et al., 2002a,b).

We have recently reported that COX-2 overexpression can be detected only in the endometriotic ovarian cyst wall and that no correlation exists between COX-2 levels and clinico-pathological features as well as symptomatology (Fagotti et al., 2004). Recently, elevated COX-2 expression in the eutopic endometrium of patients with deep endometriosis has been correlated with severe endometriosis-related dysmenorrhoea (Matsuzaki et al., 2004a).

Various therapies have been used in an attempt to treat endometriosis, including ovarian suppression therapy, surgical treatment, or a combination of these agents. The goal of the medical therapy is to induce hypoestrogenism or to antagonize estrogen action, thus leading to inhibition of endometriotic implants. However, pain recurs in about half of symptomatic women, once medical therapy is discontinued, and no data
have demonstrated a protective effect of the drugs on the incidence of recurrence after discontinuation (Vignali et al., 2002; Valle and Sciarra, 2003).

Recently, selective COX-2 inhibitors have been found to be safe and effective in the treatment of endometriosis-related pain (Cobellis et al., 2004). The effect of selective COX-2 inhibitors on endometriosis in different animal models has been also investigated, yielding inconsistent results. This target medical treatment has been shown to prevent de novo formation of endometriosis (Matsuzaki et al., 2004b), but not to influence the number and the size of the endometriosis lesions (Hull et al., 2005).

However, laparoscopic excision still remains the ‘gold standard’ approach for the management of endometriosis, and the effects may be improved with careful use of appropriate techniques and adjuvant therapies (Garry, 2004). In fact, several studies (Abbott et al., 2003; Redwine, 1991 Redwine and Wright, 2001) have demonstrated that complete laparoscopic excision of endometriosis reduces all aspects of pelvic pain and significantly improves pregnancy rate. However, 36% of these subjects showed relapse of disease within 5 years since primary surgical treatment, and required further surgery (Garry, 2004). The mechanism of relapse is still unknown, and so far no clinico-pathological or biological factors have been shown to predict the risk of relapse in patients submitted to radical ablation of severe endometriosis.

To our knowledge, no data have been reported about the role of COX-2 expression in predicting the clinical outcome in patients with endometriosis. The aim of this study was to investigate whether COX-2 immunohistochesmical status can identify patients at high risk of recurrence of endometriosis after complete laparoscopic fertility-sparing ablation.

Materials and methods

Study population

This study, updating one previously published (Fagotti et al., 2004), was carried out on the subgroup of the study population that underwent complete laparoscopic ablation for severe endometriosis. In the study period, 85 out of 101 patients (84.2%) were defined as completely ablated with no macroscopic residual endometriosis (ovarian and extra-ovarian) at the end of surgery, as primarily judged at the end of each single intervention and confirmed by a blind review of the videotapes. Incomplete ablation was performed because of the lack of patient consensus about bowel injuries during deep recto-vaginal or Douglas endometriosis resection in the remaining 16 patients. All patients underwent fertility-sparing laparoscopic surgery, and the fact that the surgical team has been stable through the years has made it possible to maintain a homogeneous surgical approach. Surgery included blunt removal of adhesions in all cases, endometrioma ablation with residual ovarian parenchyma-sparing technique in 71 cases, and removal of all macroscopic peritoneal superficial or deep endometriosis and recto-vaginal septum nodule resection in 32 cases. No laparotomic conversion and early complication were registered.

The clinical charts were available for all patients who expressed an informed consent to use their data for scientific purposes. The slides were all reviewed by a dedicated pathologist and only those cases with evident endometrial epithelium and stromal component in the surgical specimens were included in the study. Institutional review board approval was not requested because this was a retrospective study.

As previously reported, endometriosis-related symptoms, including complaints about the lower urinary and gastrointestinal tracts, were collected from the medical records and tabulated in the database (Fagotti et al., 2004).

