The involvement of the interstitial Cajal cells and the enteric nervous system in bowel endometriosis

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BACKGROUND: Our aim was to investigate the relationships between gastrointestinal symptoms and histological findings in women with bowel endometriosis. METHODS: The gastrointestinal symptoms of 362 women with endometriosis were classified according to the subgroups of the Rome II criteria. All visible endometriotic lesions of the bowel were removed; the patients were prospectively followed up for 2 years. The interstitial Cajal cells (ICC) and the enteric nervous system were immunohistochemically evaluated. RESULTS: Sixty-eight (18.8%, 95% CI 14.9–23.2) women had bowel lesions. The endometriotic lesions infiltrated the serosal layer and surrounding connective tissue in 45 cases; the subserous plexus in 11 cases; the Auerbach plexus in eight cases; the Meissner plexus in four cases. Whenever the subserous plexus was interrupted by the endometriotic lesions, the ICC were damaged. All women with endometriotic lesions reaching at least the subserous plexus reported bowel complaints. The level of infiltration into the bowel wall was correlated with severity of symptoms. Removal of lesions resulted in improvement of symptoms. CONCLUSIONS: Endometriosis-induced damage of ICC, even before muscular infiltration, may cause bowel symptoms.

Key words: bowel endometriosis/Cajal cells/enteric nervous system

Introduction

Five to 27% of women with endometriosis have intestinal involvement (Weed and Ray, 1987; Jerby et al., 1999; Redwine, 1999; Chapron et al., 2003). The sigmoid colon is most commonly involved, followed by the rectum, ileum, appendix and caecum (Redwine, 2004). The series of patients with surgically treated bowel endometriosis show that obstructive symptoms, severe pain and heavy transit disturbances are the typical pre-operative complaints (Fujimoto et al., 2001; Verspyck et al., 1997; Varras et al., 2002; Ridha and Cassaro, 2003).

It is well known that the enteric nervous system (ENS) is responsible for both bowel contractility and pain referral (Gershon et al., 1994). The ENS is divided into three nervous plexuses: the subserous or perivisceral plexus, the intramuscular or Auerbach plexus, and the submucosal or Meissner plexus. Furthermore, in the bowel, there are specialized myofibroblast cells named interstitial cells of Cajal (ICC). These cells, found throughout the gut from the oesophagus to the anus, are gastrointestinal pacemakers that generate and propagate electrical slow waves (Thuneberg, 1982; Huizinga et al., 1995). They also play other important roles in the control of gut motor activity, acting as intermediaries in the neural control of gut muscular activity (Daniel and Posey-Daniel, 1984), as spatial coordinators of gut motility (Farraway et al., 1995), and as stretch receptors (Faussone-Pellegrini, 1992). The damage of ICC has been proven to be associated with the functional loss of the spontaneous electrical slow wave and contractile activity (Ward et al., 1994, 1995; Malysz et al., 1996; Mikkelsen et al., 1998). Research into the distribution and function of ICC was greatly stimulated by discovering that ICC express c-kit and that signalling via Kit protein is necessary for development and maintenance of the ICC (Torihashi et al., 1995, 1997). Although the ENS and the ICC have a pivotal role in gut motility, several molecules may be involved in the pathogenesis of gastrointestinal symptoms. In particular, ovarian products such as progesterone, LH, hCG and relaxin (but not estrogen) may affect gastrointestinal motility (Mathias and Clench, 1998).

Previous studies evaluated the relationship between endometriosis and gastrointestinal complaints, reporting, for instance, a characteristic dysfunction of the ENS (ampulla of Vater–duodenal wall spasm) secondary to injury or lack of inhibitory control of the ENS (Mathias et al., 1998).

The group of Anaf et al., who were the first to report on the close morphological relationship between nerves and endometriotic foci by means of perineurial and endoneurial invasion...
Anaf et al. (2000), recently showed that endometriotic lesions seem to infiltrate the large bowel wall preferentially along the nerves (Anaf et al., 2004). In addition, they observed that, in all large bowel-resected specimens, a peritoneal lesion was always histologically in direct continuum with the underlying deep endometriotic lesion. This finding suggests that the infiltration of bowel wall by endometriosis might be a progressive phenomenon.

