Relationship between endometriotic foci and nerves in rectovaginal endometriotic nodules

V.Anaf1,4, Ph.Simon1, LEi Nakadi3, LFayt2, F.Buxant1, Th.Simonart2, M.-O.Peny2 and J.-C.Noel1

Departments of 1Gynaecology, 2Pathology and 3Digestive Surgery, Hospital Erasme, Université Libre de Bruxelles (ULB), Brussels, Belgium
4To whom correspondence should be addressed at: Department of Gynaecology, Hospital Erasme, 808 Route de Lennik, 1070 Brussels, Belgium. E-mail: vincent.anaf@skynet.be

The histological relationships between fibrotic tissue, endometriotic foci and nerves in the rectovaginal septum endometriotic or adenomyotic nodule were studied. This is considered to be one of the most severe forms of deep endometriosis. Masson’s trichrome staining for fibrosis detection and immunohistochemistry with the S100 monoclonal antibody for nerve detection were performed in 28 rectovaginal endometriotic nodules from patients presenting with severe dysmenorrhoea and deep dyspareunia (23 patients with no other endometriotic location or potential cause of pain at laparoscopy and ultrasonography; five patients with multiple pelvic endometriotic localizations and other potential causes of pain at laparoscopy). Patients were allocated to two groups on the basis of their preoperative pain scores for pelvic pain, dysmenorrhoea and deep dyspareunia (group 1, score ≥7; group 2, score ≤7). For each symptom, the mean number of nerves and endometriotic lesions per high-power field and the mean largest diameter of the lesions were not statistically different in groups 1 and 2. The mean percentages of nerves located within the fibrosis of the nodule and within endometriotic lesions were significantly higher in group 1 than in group 2. Among nerves located within endometriotic lesions, there was a significantly higher proportion showing intraneurial and perineural invasion by endometriosis in group 1 than in group 2. In rectovaginal endometriotic nodules, there was a close histological relationship between nerves and endometriotic foci, and between nerves and the fibrotic component of the nodule. We postulate that such topographical relationships could at least partially explain the strong association between this lesion and pain.

Key words: adenomyosis/hypogastric nerve plexus/pelvic pain/perineural invasion/rectovaginal endometriosis

Introduction

Endometriosis is defined as the presence of endometrial-like glands and stroma surrounded by a cytenogenous stroma outside the uterus. It is a frequent occurrence in women, and may be responsible for infertility. Deep infiltrating endometriosis is strongly associated with pelvic pain, severe dysmenorrhoea and deep dyspareunia. Although pain is a very important aspect of deep endometriosis, very little is known concerning the mechanisms by which this entity causes pain, and in particular the relationship between the lesion and subperitoneal nerves.

The rectovaginal septum and posterior cul-de-sac locations represent the most common pelvic locations of endometriosis after the pelvic peritoneum and ovaries. Deep infiltrating endometriosis—defined as the presence of endometriotic glands and stroma >5 mm under the peritoneum—represents a distinct entity of the disease and, as stated above, is strongly associated with pelvic pain (Cornillie et al., 1990). Rectovaginal endometriotic nodules represent a certain form of deeply infiltrating endometriosis. Three types of deep infiltrating endometriosis have been defined: the infiltrating form (type 1); the retractile form (type 2); and the rectovaginal septum nodule (type 3) (Koninckx and Martin, 1992).