The disease stage was scored according to the revised American Society for Reproductive Medicine (1997) classification. Age, infertility (failure to conceive for 1 year) and pre-operative CA125 serum levels were also recorded. In 31 patients (36.5%), candidates for assisted reproduction therapy, adjuvant therapy with GnRH analogues was performed for 6 months.

Pelvic examination and pre-operative pelvic and upper urinary tract ultrasound evaluation were routinely performed, whereas double-contrast barium enema and cystoscopy were done only when indicated by specific lower urinary and gastrointestinal symptoms (Landi et al., 2004).

At 6, 12 and 18 months after surgery, all patients were evaluated with pelvic examination, pelvic and upper urinary tract ultrasound scan, and CA125 serum levels, and were asked to complete a simple office-based questionnaire. Patients were asked to complete the same simple-office based quality of life questionnaire used before surgery, which also included a 10-point ranked ordinal scale (from 0 = none to 10 = debilitating) asking them to rate the severity of various endometriosis-related symptoms (Fagotti et al., 2004). After this follow-up period, all patients included in the study were asked annually to complete the same questionnaire and were submitted to a bimanual pelvic examination and ultrasound scan. The presence of referred endometriosis-related symptoms was not considered adequate to define a recurrence of disease that has always been objectively confirmed with medical investigation including bimanual palpation and transvaginal pelvic ultrasound. Relapse was defined as the presence of de novo symptomatic or asymptomatic endometrioma, and/or recto-vaginal septum nodule.

Immunohistochemistry

Tissue specimens were obtained from endometrioma wall (71 cases), peritoneal implants and recto-vaginal septum nodules (32 cases). Each case was submitted to more than one biopsy on all suspicious sites of endometriosis. As expected, not all the specimens were successful in identifying endometriosis. However, only cases where endometrial epithelium and stroma could be clearly identified were considered for the study. Immunohistochemistry was performed as previously described (Ferrandina et al., 2002a,b; Fagotti et al., 2004). Tissues were fixed in formalin and paraffin-embedded according to standard procedures. A 4 µm representative section from each case was deparaffinized 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 simultaneously processed 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). Section were incubated with normal rabbit serum for 15 min, then whit rabbit polyclonal antiserum against COX-2 (Cayman, Ann Arbor, MI, USA) diluted 1:300, for 1 h. Negative controls were performed using non-immunized rabbit serum or omitting the primary antiserum.

Analysis of all tissue sections was done by two different pathologists without any prior knowledge of the clinical parameters. Intensity of COX-2 in endometriosis epithelium was subjectively evaluated in a range from 0 (none) through 1 (faint) to 2 (strong) and 3 (very strong). Cases in which the intensity of staining was scored >2 were considered positive.

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Statistical analysis

Fisher’s exact test or χ²-test were used to analyse the distribution of COX-2-positive cases according to several clinico-pathological features. Statistical analysis has been done for each symptom separately, using a threshold value which corresponded to the subjective score evaluation = 5.

All medians and life-tables were calculated using the product-limit estimate and the curves were examined by means of the long-rank test (Mantel, 1996). Recurrence-free survival (RFS) analysis was performed using SOLO Statistical Software (BMDP Statistical Software Inc., Los Angeles, CA, USA).

Results

COX-2 immunostaining

The overall COX-2 positivity rate was 64.7%. COX-2 staining was scored as positive in 54 out of 71 specimens (76%) from ovarian endometriotic cysts, whereas the percentage of COX-2 positivity was 12.5% (four out of 32 cases) in peritoneal implants and recto-vaginal nodules (Table I).

In eight cases COX-2 immunostaining was performed both in peritoneal implants and recto-vaginal septum nodules from the same patient, and we found that the COX-2 score was superimposable in all but two cases (75% agreement). As previously reported (Fagotti et al., 2004), on the basis of COX-2 positivity rates, cases have been divided into two groups: group 1 (endometriomas) and group 2 (peritoneal implants and recto-vaginal nodules); the analysis of the correlations between clinico-pathological parameters and COX-2 status has followed this dichotomization.

