

Nerve Fibers on Endometriotic Lesions: Clinical Features and Review of Literature

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ABSTRACT

Objectives: The purpose of this review is to analyze the current data available on physiology, pathology, epidemiology, and finally clinical implications on the association between endometriosis and nerve fibers. **Methods:** A semantic review of literature was made of English language publications on databases PubMed and Google Scholar following a Mesh and keyword searching. The studies were finally selected by one author according to the aim of this review. **Results:** The endometriotic lesion uniformly shows a greater density of nerve fibers on the histopathological assessment when compared to normal tissue. The type of fibers within the lesions is inconstant and will diverge according to the type and location of implants. Peritoneal endometriosis presents higher sensitive lower sympathetic fibers within or near the implants. As peritoneal, the intestinal nodules show the same sensitive predominance, probably explaining the genesis of associated pain at this area. Meanwhile, uterosacral and rectovaginal septum endometriosis are usually deeper and with a higher amount of fibrotic tissue. The density of fibers is greater than normal tissue but tend to show a sympathetic predominance. Ovarian endometrioma is still understood and appears to have less nerve fibers and pain than other locations. Interestingly, the presence of deep infiltrating endometriosis lesions itself shows specific characteristics on distribution, frequency, and type of fibers regarding their locations. At present, the clear association of the histopathological findings to pain symptoms is still poorly known, and more studies are required.

Key words: Dysmenorrhea, dyspareunia, endometriosis, nerve fibers, pelvic pain

INTRODUCTION, DEFINITIONS AND EPIDEMIOLOGY

Endometriosis is a disease that affects 10% of the general female population and can reach 82% of patients with chronic pelvic pain.^[1] The diagnosis is histological and requires pelvic surgery and biopsy, but can be suspected with clinical history and pre-operative imaging. One cardinal symptom is the pain, including dysmenorrhea, deep dyspareunia, cyclic intestinal or vesical symptoms, acyclic chronic pelvic pain, and Dyschezia.^[2] All these symptoms can affect the patient's quality of life and can be responsible for absenteeism due to the severe pain.^[3]

To date, the complete knowledge on the mechanisms that cause it is still poorly understood. Several studies have failed or have just shown a weaker correlation when linked between pain scores, clinical and biochemical characteristics of the endometriotic lesions were searched.^[4,5]

As long as the experience of pain is a complex process, it is accepted that the pain symptoms related to the disease will depend not only just on the peripheral ways but also that the central nervous system is involved at least on pain associated to nodular endometriotic lesions.^[6,7]

Since the Chapron and *et al.* work in 2005, it is clearly accepted that there is no direct correlation between the

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extension or severity of the disease and the degree of pain symptoms (according to final American fertility society classification score).

Formally speaking, it is considered that a nerve fiber belongs to an endometriotic lesion when the distance to it is no >1.5 mm. The studies made by Tokushige *et al.*, published on the Human Reproduction and Fertility Sterility, respectively, demonstrated that the link between nerve fibers and endometriotic lesions certainly exists. These nerve fibers are always near or within the lesion and in higher density than in a healthy tissue [Figure 1]. Furthermore, there are probably implicated in the degree of pain symptoms since when these fibers are present on the endometriosis lesions, it is more likely the report of severe pain.^[8-10]

We present a semantic review of literature focused on the link between endometriotic lesions and nerve fibers. We start giving definitions and epidemiological information. Afterward, a review of the evidence of nerve fibers in specific endometriotic locations is analyzed. Finally, we discuss the link between nerve fibers and pain and draw conclusions.

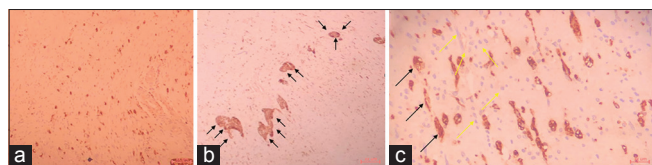


Figure 1: Histopathological/immunohistochemistry analysis of intestinal deep infiltrating endometriosis implant. Note the presence of nerve fibers (stained brown) in the endometriotic implant using Protein gene product 9.5. The arrows are pointed to nerve structures (black) and endometriotic stromal cells (yellow) (a) magnification ×10, (b) magnification ×20, (c) magnification ×40

Objective

The objective of the study was to analyze the current information related to the histopathological assessment of nerve fibers in endometriotic lesions, and their clinical relevance in Disease symptomatic status.