To the best of our knowledge, no previous study has systematically evaluated the degree of damage of the ENS and the modifications of the ICC in patients with bowel endometriosis. In the current study, we investigated the gastrointestinal symptoms of women with endometriosis undergoing surgery at our Institution. After the removal of all visible endometriotic lesions of the bowel, patients were prospectively followed-up to record changes in their bowel symptoms. Bowel histology was focused on involvement of ENS and ICC; these findings were correlated to gastrointestinal symptoms.

Materials and methods

Study population

We asked all women with chronic pain (>6 months) of suspected endometriotic origin undergoing surgery at our Institution between October 1999 and September 2003 to take part in this prospective study. The patients were informed that the study involved the surgical removal of all visible endometriotic lesions (including those on the bowel). All patients were informed of the experimental design of this study and on the complications of radical surgery of endometriosis. All patients included in the study provided a written informed consent. The study was approved by the local Institutional Review Board.

Data on age, body mass index (BMI), calculated as weight in kilograms divided by the square of height in meters), full menstrual history, previous pregnancy, and previous surgical therapies were collected.

Evaluation of patients’ bowel symptoms

In order to classify objectively patients’ bowel habits and complaints, among the available classification systems, the Rome II criteria (Thompson et al., 1999) were chosen. We found this classification to be a complete and extensive method of description and comparison of patients’ gastrointestinal symptoms. Furthermore, the timing between menstrual period and bowel complaints was investigated by using this system. These criteria are universally accepted for both research and clinical care of functional bowel disorders. Table I shows the Rome II Diagnostic Criteria for Irritable Bowel Syndrome; the complete system is available at the website: http://www.romecriteria.org.

In the case of obstructive symptoms, double contrast computed tomography (CT) scan was performed. When double contrast CT scan was suggestive for full thickness infiltration of the bowel wall, colonoscopy was performed.

The presence of typical endometriosis-related symptoms, such as dysmenorrhea and dyspareunia, was investigated but these data are not reported in the current paper.

Following surgery, a 2 year duration of follow-up was sought to assess the effects of surgical removal of endometriotic nodules on patients’ bowel symptoms. All patients attending our Institution underwent a clinical interview at 6 months, 1 and 2 years from surgery; in the remaining patients the presence of gastrointestinal symptoms was investigated by telephone interview.

Surgical procedures

During surgery, all visible endometriotic lesions were removed. Bowel lesions were removed by nodulectomy except for: (i) single lesion >3 cm in diameter; (ii) single lesion infiltrating >50% of the bowel wall; (iii) three or more lesions infiltrating the muscular layer; in these cases, intestinal resection was performed. Histological evaluation was performed on all the specimens. The anatomical distribution of these lesions was recorded.

Histological and immunohistochemical evaluation

All surgical specimens were histopathologically evaluated in a standardized fashion. The specimens were immediately fixed in 4% formaldehyde for 12 h and then embedded in paraffin.

In order to evaluate the whole thickness of the lesion, the specimens were prepared as follows. In the case of nodulectomy, the specimens were macroscopically oriented along the intestinal wall (from the serosa towards the mucosa) and cut in macro-sections of 2 mm thickness. From each macrosection, tissue blocks of ~1.5 cm length were obtained in variable number according to the size of the lesion. From each tissue block, a 5 μm section was obtained for microscopic and immunohistochemical evaluation (see below).

In the case of bowel resection, the specimen was opened longitudinally through its entire length. Two-millimetre longitudinal bands of bowel wall, reaching the two resection margins and passing through all macroscopically visible lesions, were cut. These bands were sampled in tissue blocks and 5 μm sections were obtained for microscopic and immunohistochemical evaluation. These sections were stained with haematoxylin and eosin.

The presence of clean resection margins, defined as absence of endometriosis in the specimen (including rings produced by the stapler), was investigated and recorded.

All layers of bowel wall present in the sample were evaluated starting from the subserosa. The three ganglionated plexuses of the bowel (subserous, Auerbach, and Meissner plexus) and the ICC (the pacemaker of bowel peristalsis) were immunohistochemically studied using S100 and e-kit polyclonal antibodies.

