



## Uterine innervation after hysterectomy for chronic pelvic pain with, and without, endometriosis

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### KEY WORDS

Chronic pelvic pain  
Endometriosis  
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**Objective:** Chronic pelvic pain is associated with a wide range of clinical conditions that include endometriosis. The precise cause, mechanisms of pain, and natural history are imprecise. Patterns of uterine innervation have been studied after hysterectomy for chronic pelvic pain with and without endometriosis.

**Study design:** Tissue blocks were taken from the lower one half of the uterus after hysterectomy for advanced endometriosis (n = 16 specimens; group 1) and for chronic pelvic pain without endometriosis (n = 15 specimens; group 2). The control group consisted of uteri that were removed for painless gynecologic conditions (n = 25 specimens; group 3). Tissue sections from the lower one half of the uterus were stained with anti-S100 to demonstrate patterns of innervation, and nerve fiber profiles were counted by standardized techniques; qualitative differences were also recorded.

**Results:** In uteri from women with advanced endometriosis, there were increased numbers of nerve fiber profiles compared with control specimens (group 1 vs group 3;  $P = .0013$ , Mann Whitney  $U$  test). There were also increased numbers of nerve fiber profiles in uteri that were associated with chronic pelvic pain without endometriosis (group 2 vs group 3;  $P = .04$ , Mann Whitney  $U$  test). There were no differences in nerve fiber count in uteri from groups 1 and 2 ( $P = .35$ , Mann Whitney  $U$  test). Comparing both groups of uteri with controls (groups 1 and 2 vs 3) demonstrated marked differences in nerve fiber counts ( $P = .002$ , Mann Whitney  $U$  test). Two distinctive patterns of reinnervation that were observed: disruption of nerve bundles (collateral sprouting with microneuroma formation) and ingrowth around blood vessels (perivascular nerve fiber proliferation). There were increased numbers of microneuromas (groups 1 and 2 vs 3;  $P = .001$ , chi-squared test with Yates correction) and perivascular nerve fiber proliferation (groups 1 and 2 vs 3;  $P = .008$ , chi-squared test with Yates correction) in the myometrium in chronic pelvic pain with, and without, endometriosis compared with the control group.

**Conclusion:** Nerve fiber proliferation and other features of reinnervation have been observed in the isthmus regions of uteri that were removed at hysterectomy for chronic pelvic pain with and without endometriosis. There were no quantitative differences between the groups with chronic pelvic pain and endometriosis. These observations provide an alternative explanation for the source of pain and other clinical symptoms in these clinical settings.

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Epidemiologic studies have found an annual prevalence of chronic pelvic pain of 38 per 1000 women that frequently is associated with other sensory pelvic problems including cystitis and irritable bowel syndrome.<sup>1-4</sup> Endometriosis is found in association with chronic pelvic pain and may be present in as many as 10% of women aged 15 to 45 years.<sup>1</sup> Laparoscopy demonstrates variable appearances on peritoneal surfaces in the pouch of Douglas in women with the typical symptoms of chronic pelvic pain, dysmenorrhea, dyspareunia, and subfertility; severe dysmenorrhea is the most consistent symptom in the advanced stages of the condition.<sup>5</sup> The enigma of endometriosis remains the variable relationship between symptoms and the extent of ectopic endometrium at laparoscopy, when severe pain may be associated with minor endometrial deposits; however, extensive ovarian endometriomas may have no associated symptoms. Many women are found to have chronic pelvic pain without ectopic endometrium being detected at laparoscopy; convincing evidence for the attribution of pelvic pain to the ectopic endometrial deposits is limited. Treatment with gonadotropin-releasing hormone agonists provides symptomatic relief for women with chronic pelvic pain with or without endometriosis.<sup>6</sup> One randomized, controlled trial of gonadotropin-releasing hormone agonists in women with chronic pelvic pain found that almost all women had improvements in pain scores with gonadotropin-releasing hormone agonists compared with placebo, although not all the women had endometriosis when laparoscopy was performed after the course of treatment.<sup>6,7</sup> Chronic pelvic pain with or without endometriosis may require hysterectomy with bilateral salpingo-oophorectomy for effective control of symptoms; however, recurrence of pain after surgery is not uncommon.<sup>8</sup>