METHODS

A comprehensive review of literature was carried out, conducting a total computerized search of English publications on databases PubMed and Google Scholar related to Endometriosis and nerve fibers. We included all studies found under the search of following Mesh and Keywords terms: Endometriosis AND Nerve fibers OR Nerve tissue OR Nerve endings OR Pain OR Pelvic pain. One author independently made a selection of relevant abstracts according to the aim of this review. The primary objective was to know the physiology, pathology, epidemiology, and final clinical implications of the association between nerve fibers and endometriosis.

Endometriosis and pain

Most of the patients with endometriosis describe a progression of their symptoms over their lives, usually referencing different kinds of pain and abnormal visceral sensations. This could indicate visceral hyperalgesia, finally suggesting neuropathic pain.^[11] This kind of pain, caused by an inflammation of the nervous system, affects between 0.6% and 1.5% of all the American population and could present as hyperalgesia, allodynia or just spontaneous pain.^[12]

The final origin of this neuropathic pain is still unclear, but its evidence that it is a complex process with many probable causes [Table 1]. Anaf *et al.*, in the Fertility Sterility of 2006, showed the role of immune and inflammatory factors on its occurrence.^[13]

Table 1: Possible theories and mechanisms explaining the pain in endometriosis

| Mechanism/Theory | Characteristics |
|--|---|
| Increase activity and degranulation of mast cells and macrophages Anaf <i>et al.</i> - Fertil Steril 2006 | Cyclic pain and hyperalgesia Active production of chemotactic and pro-inflammatory cytokines |
| Endo and peri-neural invasion of nerves Anaf <i>et al.</i> - Human Reprod 2000 | Direct relation with intensity of pelvic pain Accepted mechanism |
| High peritoneal fluid cytokines McKinnon <i>et al.</i> - Fertil Steril 2012 | Direct correlation with the presence of painful implants |
| Higher density of nerve fibers Anaf <i>et al.</i> - Gynecol Obstet Invest 2011 | Due neuron differentiation of undifferentiated cells and “Nerve Sproud Effect” Related to severe neuropathic pain Probably direct correlation between fiber density and pelvic pain |
| Anatomical location of implants Wang - Human Reprod 2009 | Alteration of normal position and range of movements of fixed structures |
| Cajal cell damage Remorgida - Human Reprod 2005 | Bowel symptoms in intestinal endometriosis |

Meanwhile, Witzel *et al.* talked about the “nerve sprouting,” the increased numbers of nerve fibers in the vicinity of injured nerve structures as the main cause of neuropathic pain.^[14]

Recently, it has been demonstrated the association between nerve fibers and angiogenic - neurotrophic factors in endometriotic lesions. In 2014, Morotti *et al.* published a nonsystematic review of the literature and concluded that, even if the link between them have not already been demonstrated, certainly there is an increased expression of neurologic factors and nerve fibers on the endometriotic lesions. Furthermore, the peritoneum plays a role in these changes.^[15]

Peritoneal endometriosis [Table 2]

In 2001, Tulandi *et al.* described for the very 1st time the presence of the nerve fibers in endometriotic peritoneal lesions and found no difference in the amount of them when compared to a healthy peritoneum. In patients with endometriosis, 79.6% presented positive antibody against

neurofilament (NF) protein, frequently used to assess the presence of nerve fibers structures on tissues. Furthermore, he showed that the distance between these nerve fibers to the endometrial glands was shorter in women with pelvic pain rather than the asymptomatic, suggesting a functional role of these nerve structures in the disease-related pain symptoms.^[16]

Main histopathological markers of nerve fibers are show in Table 3.

