



Mechanisms of peripheral sensitization in endometriosis patients with peritoneal lesions and acyclical pain

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Abstract

Purpose Endometriosis (EM) is one of the most frequent differential diagnoses concerning chronic pelvic pain. Women under hormonal therapy (HT) often benefit from it but sometimes suffer a setback and develop acyclical pelvic pain. Due to the assumption that mechanisms of neurogenic inflammation are involved in the generation of chronic pelvic pain, we aimed to investigate the expression of sensory nerve markers in EM-associated nerve fibers of patients with/without HT.

Methods Laparoscopically excised peritoneal samples from 45 EM and 10 control women were immunohistochemically stained for: PGP9.5, Substance P (SP), NK1R, NGFp75, TRPV-1, and TrkA. Demographics and severity of pain were documented.

Results EM patients showed a higher nerve fiber density (PGP9.5 and SP) and increased expression of NGFp75, TRPV1, TrkA, and NK1R in blood vessels and immune cells compared with controls. Patients with HT have cycle-dependent pelvic pain but suffer from acyclical pelvic pain. Interestingly, reducing NK1R expression in blood vessels under HT was observed. A correlation between dyspareunia severity and nerve fibers density and between NGFRp75 expression in blood vessels and cycle-dependent pelvic pain severity was observed.

Conclusion Patients under HT have no ovulation and no (menstrual) bleeding, which correlate with inflammation and cyclical pain. However, acyclical pain seems to be due to peripheral sensitization once it is present under treatment. Neurotransmitters, like SP and their receptors, are involved in mechanisms of neurogenic inflammation, which are relevant for pain initiation. These findings indicate that in both groups (EM with/without HT), neurogenic inflammation is present and responsible for acyclical pain.

Keywords Endometriosis · Nociceptive markers · Nociceptive receptors · Hormonal treatment · Pelvic pain

What does this study add to the clinical work

Endometriosis (EM) is one of the most frequent differential diagnoses concerning chronic pelvic pain. Our findings indicate that in EM patients with and without hormonal therapy, neurogenic inflammation is present and responsible for acyclical pain.

Introduction

Characterized by the ectopic deposition and growth of endometrial-like tissues, endometriosis (EM) is an estrogen-dependent and inflammatory disorder [1]. EM lesions infiltrate adjacent organs (e.g., genitals, bladder, intestine, abdomen), resulting in inflammation, formation of scar tissue, and functional impairments of affected organs [1, 2]. The symptoms often affect patients' psychological and social well-being and impose a substantial economic burden on society. For this reason, EM is considered a disabling condition that may significantly compromise social relationships, sexuality, and mental health [3–5]. Approximately 10% of women of reproductive ages are affected, i.e., 2 million women in Germany and 270 million worldwide, and 30–50% of them suffer from infertility [6]. Despite its negative impact on the quality of a patient's life, many issues related to EM remain unclear.

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Due to the duration of pain and dissemination of endometriotic lesions, the associated symptoms showed a wide variation including cyclic and acyclic lower abdominal pain, dysmenorrhoea, dyspareunia, dyschezia, dysuria and sub- or infertility. The pathogenesis of pain generation is very complex [1, 7, 8]. Recent evidence demonstrates that the peripheral nervous system plays an important role in the pathophysiology of this disease. Our group focus on understanding pain generation due to peritoneal lesions and already demonstrated wide changes in the innervation in the EM-affected peritoneum [9]. The higher density of sensory nerve fibers, the lower density of sympathetic nerve fibers, the release of proinflammatory neurotransmitters like SP and CGRP as well as periendometriotic inflammation suggested neurogenic inflammatory reaction in this tissue. Peripheral sensitization of EM-associated nerve fibers might be one key player in the modulation and severity of pain [10].

Because the pathogenesis of EM is still unresolved, no causal treatment options are available. The primary treatment goals are to relieve pain and eliminate fertility issues in women who wish to conceive [11]. Hormonal therapy is the first line of treatment for women with EM [12]. This treatment decreases the production of the estrogen-induced release of prostaglandins and consequently inflammation [13]. With continuous hormonal treatment, dysmenorrhoea may be reduced compared to cyclic use, but the incidence of erratic bleeding may increase, and safety issues have not been fully studied [12]. Also, the development of acyclical pain under hormonal treatment is possible and described. We observed a high grade of inflammation especially in peritoneal lesions of symptomatic patients under hormonal treatment [7]. Taking together, we aimed to understand this pathway of pain generation in more detail and investigate the expression of sensory nerve markers in the periphery of peritoneal EM under the influence of hormonal therapy. The characteristics of tissue were also analyzed concerning the acyclic pelvic pain experience of EM patients.

Methods

Patients

This prospective study enrolled 55 women. Forty-five EM patients, who underwent laparoscopy due to symptomatic EM with excision of endometriotic lesions, were included. The diagnosed EM was staged according to the revised classification of the American Society of Reproductive Medicine (rASRM) as I: minimal, II: mild, III: moderate, and IV: severe. In the analysis, two stages had been considered: mild (rASRM I and II) and severe (rASRM III and IV). Ten control samples were collected from women

without EM, who had undergone laparoscopy for benign gynaecological presentations such as nonendometriosis associated with ovarian cysts, uterine fibroids, hydrosalpinx, pelvic pain, peritonealized tissue or the unfulfilled wish to have children.

Patients were selected based on clinical intraoperative and subsequent histopathologic findings. All patients had been given a complete gynaecological examination. The severity of pain was documented using a standardized questionnaire with a visual analog scale (VAS). The pain intensity was determined with the help of a visual numerical analog scale (0 = no pain, 10 = strongest imaginable pain). The women were divided into two groups based on the pain scale: moderate pain (0–5 on the scale) and severe pain (6–10 on the scale).

The study was approved by the Institutional Review Board of the Charité University Medical Centre (Ethic vote EA4/036/12). All patients gave their consent.

Sample collection and immunohistochemistry of peritoneal endometriotic lesions processing

All the surgically excised lesions (EM patients) and healthy peritoneum (control samples) were immediately fixed in buffered formalin 4% for 12 h and thereafter embedded in paraffin. Two μm thickness sections were immunohistochemically stained with antibodies (Supplemental Table I) against the nerve fibers markers: protein gene product 9.5 (PGP9.5), Substance P (SP); and nociceptive receptors: Neurokinin-1 Receptor (NK1R), Nerve Growth Factor Receptor p75 (NGFp75), Transient Receptor Potential Vanilloid 1 (TRPV-1), and Tropomyosin Receptor Kinase A (TrkA).

Negative control sections were processed by omitting the specific primary antibody. A skin incision and a tissue section of peritoneal EM with large nerve incisions were used as the positive control. Staining was detected using an axiophot (Carl Zeiss, Göttingen, Germany) microscope. Photomicrographs were taken at different magnifications (100 and 400) and were further processed using Adobe Photoshop (Adobe Systems, Unterschleissheim, Germany).