According to this classification, rectovaginal endometriotic nodules—as investigated in the present study—represent the deepest and most severe lesions. They are spherical in shape, located deep in the rectovaginal septum, and are often only visible as small typical peritoneal lesions at laparoscopy. According to other authors, this type of lesion should not be considered as endometriosis, but rather represents adenomyosis or an adenomyoma, a circumscribed nodule composed of endometrial-like glands, scanty stroma and abundant smooth muscle (Donnez et al., 1995). These lesions are often more palpable than visible. In gynaecological terms, there may exist dysmenorrhoea which appears progressively, dyspareunia, menometrorrhagia and lower pelvic pains that may radiate posteriorly to the sacrococcygial region. These symptoms are initially cyclical, but tend to become continuous as the lesion increases. Clinical examination reveals a nodule in the posterior fornix or in the pouch of Douglas, which can become completely filled in certain cases, creating the sensation of a tumour. In the digestive tract, a rectal syndrome may occur completely filled in certain cases, creating the sensation of a tumour. In the digestive tract, a rectal syndrome may occur.
Nevertheless, the exact mechanism by which endometriotic nodules produce chronic pelvic pain and an exacerbation of the pain at palpation remains unclear. In order to clarify this issue, we have analysed the relationship between nerve invasion by endometriotic foci, its concomitant fibrosis, and the intensity of pelvic pain.

Materials and methods

Patients
Between June 1996 and January 1998, 29 consecutive non-menopausal Caucasian French-speaking patients (mean age 30 years; range 20–41 years) underwent laparoscopic resection of a rectovaginal endometriotic nodule. The indication for surgery was pain in all 29 patients, and pain associated with infertility in 18 patients. All patients were heterosexual, and none had been treated for any psychiatric disorder or had been sexually abused. No patients took psychotropic drugs. Twenty-four patients (83%) were married, and five (17%) had a regular sexual partner. All patients presented with severe dysmenorrhea and deep dyspareunia of more than 2 years duration; 18 patients [gravity 0, parity 0 (n = 13); gravity 1, parity 0 (n = 3); and gravity 1, parity 1 (n = 2)] were infertile (62%). Among the other 11 patients, six (38%) were gravity 1, parity 1, four were gravity 2, parity 1, and one was gravity 2, parity 2. In all patients, a physical examination revealed the presence of a bulky induration in the posterior fornix which appeared as a dark blue nodule at speculum. The lesion was exquisitely painful at palpation in all 29 patients, among whom 24 (83%) presented with only a rectovaginal endometriotic nodule as the unique cause of pain at physical examination and laparoscopy, and no endovaginal ultrasonographical suspicion of uterine adenomyosis. The other five patients (17%) presented with other potential causes of pain at laparoscopy: peritoneal endometriosis (n = 5), pelvic adhesions (n = 5), ovarian endometriomas (n = 4), hydrosalpinx (n = 2), uterine myomata (n = 1) and uterine adenomyosis confirmed at histology (n = 2). In those five patients, concomitant laparoscopic surgery included adhesiolysis (n = 5), CO2 laser peritoneal endometriosis vaporization (n = 5), ovarian endometriomeotomy (n = 4), salpingectomy (n = 1), salpingonec-tomy (n = 1) and laparoscopically assisted vaginal hysterectomy (n = 3).

According to the revised classification of endometriosis of the American Fertility Society (AFS), and not including one patient who was lost to follow-up (see below), 10 patients had endometriosis stage 1, 13 had stage 3 and five had stage 4. With regard to the revised AFS scores, 10 patients had a score of <15, and 18 patients had a score >15.

The patients with stage 1 endometriosis were those who presented a partial posterior cul-de-sac obliteration, while those with stage 3 and stage 4 had a complete posterior cul-de-sac obliteration.

All 29 operated patients had stopped taking any hormonal medication for at least 3 months before surgery.

In order to rule out involvement of the digestive mucosa and/or a stricture of the rectum, all patients underwent a rectosigmoidoscopy and a barium enema. In 17 patients (59%), the barium enema showed the presence of a retractile perivisceritis on the anterior rectal wall. None of the 29 patients presented either rectum calibre reduction at barium enema, nor red or dark-blue mucosal or submucosal areas at rectosigmoidoscopy. Therefore, neither discoid anterior rectal wall resection nor anterior rectal resection with end-to-end or lateroterminal anastomosis were performed. It must be stressed that rectal endoscopic ultrasonography (EUS) was not performed preoperatively in these patients. The use of preoperative rectal EUS was recently proposed (Chapron et al., 1998) in order to select patients with deep digestive wall endometriotic infiltration. Those patients are probably better candidates for bowel resection than conservative digestive surgery (Chapron et al., 1998). Therefore, we cannot totally exclude that some of our patients had deep endometriotic infiltration of the bowel muscularis or submucosa.