Sampson originally proposed the theory of retrograde menstruation, where endometrium passes into the peritoneal cavity in a reverse direction along the Fallopian tubes.<sup>9</sup> This process may be observed in asymptomatic women with normal menstrual cycles; an explanation of the reason that some women experience symptoms of endometriosis and other women do not has not been resolved.<sup>10</sup> Defects in both cell-mediated and humoral immunity have been demonstrated, though they do not explain the rate of tissue deposition if endometrium were behaving as a transplanted graft.<sup>11</sup> Inoculation of endometrial cells in the cornea has achieved successful implantation, and primary tissue injury along the needle track may have contributed to these results.<sup>12</sup> Coelomic metaplasia, vascular spread, and genetic theories have also been proposed to explain endometriosis, although none have been substantiated fully.<sup>13-15</sup>

We have proposed that processes of denervation succeeded by reinnervation cause chronic pelvic pain, dysmenorrhea, menorrhagia, dyspareunia, and subfer-

tility in severe endometriosis.<sup>16</sup> The nerve supply is delivered with the uterine arteries in the parametrial tissues at the level of the uterine isthmus and secondarily within the fascial supports, which include the uterosacral-cardinal ligament complex.<sup>17</sup> These ligaments suspend the lower genital tract through variable insertions into the cervix and upper vagina that are demonstrated readily at laparoscopy.<sup>18</sup> Asymmetric damage to the uterine supports is frequently observed in parous women with chronic pelvic pain that is associated with difficult intrapartum episodes and typical features of reinnervation.<sup>19,20</sup> This study investigates patterns of uterine innervation after hysterectomy for chronic pelvic pain with, and without, endometriosis.

## Methods

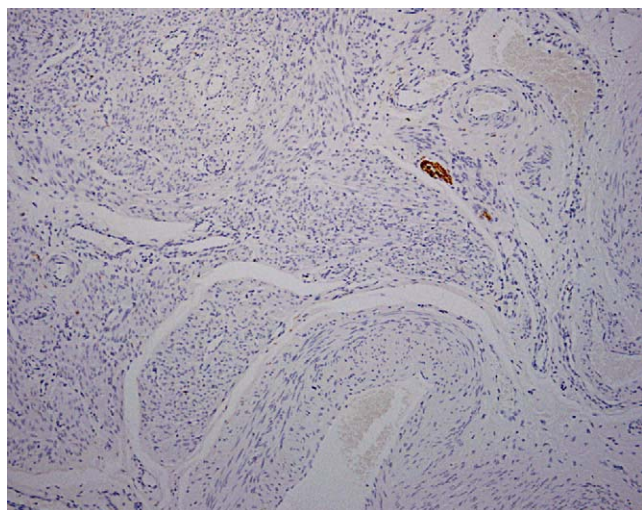
### Patients

Samples from the lower one half of the uterus of 16 consecutive patients with advanced endometriosis (revised American Fertility Society, grades III-IV) who underwent hysterectomy for endometriosis (group 1; mean age, 41.5 years [range, 27-53 years]; nulliparous, 8/16 samples; multiparous, 8/16 samples) were collected. The diagnosis of endometriosis was confirmed by histologic examination in 14 of 16 specimens. Eight uteri had incidental fibroid tumors, and 2 uteri had mild adenomyosis. Samples were also collected from 15 uteri that were removed at hysterectomy for chronic pelvic pain without endometriosis (group 2; mean age, 39.5 years [range, 31-46 years]; mean parity, 1.6 [range, 0-3]). Seven uteri had associated small, fibroid tumors (<3 cm), and 2 uteri had mild adenomyosis.

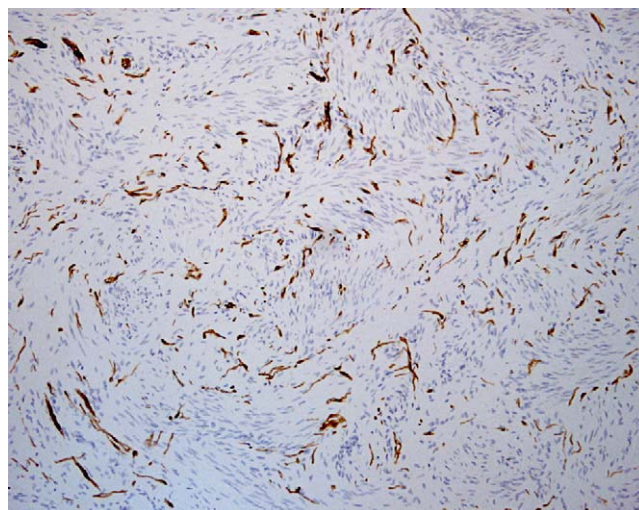
Control samples (group 3) were obtained from 2 sources: 17 parous uteri that had been removed for painless gynecologic indications (mean age, 42.0 years [range, 34-47 years]; mean parity, 2.4 [range, 1-4]; 4 for ovarian cysts, 4 during reconstructive surgery, 6 for menstrual dysfunction, 3 for miscellaneous indications) and 8 nulliparous uteri (mean age, 40 years [range, 30-52 years]; removed during surgery for single fundal fibroid tumors (4/8 samples) and for incidental reasons (4/8 samples) from the tissue archive. Tissue blocks were collected in the sagittal plane from the lower one half of the uterus to include both the endometrium and the serosal margin. All tissue samples were fixed in 10% phosphate buffered formalin then processed into paraffin wax. Ethics approval was obtained from the Local Research Ethics Committee, and each woman gave consent for the tissue samples to be studied.