Determination of nerve fiber density

The density of PGP9.5 and SP-positive nerve fibers was assessed by counting the number of immunostained nerves proximal to the endometriotic lesions (epithelial, stromal, and smooth muscle cells) and in the distal area at 1 mm^2 .

The “hotspot” method [14] was used to determine the nerve fiber density of the control tissue. The immunostained section was scanned at low magnification (10 \times), and the

tissue area with the greatest number of nerves (“hotspot”) was selected. Five hotspots were evaluated and averaged for each control. The density was measured by the sequential assessment of two investigators. In cases of discrepant results, both observers repeated the analysis together and reached a consensus.

Statistical analysis

Statistical analysis was performed using Graphpad Prism 9 and non-parametric (Mann–Whitney-*U*, Wilcoxon, Kruskal–Wallis or Spearman correlation test). χ^2 and Fisher’s exact tests were used for the qualitative variable. Statistically significance was assumed for $p < 0.05$.

Results

Patients’ characteristics

Demographic and clinical variables for the 55 women recruited for this study are summarized in Table 1. Our case group comprised 45 EM patients, 20 (44.44%) presented minimal to mild EM (rASRM I and II) and 25 (55.56%) moderate to severe (rASRM III and IV). Twenty-three (23/45) of them were under hormonal therapy at the time of the surgery. Of these 23, 8 women received progestin-only therapy, 10 a combined progestin–estrogen, and in 5 EM patients, the preparation taken could no longer be determined. The mean age of the EM patients was 31.1 (19–53) years. EM patients who took hormones were on average younger (28.1) than women who did not take hormonal preparations (34.3; $p = 0.0037$).

The control group was a compost of ten patients, four of them received hormonal therapy. In one case, it was a pure progestin therapy, in two others, a combined progestin–estrogen therapy and in one other case, the product ingested could no longer be determined. Women in the control group were on average 32.1 (20–46) years old. No significant difference in age between the non-EM patients who took hormones (32.5) and the ones who did not take (31.8) was observed.

Pain characterization

Forty-two EM patients (95.5%) reported pelvic pain. One patient under hormonal treatment reported no pelvic pain. In two cases, no statement was made, these women were not taking any hormones. In the control group, six patients (54.5%, 3 under hormonal therapy) stated to suffer from pelvic pain. Three (27.3%) denied suffering from this pain

(one under hormonal therapy), and in two cases (18.2%), no information was given. All pain and patient characterization are summarized in Table 1.

Cycle-dependent pelvic pain (CDPP)

Of the 42 EM patients reporting pelvic pain, 15 (33.3%) communicate suffering from CDPP. Four of them were on hormonal therapy and eleven were not. A statistical difference ($p = 0.0096$) could be seen in the hormonal therapy and this pain. No CDPP was reported in seven EM patients (one without hormonal therapy). For 23 patients with EM (13 positives for hormone therapy), this information was missing. In the control group, three (27.3%) women reported CDPP. Two (18.2%) of them were on hormonal therapy. In seven cases (70%), this statement was missing.

Regarding the strength of the CDPP, EM patients (data from 13 women—10 negatives for hormonal therapy) suffered on average from a pain level of 5.5 (2–9). No difference in the average pain severity was found between EM patients who were on hormonal treatment (6.7; 4–7) and those who were not (5.1; 2–8). In the control group (data from three patients), the severity of the CDPP was an average of 6 (4–9) and did not differ from the group of EM patients.

Cycle-independent abdominal pain (CIAP)

Only ten (22.2%) EM patients describe suffering from CIAP with an average severity of 5.7 (2–8). Five EM patients under hormonal therapy report on average a CIAP of 5.2 (4–7) and for the five patients without hormonal therapy, a pain average of 6.3 (2–8). Two (20%) women under hormonal therapy from the control group also describe feeling this pain in severity 2 and 3.

Dysmenorrhea

Dysmenorrhea or painful bleeding in cyclical modus of combined pills (withdrawal bleeding) was a symptom communicated from 39 (86.7%) EM patients, with 21 under hormonal therapy. Two patients (4.4%—1 receiving hormonal treatment), said do not suffer from this and the other four (8.9%) did not answer. In the control group, dysmenorrhea was expressed as a symptom for four women (40%), two of these being on hormonal therapy. Two patients (20%, 1 taking hormones) affirmed no painful bleeding in the cyclical modus of combined pills. This information was missing for four patients (40%) in the control group, two under the hormonal treatment.

The severity of the dysmenorrhea or painful bleeding was informed for 21 EM patients (46.7%), 9 of them were under

Table 1 Characteristics of the study population

Group	Nr	Age	rASRM	Hormonal therapy	Pain characterization (pain intensity)				Dysmenorrhea	Dyspareunia	Dyschezia	Dysuria
					Pelvic pain	Cycle-dependent pelvic pain	Cycle-independent pelvic pain					
EM patients	H-	1	35	Severe	-	Yes	NA	NA	Yes (NA)	NA	NA	NA
		2	33	Mild	-	Yes	Yes (3)	No	Yes (3)	Yes (2)	No	No
		3	32	Severe	-	Yes	Yes (NA)	NA	Yes (3)	Yes (10)	No	No
		4	39	Mild	-	Yes	Yes (2)	NA	Yes (3)	Yes (NA)	Yes (NA)	NA
		5	43	Severe	-	Yes	NA	NA	Yes	NA	NA	NA
		6	25	Severe	-	Yes	Yes (8)	Yes (6)	Yes (7)	Yes (8)	No	No
		7	29	Severe	-	Yes	Yes (5)	No	Yes (7)	Yes (1)	No	No
		8	36	Severe	-	Yes	Yes (5,5)	Yes (7,5)	Yes (5,5)	Yes (4,5)	No	No
		9	53	Severe	-	Yes	NA	NA	Yes (NA)	NA	Yes (NA)	NA
		10	27	Severe	-	Yes	Yes (3)	No	Yes (3)	Yes (3)	Yes (3)	No
		11	32	Severe	-	Yes	Yes (5)	No	Yes (7)	Yes (2)	Yes (6)	No
		12	42	Severe	-	Yes	NA	NA	NA	No	Yes (NA)	No
		13	26	Severe	-	Yes	NA	NA	Yes (NA)	Yes (NA)	Yes (NA)	Yes (NA)
		14	30	Severe	-	NA	NA	NA	NA	NA	NA	NA
	H+		15	38	Severe	-	Yes	Yes (5)	Yes (2)	Yes (4)	Yes (2)	Yes (4)
		16	29	Mild	-	Yes	NA	NA	Yes (NA)	Yes (NA)	No	Yes (NA)
		17	28	Mild	-	Yes	Yes (6)	Yes (8)	Yes (8)	Yes (3)	No	Yes (5)
		18	22	Severe	-	Yes	Yes (9)	Yes (8)	Yes (9)	Yes (9,5)	No	Yes (6)
		19	42	Severe	-	Yes	NA	NA	Yes (NA)	NA	NA	NA
		20	38	Mild	-	Yes	No	No	Yes (3)	Yes (2)	Yes (5)	No
		21	27	Mild	-	Yes	NA	NA	NA	NA	NA	NA
		22	49	Severe	-	NA	NA	NA	No	NA	NA	NA
		23	22	Mild	E, POP	No	NA	NA	NA	NA	NA	NA
		24	26	Mild	COC	Yes	No	Yes (6)	Yes (0)	No	No	No
		25	19	Mild	COC	Yes	NA	NA	Yes (NA)	Yes (NA)	Yes (NA)	NA
		26	35	Mild	COC	Yes	Yes (7)	Yes (7)	Yes (8)	Yes (7)	Yes (5)	No
		27	28	Severe	NA	Yes	NA	NA	Yes (NA)	Yes (NA)	No	No