All patients provided their informed consent for this study.

Pain evaluation
In total, 28 patients (97%) answered mailed questionnaires, one in the preoperative and one in the postoperative phase after a mean (± SD) follow-up of 21.0 ± 2.3 months. One patient had moved abroad and was lost to follow-up.

The designer and corresponding author (F.B.) of the questionnaires was unknown to the patients, and had not participated at either the pre- or postoperative consultation, or at the operations.

The questionnaires were designed to obtain information about the intensity of pre- and postoperative pain, using visual analogue pain scales for pelvic pain, dysmenorrhea and deep dyspareunia. The pain scales were ungraded lines of 10 cm length. ‘No pain’ was indicated at the left side of the scale, and ‘the maximal pain you could imagine’ at the right side of the scale.

Comparison of pre- and postoperative pain scores was made using the non-parametric Wilcoxon signed-rank test. Patients were allocated arbitrarily to two groups according to the intensity of their preoperative pain (group 1, pain score >7; group 2, pain score ≤7).

Preoperative data
Five patients (17%) had undergone diagnostic laparoscopy for pelvic pain during the past 5 years. A review of the charts and operative protocols mentioned the presence of a pouch of Douglas obliteration in four cases, though the underlying lesion had not been removed. Twelve patients (41%) had previously received different regimens of hormonal therapies composed of gonadotrophin-releasing hormone (GnRH) agonists, danazol or progestagens, and of those patients, seven (58%) were improved under medical therapy, although symptoms recurred rapidly after treatment interruption. Before undergoing laparoscopic resection of the rectovaginal nodule, the mean duration of symptoms was 40.4 (range 30–52) months for chronic pelvic pain, 42 (range 36–59) months for cyclical dysmenorrhea, 36 (range 24–48) months for deep dyspareunia, and 28 (range 21–42) months for cyclical rectal pain. Neither GnRH agonists, danazol nor progestagens were prescribed after the intervention, and such medications had been stopped for at least 3 months before surgery.

Surgical technique for the resection of the rectovaginal endometriotic nodule
All 29 patients underwent the same surgical procedure for excision of the rectovaginal endometriotic nodule, performed by the same two surgeons (V.A. and Ph.S.). The surgical technique used was described previously (Donnez et al., 1995). In brief, the patients were placed in the dorsal decubitus position, and three suprapubic trocars (one in the midline and two laterally) each of 5 mm diameter were inserted. The CO2 laser was connected to the laparoscope. A cannula was inserted in the uterus, a sponge on a forceps applied against the nodule, and a probe (Hegar 18) placed in the rectum. When the nodule was clearly identified by palpation, the plane between the posterior aspect of the nodule and the anterior rectal wall was dissected with help from the CO2 laser until the healthy vagina was reached under the inferior edge of the lesion. The vagina was then incised with the CO2 laser, and the nodule circumscribed and removed transvaginally. No systematic laser uterine nerve ablation (LUNA) procedure was performed, but any residual induction in the

Subperitoneal nerves and rectovaginal septum endometriotic nodules

Chapron et al., 1998) in order to select patients with deep digestive wall endometriotic infiltration. Those patients are probably better candidates for bowel resection than conservative digestive surgery (Chapron et al., 1998). Therefore, we cannot totally exclude that some of our patients had deep endometriotic infiltration of the bowel muscularis or submucosa.
surrounding tissues was removed transvaginally. The colpotomy was then closed sagitally using a Catgut 1 (Ethicon, Neulilly, France) running suture. The patients were discharged from hospital on day 2 or 3 postoperatively, and advised to avoid sexual intercourse during the subsequent 3-week period. All patients had their first sexual intercourse within 2 months postoperatively.