### Immunohistochemistry

Three-micron sections were cut from the tissue blocks and stained with hematoxylin and eosin and anti-S100



**Figure 1** Normal myometrium is innervated sparsely with small nerve bundles that are distributed with blood vessels. There are nerve fiber concentrations at the endometrial-myometrial interface and in the subserosal layers. Nerves are stained with anti-S100 (objective magnification,  $\times 10$ ).



**Figure 2** Reinnervation in the myometrial stroma after hysterectomy for advanced endometriosis. Widespread, chaotic reinnervation was observed in the lower one half of the uterus (objective magnification,  $\times 10$ ).

protein. Deparaffinized sections were pretreated with trypsin (Difco 215230; DakoCytomation A/S, Glostrup, Denmark) before polyclonal anti-S100 protein (Dako Z0311; DakoCytomation A/S) was applied. Endogenous peroxidase activity was eliminated by the application of a commercial peroxidase blocking solution (Dako S2023). We stained the sections for S100 to detect nerves along with 2 positive controls on each slide. The sections were stained on an automated immunohistochemical stainer with detection kit (Dako K5001; ChemMate TM; DakoCytomation A/S). The nuclei were counterstained with hematoxylin.

### Tissue analysis

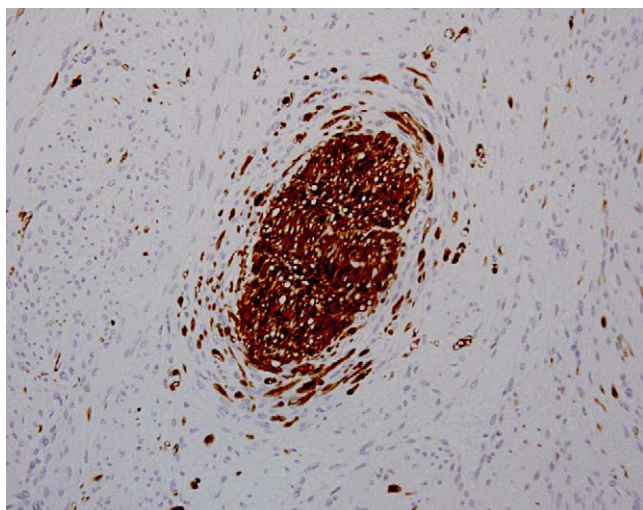
Tissue sections were examined on a microscope with both semiquantitative and qualitative techniques. Immunoreactive nerve profiles were counted in 6 random grids that measured  $1 \text{ mm}^2$  each, at least 2 mm apart. To ensure representative sampling across the section, myometrial sections were divided into inner and outer halves, and a total of 6 grids were counted, with 3 grids in each half of the section. The observers were blind to the source of the material and counted representative fields rather than areas of high nerve fiber density. Areas of artifact, or those areas that were associated with high vessel density, were avoided along with foci of adenomyosis or leiomyomas. To ensure consistency, similar random fields from the same section were re-counted by a second observer. Further blocks were taken from the upper half of the uterus in a subset of cases to exclude reinnervation at this site.

Tissue sections were also examined for the presence of microneuromas and perivascular nerve fiber proliferation. In morphologic terms, microneuromas consisted of a central nerve trunk, with splaying of individual nerve fibers, surrounded by further nerve fibers in the myometrial stroma.<sup>21,22</sup> Differing degrees of perivascular nerve fiber proliferation were noted in several sections. It was recorded if individual nerve fibers were seen within the adventitia of the artery in  $\geq 1$  layers, over one half of the circumference of the vessel. Statistical analysis was performed by standard, nonparametric techniques.