The purified immunoglobulin fraction of rabbit polyclonal anti-serum for cow S-100 protein (Dako, Denmark) was used for immunohistochemical detection of ganglionic and nervous cells (ENS). S100 is a calcium-binding protein closely associated with glial cells.

### Table 1. Rome II diagnostic criteria for irritable bowel endometriosis

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom 1</td>
<td>Abnormal stool frequency (for research purposes, ‘abnormal’ may be defined as more than three bowel movements per day and fewer than three bowel movements per week)</td>
</tr>
<tr>
<td>Symptom 2</td>
<td>Abnormal stool form (lumpy/hard or loose/watery stool)</td>
</tr>
<tr>
<td>Symptom 3</td>
<td>Abnormal stool passage (straining, urgency, or feeling of incomplete evacuation)</td>
</tr>
<tr>
<td>Symptom 4</td>
<td>Passage of mucus</td>
</tr>
<tr>
<td>Symptom 5</td>
<td>Bloating or feeling of abdominal distension</td>
</tr>
</tbody>
</table>

At least 12 weeks, which need not be consecutive, in the preceding 12 months of abdominal discomfort or pain that has two of three features: 1. Relieved with defecation; and/or 2. Onset associated with a change in frequency of stool; and/or 3. Onset associated with a change in form (appearance) of stool

Symptoms that cumulatively support the diagnosis of IBS:

1. Abdominal pain or discomfort associated with the passage of stool; and
2. The symptom is present on most days; and
3. The exacerbation is associated with a change in stool frequency or a change in stool form; and
4. The symptom is present for at least 12 weeks; and
5. The symptom is not better explained by another current diagnosis; and
6. The symptom is not better explained by another current diagnosis.
(Stefansson et al., 1982). In addition to Schwann cells, it can be used to visualize neuronal structures within the peripheral nerves, with a distribution similar to that seen by neurofilament protein antibodies (Sato et al., 1989).

A rabbit polyclonal antibody against c-kit (Dako, Japan) was used for selective identification of ICC. The c-kit gene encodes for a receptor for a growth factor termed Stem Cell factor; this receptor contains an internal tyrosine kinase component and is essential for the normal development of ICC and the rhythmic activity in the gastrointestinal tract (Maeda et al., 1992; Huizinga et al., 1995; Kluppel et al., 1998; Liu et al., 1998; Wu et al., 2000). ICC c-kit reactivity was evaluated not only at the level of the endometriotic lesion but also in the macroscopically normal bowel wall.

For immunohistochemistry, the 5-µm sections obtained were placed on glass slides, deparaffinized in xylene, hydrated in graded ethanol and immersed in 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. Slides in citrate buffer (pH 6.5) were then microwaved for two cycles of 5 min at 460°C to enhance antigen detection. The dilutions of the primary antibodies were: 1:200 for S100 and 1:50 for c-kit. Immunohistochemical staining was performed on deparaffinized sections with linked streptavidin–biotin (LSAB) complex technique using an LSAB detection kit (Dako, USA) with diaminobenzidine as the chromogen.

When large specimens of bowel were available, sections of ‘endometriosis free’ wall were used as internal controls (intra-patient control) as well as bowel specimens from other patients undergoing resections for other gastrointestinal indications (inter-patients control).

Based on ENS anatomy, the bowel specimens were histologically classified into four stages: stage 0, the specimen is composed only of peritoneum and subserosal dense connective with endometriotic foci (lesions not reaching the subserous plexus); stage 1, endometriotic foci are placed in the subserous fat tissue or adjacent to the neurovascular branches (subserous plexus), rarely involving the external muscle layer; stage 2, the muscular wall and the Auerbach plexus are deeply infiltrated by endometriotic lesions; stage 3, the infiltration has reached the submucosal (Meissner) nervous plexus or the mucosa.

The section showing the deepest lesions was used to histologically classify the case.

**Statistical analysis**

Student’s t-test and Mann–Whitney U-test were used to compare the baseline characteristics of the patients. The χ²-test was used to compare the presence of an improvement of symptoms at follow-up between different groups of patients. All analyses were performed using the Statistical Packages for the Social Sciences (SPSS, USA). P < 0.05 was considered statistically significant.