**Histological and immunohistological study**

After CO2 laser laparoscopic excision, the greatest diameter of each excised specimen was measured in groups 1 and 2, using a graduated scale. The rectovaginal nodules were fixed immediately in 10% phosphate-buffered formaldehyde for 12 h and then embedded in paraffin. For each specimen, four serial sections each of 4 µm were prepared. The first and second sections were stained with haematoxylin and Masson’s trichrome respectively, as described previously (Masson, 1929). Masson’s trichrome staining is a specific staining for the detection of collagen fibres. The third section was used as a negative control. The final section was coated on silanated glass for S100 immunohistochemistry. Briefly, the sections were deparaffinized and the endogenous peroxidase blocked with 0.6% hydrogen peroxide–methanol for 30 min. Tissues were then incubated overnight at 4 °C with the primary antibody S100 (clone 15E2E2; dilution 1/100; Biogenex, San Ramon, CA, USA). The Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) was used for the subsequent steps according to the manufacturer’s instructions. Chromogenic development was accomplished using diaminobenzidine–hydrogen peroxide. Slides were then slightly counterstained with haematoxylin, dehydrated and coverslips applied.

S100 protein is a highly sensitive marker for myelinated nerves which are normally present in the rectovaginal septum. S100 monoclonal antibody is directed against an acidic, dimeric calcium-binding protein (mol. wt 21 000 Da) composed of different combinations of α and β subunits, and is present in the nucleus and cytoplasm of Schwann cells. S100 protein is structurally similar in terms of its calcium-binding domains to calmodulin, an important transducer of calcium-mediated signals.

To control for non-specific binding of the primary antibody, non-immune mouse serum at the same concentration as the primary antibody preparation was substituted as the first layer of the serial sections for S100 immunohistochemistry.

Positive controls for S100 staining were large biopsies of the upper third of the unaffected rectovaginal septum in non-menopausal patients (n = 6) [mean age 32 (range 24–39) years] with (n = 3) and without (n = 3) laparoscopically proven endometriosis. A nerve was considered as ‘encapsulated’ in fibrotic tissue when it was totally surrounded by Masson’s trichrome-positive tissue. The number of nerve structures encapsulated in fibrotic tissue was determined in groups 1 and 2 by calculating the mean number of S100-positive nerve structures located within Masson’s trichrome-positive areas in at least 10 randomly selected non-overlapping (×40) high-power fields (hpf). Similarly, in each group the total number of S100-positive nerve structures was calculated, and the respective percentages of nerves situated within endometriotic glands and/or stroma. Results were expressed as the mean (± SD) number of nerves per ×40 hpf and mean (± SD) percentages of nerves located in fibrosis and within endometriotic lesions. In each studied field of lesions from groups 1 and 2, each nerve structure was then examined histologically at ×400 in order to detect the presence of perineurial or endoneurial invasion by stromal and/or glandular endometriotic cells.

Perineurial invasion was defined as the presence of stromal cells that have penetrated the perineurium which is composed of concentric flattened cells separated by layers of collagen at the periphery of the myelinated neural cells (Sternberg, 1992). Endoneurial or intrafascicu-

<table>
<thead>
<tr>
<th></th>
<th>Before surgery</th>
<th>After surgery</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic pain</td>
<td>5 (5–10)</td>
<td>1 (0–5)</td>
<td>&lt; 0.001a</td>
</tr>
<tr>
<td>Dysmenorrhoea</td>
<td>9 (5–10)</td>
<td>1 (0–5)</td>
<td>&lt; 0.0001a</td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>7 (5–10)</td>
<td>1 (0–5)</td>
<td>&lt; 0.0001a</td>
</tr>
</tbody>
</table>

Values are median (range).

*Pre- and postoperative scores were compared using the non-parametric Wilcoxon signed-rank test.