Later, in 2006, 40 women with histological confirmation of peritoneal endometriosis went to a histopathological analysis of their peritoneal lesions, and the results proved that the density of the nerve fibers on peritoneal lesions was greater than normal peritoneum in patients without endometriosis. Furthermore, these fibers were nearest to endometriotic glands and blood vessels, containing a mixture of cholinergic, adrenergic, sensory A and B nerve fibers, making them a special kind of fiber.^[8]

1 year later, Mechsner *et al.* studied a total of 106 endometriotic lesions using NF as a marker and confirmed the previous results of Tulandi: 74% of nerve fibers within these lesions, and no differences in the proportion when compared to a healthy peritoneum.^[9] One interesting point of the study was the detection of glycoprotein component 43 (GP -43), a specific marker for newly grown fibers inside the endometriotic lesions. It appears constantly near to immature

Table 2: Main characteristics in peritoneal endometriosis

| Location | Characteristics |
|---|---|
| | 74–79% positive nerve fibers |
| Peritoneum | Higher sensorial lower sympathetic fibers |
| Probably direct correlation between fiber density and pelvic pain | |

Table 3: Immunohistochemistry markers for nerve fibers in endometriosis

| Immunohistochemistry marker | Characteristics |
|-----------------------------|---|
| S-100 | Acidic protein, 100% soluble in ammonium sulfate at neutral pH. Common marker of neural tissue/lesions and melanoma |
| PGP 9.5 | Member of ubiquitin hydrolase family proteins confined to neural and neuroendocrine cells. Act as a specific marker of neural and nerves sheath differentiation |
| NF | Major citoesqueleto element in nerve axons and dendrites. Consist of three distinct polypeptides (neurofilament triplet) |
| GP 43 | Concanavalin A-binding glycoprotein of 43.000 Dalton |
| NGF | Neurotrophic factor and neuropeptide, primarily involved in the regulation of growth, maintenance, pro-liberation, and survival of neurons |
| SP | Undecapeptide member of tachykinin neuropeptide family released from the terminals of sensory fibers. Act as a neurotransmitter and neuromodulator |
| TH | Enzyme responsible for catalyzing the conversion of the amino acid L-Tyrosine to L-3,4-dihydroxyphenylalanine. Is present in peripheral sympathetic neurons |
| TGF-b | Multifunctional cytokine. When activated, leads to the activation of regulatory proteins, inducing differentiation, chemotaxis, proliferation, and activation of immune cells |
| NPY | 36 amino-acid neuropeptide produced mainly by neurons of the sympathetic system and act as a strong vasoconstrictor and promote growth of fat tissue |
| CGRP | 37 amino-acid peptide produced in both peripheral and central neurons. Act as a strong vasodilator and regulate the transmission of nociception |
| TrKa-A | Specific transmembrane receptor of the NGF |

NF: Neurofilament, GP-43: Glycoprotein protein 43, PGP 9.5: Protein gene product 9.5, SP: Anti-substance-P, TH: Tyrosine hydroxylase, NGF: Nerve growth factor, TGF-B: Transforming growth factor-beta, NPY: Anti-neuropeptide Y, CGRP : Calcitonin gene-related peptide, TrKa-A : NGF high affinity receptor-tyrosine kinase A

blood vessels and shows a clear difference between the mature fibers. They are generally accompanied by mature blood vessels and rarely in contact with endometriotic glandular or stromal tissue.

With these findings, he suggested that nerve fibers related to endometriosis lesions are newly and probably following a process of neuroangiogenesis.