Table 1 (continued)

Group	Nr	Age	rASRM	Hormonal therapy	Pain characterization (pain intensity)				Dyspareunia	Dyschezia	Dysuria
					Pelvic pain	Cycle-dependent pelvic pain	Cycle-independent pelvic pain	Dysmenorrhea			
	28	35	Mild	POP	Yes	NA	NA	Yes (NA)	NA	NA	NA
	29	26	Mild	NA	Yes	NA	NA	Yes (NA)	No	NA	Yes (NA)
	30	23	Mild	POP	Yes	NA	NA	Yes (NA)	Yes (NA)	NA	NA
	31	30	Severe	POP	Yes	Yes (5)	No	Yes (7)	Yes (3)	Yes (7)	No
	32	22	Severe	POP	Yes	No	Yes (4)	Yes (6)	Yes (7)	Yes (8)	Yes (5)
	33	39	Mild	COC	Yes	NA	NA	Yes (NA)	Yes (NA)	No	NA
	34	32	Mild	POP	Yes	No	NA	Yes (6)	Yes (NA)	No	No
	35	36	Mild	COC	Yes	NA	NA	Yes (NA)	Yes (NA)	Yes (NA)	No
	36	27	Mild	NA	Yes	NA	NA	Yes (NA)	NA	NA	NA
	37	29	Severe	POP	Yes	No	NA	Yes (0)	Yes (5)	No	No
	38	23	Severe	COC	Yes	No	Yes (4)	Yes (10)	Yes (6)	Yes (8)	No
	39	25	Severe	COC	Yes	NA	NA	Yes (NA)	NA	No	No
	40	24	Severe	NA	Yes	Yes (NA)	Yes (5)	No	Yes (NA)	No	No
	41	27	Mild	NA	Yes	NA	NA	Yes (NA)	NA	Yes (NA)	NA
	42	30	Severe	COC	Yes	NA	NA	Yes (NA)	NA	NA	Yes (NA)
	43	24	Mild	POP	Yes	No	No	Yes (4)	No	Yes (3)	No
	44	35	Mild	POP	Yes	NA	NA	Yes (NA)	NA	NA	NA
	45	30	Severe	COC	Yes	Yes (8)	NA	Yes (8)	Yes (NA)	No	Yes (NA)
Control	C1	26	-	-	Yes	NA	NA	No	NA	NA	NA
	C2	38	-	-	NA	NA	NA	NA	NA	NA	NA
	C3	45	-	-	NA	NA	NA	NA	NA	NA	NA
	C4	27	-	-	Yes	Yes (9)	No	Yes (3)	Yes (9)	No	No
	C5	28	-	-	No	NA	NA	Yes (NA)	Yes (NA)	NA	NA
	C6	27	-	-	Yes	NA	NA	NA	NA	NA	NA
	C7	46	-	NA	No	NA	NA	No	NA	NA	NA
	C8	43	-	POP	Yes	NA	NA	NA	NA	NA	NA
	C9	20	-	COC	Yes	Yes (5)	Yes (2)	Yes (NA)	NA	No	NA
	C10	21	-	COC	Yes	Yes (4)	Yes (3)	Yes (4)	Yes (4)	Yes (4)	Yes (2)

H+ hormonal therapy, H- no hormonal therapy, mild rASRM I and II, severe rASRM III and IV, E estrogen, POP progestogen, COC combined pills, NA no information

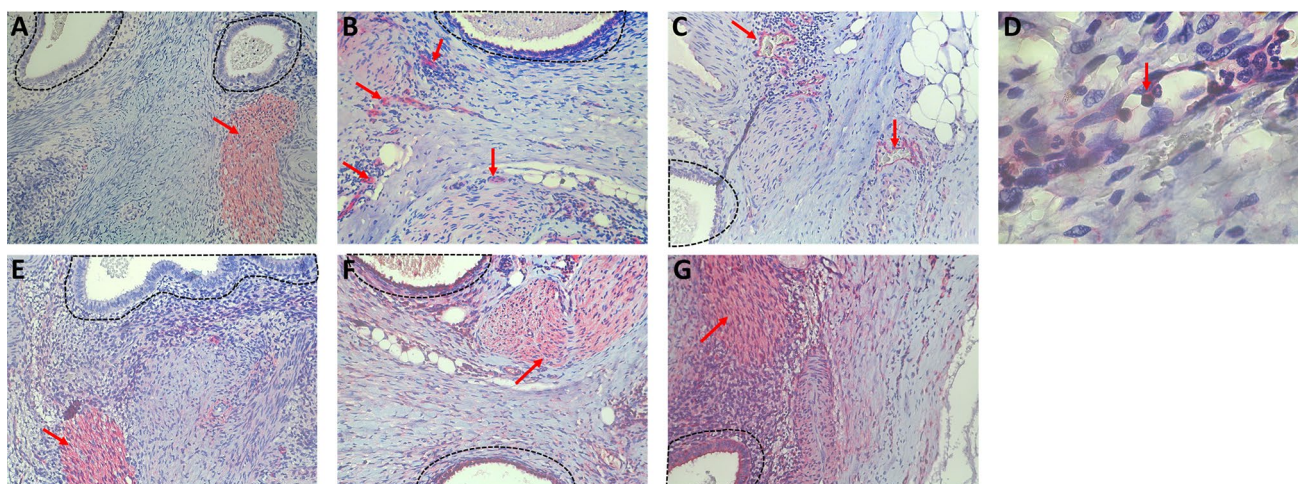


Fig. 1 Expression of PGP9.5 (A), Substance P (B), NK1R-positive blood vessels (C), immune cells (D), NGFRp75 (E), TRPV1 (F), and TrkA (G) (red arrow) surrounding the endometriotic lesion (black

marked) was counted. All pictures are in 400× magnification, except D, which is in 1000× magnification

hormonal therapy. No statistical difference was found in the severity of the dysmenorrhea between EM patients who were under hormonal therapy (5.4; 0–10) and those who were not (5.2; 3–9). In the control group, two (20%) women reported the severity of this symptom. One patient who was taking hormones reported a pain level of 4 while the other, who was not taking hormones, reported a pain level of 3.