Correlation with clinico-pathological parameters

Patient characteristics are shown in Table I. Mean ± SD age was 31.9 ± 6.0 years, while mean ± SD CA125 levels were 70.6 ± 40.5 IU/ml and mean ± SD ASRM score was 52.8 ± 30.6. Seventy-seven patients (90.6%) were scored as stage III–IV. The mean ± SD diameters were 4.2 ± 2.1 and 1.5 ± 1.1 cm (based on the ultrasound findings) for the endometrioma and for the RV septum nodules respectively.

In our study population, an irregular use of non-steroidal anti-inflammatory drugs (NSAID) during menses in 95.9% of patients was referred.

As previously reported (Fagotti et al., 2004), no significant association between COX-2 expression in endometriosis tissues and clinico-pathological characteristics was found.

At median follow-up of 54 months (range 10–102), endometriosis recurred in 21 patients (24.7%), and in 18 (85.7%) of these cases almost one of the endometriosis-related symptoms was referred. In our population, three different patterns of recurrence were clinically documented: three patients (14.3%) showed an isolated RV recurrence, while endometrioma or both endometrioma and RV nodule were found in 17 (80.1%) and 1 patients (5.6%), respectively. During the follow-up period 13 out of 21 (62%) relapsed patients were submitted to operative laparoscopy because of the severity of their symptoms and/or infertility. In all these cases definitive histopathology examination confirmed the presence of endometriosis. Surgery was not performed in the remaining eight patients because of the lack of consensus in five cases, and the absence of endometriosis related symptoms. In this group of non-operated patients the bimanual-palpation and the pelvic ultrasound repeated during the follow-up period confirmed the presence of the relapse.

Analysing the correlation between the clinico-pathological characteristics and the rate of relapse, no statistically significant correlation except between COX-2 expression in endometriomas was found by the univariate analysis. In particular, patients with COX-2-positive endometriotic cysts showed a lower relapse rate than patients with COX-2-negative endometriotic cysts (16.7 versus 41.2%; P = 0.036) (Table I). In our population, the diameter of the endometrioma and of the extra-ovarian lesions was not correlated with the relapse rate (data not

<table>
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<th>Variable</th>
<th>Cases n (%)</th>
<th>Relapse n (%)</th>
<th>P*</th>
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<tr>
<td>Age (years)</td>
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<tr>
<td>≤30</td>
<td>45 (55.6)</td>
<td>7 (22.2)</td>
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<td>&gt;30</td>
<td>86 (61.5)</td>
<td>21 (35.4)</td>
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<td>≤5</td>
<td>54 (76)</td>
<td>4 (7.4)</td>
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<tr>
<td>&gt;5</td>
<td>54 (63.5)</td>
<td>11 (20)</td>
<td>NS</td>
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<tr>
<td>ASRM stageb</td>
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<tr>
<td>I–II</td>
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<td>3 (37.5)</td>
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<tr>
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<td>9 (16.7)</td>
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<td>Positive</td>
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<td>9 (16.4)</td>
<td>0.0152</td>
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</table>

*P values calculated by χ² or fisher’s exact test.

a According to American Society for Reproductive Medicine (1997).

bNS = not significant.
A similar but not statistically significant trend was shown in the group of patients in which the expression of COX-2 was evaluated in the peritoneal implants and recto-vaginal nodules. In fact, COX-2-positive cases did not show any clinical relapse, whereas a relapse rate of 35.7% was registered in the COX-2-negative patients (Table I). Moreover, taking together the two subgroups of patients, and considering as positive the 12 patients with positive COX-2 endometriomas and/or peritoneal implants/recto-vaginal septum nodules, we found that COX-2-positive patients showed a significantly lower relapse rate with respect to COX-2-negative cases (16.4 versus 40%; \( P = 0.0152 \)) (Table I). The only parameter retaining a statistically independent value by multivariate analysis was the COX-2 overexpression in the endometrioma (data not shown).