The relation of peritoneal endometriotic implants and pelvic pain was described by Meschner in 2009. Studying 51 patients, he found that the group who assess higher pelvic pain scores and dysmenorrhea (82.6%) had considerably more levels of a NF protein gene product 9.5 (PGP 9.5), but this does not apply to dyspareunia, dysuria, dyschezia, or other types of pain. In this study, there was no correlation between the activity of the lesions assessed on surgery (white, red, and black lesions) and the presence of “endometriotic nerve fibers” within or near it.^[10]

It appears that these lesions had a higher amount of sensory innervations rather than normal tissue. In 2012, 60 patients and 40 endometriotic lesions were studied. Using anti-substance P (SP) as sensory innervation marker, tyrosine hydroxylase (TH) as adrenergic, and PGP-9 as a pan-neuronal immunohistochemical marker, investigators found a higher density of sensory and a lower sympathetic nerve fibers in these lesions compared to a healthy peritoneum. There was no difference in the results when analyzed according to the American society of reproductive medicine stage and menstrual cycle itself.^[17] The same group published a second analysis of this data and showed that the interleukin 1 (IL-1) and nerve growth factor (NGF) expression (proteins involved in fibroblast proliferation, growth, maintenance, and survival of some neurons) on the endometriotic cells were associated with a higher ratio of sensory nerve fibers among others. Finally, his group hypothesized that this NGF and IL-1 have an important role on the development and maintenance of the endometriotic sensory nerve fibers. Posteriorly, Arnold and Pongratz, showed a reduction of the density in the normal peritoneum of both patients, with or without endometriosis. In the endometriosis group, the rate of sympathetic nerves was significantly lower within peritoneal lesion compared to a healthy peritoneum, also seen in other chronic inflammatory diseases.^[18,19]

It is well known that neurons implied in the peritoneal endometriosis nerve fibers are modulated by different calcium-binding proteins as calbindin, calretinin, and parvalbumin. These proteins act as central and peripheral sensibilizers to these neurons, probably representing a link between the development of new nerve fibers and the pain modulation in women with endometriosis. When compared to a normal healthy distant peritoneum, the measure of these proteins proximal to the endometriotic peritoneal lesions

was significantly higher.^[20] Among these calcium-binding proteins, just calretinin was significantly higher on peritoneal fluids of patients with endometriosis and pain symptoms.

Other modulator possibly implied in pain pathways is the transformation growth factor-beta (TGF-B), who plays an important role in the immune response, angiogenesis, and endometrial stromal cell proliferation.^[21] Tamburro and his group published a study of 35 patients and found an elevated intensity of TGF-B in nerve fibers of women with higher pain (dysmenorrhea) scores and active “red peritoneal appearance” lesions in recent surgery.^[22]

Finally, it has been previously described that a higher macrophage density certainly exists on patients affected by the disease. This finding correlated positively with the amount of nerve fibers, both in peritoneal endometriotic lesions and in the healthy peritoneum of women with endometriosis. Thus, they are significantly higher in the global group of endometriotic patients when compares to women without the disease.^[23]

Uterosacral and rectovaginal septum endometriosis

Although the uterosacral ligaments (USL) and rectovaginal septum are accepted as painful locations, the mechanism associated to the pelvic pain and dysmenorrhea is still unclear.^[24,25]

On histological analysis, these lesions are usually deeper and with high rates of fibrosis associated when compared to other locations.

The first to directly study the nerve fibers on these sites was Anaf *et al.* in 2000, publishing his results of measurement of nerve fibers in 28 women with rectovaginal nodules.^[26] He found that patients with more pain on the visual analog scale (up to seven) applied specifically to dyspareunia and dysmenorrhea, had significantly more nerve fibers density within the endometriotic nodule when compared to the group with lower pain symptoms. He also showed that the group of women with pain had more intra- and peri-neural infiltration associated, probably related to this elevated pain scores.

There is evidence that supports the fact that even the immune system can modulate a possible cellular and molecular mechanism for pain on deep infiltrating endometriosis (DIE) lesion at these locations, and not just attribute it to knowing that certainly occurs. Anaf *et al* found a higher mast cell activity in these implants, closer to the nerve fibers location (< 25mm).^[13]

Opposite to the previously described on peritoneal lesions, the USL appears to have more sympathetic nerve fibers. According to Kelm *et al.* in 2008, and despite the methodological bias as a fact, these lesions show an increased

density of sympathetic and parasympathetic nerve fibers when compared to specimens from healthy women.^[27]