Dyspareunia

Twenty-seven (60%, 13 on hormonal therapy) EM patients reported dyspareunia. Four women (8.9%, 3 under hormonal treatment) stated no pain during sexual intercourse. This information was not given for 14 EM patients (31.1%, 7 under hormonal treatment). No information was given for six women (60%, two received hormones, four did not) from the control group. Three patients (27.3%, one taking hormones) suffered from dyspareunia. Only one control patient (10%), who was under hormonal therapy, affirmed not to have this pain.

EM patients reported an average degree of dyspareunia of 4.7 (1–10). Five patients, who were under hormonal therapy, reported a mean pain score of 5.6 (3–7). The other 11, who were not taking hormones, reported an average pain of 4.3 (1–10). Two patients from the control group (one patient in hormonal therapy) reported a pain score of 4 and 9.

Dyschezia

Dyschezia was indicated as a symptom in 16 (35.5%) patients with EM, 8 of them on hormonal therapy. Another 16 women (35.5%) stated that they did not have dyschezia. Of these, eight women took hormones. Thirteen EM patients

(28.9%) did not answer this question. In the control group, one woman who received hormones suffered from dyschezia severity 4. Two women, one of them taking hormones, denied suffering from this condition (20%). In seven cases (70%, two did take hormones), the corresponding information was lacking in the questionnaire.

A mean pain severity of 5.4 (3–8) concerning dyschezia was given in 16 EM patients (35.6%). Eight patients who were on hormone therapy estimated pain intensity to be a mean of 6.2 (3–8). However, the other eight women who did not receive hormones reported an average pain of 4.5 (3–6).

Dysuria

Eight (17.8%) EM patients suffered from dysuria, four of them were on hormone therapy. In this group (only three patients answered this question), the average severity of the dysuria was given as 5.3 (5–6). In 21 cases (46.7%, 11 on therapy), the dysuric pain was denied and in 16 cases (35.6%), the information was missing. One control patient affirmed suffering from dysuria with a severity of 2, another denied it. Both were under hormonal treatment. In eight cases (80%), this information was missing.

Immunohistochemistry results

All the results are presented as median, 25–75% percentile. Figure 1 shows the immunohistochemistry results as an example.

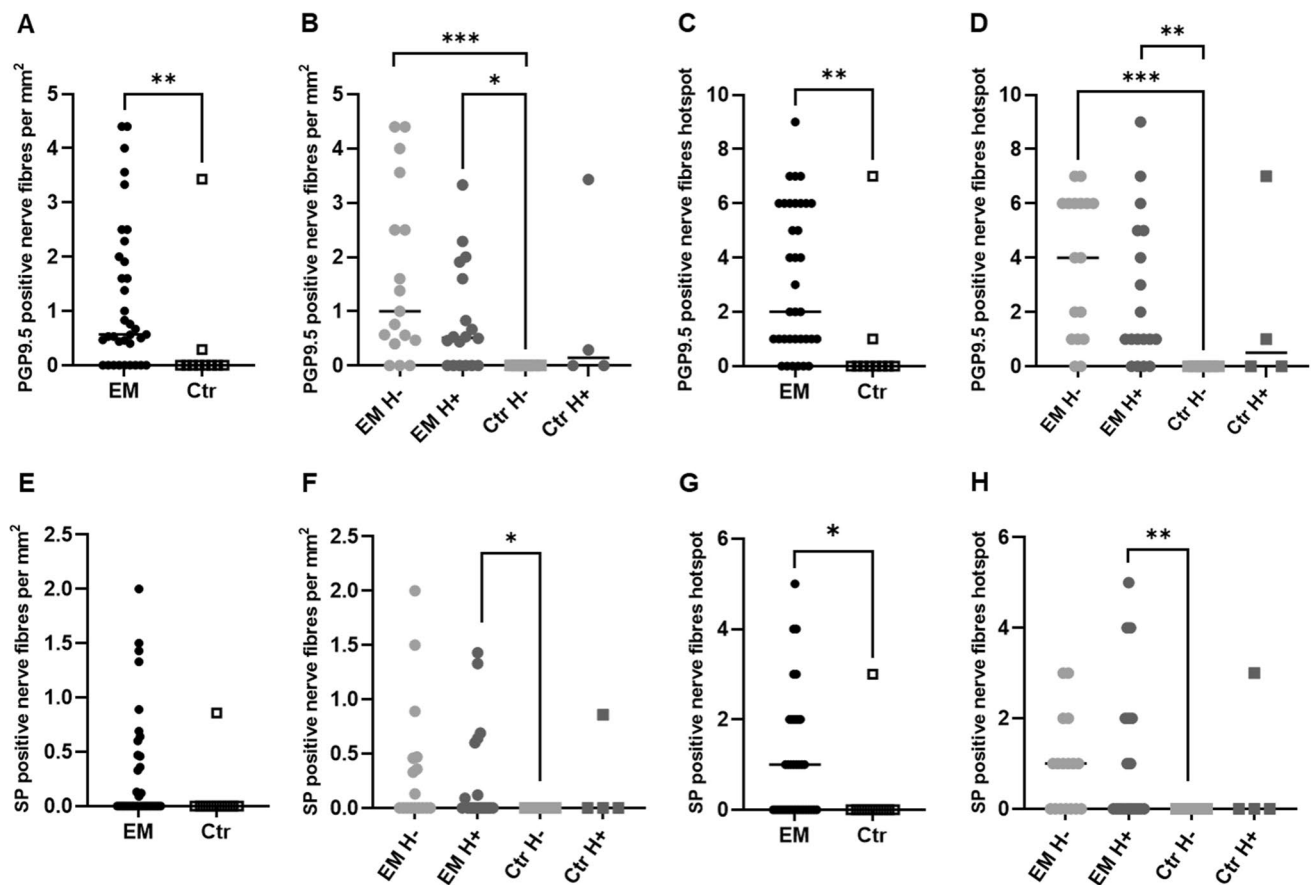


Fig. 2 Nerve fibers density in endometriotic lesions and healthy peritoneum. PGP9.5-positive nerve fibers per mm^2 (A–B) and hotspot (C–D). Substance P (SP)-positive nerve fibers per mm^2 (E–F) and hotspot (G–H). EM endometriosis patients, Ctr control, H+ under

hormonal treatment, H– without hormonal treatment; all the results are presented as median, 25–75% percentile. Mann–Whitney test and Kruskal–Wallis with Dunn’s multiple comparison tests. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Nerve fibers density in endometriotic lesions