**Results**

There was a significant difference between pre- and postoperative pain scores (after a mean follow-up of 21.0 ± 2.3 months) for the three symptoms (Table I). There was no statistically significant difference between the mean largest diameter of the nodules in groups 1 and 2 for the three symptoms studied (Table II). S100 immunohistochemistry performed on the clinically unaffected rectovaginal septum biopsies of patients with or without pelvic endometriosis showed the presence of numerous nerve structures in the loose areolar tissue located between the posterior vaginal wall and the subperitoneal anterior rectal wall muscularis. Masson’s trichrome staining was performed in the same biopsies and was negative. None of the clinically unaffected cases showed either endometriotic foci or fibrosis in the rectovaginal septum.

There was no significant difference between the mean number of nerve structures (per ×40 hpf) in groups 1 and 2 for the different symptoms, or between the mean number of endometriotic lesions in groups 1 and 2 for the different symptoms (Table II). The mean (± SD) percentages of nerves located within the fibrosis and within the endometriotic lesions, i.e. intraglandular (Figures 1 and 2) were significantly higher in group 1 than in group 2. Among the nerve structures located within endometriotic lesions, the proportions of nerves showing perineurial and endoneurial invasion by endometriotic glands or stroma were higher in group 1 than in group 2 for the three different symptoms (Figures 2 and 3; Table II). The five patients who had more than one endometriotic localization had a preoperative pain score >7 (group 1) for each of the three symptoms. In those patients, the mean number of nerves and endometriotic lesions was not significantly different from that of the other patients of group 1 for each symptom. In addition, the mean percentages of nerves located in fibrosis, and the proportion of intra-endometriotic nerves showing

---

**Table I. Pre- and postoperative pain scores for 28 patients**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Before surgery</th>
<th>After surgery</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic pain</td>
<td>5 (5–10)</td>
<td>1 (0–5)</td>
<td>&lt; 0.001a</td>
</tr>
<tr>
<td>Dysmenorrhoea</td>
<td>9 (5–10)</td>
<td>1 (0–5)</td>
<td>&lt; 0.0001a</td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>7 (5–10)</td>
<td>1 (0–5)</td>
<td>&lt; 0.0001a</td>
</tr>
</tbody>
</table>

Values are median (range).

*Pre- and postoperative scores were compared using the non-parametric Wilcoxon signed-rank test.
Table II. Histological and immunohistological results for 28 patients according to the preoperative pain scores

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Pelvic pain</th>
<th>Dysmenorrhoea</th>
<th>Deep dyspareunia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1 (n = 18)</td>
<td>Group 2 (n = 10)</td>
<td>Group 1 (n = 19)</td>
</tr>
<tr>
<td>Lesion diameter (mm)</td>
<td>23.0 ± 2.0</td>
<td>22.8 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.6 ± 1.8</td>
</tr>
<tr>
<td>No. of nerves per ×40 hpf</td>
<td>18.1 ± 2.0</td>
<td>17.6 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.2 ± 1.2</td>
</tr>
<tr>
<td>No. of lesions per ×40 hpf</td>
<td>7.6 ± 2.1</td>
<td>7.2 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5 ± 1.0</td>
</tr>
<tr>
<td>Intrafibrinous nerves (%)</td>
<td>41.2 ± 6.0</td>
<td>24.0 ± 4.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.5 ± 6.5</td>
</tr>
<tr>
<td>Intraglandular nerves (%)</td>
<td>39.2 ± 7.0</td>
<td>24.3 ± 5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.6 ± 6.9</td>
</tr>
<tr>
<td>Perineurial invasion (%)</td>
<td>29.0 ± 5.7</td>
<td>13.8 ± 7.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.0 ± 6.0</td>
</tr>
<tr>
<td>(% of intra-endometriotic nerves)</td>
<td>35.8 ± 7.8</td>
<td>12.3 ± 6.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.0 ± 8.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
Comparison of the data was performed using Student’s t-test (two-tailed). *Not significant; <sup>b</sup>P < 0.001; <sup>c</sup>P < 0.01.
Group 1: pain score >7; group 2: pain score =7.

hpf = high-power field.

Figure 1. Perineurial invasion by endometriotic stroma in rectovaginal septum endometriotic nodule section. The nerve appears grey in the centre of the photograph. (Haematoxylin and eosin staining; scale bar = 100 μm.)