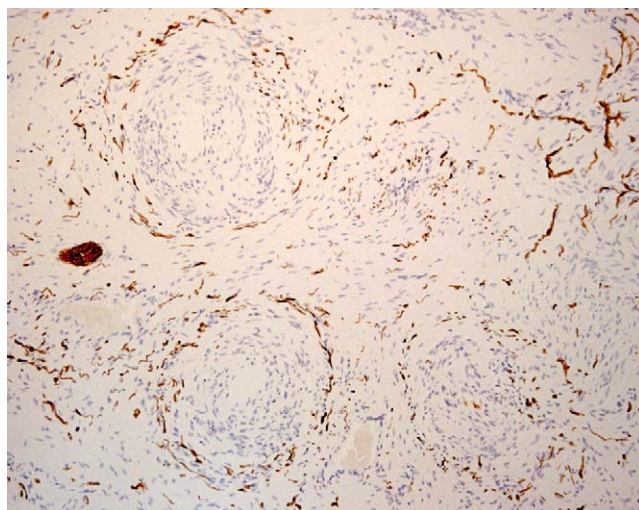
### Results

Increased numbers of nerve profiles were observed in the myometrium of the lower half of the uterus in endometriosis (group 1 vs group 3;  $P = .0013$ , Mann Whitney  $U$  test) and chronic pelvic pain (group 2 vs group 3;  $P = .04$ ) compared with controls (Table; Figures 1-4). There were no significant differences in the nerve counts between these 2 groups (group 1 vs group 2;  $P = .35$ , Mann Whitney  $U$  test).

A comparison of uteri from women with chronic pelvic pain with and without endometriosis (groups 1 and 2) with control uteri (group 3) demonstrated clear differences in nerve fiber counts ( $P = .002$ , Mann Whitney  $U$  test; Table). In addition, increased numbers of microneuromas (Figures 3 and 4) were observed in the combined group (groups 1 and 2), compared with the control group (group 3; 16/31 vs 2/25;  $P = .001$ , chi-squared test with Yates correction). Perivascular nerve



**Figure 3** Microneuroma formation in the myometrial stroma with disruption of the nerve bundle and perineural nerve fiber proliferation (collateral sprouting) against a background of myometrial nerve fiber proliferation (objective magnification,  $\times 20$ ).



**Figure 4** Perivascular nerve fiber proliferation with nerves regrowing along small arteries after hysterectomy for stage IV endometriosis (objective magnification,  $\times 10$ ).

**Table** Differences in myometrial innervation in the lower one half of the uterus in endometriosis (group 1), chronic pelvic pain without endometriosis (group 2), and control subjects (group 3)

Variable	Group			P value*
	1 (n = 16)	2 (n = 15)	3 (n = 25)	
Verve fiber profiles per 0.5 mm <sup>2</sup> †	31.5 (17-53)	22 (14-66)	12 (6-29)	.002, Mann Whitney
Microneuromas (n)	9	7	2	.001, chi-squared, Yates correction
Perivascular nerve fiber proliferation (n)	13	9	8	.008, Fisher's exact test

\* Groups 1 and 2 versus 3.

† Data are given as median (interquartile range).

fiber proliferation (Figures 3 and 4) was also more frequent in chronic pelvic pain with or without endometriosis (22/31 vs 8/25;  $P = .008$ , Fisher's exact test).

Observations in the uterine cervix showed some features of reinnervation, although there were no differences in nerve counts among the 3 groups. There were no signs of abnormal innervation in the uterine fundus.

## Comment

Nerve fiber proliferation in the lower half of the uterus has been observed in women with chronic pelvic pain with and without endometriosis that may contribute to clinical symptoms in both groups. There were no quantitative differences in nerve counts that suggested a similar cause for chronic pelvic pain, irrespective of the presence of ectopic endometrium. Nerve fiber proliferation was extensive, chaotic, and, in some blocks, asymmetric, affecting one half of the uterus to a greater degree.<sup>21</sup> Other features that were associated with reinnervation included collateral sprouting of nerve

bundles (Figures 3 and 4) and perivascular nerve fiber proliferation (Figures 3 and 4), both of which were more common in the groups with chronic pelvic pain.

Traumatic disruption of nerve bundles initiates processes of axonal regeneration along the line of the nerve bundle results in the appearance of collateral sprouting, which has also been termed *microneuroma formation*.<sup>21,22</sup> Varying degrees of collateral sprouting have been reported in the uterus and attributed to the traumatic effects of parturition.<sup>21</sup> In this study, we found microneuromas in the myometrium that were typical of the appearances after traumatic injury.<sup>23</sup> Nerve fiber proliferation was seen to varying degrees around the circumference of the vessel and, in severe cases, in concentric layers. This observation has been reported previously in the vulva<sup>24</sup> and may contribute to cyclic, gynecologic symptoms in which increases in blood flow in the second half of the menstrual cycle are associated with the severe dysmenorrhea that may respond to treatment that reduces pelvic blood flow.<sup>6,8</sup> Perivascular nerve fiber proliferation may represent the consequences of injury to branches of the uterine

neurovascular bundles through processes of straining (eg, constipation, second stage of labor, repetitive lifting).<sup>25</sup>