Using anti-PGP9.5 and anti-SP, nerve fibers were detected in peritoneal specimens from women with EM and healthy peritoneum from women without EM. PGP9.5 nerve fibers density was significantly increased in endometriotic lesions (0.57, 0.0–2.0) compared to healthy peritoneum (0.0, 0.0–0.072; $p = 0.0079$) both in the view of nerve fibers per mm^2 and in the hotspot image (EM: 2.0, 1.0–6.0; Control: 0.0, 0.0–0.25; $p = 0.0017$) (Fig. 2A and C). EM patients showed significantly more SP-positive nerve fibers in the hotspot view (EM: 1.0, 0.0–2.0; control: 0.0, 0.0–2.0; $p = 0.0393$) but no difference in the density of SP-positive nerve fibers per mm^2 (EM: 0.0, 0.0–0.47; control: 0.0, 0.0–0.86; $p = 0.0874$) (Fig. 2E and G).

When the hormonal therapy is taken into consideration, statistical significance is observed in the EM group with (EM H+) and without hormonal intake (EM H–) compared with the control without treatment (Ctr H–) for PGP9.5 both in the nerve fibers per mm^2 (EM H+: 0.51, 0.0–1.7;

$p = 0.0123$; EM H–: 1.0, 0.4–3.0; $p = 0.0001$; Ctr H–: 0.0, 0.0–0.0) and in the hotspot image view (EM H+: 1.0, 0.7–5.0; $p = 0.0028$; EM H–: 4.0, 1.0–6.0; $p < 0.0001$; Ctr H–: 0.0, 0.0–0.0) (Fig. 2B and D).

No correlation could be seen between the nerve density and the rASRM stages. The PGP9.5 (hotspot view, $r = 0.728$; $p = 0.0029$) and SP (hotspot and nerven/ mm^2 , $r = 0.5741$; $p = 0.0278$ and $r = 0.7118$; $p = 0.004$, respectively) nerve fibers density correlated with dyspareunia pain levels (Table 2).

Increased expression of NK1R in blood vessels and immune cells of EM patients

EM patients presented more NK1R-positive stained vessels than the control group (EM: 16.0, 2.0–32.0; Ctr: 4.5, 0.75–7.75; $p = 0.0302$) (Fig. 3A). A statistical difference could also be found when the treatment was taken into account. EM patients under hormonal therapy showed fewer NK1R-positive stained vessels compared with EM patients without treatment (EM H+: 11.0, 0.0–24.0;

Table 2 Correlation analysis

		P value	
Hormonal therapy in EM ^a	Cycle-dependent pelvic pain	0.0096*	
	Cycle-independent lower pain	0.377	
	Dysmenorrhea	0.9153	
	Dyspareunia	0.3159	
	Dyschezia	>0.9999	
	Dysuria	0.9087	
Pain level in EM and Control ^a	Cycle-dependent pelvic pain	0.6866	
	Cycle-independent lower pain	0.356	
	Dysmenorrhea	0.2105	
	Dyspareunia	>0.9999	
	Dyschezia	>0.9999	
	Dysuria	>0.9999	
Pain level and hormonal therapy ^a	Cycle-dependent pelvic pain	0.5846	
	Cycle-independent lower pain	0.2308	
	Dysmenorrhea	0.1009	
	Dyspareunia	0.551	
	Dyschezia	0.2745	
	Dysuria	>0.9999	
Pain level and rARSM ^a	Cycle-dependent pelvic pain	0.703	
	Cycle-independent lower pain	0.076	
	Dysmenorrhea	0.628	
	Dyspareunia	0.404	
	Dyschezia	0.106	
	Dysuria	0.022*	
Cycle-dependent pelvic pain and nerve fibres density/ nerve fibres receptors ^b	NGFR	Hotspot	0.7461
		Nerve fibres/ mm ²	0.8456
		Blood vessels/ mm ²	0.0334*
	NK1R	Blood vessels/ mm ²	0.2129
		Immune cells/ mm ²	0.6101
	PGP9.5	Hotspot	0.8502
		Nerve fibres/ mm ²	0.5730
	SP	Hotspot	0.3114
		Nerve fibres/ mm ²	0.7346
	TrkA	Hotspot	0.8476
		Nerve fibres/ mm ²	0.9604
		Blood vessels/ mm ²	0.6203
	TRPV1	Hotspot	0.3552
		Nerve fibres/ mm ²	0.7353
		Blood vessels/ mm ²	0.2547
Cycle-independent lower pain and nerve fibres density/ nerve fibres receptors ^b	NGFR	Hotspot	0.7905
		Nerve fibres/ mm ²	0.6111
		Blood vessels/ mm ²	0.6299
	NK1R	Blood vessels/ mm ²	0.2234
		Immune cells/ mm ²	0.2682
	PGP9.5	Hotspot	0.4417
		Nerve fibres/ mm ²	0.3664
	SP	Hotspot	0.7659
		Nerve fibres/ mm ²	0.9865
	TrkA	Hotspot	0.6990
		Nerve fibres/ mm ²	0.7947
		Blood vessels/ mm ²	0.5098
	TRPV1	Hotspot	0.5463
		Nerve fibres/ mm ²	0.4317
		Blood vessels/ mm ²	0.5088
Dysmenorrhea and nerve fibres density/ nerve fibres receptors ^b	NGFR	Hotspot	0.9351
		Nerve fibres/ mm ²	0.4078
		Blood vessels/ mm ²	0.7560
	NK1R	Blood vessels/ mm ²	0.3581
		Immune cells/ mm ²	0.3963
	PGP9.5	Hotspot	0.2640
		Nerve fibres/ mm ²	0.4078
	SP	Hotspot	0.9358
		Nerve fibres/ mm ²	0.1580
	TrkA	Hotspot	0.3239
		Nerve fibres/ mm ²	0.3997
		Blood vessels/ mm ²	0.1797
	TRPV1	Hotspot	0.9879
		Nerve fibres/ mm ²	0.2799
		Blood vessels/ mm ²	0.9986

Table 2 (continued)