**Results**

Out of 504 patients meeting the inclusion criteria for the study, 423 agreed to participate in the study. The presence of endometriosis was histologically confirmed in 362 women. Sixty-eight out of these 362 patients (18.8%, 95% CI 14.9–23.2) were found to have bowel involvement at surgery; none of these patients had previously had surgical excision of bowel endometriosis, although some of them (n = 15, 22.1%) had a history of previous surgery for endometriosis. The mean (± SD) age was similar in women with and without bowel involvement (33.1 ± 5.7 and 33.3 ± 4.3 years respectively). No significant difference was observed in the BMI (mean ± SD) of women with and without bowel involvement (21.1 ± 2.8 and 21.2 ± 2.9 kg/m² respectively; P = 0.757). However, the BMI of women with stage 1, 2 and 3 bowel endometriosis was significantly lower than that of other subjects included in the study (20.4 ± 1.7 and 21.3 ± 2.9 kg/m² respectively; P = 0.011). Fifty-nine (86.7%) women with bowel involvement and 220 (74.8%) women without bowel endometriosis were nulliparous.

All cases of stage 2 and 3 bowel endometriosis were identified at double contrast CT scan; endometriotic lesions appeared as extrinsic lesions compressing and infiltrating bowel wall; focal regions of bowel wall thickening were typically identified and in some cases were associated with a narrowed lumen of the bowel. Double contrast CT scan could not discriminate between stage 2 and stage 3 bowel lesions. Colonoscopy revealed the presence of an extrinsic compression of bowel lumen in two patients.

Forty-eight women had their deepest lesion on the sigmoid, 12 on the rectum and eight on the small bowel. Bowel segmental resection was performed in nine cases. During surgery a single lesion > 3 cm in diameter was found in three patients; a single lesion infiltrating ≥ 50% of bowel wall in four patients, and three or more lesions infiltrating the muscular layer in two patients. Eighty-four nodules were removed in the remaining 59 women. At histology, 24 (35.3%) patients had more than one bowel lesion.

The major complications experienced in the 362 procedures performed are listed in Table II.

**Evaluation of depth of endometriotic invasion**

In the majority of the patients (45/68, 66.2%) endometriotic lesions were confined at the level of the serosal layer and surrounding connective tissue (stage 0). Eleven women had the subserous plexus infiltrated by endometriosis (stage 1). In eight patients the Auerbach plexus was disrupted by endometriotic glands and the muscular wall was deeply infiltrated (stage 2); hypertrophic or degenerative changes of the ganglionic component of the plexus were occasionally (n = 3) seen at the site of the endometriotic lesions. No correlation was observed at histology between nerve disruption and the degree of reactive and inflammatory changes. In four cases the infiltration reached the submucosal Meissner plexus or the mucosa itself (stage 3); these lesions were characterized by large endometriotic foci with massive tissue reaction, haemorrhage, and inflammatory reaction involving the whole thickness of the wall (Figure 1).
Figure 1. The purified immunoglobulin fraction of rabbit antiserum for cow S-100 protein was used for immunohistochemical detection of ganglionic and nervous cells. (A) Meissner plexus disarranged and interrupted by an endometriotic focus located in the submucosa (×10). (B) Auerbach plexus interrupted by an endometriotic focus (×25). (C) Auerbach plexus far from endometriotic foci has a hypertrophic reaction associated with degenerative changes (×25). (D) Auerbach plexus far from endometriotic foci with severe degenerative changes and fibres vanishing.

Figure 2. Antibody against c-kit was used for selective identification of interstitial cells of Cajal. (A) Normal enteric muscular wall, Auerbach plexus and Cajal cells are shown by c-kit immunostaining (×10). (B) Normal enteric muscular wall, Cajal cells are far from the Auerbach plexus (×25). (C) Rare Cajal cells are shown near an endometriotic focus deeply located in the enteric muscular layer (×25). (D) Unstained Auerbach plexus and rare Cajal cells in the muscular wall far from endometriotic foci (×25).
In all specimens with endometriotic infiltration of bowel wall, the deep lesions were in direct continuity with serosal lesions.