Patterns of reinnervation in a retrospective survey of stored uteri have been reported,<sup>21</sup> though there are no other quantitative studies for comparison. It is important to note that the quantitative analysis in this report refers to the mean nerve count in random fields by blinded observers; peak nerve counts may have emphasized the differences although the precise orientation of some of the tissue blocks was unknown (Figures 3 and 4). Both adenomyosis and fibroid tumors were recorded in the study groups (groups 1 and 2); for the most part, these were incidental pathologic findings that were avoided during the quantitative analysis and did not contribute to differences in myometrial nerve counts. The selection of nulliparous control samples in this series was limited because it is relatively unusual to remove nulliparous uteri. Preliminary studies within our group confirm that uteri that have been removed for single large fibroid tumors (4/8 nulliparous control samples) have normal innervatory patterns; however, 2 nulliparous uteri that had been removed for menstrual disorders showed minor increases in nerve fiber counts. There were several uteri with increased nerve fiber counts in the parous control samples that may have been caused by previous vaginal delivery. In the endometriosis group, 2 parous uteri were removed in association with endometriotic cysts. Myometrial nerve counts were similar to parous control samples, which suggests the possibility of a different cause for ovarian endometriomas in these circumstances. Each of these 3 features would serve to reduce the quantitative and qualitative differences between the control and study groups.

This study was not large enough to differentiate the effects of parity, although these preliminary observations suggest that perivascular nerve fiber proliferation was more frequent in nulliparous uteri and that micro-neuromas were more frequent in parous uteri from women with chronic pelvic pain. Several nulliparous women in this study gave a history of prolonged constipation although this observation was not examined formally in this study; most of the parous subjects had difficult intrapartum episodes in their first labor. That these histologic findings were present occasionally in the control group suggests a universal pattern of this kind of injury, such as excessive abdominal straining that may occur during daily activities. Injury to the parametrial tissues and uterosacral-cardinal ligaments during the second stage of labor may account for injuries to nerve bundles in parous women.<sup>19,20</sup> Prolonged maternal voluntary efforts that complicated malpositions, big babies, and operative vaginal delivery may be significant in this respect.

Successful implantation of endometrium has followed needle inoculation in the cornea,<sup>12</sup> and endometriosis is also found in abdominal and perineal incisions after

cesarean delivery and episiotomy, respectively.<sup>1</sup> Adherence of ectopic endometrium may reflect its availability at the time of tissue injury. The reason that some women with chronic pelvic pain have endometriosis and other women do not, may be attributable to the availability of ectopic endometrium at the time of the injury. Avulsion of the uterine supports during single intrapartum episodes or recurrent abrasions and petechial hemorrhages to the uterosacral ligaments that were caused by prolonged constipation may provide injured tissue surfaces. The choice of breast or bottle feeding may influence the availability of endometrium during tissue repair in the early puerperium. These observations may explain the positive response to treatment with gonadotropin-releasing agonists in women with chronic pelvic pain with or without endometriosis.<sup>6</sup> Disruption of uterine innervation also interrupts normal fundocervical polarity of uterine contractility and may promote retrograde menstruation.<sup>25</sup> The spectrum of intrapelvic manifestations of the condition may be further extended by pathologic processes that cause tissue injury without accompanying reinnervation, such as pelvic infection or serosal damage to small bowel in Crohns disease.

Nerve fiber proliferation has been reported in all female pelvic viscera and may account for common clinical presentations that include pelvic pain, dysmenorrhea, dyspareunia, vulval pain, and urinary and bowel urgency.<sup>20-29</sup> Denervation that is caused by injuries to uterine neurovascular bundles and myofascial supports is succeeded by reinnervation that may provide an explanation for some forms of chronic pelvic pain that is associated with endometriosis. Retrograde delivery of ectopic endometrium to injured peritoneal surfaces may determine the varying laparoscopic appearances of different stages of the condition; processes of denervation-reinnervation in the uterine isthmus and myofascial supports may account for some clinical symptoms. If neurologic processes such as those described in this article contribute to the cause of the condition, then it may explain the response to treatment that reduces pelvic blood flow in the second half of the menstrual cycle. It may also have some bearing on the recurrence of pain after hysterectomy with bilateral oophorectomy or other surgery that is directed at the interruption of nerve pathways. In many women, ectopic endometrium may represent an epiphenomenon to underlying processes of denervation-reinnervation that may be responsible for persistent dysmenorrhea and chronic pelvic pain.

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