In addition, studying 34 DIE lesions of USL, pouch of Douglas, peritoneal sidewall and rectum, Wang found that the density of nerve fibers was significantly higher at these sites compared to peritoneal lesions.^[28] Specifically, he found that the rectal nodules had the highest density of nerve fibers among all. However, the association of these results to clinical pain still in doubt.^[29]

Intestinal endometriosis

Anaf *et al.*, in 2004, published on the Human Reproduction the first study related to nerve fibers density in a bowel endometriosis lesion. Using various immunohistochemistry markers, he found that 53% of the bowel endometriotic nodules were directly invading nerve fibers (or somewhere along their pathways) [Figures 2 and 3].^[30] Posteriorly, the group of Genova led by Ferrero study anatomopathological samples of colorectal endometriosis, and found that the density of sympathetic nerve fibers in the endometriotic nodule was significantly lower (in mucosal and muscular layers) compared to healthy bowel areas. Besides, when assessing the density of sensory fibers near to endometriotic areas and comparing these to healthy bowel tissue, no differences were found. This knowledge allows us to think that this kind of lesions may exist over an environment of an “autonomic-sensorial imbalance” during chronic inflammation process, with greater sensorial rather than sympathetic nerve fibers.

Later, Wang measured the amount of nerve fibers (using NF and PGP 9.5) in the normal bowel, DIE bowel, and no DIE bowel endometriotic lesions. He found that DIE bowel lesions had significantly higher density of nerve fibers when compared to other groups. No differences were found analyzing different intestinal locations. In addition, he concluded that they probably contain a mixture of sensory A and C, adrenergic, and cholinergic fibers within the lesion.^[31]

Finally, even in the presence of a considerable number of studies assessing bowel endometriosis, no one has correctly evaluated the correlation between microscopically and immunohistochemical findings with clinical pain: Thus, their real association remains uncertain.

Major features of posterior compartment endometriosis are show in Table 4.

Ovarian endometriosis [Table 5]

Although initial investigations did not find nerve fibers in samples of ovarian endometriomas submitted to cystectomy,^[32] a later experience will come out to confirm its real existence. Once more, Anaf *et al.* was the first to describe it. The study published by his group in the Human Reproduction in 2006 and Odigiri on the Fertility and Sterility in 2009, using NGF and neural cell

adhesion molecule as markers, showed that endometriomas have a higher density of fibers and mast cells than normal peritoneum, and even peritoneal endometriotic lesions.^[33,34] When the endometrioma tissue is compared to normal ovarian samples, a higher density of nerve fibers is observed too.^[35]

Like other specific sites, ovarian endometriosis nerve fibers show a mixture of sympathetic, parasympathetic and sensory fibers, but interestingly, they do not show the sensorial-sympathetic imbalance (to sensorial) seen in peritoneal and bowel endometriosis.^[35,36] This suggests that ovarian disease had different qualitative neuroangiogenic properties that could explain their clinical presentation.

Zhang and his group evaluated 29 women with ovarian endometriomas with and without pain symptoms and found a significantly higher density of fibers on symptomatic (40%) compared to non-symptomatic (19%) women's. Furthermore, he did not find differences between a solitary lesion or if it is associated to other endometriotic implants. These certainly enhance the role of the nerve fibers by their own in the genesis of disease-related pain symptoms.^[36]

In 2012, Liu studied the role of specific nociceptors in patients with endometriosis. He found that the nerve density

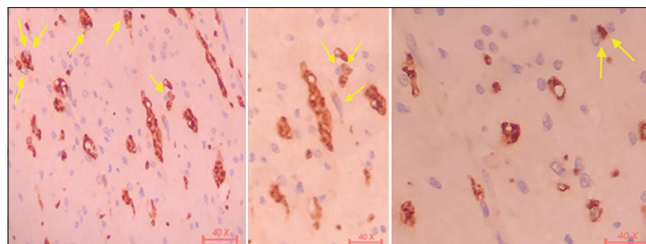


Figure 2: Closer view of the endometriotic nerve fibers. (Sequence of three figures). See the presence of nerve fibers in a deep infiltrating endometriosis bowel implant (stained with protein gene product 9.5) in a magnified field. Note the closer histological relationship between stromal cells and nerve fibers, in some cases apparently invading the nerve (yellow arrows)