**Interstitial Cajal cells**

Out of 11 patients with stage 1 bowel endometriosis, seven (63.6%) had c-kit unreactive ICC; all these patients had the extramural plexus and nerves interrupted by the endometriotic lesions. In all the patients with stage 2 (n = 8) and stage 3 (n = 4) bowel endometriosis, ICC were c-kit unreactive.

The extension of c-kit unreactivity (i.e. limit between c-kit unreactivity and c-kit reactivity) around the endometriotic nodule was measured whenever the surgical specimen and the anatomical distribution (n = 18) of the lesions allowed this detection. In the case of single endometriotic lesions, c-kit unreactivity was found to spread up to 5 cm from the nodule. In case of multiple lesions, an overlap between different areas of c-kit unreactivity was observed; this phenomenon caused a large segment of bowel to be involved (‘bridging pattern’) (Figure 2).

**Patients’ symptoms before surgery and at follow-up**

The prevalence of bowel symptoms was similar in women with endometriotic lesions confined at the level of the serosal layer and surrounding connective tissue (stage 0) and in patients without bowel endometriosis. All women with stage 1, 2 and 3 bowel endometriosis reported bowel complaints (Table III). Patients with stage 2, but not those with stage 1 and 3, reported a worsening of their symptoms during menstruation. The four stage 3 patients reported persistent nausea (n = 3), vomiting (n = 2) and subocclusive crisis (n = 1).

Table IV shows the follow-up after surgery. So far we have obtained a follow-up from 97.0% (130/134) patients

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### Table III. Patients’ symptoms according to the presence of bowel endometriosis

<table>
<thead>
<tr>
<th>Gastrointestinal symptoms</th>
<th>Women with bowel endometriosis</th>
<th>Women with bowel endometriosis</th>
<th>Women without bowel endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage 0 (n = 45)</td>
<td>Stage 1, 2, 3 (n = 23)</td>
<td>(n = 294)</td>
</tr>
<tr>
<td>Irritable bowel syndrome predominantly diarrhoea</td>
<td>2 (4.4)</td>
<td>4 (17.4)</td>
<td>16 (5.4)</td>
</tr>
<tr>
<td>Irritable bowel syndrome predominantly constipation</td>
<td>1 (2.2)</td>
<td>3 (13.0)</td>
<td>9 (3.1)</td>
</tr>
<tr>
<td>Functional abdominal bloating</td>
<td>2 (4.4)</td>
<td>2 (8.7)</td>
<td>7 (2.4)</td>
</tr>
<tr>
<td>Functional constipation</td>
<td>2 (4.4)</td>
<td>3 (13.0)</td>
<td>22 (7.5)</td>
</tr>
<tr>
<td>Functional diarrhoea</td>
<td>2 (4.4)</td>
<td>4 (17.4)</td>
<td>9 (3.1)</td>
</tr>
<tr>
<td>Unspecified functional bowel symptoms</td>
<td>6 (13.3)</td>
<td>2 (8.7)</td>
<td>3 (1.0)</td>
</tr>
<tr>
<td>Functional abdominal pain syndrome</td>
<td></td>
<td>4 (17.4)</td>
<td>21 (7.1)</td>
</tr>
<tr>
<td>Unspecified functional abdominal pain</td>
<td>1 (2.2)</td>
<td>1 (4.3)</td>
<td>8 (2.7)</td>
</tr>
</tbody>
</table>

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### Table IV. Follow-up data for patients with bowel symptoms

<table>
<thead>
<tr>
<th>Patients at follow-up (n)</th>
<th>Symptoms [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free of symptoms</td>
</tr>
<tr>
<td>Women with stage 0 bowel endometriosis</td>
<td>6 months (n = 16)</td>
</tr>
<tr>
<td></td>
<td>1 year (n = 12)</td>
</tr>
<tr>
<td></td>
<td>2 years (n = 5)</td>
</tr>
<tr>
<td>Women with stage 1 bowel endometriosis</td>
<td>6 months (n = 11)</td>
</tr>
<tr>
<td></td>
<td>1 year (n = 8)</td>
</tr>
<tr>
<td></td>
<td>2 years (n = 3)</td>
</tr>
<tr>
<td>Women with stage 2 bowel endometriosis</td>
<td>6 months (n = 8)</td>
</tr>
<tr>
<td></td>
<td>1 year (n = 5)</td>
</tr>
<tr>
<td></td>
<td>2 years (n = 2)</td>
</tr>
<tr>
<td>Women with stage 3 bowel endometriosis</td>
<td>6 months (n = 4)</td>
</tr>
<tr>
<td></td>
<td>1 year (n = 3)</td>
</tr>
<tr>
<td></td>
<td>2 years (n = 1)</td>
</tr>
<tr>
<td>Women without bowel endometriosis</td>
<td>6 months (n = 91)</td>
</tr>
<tr>
<td></td>
<td>1 year (n = 59)</td>
</tr>
<tr>
<td></td>
<td>2 years (n = 27)</td>
</tr>
</tbody>
</table>