Figure 2. Nerve located within the endometriotic stroma. The nerve is stained dark-brown. (S100 immunohistochemistry; Mayer’s haematoxylin counterstaining; scale bar = 100 μm.)

Figure 3. Intraneurial invasion by endometriotic stroma. The nerve fascicle is dissected by endometriotic stromal cells. (S100 immunohistochemistry; Mayer’s haematoxylin counterstaining; scale bar = 200 μm.)

intraneurial invasion were not statistically significantly different from those of the other patients in group 1 for the three symptoms. Nevertheless, the proportion of intra-endometriotic nerves showing perineurial invasion was significantly higher in those patients with multiple endometriotic lesions for pelvic pain, dysmenorrhoae and deep dyspareunia (P < 0.05).

When patients were classified into three groups according to the revised AFS endometriosis stage (Table III), no statistically significant inter-group difference was found for the mean number of nerves and endometriotic glands per hpf. A statistically significant difference was found for the other histological results when stage 1 patients were compared with stage 3 and 4 patients. These significant differences were not found between patients with a complete cul-de-sac obliteration (stage 3 and 4), with the exception of the percentages of perineurial invasion (P < 0.02).

Similar differences were found when the results were compared according to revised AFS endometriosis scores (Table IV).
suggested that both fibrotic reaction and functional glands were required for pain mediation (Sturgis and Call, 1954). In the particular case of rectovaginal septum endometriotic nodule, the fixed position of the posterior pelvic structures (upper vagina, cervix and rectum) could also represent a cause of pain occurring during intercourse or at defaecation. All 29 patients presented with an important exacerbation of pain when pressure was exerted on the nodule at physical examination. This lesion appeared to be a ‘pain-triggering zone’ at vaginal palpation, suggesting a close relationship between the endometriotic lesion and nerve structures. However, the relationship between nerves and rectovaginal endometriotic nodules has, until now, not been studied.

S100 immunohistochemical staining performed on the clinically unaffected rectovaginal septum and the rectovaginal endometriotic nodules showed the presence of numerous nerve structures. Pain impulses from that region, the uterus and cervix are transmitted through afferent sympathetic nerve fibres that course through the uterosacral ligaments and the posterolateral pelvis, and come together in the midline as the superior hypogastric plexus, en route to the dorsal horn of the spinal cord (Frankenhauser, 1864). Painful stimuli are then processed, modulated, and centrally transmitted to higher levels.

In both groups 1 and 2, a major proportion of the nerves were totally surrounded by fibrosis (Table II). Fibrous tissue, which is absent in the unaffected rectovaginal septum, possibly results from an inflammatory process that follows cyclical bleedings in the rectovaginal endometriotic nodule. It is of course difficult to demonstrate that the fibrosis surrounding the nerves exerts pressure on the nerve fascicles rather than being apposed to them. However, the rigid aspect of the lesion at palpation and the retractile perivisceritis that was often visible at barium enema strongly suggested that a ‘compression phenomenon’ may occur in this type of lesion. Neurophysiological and electron microscopy studies have demonstrated that induced perineural fibrosis results in morphological changes as well as functional nerve changes (compound action potentials and motor nerve conduction velocity decrease, fast axonal transport decrease). Such nerve morphological and functional changes are generally present in ‘compression neuropathy’ and associated with hyperaesthesia (Ando, 1990; Sommer et al., 1995; Bai et al., 1999). There are a number of examples of pain caused by the compression of peripheral somatic (disca hernia) or autonomic nerves (chronic pancreatitis), and the frequent entrapment of nerve structures within the fibrotic tissue of the nodule may represent one of the aetiopathogenic mechanisms of pain.

