Deeply infiltrating pelvic endometriosis: histology and clinical significance

Freddy J. Cornillie, Ph.D.†
Didier Oosterlynck, M.D.*
Joseph M. Lauweryns, M.D., Ph.D.‡
Philippe R. Koninckx, M.D., Ph.D.*

Catholic University of Leuven, Leuven, Belgium

In 179 consecutive laparoscopies for infertility (n = 105), pain (n = 60), or both problems (n = 14), endometriosis was diagnosed in 77%, 82%, and 86%, respectively. Eighty implants with positive histology and with careful assessment of depth were sampled by CO₂ laser excision from 53 patients. Deep (≥5 mm), intermediate (2 to 4 mm), and superficial (<1 mm) infiltration was found in 48%, 35%, and 17% of implants, respectively. Deep infiltration was observed in the pouch of Douglas (55%) and at the uterosacrals (34%), but was absent from the ovarian fossas. Deep implants were found to be active in 68%. At an intermediate depth, however, only 25% of implants were active, whereas 58% of superficial foci showed activity. Deep implants were in phase with the endometrium in 74%. At an intermediate depth, however, only 38% showed regular cyclicity, whereas 57% of superficial implants were in phase with the cycle. Deep infiltration occurred through loose connective tissue septa into the fibromuscular tissue and was always stopped at the underlying fat tissue. Very deep implants (>10 mm) were found exclusively in patients with pain; superficial implants, on the contrary, were found most frequently in patients with infertility (83%).

The macroscopic appearance of pelvic endometriosis is variable. Pigmented, nonpigmented, and subtle lesions were observed. Pigmented endometriotic plaques are the typical powder burn lesions; they contain hemosiderin-laden tissue embedding inactive endometriotic glands and fibrous stroma. Nonpigmented endometriotic implants, differing from typical and pigmented lesions, were also found, and by histology it was shown that they contain active endometriotic glands and stroma. Subtle endometriotic lesions, either nonpigmented or pigmented and sometimes only suspected for bearing disease, were detailed. It was shown by light microscopy, and by both scanning and transmission electron microscopy, that these small lesions were composed of highly differentiated endometrial glands and a large amount of well-vascularized ectopic stroma, suggesting that these minimal lesions are active implants. These subtle endometriotic lesions are superficial and small implants, sometimes not exceeding 1 mm in diameter.

Recently, it was demonstrated with the use of laser excisional techniques that 33% of the patients with histologically proven endometriosis had lesions penetrating deeper than 4 mm. Therefore the present study was undertaken to investigate systematically the histological characteristics and the activity of deeply infiltrating pelvic endometriosis.
MATERIALS AND METHODS

Patients and Biopsies

From October 1988 to July 1989, 179 consecutive laparoscopies were performed for infertility (n = 105), pelvic pain (n = 60), or both (n = 14). Macroscopically, endometriosis was diagnosed in 77%, 82%, and 86% of these groups, respectively. The pelvic implants were excised with a CO₂ laser (Sharplan 1060; Laser Industries, Ltd., Tel Aviv, Israel), and the depth of infiltration of endometriosis was accurately assessed during and after excision by comparing the depth of excision and the height of the biopsy with the graded tip of a second puncture instrument. For this, either a Nehzat probe (Cabot Medical, Langhorne, PA), with tip perforated by holes of 1.2 mm diameter each, or any other graded palpator was held adjacent to the lesion. Biopsies were taken from all lesions penetrating deeper than 3 mm (n = 110 women); in addition, and as part of an ongoing study to compare the laparoscopic appearance with the morphology of implants, most of the minimal as well as all of the suspected lesions were biopsied. The laparoscopic diagnosis of endometriosis was confirmed by histology in 84 patients (76%). Women with ovarian endometriosis only and women using medical suppressive therapy for endometriosis were excluded. This study thus comprises 53 normally-cycling women and 80 histologically proven endometriotic implants with a careful assessment of the depth of infiltration of the disease. Endometrial biopsies were taken with a Novak curette (s.a. Manufacture Belge de Gembloux, Gembloux, Belgium).

Morphology

Biopsies were fixed in phosphate-buffered formalin, dehydrated through alcohols, and embedded in paraffin. The deep implants were divided in two tissue blocks, from which at least two sections were made perpendicularly to the peritoneal surface, and were stained with hematoxylin and eosin. All biopsies were studied by one of the authors (F.J.C.) and endometriosis was diagnosed only when ectopic glands together with stroma were found. Endometriotic implants were considered proliferative when epithelial and/or stromal mitoses were present; pseudostratification only of the glandular nuclei was not used as a hallmark of proliferation. Secretory transformation was diagnosed when intracellular glycogen pools (subnuclear and perinuclear vacuoles) together with intraluminal secretions were found, but not when only predecidual-like transformation of the ectopic stroma was seen. Active implants thus reveal the presence of mitoses or glycogen accumulation or both. Implants were considered inactive when they lacked any epithelial differentiation and when the ectopic stroma was scanty or replaced by fibrous tissue.