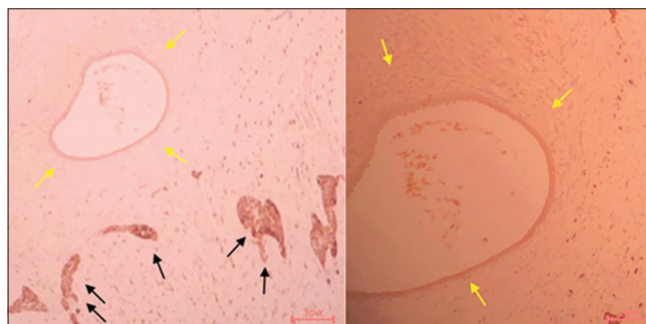


Figure 3: Far view of nerve fibers in intestinal deep infiltrating endometriosis implant. Note the presence of nerve fibers (black arrows) within the stromal cells. Furthermore, a clear endometriotic gland is seen (yellow arrows)

of the transient receptor potential cation channel - 1, which had the function of transmitting heat and pain sensations, was significantly higher in ectopic endometrial tissue. Interestingly, this directly correlated with severity of pain symptoms referred to by the patients.^[37]

Endometriotic lesions, nerve fibers, and hormones

The effect of hormones on nerve physiology has already been studied. Progesterone has a role promoting myelination of nerve structures. Therefore, theoretically, this would protect them against neurodegenerative events generated by any physical trauma.^[38] Meanwhile, in animal models, estrogens have demonstrated an important effect improving sympathetic innervations of nerve fibers, allowing a nerve regeneration and final re-innervation of harm tissues.^[39]

The hormonal effect over the endometriotic lesions was studied by Signorile *et al.* and his group of Italy. He demonstrated the presence of estrogen and progesterone receptors in rectovaginal endometriotic tissue, and in their own nerve fibers, suggesting that these hormones might have a place in maintaining the disease.^[40] Furthermore, it has been hypothesized that hormones, specifically estrogens, might have a duty in the promotion and maintenance of the related pain symptoms.^[41] This is the background that places the hormone treatment as a therapeutic tool causing a downregulation of cellular pathways of NGF, leading to an inhibition of NGF associated nociceptive effects, and finally reducing the pain.

Table 4: Main characteristics in posterior compartment endometriosis

| Location | Characteristics |
|---|---|
| | Deeper lesions with high rate of fibrosis |
| Uterosacral ligament | Higher sympathetic/lower sensorial fibers |
| Rectovaginal septum | Pain probably related to peri-neural nerve invasion, fibrotic tissue and nerve compression 53% positive nerve fibers |
| Bowel | Higher sensorial/lower sympathetic fibers |
| Association between nerve fibers and pain uncertain | |

Table 5: Main characteristics in ovarian endometriosis

| Location | Characteristics |
|---|--|
| | High nerve fibers and mast cells |
| Ovarian endometrioma | No differences in sensorial/sympathetic fibers |
| Probably direct correlation between fiber density and pelvic pain | |

Some studies, as published by Wang on the Fertility and Sterility in 2011, showed no difference in the density of nerve fibers in different moments of the menstrual cycle.^[42]

Contrasting this, Tokushige published her experience with over 22 women with peritoneal endometriotic lesions treated with progestin or combined oral contraceptive, and found a significant decrease of nerve fibers density when compared to non-treated patients. Nevertheless, this work had the bias of not assessing pain symptoms referred, so they cannot prove that hormone treatment has real clinical effects.^[43]

For the present, the effect of medical - hormonal treatment over the pain symptoms and nerve fibers density remains controversial.

DIE, nerve fibers, and pain

DIE is defined as an infiltration of peritoneum surface deeper than 5 mm, affecting between 4% to 37 % of total endometriotic patients, with the urinary and intestinal tract involvement as the most severe presentations.