*Or return to baseline symptoms after an initial improvement.
with bowel symptoms at 6 months after surgery, from 87 patients at 1 year, and from 38 patients at 2 years.

Of patients with stage 1, 2 and 3 bowel endometriosis, 87.5% were completely free of symptoms or had a considerable improvement of symptoms at 1 year from surgery; this percentage is significantly higher than those observed in women with stage 0 bowel endometriosis and in subjects without bowel lesions (25.0 and 16.9% respectively; $P < 0.001$).

The six patients with stage 1, 2 and 3 bowel endometriosis who reached the 2 year control were still having benefit from surgery.

Discussion
To the best of our knowledge, this is the first report on ICC involvement in patients with bowel endometriosis. Antibodies to c-Kit provided pathologists with the opportunity to identify ICC and understanding their anatomical distribution in the gastrointestinal muscles. Many studies have revealed that ICC are reduced in number or lost in a variety of cases with motor dysfunction, including pseudo-obstruction, achalasia, ulcerative colitis, infantile pyloric stenosis, Chagas’ disease, diabetes, slow-transit constipation, and idiopathic gastric perforation (Sanders et al., 1999; Vanderwinden and Rumessen, 1999). For the first time, we observed, around the endometriotic bowel lesions, the disappearance of c-kit reactivity, which is universally considered a marker of ICC function. Interestingly, ICC were functionally damaged whenever subserous plexus and nerves were interrupted by the endometriotic lesions. The damage of ICC was observed even at distance from the involved area, and, most importantly, even in the absence of muscular alteration. It has been shown that ICC do not die when c-kit signalling is blocked, rather they redifferentiate into a smooth muscle-like phenotype. Importantly, it is now recognized that loss or damage to the ICC can cause serious motor dysfunction (Sanders et al., 2002).

Since many aspects of ICC pathophysiology remain unclear, we cannot provide a final explanation on the finding of extended ICC unreactivity around the endometriotic lesion. It can be hypothesized that ICC damage is caused by a loss of neurotropism secondary to the ENS damage. In line with this hypothesis, it has been shown in a mouse model that neurons induce c-Kit-positive precursor cells to become ICC (Torihashi et al., 1999). Thus, it is possible to postulate that any damage to the ENS might translate into ICC alterations. This hypothesis is consistent with our finding that ICC unreactivity was present only when ENS was damaged. Alternatively, inflammatory bioactive substances produced by the endometriotic lesion may damage ICC. In a canine model, it has been proven that inflammation can induce reduction in ICC density and damage in ICC function (Lu et al., 1997). As endometriotic infiltration of bowel wall is associated with an inflammatory reaction, ICC unreactivity may be caused by locally released inflammatory molecules. It is also possible that these two hypotheses mutually contribute to the establishment of the observed findings. Finally, we cannot exclude that ICC are directly invaded by endometriotic stromal cells; this hypothesis could be immunohistochemically tested by using CD10 antibodies, which represent a sensitive marker of endometrial stroma both at eutopic and ectopic sites (McCluggage et al., 2001; Sumathi and McCluggage, 2002; Toki et al., 2002). To further complicate the scenario, it is still unclear whether unreactive ICC can recover c-kit reactivity and thus their function.