Statistics

Statistical significance was evaluated by the χ² and Student’s t-tests and by the Spearman correlation test.

RESULTS

Accuracy of Laparoscopic Assessment of Depth of Infiltration

The depth of infiltration was measured on the microscopic slides when the peritoneal mesothelium together with the whole subperitoneal thickness of the implant was included in the sections. Up to a depth of infiltration of 5 mm, all laparoscopic and histological measurements corresponded within 1 mm (n = 15). For endometriosis deeper than 8 mm, the laparoscopic estimation of depth was systematically 1 to 2 mm greater than the microscopic measurement. With the exception of one surgical misjudgement, all excised deep biopsies contained endometriotic elements up to the bottom of the blocks.

Depth of Infiltration

Infiltration of endometriotic implants was superficial (≤1 mm), intermediate (2 to 4 mm), or deep (≥5 mm) in 17%, 35%, and 48%, respectively (Table 1). Deep disease was found exclusively in the pouch of Douglas (55%), at the uterosacralis (34%), and the uterosacral fold (11%), but not in the ovarian fossas (Fig. 1). Five endometriotic implants showed very deep penetration (≥10 mm); 4 of these (80%) were taken from the cul-de-sac, whereas another was diagnosed at the right uterosacral ligament.

Table 1  Depth of Infiltration of all Positive Specimens Excised During Laparoscopy

<table>
<thead>
<tr>
<th>Depth (mm)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>lesions (%)</td>
<td>17</td>
<td>13</td>
<td>17</td>
<td>5</td>
<td>13</td>
<td>6</td>
<td>3</td>
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<td>14</td>
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Activity of Endometriotic Implants
at Different Depths

The activity and cyclicity of implants was significantly different at different depths of infiltration (Fig. 2). Superficial and deep lesions showed cellular activity in 58% and 68% of the implants, respectively, whereas implants with an intermediate depth of infiltration were active in 25% only ($P < 0.0001$).

Superficial and deep pelvic implants showed cellular differentiation in phase with the eutopic endometrium in 57% and 74%, respectively. Only 38% of the implants at an intermediate depth, however, were in phase with the endometrium ($P < 0.0001$). No significant difference was found, neither for the activity nor for the cyclicity, between depths of infiltration ranging from 5 mm up to the deepest lesion found (15 mm). Indeed, in lesions of 5 to 9 mm and of 10 mm or deeper, activity was found in 68% and 69%, whereas they were in phase with the endometrium in 73% and 67%, respectively.

In one patient with superficial and deep implants, both were proliferative and in phase with the endometrium. In eight patients, intermediate and deep endometriosis was diagnosed. A similar differentiation in both types of implants was seen in five women, whereas in three patients the differentiation was in phase with the endometrium in the deep implants only.

Histopathology of Infiltrating Endometriotic Lesions

Deep, infiltrating endometriotic implants revealed peculiar histological characteristics. Infiltration occurred into subjacent fibromuscular tissue along the loose connective tissue septa (Fig. 3). These loose septa were completely filled with ectopic endometrial stromal cells embedding small active glands with regular cyclicity. Strikingly deep penetration was arrested at the border of the underlying fat tissue. Deep biopsies usually contained nerve fibers, and lymph follicles were occasionally present in the ectopic stroma. Hyperplasia of the fibromuscular tissue around the implant was generally observed and perivascular mononuclear inflammatory cells were usually present.

Cystic transformation of endometriotic glands was a common observation. The flattened or cuboidal lining epithelium was never proliferative nor secretory, although intraluminal secretory-like substances could be found. Bleeding into the lumen

Figure 1 Depth of infiltration of endometriotic implants at the ovarian fossas (■), the uterosacral (□), the uterovesical fold (□), and the pouch of Douglas (□).

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Figure 2 Activity and cyclicity of pelvic endometriotic lesions at different depths. (a), Active (■) and inactive (□) implants as well as foci with cystic glands (□) are shown. (b), Implants in phase (■) and out of phase (□) with the endometrium are shown.
of such microcysts (diameter 500 to 2,000 μm) was occasionally seen, indicating that these glandular cysts were comparable with microendometriomas. Since mitoses and glyogen were absent from the lining epithelium of the microcysts, these glands were, however, considered inactive. Inactive microendometriomas were observed at all different depths of infiltration, but occurred more frequently at 2 to 4 mm below the peritoneum.

Depth of Infiltration of Endometriosis Related to Age and Indication for Laparoscopy

No correlation was found between the age and the maximum depth of penetration of disease. The presence of very deep endometriosis strongly correlated with pelvic pain ($P < 0.0001$, Fig. 4). Indeed, all patients with very deep lesions (>10 mm) suffered from severe pelvic pain. In women with superficial (≤1 mm), intermediate (2 to 4 mm), or deep (5 to 10 mm) infiltration, pain was found in 17%, 53%, and 37%, respectively.