General histopathological analysis shows that DIE nerves fibers usually expresses TH, SP, anti-neuropeptide Y, and calcitonin gene-related peptide markers, without significantly density differences among all. This would indicate that DIE lesions, as itself, had a mixture of sympathetic and sensory nerve fibers no matter their anatomical location.^[31] Likewise, it is known that endometriotic glands and estroma generally express NGF and NGF high-affinity receptor, growth factor needed for survival and maintenance of sensory and sympathetic neurons. This information is essential to search and read the results of any study related to endometriosis, pain symptoms, and nerve fibers.

In 2004, a couple of case reports by Quinn found a uniform higher amount of nerve fibers in USL DIE lesions, suggesting that these lesions might be special.^[44,45]

In the same path, Wang demonstrated in 2009 that DIE Implants contain more nerve fibers than healthy peritoneum, enhancing the link between nerve fibers and DIE lesions.^[31]

Later, McKinnon *et al.*, in 2012, strengthened the concept of a DIE lesion as an individual category related to high nerve fibers density and pain, finding an uniformly high density of fibers in DIE Implants no matter their anatomical location.^[29]

Finally, many studies have demonstrated that DIE lesions have a large number of nerve fibers when compared to no DIE lesions, but the reason as to why this occurs remains unclear. One hypothesis refers to the fact that this type of endometriosis could represent a further step in the disease and may have a higher potential of promoting nerve growth when compared to other types of lesions. Furthermore, many investigators think that a pro-inflammatory reaction of the DIE implant leads to more neuroangiogenesis than other kinds of lesions.

The direct association of this higher nerve density and the presence of clinical pain is more clear for peritoneal endometriosis, but still uncertain for other sites.^[29]

Evidence coming from Anaf *et al.* supports the fact of increased activity of mast cells in DIE nodule and their probably relationship to pain symptoms as cyclic pain and hyperalgesia, commonly seen in the disease.^[13]

DISCUSSION

The complete knowledge of pain mechanism caused by endometriosis is still unknown, and it is probably multifunctional, requiring the interaction of the endometriotic lesion with different nerves structures and mediators. Initial studies of Sturt in the 90s showed that functional glands and fibrotic reaction are both needed for pain mediation and the development of the peritoneal endometriosis.^[46]

It is known that the anatomical location of the implant will have a specific correlation with pain.^[31]

Besides, the chronic cyclic intra-abdominal bleeding found in endometriosis could explain the classical dysmenorrhea referred to by the patients. In cases where the disease compromises fixed structures as cervix, upper vagina and rectosigmoid, specific symptoms as Dyschezia and dyspareunia would arise due to the alteration of its natural position and the limitation of their range of movements.

Nowadays, two big mechanisms of the interaction between endometriosis and nerves have been described with the purpose to explain the genesis of pain symptoms.

The first is the direct contact between endometriosis and the nerve fiber. Anaf demonstrated that the invasion of the endo-neural or peri-neural tissue by the disease is strongly correlated with pain.^[26] This neural invasion finally creates a nerve lesion, similarly seen in other painful gynecologic and extra-gynecologic conditions such as chronic pancreatitis, pancreatic carcinoma, and carcinoma of the Bartholin gland.^[47] Furthermore, in 2005, Remorgida *et al.* described that bowel endometriosis can lead to Cajal cell damage, responsible of many bowel symptoms.^[48]

Second, it is well accepted that endometriosis is an inflammatory process, with abnormal function of immune cells and increased activity of macrophages, which can interact and enhance the growth of nerve endings within the lesions.^[49] Likewise, active production of chemotactic molecules, macrophages, pro-inflammatory cytokines, mast cells activity, and recounting immune cells is usually found. All these processes could explain the maintenance, but mostly, the advance of the disease.^[50]

As well, this it is probably a direct cause of pain, by the forthright effect of the neurotropic and neuroprotective cytokines activity. In the same way and reinforcing this concept, it is well documented that anti-inflammatory medications are effective in reducing the pain, enhancing the role of inflammation in their pathophysiology.^[51,52]