We believe that the finding of unreactive ICC cells even in the absence of muscular infiltration by endometriosis (stage 1) is of paramount importance in the understanding of patients’ complaints. In fact, in the most advanced stages, there is always a certain degree of muscular damage, fibrosis and inflammatory reaction which may contribute to the symptoms reported by the patients pre-operatively. Therefore, in these cases, it is difficult to discriminate the relative contribution of nerves and muscles to the origin of bowel alterations. In stage 1 of our classification, endometriotic lesions do not infiltrate the muscles; we believe that the complaints reported by these patients are a consequence of the altered peristalsis secondary to ICC unreactivity. Clinical follow-up seems to confirm this hypothesis, as this group of patients had persistent improvement of symptoms after surgery. This observation is consistent with the fact that reports of women having been treated for irritable bowel syndrome for a long time before a final diagnosis of endometriosis was made have been recently published (Lea and Whorwell, 2003).

For the first time we also observed a correlation between patients’ symptoms, menstrual cycle and histological data. A triple pattern of gastrointestinal symptoms and histological findings may be suggested: (i) alterations in bowel transit, not worsened at menstrual cycle (associated with a damage of subserous plexus along with ICC unreactivity); (ii) painful bowel symptoms worsened during the menstrual cycle (associated with a damage of the Auerbach plexus); (iii) continuous severe painful bowel symptoms up to occlusions, the severity of the symptoms being unrelated to the menstrual cycle (associated with a damage of the Meissner plexus).

We are aware that the limited number of patients included in our study (only 23 women with ‘true’ bowel endometriosis) does not allow us to propose this triple pattern as a clinical entity. However, we believe that it may represent an interesting working hypothesis for further investigations.

The limited number of patients included in the current study and the study design (absence of control group, lack of randomization, and limited follow-up) do not allow us to draw definitive conclusions on the clinical management of patients with bowel endometriosis. However, the evident improvement recorded at follow-up in symptoms of patients with stage 1, 2 and 3 bowel endometriosis supports the pathogenic role of the bowel endometriotic lesions upon gastrointestinal complaints. Further studies should evaluate whether medical or surgical treatment should be offered to these patients.

In our series, all deep endometriotic lesions were always in direct continuity with the superficial lesions. This finding is in line with the previous observation of Anaf et al. (2004) that the infiltrating large bowel endometriotic lesions...
originate from the progression or invasion of lesions primarily located on the serosa of the large bowel.

We agree with the observation of Chapron et al. (2003) that lesions limited to the serosa should not be considered true bowel endometriosis. This belief is supported by the fact that symptoms before surgery and at follow-up were similar in subjects with stage 0 lesions and in those without endometriotic lesions of the bowel but significantly different from those of women with stage 1, 2 and 3 bowel endometriosis.

Another limit of our study is in the use of Rome II criteria (originally designed for functional bowel disorders) in both patients with and without organic bowel pathology. However, we decided to use this system as it provides a standardized framework to categorize gastrointestinal disorders and allows comparisons between groups of patients; this approach gave us the opportunity to correlate patients’ symptoms to histological findings.

As we have no proof of radical extirpation of endometriosis when laparoscopic nodulectomy was performed, the possible influence of a residual disease on bowel function cannot be determined. However, as all the patients with stage 1, 2 and 3 bowel endometriosis reported improvement of bowel symptoms at 1 year (n = 16) and 2 year (n = 6) follow-up, these results seem to suggest that, at least within the first year, nodulectomy can be considered an effective treatment for bowel endometriosis.

Finally, a placebo effect of surgery on bowel symptoms cannot be excluded. In fact, improvements of bowel habits were temporarily reported both by patients with and without bowel endometriosis. However, at 1 year follow-up, the improvement of symptoms was still present in all patients with stage 1, 2 and 3 bowel endometriosis, while it was present only in 33.3% of women with stage 0 bowel endometriosis and in 27.1% of subjects without bowel endometriosis.

Considering that women with endometriosis, besides pain, might suffer from infertility and that they do not usually have a life-threatening condition, the clinician must always weigh-up the risk of potential complications of surgery against the benefit of potential improvements in bowel function. However, at 1 year follow-up, the improvement of symptoms was still present in all patients with stage 1, 2 and 3 bowel endometriosis, while it was present only in 33.3% of women with stage 0 bowel endometriosis and in 27.1% of subjects without bowel endometriosis.

In conclusion, our data support the role of ICC in the pathophysiology of bowel endometriosis. Further studies are needed to fully evaluate the clinical implications of this finding.

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