Dyspareunia and nerve fibres density/ nerve fibres receptors ^a	NGFR	Hotspot	0.0991
		Nerve fibres/ mm ²	0.1937
		Blood vessels/ mm ²	0.6278
	NK1R	Blood vessels/ mm ²	0.5823
		Immune cells/ mm ²	0.8075
	PGP9.5	Hotspot	0.0029**
		Nerve fibres/ mm ²	0.0659
	SP	Hotspot	0.004**
		Nerve fibres/ mm ²	0.0029**
	TrkA	Hotspot	0.4392
		Nerve fibres/ mm ²	0.1720
		Blood vessels/ mm ²	0.2187
	TRPV1	Hotspot	0.8811
		Nerve fibres/ mm ²	0.8315
		Blood vessels/ mm ²	0.9921
Dyschezia and nerve fibres density/ nerve fibres receptors ^b	NGFR	Hotspot	0.2829
		Nerve fibres/ mm ²	0.1263
		Blood vessels/ mm ²	0.6422
	NK1R	Blood vessels/ mm ²	0.6975
		Immune cells/ mm ²	0.9926
	PGP9.5	Hotspot	0.6153
		Nerve fibres/ mm ²	0.3081
	SP	Hotspot	0.0542
		Nerve fibres/ mm ²	0.1275
	TrkA	Hotspot	0.3313
		Nerve fibres/ mm ²	0.2546
		Blood vessels/ mm ²	0.9908
	TRPV1	Hotspot	0.0826
		Nerve fibres/ mm ²	0.7677
		Blood vessels/ mm ²	0.4181
Dysuria and nerve fibres density/ nerve fibres receptors ^b	NGFR	Hotspot	0.6985
		Nerve fibres/ mm ²	0.6103
		Blood vessels/ mm ²	0.2279
	NK1R	Blood vessels/ mm ²	0.1544
		Immune cells/ mm ²	0.1397
	PGP9.5	Hotspot	0.8971
		Nerve fibres/ mm ²	0.9706
	SP	Hotspot	0.6985
		Nerve fibres/ mm ²	0.6324
	TrkA	Hotspot	>0.9999
		Nerve fibres/ mm ²	0.6691
		Blood vessels/ mm ²	0.2868
	TRPV1	Hotspot	>0.9999
		Nerve fibres/ mm ²	>0.9999
		Blood vessels/ mm ²	>0.9999
rARSM and nerve fibres density/ nerve fibres receptors ^b	NGFR	Hotspot	0.3202
		Nerve fibres/ mm ²	0.4664
		Blood vessels/ mm ²	0.4483
	NK1R	Blood vessels/ mm ²	0.8568
		Immune cells/ mm ²	0.7104
	PGP9.5	Hotspot	0.2244
		Nerve fibres/ mm ²	0.4243
	SP	Hotspot	0.3644
		Nerve fibres/ mm ²	0.7765
	TrkA	Hotspot	0.1818
		Nerve fibres/ mm ²	0.2018
		Blood vessels/ mm ²	0.9271
	TRPV1	Hotspot	0.7402
		Nerve fibres/ mm ²	0.5557
		Blood vessels/ mm ²	0.1895

Bold values highlight the significant correlations

Analyses were made with ^a χ^2 or Fisher and ^bSpearman correlation

* $p < 0.05$; ** $p < 0.005$

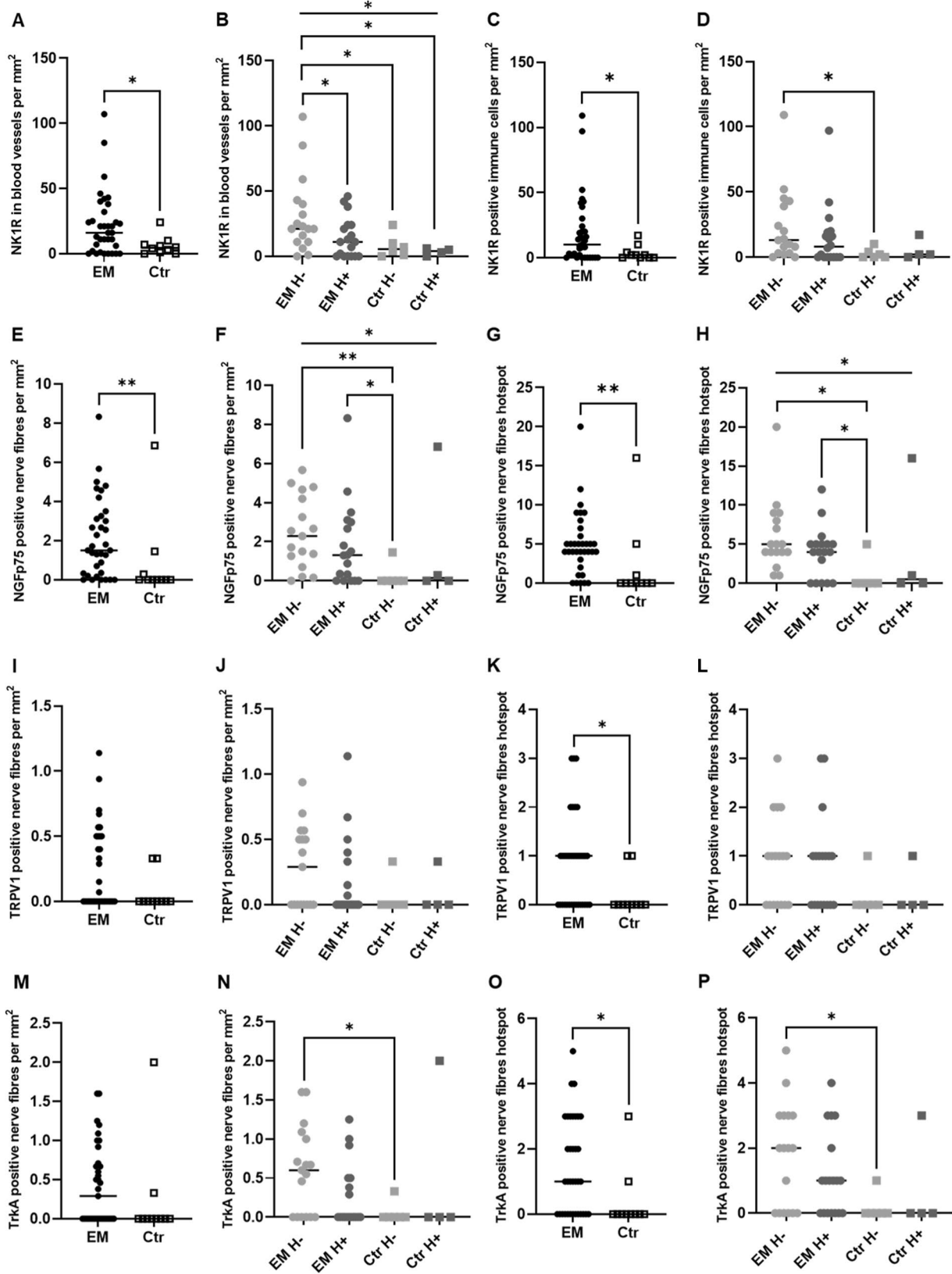


Fig. 3 Endometriosis patients showed increased expression of nociceptive markers. NK1R-positive nerve fibers per mm² (A–B) and hotspot (C–D); NGFp75-positive nerve fibers per mm² (E–F) and hotspot (G–H); TRPV1-positive nerve fibers per mm² (I–J) and hotspot (K–L); and TrkA-positive nerve fibers per mm² (M–N) and hotspot (O–P). EM endometriosis patients, Ctr control, H+ under hormonal treatment, H– without hormonal treatment; all the results are presented as median, 25–75% percentile. Mann–Whitney test and Kruskal–Wallis with Dunn’s multiple comparison tests. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