Along with this and following the results presented by Anaf *et al.* on the fertility and sterility, apparently there is a relationship between nerves, local degranulating of mast cells, and pain symptoms. In DIE implants there is evidence of increased activity of these cells with larger de-granulation events, which might contribute in the genesis of the cyclic pain and hyperalgesia.^[13]

On the other hand, the relationship between nerve fibers and their relation to pain symptoms was also studied by Anaf *et al.* Analyzing 31 DIE vaginal nodules with NF marker, they found that the nerve density is 2–3 times higher within the nodule, when compared to a healthy vagina tissue. Once again, this emerged as a new and direct explanation of the severe neuropathic pain associated to these lesions.^[53]

Multiple theories have been born trying to explain this nerve high density. One of them states that this increase might occur due to the persistence of poorly differentiated (or undifferentiated) cells inside the endometriotic lesions. Aforementioned cells could be turned into neurons under the influence of a specific stimulus, finally leading to a mature nerve fiber.^[54]

Other explanations arise on the potential effect of neurotrophins on nerve regeneration. As follows, when a nerve injury occurs, neurotrophins allow an increase of several sprouts from one axon, raising the final number of them. Conclusively, there will be a large number of fibers due to this “sprout effect,” explaining the high density of nerve fibers frequently seen.^[55]

In 2012, McKinnon *et al.* published an observational study of 48 endometriotic lesions searching for a relationship between endometriosis, pain symptoms, and peritoneal fluid cytokine concentrations. He found that a vast majority of painful endometriotic implants has nerve fibers in its histopathological analysis and also a higher level of cytokines in peritoneal fluid. Among all anatomical locations, the rectovaginal septum shows a significantly higher concentration of fibers,

peritoneal fluid cytokines, and pelvic pain. Furthermore, patients with ovarian lesions reported less pain symptoms and were less likely to find nerve fibers within it. These results certainly correlate with what we usually see in our clinical practice.^[29]

It is strongly accepted that DIE lesions have more nerve fibers and severe pain than no DIE lesions, especially in the rectovaginal area.^[26,31]

Although peritoneal implants are not usually related to severe pelvic pain or higher density of fibers when compared to other pelvic areas, it has been specifically described that the proximity of the fibers to these lesions is directly associated with dysmenorrhea.^[10,29]

The rectovaginal nodule is usually associated to severe pain in the clinical practice, and this correlation has also been found in the literature.^[25] This septum is in close proximity to the sympathetic nervous system, and in such a way, is consistently highly innervated. Thus, it is probable that all DIE lesion in this area directly affects the nerve or their pathways regarding its size, causing pain by direct invasion of the nerve.^[29]

In the ovarian endometrioma, the alliance between pain and nerve fibers is contradictory and remain as an unclear affair. While some studies find a higher amount of nerves within it, others show a lower density of fibers (using S-100 immunohistochemical marker)^[13] or no nerves using NF.^[32] Although the possible bias of the studies, both nerve fiber density and pain symptoms appear to be lower than other anatomical locations.

The in-depth understanding of the biology and nature of the pain symptoms, their mechanisms, biology's paths, mediators, and their final correlation with histopathological findings will be relevant for the clinician, since it will allow an adequate understanding of the disease, therapeutic approach and final benefit of the medical intervention on a patient's quality of life.

It is necessary to confirm the final association between the histopathological results and endometriosis-related - clinical symptoms through large-scale studies with adequate internal validation. Similarly, it will be interesting to know the force of this association in different anatomical locations, with the aim to offer a rationale and individualized treatment of the disease.

CONCLUSION

The endometriotic lesion shows a greater density of nerve fibers on the histopathological assessment when compared to normal tissue. Although the type of nerve fibers within

the lesions, tend to be more sensorial than normal healthy tissue, there are inconstant and will vary according to the type and site of lesions. The DIE lesions had a greater density of nerve fibers than no DIE lesions, regarding its location. At present, the clear association between these histopathological findings to pain symptoms is still poorly known and more studies are needed.

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