Carcinomas arising in various organs exhibit a capacity, and in some instances a proclivity, to invade around (perineural invasion) and into (endoneurial or intrafascicular invasion) nerves (Sternberg, 1992). This phenomenon is frequently observed in pancreatic carcinomas, but less frequently in invasive mammary carcinomas, where it is estimated at about 10% of invasive duct carcinomas (Rosen, 1998). Adenoid cystic carcinomas of the Bartholin’s gland are also characterized by the frequent presence of perineural and endoneurial invasion, and this may explain why such lesions are often painful before

### Table III. Histological and immunohistological results for 28 patients according to revised AFS endometriosis stage

<table>
<thead>
<tr>
<th>Stage</th>
<th>(n = 10)</th>
<th>Stage 3</th>
<th>(n = 13)</th>
<th>Stage 4</th>
<th>(n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of nerves per ×40 hpF</td>
<td>18.2 ± 0.9a</td>
<td>17.5 ± 1.0a</td>
<td>18.0 ± 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of lesions per ×40 hpF</td>
<td>7.6 ± 0.9a</td>
<td>7.5 ± 1.0a</td>
<td>7.6 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrafibrinous nerves (%)</td>
<td>25.7 ± 6.8ab</td>
<td>41.1 ± 6.2a</td>
<td>37.6 ± 8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraglandular nerves (%)</td>
<td>27.1 ± 5.9bc</td>
<td>38.3 ± 6.6a</td>
<td>40.0 ± 6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perineural invasion (% of intra-endometriotic nerves)</td>
<td>14.0 ± 7.0ab</td>
<td>27.85 ± 4.5a</td>
<td>35.5 ± 8.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endoneurial invasion (% of intra-endometriotic nerves)</td>
<td>13.8 ± 9.0ab</td>
<td>33.4 ± 6.3a</td>
<td>33.6 ± 8.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aNot significant; bP < 0.001; cP < 0.01; dP < 0.02.

On the left side of the ‘i’ (first column): stage 1 results are compared with stage 3 results.

On the right side of the ‘i’ (first column): stage 1 results are compared with stage 4 results.

Comparisons of stage 3 with stage 4 results are expressed in the second column.

hpF = high-power field.

### Table IV. Histological and immunohistological results for 28 patients according to revised AFS endometriosis scores

<table>
<thead>
<tr>
<th>Group 1 (score ≤15)</th>
<th>(n = 10)</th>
<th>Group 2 (score &gt;15)</th>
<th>(n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of nerves per ×40 hpF</td>
<td>18.2 ± 0.9</td>
<td>17.8 ± 1.1a</td>
<td></td>
</tr>
<tr>
<td>No. of lesions per ×40 hpF</td>
<td>7.6 ± 0.9</td>
<td>7.5 ± 1.0a</td>
<td></td>
</tr>
<tr>
<td>Intrafibrinous nerves (%)</td>
<td>25.7 ± 6.8</td>
<td>39.6 ± 7.6a</td>
<td></td>
</tr>
<tr>
<td>Intraglandular nerves (%)</td>
<td>27.1 ± 5.9</td>
<td>38.8 ± 6.5a</td>
<td></td>
</tr>
<tr>
<td>Perineural invasion (% of intra-endometriotic nerves)</td>
<td>14.0 ± 7.0</td>
<td>29.4 ± 5.6a</td>
<td></td>
</tr>
<tr>
<td>Endoneurial invasion (% of intra-endometriotic nerves)</td>
<td>13.8 ± 9.0</td>
<td>35.0 ± 8.4b</td>
<td></td>
</tr>
</tbody>
</table>

aNot significant; bP < 0.001; cP < 0.01.

hpF = high-power field.

### Discussion

Recently, large series of laparoscopic resection of rectovaginal endometriotic nodules have shown good results in terms of the relief of chronic pelvic pain and reduction in deep dyspareunia and severe dysmenorrhoea (Nezhat et al., 1992; Domene et al., 1995). Unfortunately, the outcomes of retrospective studies rarely involve the woman’s view, but are often based on the surgeon’s opinion. Using mailed preoperative and postoperative questionnaires (after mean follow-up of 21.0 ± 2.3 months), we were able to demonstrate a significant difference between pre- and postoperative pain scores. These results strongly suggest that the resection of such lesions significantly relieves pelvic pain, dysmenorrhoea and deep dyspareunia.