Kaplan–Meier curves for recurrence-free survival (RFS) in group 1 (Figure 1A) and group 2 (data not shown) were analysed. A trend for a longer RFS for COX-2-positive patients was shown in both groups, although the difference was not statistically significant. However, when considering the entire study population, median RFS was not reached in COX-2-positive patients but was reached after 52 months for COX-2-negative patients, i.e. 2 year RFS was 93% in the COX-2-positive patients compared with 67% in the COX-2-negative patients (Figure 1B) (\( P = 0.0158 \)).

**Discussion**

We have recently reported a higher expression of COX-2 in the endometriotic ovarian cyst wall than in extra-ovarian localizations (Fugotti et al., 2004). However, we failed to find any significant correlation between COX-2 positivity and clinicopathological characteristics and symptoms in the study population (Fugotti et al., 2004). In the present study, we analysed the prognostic role of COX-2 immunohistochemical status in the subgroup of patients who underwent complete laparoscopic ablation of severe endometriosis.

Recently, in a large and prospective series, the favourable role of laparoscopic complete excision of endometriosis in the reduction of all aspects of pelvic pain and in the improvement of the quality of life and sexual function of women with endometriosis has been documented (Abbott et al., 2003). Although operative laparoscopy can be considered the ‘gold standard’ approach in the management of severe endometriosis, 20–40% patients still show subjective or objective relapse of disease during follow-up and require further surgery (Redwine and Wright, 2001; Abbott et al., 2003). Similarly, in our series 24.7% of patients undergoing a complete laparoscopic ablation of severe endometriosis showed an objective relapse during the follow-up period. There are few data in the literature about the relapse rate after radical ablation with conservative surgery for severe endometriosis. In particular there is a lack of randomized trials assessing the difference of radical versus non-radical ablation of severe endometriosis in terms of relapse rate. Ours is a very homogeneous population in which every relapse should be considered as a *de novo* endometriosis, making the type of surgery not relevant in determining the relapse risk.

To date, no clinico-pathological factors have been shown to predict the risk of relapse in patients undergoing fertility-sparing complete ablation of severe endometriosis. Moreover, to our knowledge there are no data that correlate the biology of primary endometriosis and its trend to develop a clinical recurrence after complete ablation.

COX-2 overexpression has usually been associated with worse clinical outcome in several human malignancies and associated with clinico-pathological characteristics of tumour aggressiveness (Huang et al., 1998; Sheehan et al., 1999; Tucker et al., 1999; Ferrandina et al., 2000a,b).

We reported a significant association between COX-2 overexpression and a lower relapse rate and a better recurrence-free survival. It remains to be explained why, although COX-2 overexpression seems to characterize more aggressive tumours due to its involvement in the angiogenic and inflammatory pathways (Vane et al., 1994; Daniel et al., 1999), it behaves as a marker of better RFS in patients submitted to complete endometriosis ablation. It is conceivable that an accurate evaluation of the molecular mechanisms involved in the COX-2 network should be performed to substantiate these findings.

Patients with severe endometriosis often report a chronic use of NSAID in the treatment of the endometriosis-related symptoms. Therefore, the possibility that the overexpression of COX-2, which is the target of NSAID, might identify patients more likely to benefit from NSAID treatment and to exhibit better outcomes in COX-2-overexpressing patients cannot be excluded. On the other hand, the absence of a specific target in COX-2-negative patients might justify the
worse prognosis of this subgroup of patients with severe endometriosis.

In conclusion, our data have substantiated the hypothesis that patients with COX-2-overexpressing endometriosis show a better clinical outcome in terms of relapse rate and RFS. Thus, a randomized controlled trial might be performed to verify whether the use of selective COX-2 inhibitors in the adjuvant setting is able to further improve the clinical outcome of patients with COX-2-positive endometriosis.

References


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