DISCUSSION

Excision of deep endometriosis with the CO$_2$ laser was first reported by Martin et al.$^7$ They demonstrated superficial (≤1 mm), intermediate (2 to 4 mm), and deep (>5 mm) infiltration of endometriosis in 11%, 56%, and 33% of their patients, respectively. These data are largely comparable with our findings showing 17%, 35%, and 48% of the lesions at these depths, respectively. Furthermore, very deep lesions (>10 mm) were identified in 8% of the patients by Martin et al.$^7$ and in 11% of the patients in our study. The true incidence of deep endometriosis in women with infertility and/or pain, however, is probably lower since a selection bias existed in the present study. Indeed, infiltrating implants were preferentially sampled for histopathological examination, whereas subtle and superficial foci were vaporized in some women.

Histopathological examination confirmed the laparoscopic judgement of depth of infiltration during laser excision. Indeed, except for one clinical misjudgement, all deep excisions contained endometriotic glands and stroma from the top (peritoneal surface) to the bottom (fat tissue) of the blocks. Furthermore, rupture of microcysts and leakage of chocolate-like substance from these structures proved the presence of deeply penetrating endometriosis during excision. Although the limits between endometriotic and healthy tissue are often difficult to judge, the good correlation found between laparoscopic estimations and microscopic measurements proved that accurate assessment of this depth is possible during laparoscopy. The systematic overestimation for very deep implants (>8 mm) can be explained as follows. Superficial and intermediate implants are excised under the lesion because this is technically easy. Deep endometriosis, however, is obviously excised with great care and prudence. Because the surgeon continues to fear for excising too deeply, he tends to vaporize the bottom of the lesion remaining at the border of the endometriotic and the underlying healthy tissue. Histological specimens of very deep implants therefore do not contain the “real” bot-

Figure 4 Depth of infiltration of endometriosis in patients with infertility (●), pain (□), or both (□).
tom of the lesion. It could be argued that the depth of infiltration was underestimated due to incomplete excision. In all patients, however, excision was performed down to macroscopically normal tissue lacking any palpable induration.

To the best of our knowledge, this is the first demonstration that deep endometriosis is a very active disease, in phase with the eutopic endometrium. Deep endometriosis is clearly different from disease with intermediate depth, which was less active (25%) and was less in phase with the cycle (38%). Also the superficial implants were rather active (58%), but less regular cyclicity was found than in deep lesions (57% versus 74%). It may thus be concluded that deep endometriosis is an active disease with a good response to steroid hormones. This finding was also evident in women with lesions at different depths. Indeed, nine women had deep and superficial or intermediate implants; activity in phase with the endometrium was seen in all implants of four patients. Proliferation in phase with the cycle was observed in the deep lesions only of three other patients. This shows that deep implants can be active, whereas other (intermediate) implants remain inactive in the same patient.

The question therefore remains why implants at 2 to 4 mm are less active than superficial and deep implants. To explain this difference, the following hypothesis is suggested. Superficial implants are initial stages of disease resulting from the implantation of fresh and viable regurgitated endometrial cells. Local factors may regulate initial ectopic growth and superficial pelvic infiltration. However, at an intermediate depth, many implants would burn out and become inactive, whereas some lesions still infiltrate deeper. Indeed, the histological pattern of activity and cyclicity changes at about 5 mm beneath the peritoneal surface. Peritoneal fluid (PF) is known to contain concentrations of estradiol and progesterone (P) which, compared to serum, are 10 to 100 times higher during the luteal phase and 2 to 3 times higher during the follicular phase. We therefore suppose that superficial and intermediate implants are partly influenced by steroids from the PF, whereas, because of impaired diffusion at greater depths, deep implants are mainly under the control of circulating steroids. This gradient of steroid influences from the PF and plasma explains why deep endometriosis is active. Endometriotic implants infiltrating <5 mm are active when they are initial (i.e., superficial) disease or inactive when they are older and burning out.

Progesterone from PF may thus inhibit growth of superficial lesions which finally burn out. Involution of superficial endometriotic foci was demonstrated by histology and electron microscopy during medical suppressive therapy with progestogens and antiprogestins and, during pregnancy, P may inhibit growth of endometriotic implants.

Deep infiltration of endometriosis was found at the base and between the uterosacral and, to a lesser degree, at the uterovesical fold, but not at the ovarian fossa. In healthy women, large bundles of fibromuscular tissue, separated by prominent loose septa are present under the peritoneal lining in these areas. Since deep endometriosis infiltrates through the septa, we suggest that the presence of loose connective tissue permits infiltration. Moreover, deep penetration was always stopped at the border with the underlying fat tissue, which further supports this hypothesis, since fat tissue contains very few intercellular loose septa.

The strong correlation between deep endometriosis and pelvic pain is striking. We demonstrated indeed that all patients with very deep implants (>10 mm) suffered from severe pelvic pain. Deep biopsies contained nerve fibers and inflammatory cells within and around the implants. The latter findings may explain why deep implants cause pain.

In conclusion, deep endometriosis is an active disease and is histologically different from endometriosis with superficial and intermediate infiltration. Its localization is limited to pelvic areas with fibromuscular tissue such as the pouch of Douglas, the uterosacral, and the uterovesical fold. Finally, deep endometriosis is strongly correlated with pelvic pain.

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