EM H–: 21.0, 11.0–41.5; $p = 0.0465$) (Fig. 3B). Also, the EM group of patients without treatment differ from the control group ($p = 0.0025$), a difference that was not maintained when the EM patients with hormonal therapy were evaluated ($p = 0.3312$) (Fig. 3B). Regarding immune cells NK1R-positive, EM patients also have an increased amount of positive cells compared with the controls (EM: 10.0, 0.0–24.0; Ctr: 2.0, 0.0–5.5; $p = 0.0415$) (Fig. 3C). This statistical difference was maintained when EM without treatment was compared with controls also without hormonal therapy (EM H–: 13.0, 3.0–41.0; Ctr H–: 1.0, 0.0–5.5; $p = 0.0184$) (Fig. 3D).

No correlation could be seen between the NK1R expression and the rASRM stages. The NK1R-positive stained vessels and immune cells also did not correlate with the pain levels (Table 2).

EM patients showed increased expression of nociceptive markers

NGFRp75 staining showed a significant difference between EM patients and the control group when looking at the hotspot (EM: 4.0, 3.0–6.0; Ctr: 0.0, 0.0–5.0, $p = 0.0064$) and nerve fibers per mm² (EM: 1.5, 0.33–3.26; Ctr: 0.0, 0.0–0.58; $p = 0.0067$) (Fig. 3E and G). EM patients under hormonal therapy and without also presented increased NGFRp75-positive nerve fibers in the hotspot (EM H+ : 4.0, 0.0–5.0; EM H–: 5.0, 4.0–8.5; Ctr H–: 0.0, 0.0–1.25) and per mm² view (EM H+ : 1.31, 0.0–3.0; EM H–: 2.29, 0.98–4.43; Ctr H–: 0.0, 0.0–0.36) when compared with controls without treatment (hotspot $p = 0.0429$ and 0.0017; mm² $p = 0.0285$ and 0.0011) (Fig. 3F and H). No difference was observed in the NGFRp75 stained vessels (data not shown).

When looking at TRPV1-positive colored nerves, EM patients showed more nerve fibers than the control group in the hotspot view (EM: 4.0, 3.0–6.0; Ctr: 0.0, 0.0–2.0; $p = 0.039$) (Fig. 3E), but not when looking at positively colored fibers per mm² (EM: 1.5, 0.33–3.26; Ctr: 0.0, 0.0–0.58; $p = 0.7520$) (Fig. 3I and K) or taking into consideration the hormonal treatment (Fig. 3J and L). The analysis of TRPV1-stained vessels also showed no significant difference between these two groups (data not shown).

Women with EM showed an increased amount of TrkA colored nerve fibers compared to the control patients when looking at the hotspot view (EM: 1.0, 0.0–3.0; Ctr: 0.0, 0.0–0.25; $p = 0.0242$) (Fig. 3O), which was not the case when the nerve fibers per mm² ($p = 0.1079$) (Fig. 3M) and the stained blood vessels (data not shown) were analyzed. In addition, a statistical difference was only obtained between EM and control patients without hormonal treatment for the hotspot (EM H–: 2.0, 0.0–3.0; Ctr H–: 0.0, 0.0–0.25; $p = 0.0313$) and nerve fibers per mm² (EM H–: 0.6, 0.0–1.04; Ctr H–: 0.0, 0.0–0.08, $p = 0.0234$) (Fig. 3N and P).

No correlation could be seen between NGFRp75, TRPV1, and TrkA expression and the rASRM stages. However, a correlation between NGFRp75 stained blood vessels and the severity of the cycle-dependent pelvic pain was observed ($r = -0.5398$; $p = 0.0334$) (Table 2).

Discussion

The majority of research into the mechanisms underlying pain in EM has focused on the endometriotic lesions with the estrogen-dependent cyclical release of pain mediators as the primary source of EM-associated pain [10, 15]. This reflects the typical nociceptive pain, which disappears with the end of the menstrual bleeding and is the reason why hormonal treatment and nonsteroidal anti-inflammatory substances (NSAP) work, especially at the beginning of the disease. However, not seldom do patients experience a shift from cyclical to more acyclical pain or develop acyclical pain under hormonal treatment. This suggests the involvement of additional complex mechanisms.

Although lesion-specific pain is undoubtedly essential for the induction of EM-associated pain, lesion removal does not provide pain relief in all cases [7]. Furthermore, only a marginal association exists between lesion size or disease stage and the severity of pelvic symptoms [16]. A more recent understanding of the mechanisms underlying the development of a chronic pain state in EM implicates cyclical bleeding from lesions and subsequent inflammation at both lesion sites and in the peritoneal cavity. These proinflammatory responses then result in sensory nerve activation and altered activation of nociceptive pathways. The complexity of peripheral and central sensitization makes research in this field very difficult.

In this study, we focused on peripheral sensitization of EM-associated nerve fibers: we investigated (i) the nerve fiber density of sensory nerve fibers in symptomatic EM patients, (ii) analyzed the expression of the SP and their receptor NK1R and (iii) the expression of nociceptive receptors and compared the findings between EM patients and