The exact mechanism by which this lesion causes pain is still unknown, but numerous theories have been proposed to explain pain mediation by endometriotic tissue, including production and release of prostaglandins; inflammatory mediators such as kinins, histamine, interleukins, etc., fibrosis and cyclical haemorrhage. An early theory of pain production...
they become palpable (Rosai, 1996; Anaf et al., 1999). The finding of epithelial invasion of the perineural and endoneurial space was at one time considered evidence of malignant disease. The theoretical basis for the viewpoint that perineural invasion was evidence for malignancy was weakened when it was concluded that the perineural space is not a lymphatic space, and that involvement may represent extension into the tissue plane of least resistance (Rodin et al., 1967). Descriptions of perineural invasion by benign lesions such as endometriosis are much less frequent. In 1956, a case of breast chronic cystic disease was described in which normal mammary ducts were found within the perineural space (Ackerman, 1957), while others (Taylor and Norris, 1967) later demonstrated perineural invasion in 2% of breast biopsies, essentially in patients with sclerosing adenosis. Interestingly, as in rectovaginal septum endometriotic nodules, the pain produced by sclerosing adenosis of the breast is also characterized by an exacerbation when pressure is applied to the lesion (Preece et al., 1979). Perineural and endoneurial invasion have also been described in chronic pancreatitis, another very painful benign disease (Bartow et al., 1981). The involvement of somatic pelvic nerves by endometriosis is a rare, but well-described, clinical and pathological entity (Head et al., 1962; Baker et al., 1966; Forrest and Brooks, 1972). Cyclical sciatica caused by biopsy-proven endometriosis was first described in 1955 (Denton and Sherrill, 1955). Symptomatic deposits of endometrial tissue involving the sciatic nerve may be identified radiographically and surgically. Catamenial pain, weakness and sensory loss represent the most frequent symptoms of endometriotic involvement of the sciatic, femoral or lumbosacral nerve roots (Zager et al., 1998). In the available literature, perineural invasion of the autonomic nervous system by endometriosis has been described in only one case of uterine sarcomatous painful nodule discovered after total hysterectomy with bilateral adnexectomy for endometrial cancer (Roth, 1973). Such observations associated with the frequent presence of perineural and endoneurial involvement in rectovaginal endometriotic or adenomyotic lesions (Figure 3) suggest a certain ‘neurotropism’ of deep infiltrating endometriosis.

In conclusion, we have demonstrated that in rectovaginal septum endometriotic nodules, a high proportion of the nerves are encapsulated in the nodular fibrosis of the nodule and that there is a close morphological relationship between nerves and endometriotic foci by means of perineural and endoneurial invasion. In addition, patients with the highest preoperative pain scores display higher proportions of nerve encapsulation in fibrosis and endometriotic lesions and more frequent peri- and endoneurial invasion than patients with lower preoperative pain scores (Table II). Similar histological results were found when stage 1 endometriosis patients were compared with stage 3 and 4 patients, as well as when patients with a pain score <15 were compared with those with a score >15 (Tables III and IV). From a methodological point of view, we recognize that we were unable to provide adequate controls. Theoretically, the most adequate controls should be rectovaginal endometriotic nodules from women with no history of pelvic pain, dysmenorrhea or deep dyspareunia. However, in our experience—as well as in the available literature—the presence of a rectovaginal endometriotic nodule is always associated with pelvic pain, dysmenorrhea and/or deep dyspareunia (Cornillie et al., 1990; Koninckx and Martin, 1992; Nezhat et al., 1992; Donnez et al., 1995; Clayton et al., 1999; Porpora et al., 1999). In addition, it would be unethical to remove a rectovaginal septum endometriotic nodule in an asymptomatic patient. We postulate that the topographical relationship between nerves and endometriotic foci and between nerves and fibrosis could at least partly explain the strong association between rectovaginal endometriotic nodules and pain.

References


Received on January 31, 2000; accepted on May 11, 2000