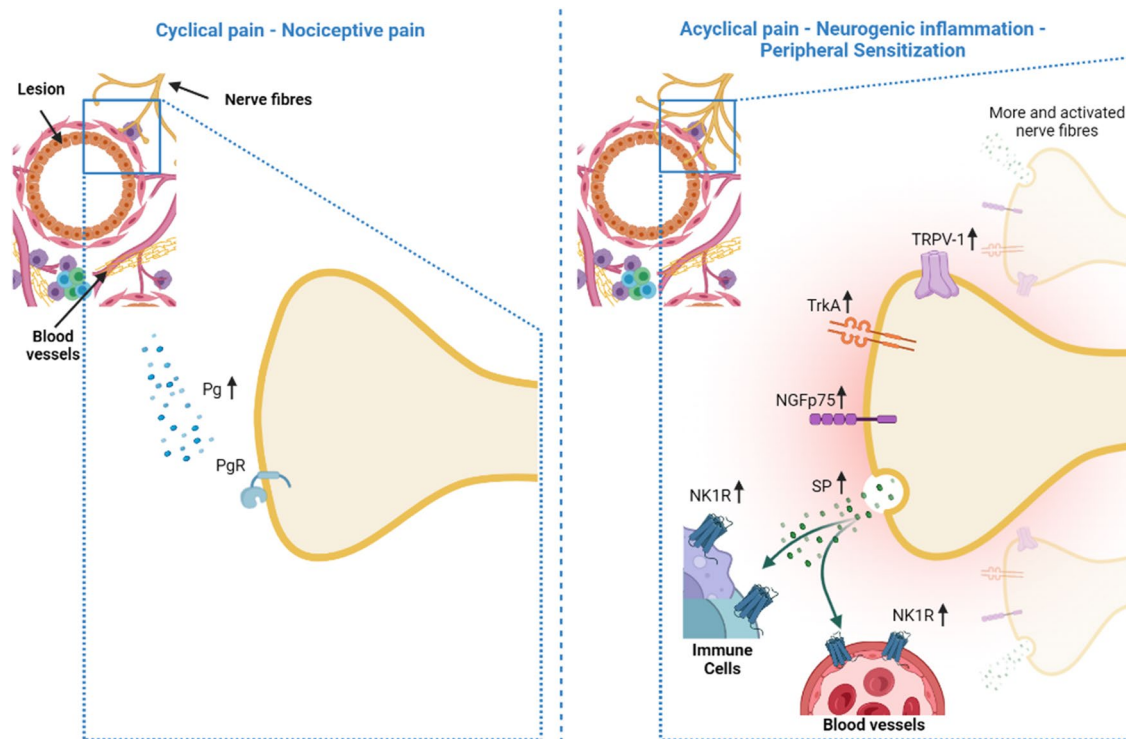


Fig. 4 Summary of the cyclical and acyclical pain expression of nociceptive markers. In the cyclical pain (left), only the prostaglandin (Pg) and their receptor (PgR—prostaglandin receptor) are involved in the pain, in the nociceptive pain. In the acyclic pain (right), we have

more and activated nerve fibers, increased expression of TRPV-1, TrkA, NGFp75 in the nerves, increased release of substance P (SP) and increased expression of NK1R in immune cells as well as in blood vessels

controls and between patients using or not using hormonal treatment.

We demonstrated the presence of sensory nerve fibers in peritoneal endometriotic lesions in 45 women with confirmed symptomatic EM. The density of nerve fibers in peritoneal endometriotic lesions was much greater than in normal peritoneum in women with no EM, both in nerve fibers per mm² and in hotspot image. EM patients also showed significantly more SP-positive nerve fibers in the hotspot view. Taken together, our data confirm the high innervation of the endometriotic lesion already seen by different groups [17–20]. These sensitive nerve fibers typically function as nociceptors, implicating them strongly in the generation of EM-associated pelvic pain [21, 22]. This supports the correlation between dyspareunia and the density of nerve fibers using anti-PGP9.5 and anti-SP seen in this study. The higher nerve fiber density goes in line with higher sensitivity in the case of mechanically stretching of the tissue during intercourse. There was no difference in the nerve fiber density between patients with and without hormonal treatment.

Neurogenic inflammation is caused by releasing the neurotransmitters from sensitive nerve endings, through interaction with immune cells [15] and might be the main source for a shift from cyclical to acyclical pain,

or a reason for the development of acyclical pain under hormonal treatment. The expression of NK1R (receptor for SP) is reported to be upregulated by estrogen and TNF- α [19]. As local production of TNF- α and estrogen is increased in endometriotic lesions, NK1R expression would be and has been reported to be elevated [23]. In our study, NK1R could be detected in blood vessels but also immune cells at higher levels when compared with control patients. NK1R activation is involved in ERK1/2 protein (MAPK), p38 MAPK, NF- κ B, PI3K, Akt, Src, EGFR and Rho/Rock signaling pathways in different cell types [24]. Importantly, all these proteins have been implicated in the development of EM [19]. To the best of our knowledge, this is the first study demonstrating this finding in peritoneal endometriotic lesions and gives the strong hint for evidence of neurogenic inflammation due to EM-associated nerve fibers.

Increased levels of neurotrophins such as NGF and their receptors NGFRp75 and TrkA are also seen in endometrial biopsies of women with EM [17, 25]. The greatly increased expression of NGFRp75 and TrkA by endometriotic lesions may also play a role in inducing the ingrowth of nerve fibers into endometriotic tissue and may play a primary role in setting up the mechanisms for the generation of pain [18,

26, 27]. These effects are exacerbated by increased levels of circulating estrogen in EM patients, as estrogen can enhance NGF activation of NGFRp75 and TrkA [28]. This is important as its downstream target is the well-known nociceptive channel TRPV1. The TRPV1 receptor is the most important activator of silent C-fibers. It was found to be upregulated in endometriomas and ectopic endometrial cells [29, 30], as well as in our EM samples suggesting the peripheral sensitization of the nerve fibers. This was, in all symptomatic patients, upregulated, independent from the use of hormonal treatment. An increase in the density of nerve endings throughout lesions and enhanced excitability of nerves provide the basis for increased nociception at lesion sites [10].

Pain severity was assessed to determine if there could exist a correlation between nerve fiber density, receptors expression and hormonal therapy. EM is associated with sexual pain, specifically, pain with deep penetration (dyspareunia). The etiology of dyspareunia in EM seems to be multifactorial [31], but a higher density of nerve fiber bundles around the endometriotic lesion, compared to patients without dyspareunia, was already confirmed [32]. Now, we show a correlation between this pain severity and the density of the nerve fibers. Along these lines, we indicate the correlation between NGFRp75 expression in blood vessels and the cycle-dependent pelvic pain severity. As commented above, this effect is aggravated by increased levels of estrogen in EM patients, as estrogen can enhance NGFRp75 activation [28].

It has been shown that traditional hormone therapies that alleviate EM-associated pain, including progestogens and oral contraceptives, significantly reduced nerve fiber density in ectopic endometrium [33]. What we observed was a reduction of the cycle-dependent pelvic pain in EM patients to the treatment and the marginally reduced expression of NK1R in the blood vessels of these patients compared to those that did not receive the hormonal therapy. Since patients under hormonal therapy do not ovulate or bleed, makes sense that they also have no or less cyclic pain. As NK1R is related to inflammation (vasodilatation and interleukins release induction) [34], this makes us hypothesize that the hormonal intake is efficient only against the inflammation due to the EM but not to the pain itself as the other markers did not decrease with the treatment and the patients still suffer from acyclical pain (Fig. 4). This shows that neurogenic inflammation is present, and therefore causes the peripheral sensitization of the sensory nerve fibers.

Patients under hormonal therapy have no ovulation and no (menstrual) bleeding, which are typically associated with inflammation and cyclical pain. However, acyclical pain seems to be due to peripheral sensitization once it is present under the treatment. Neurotransmitters, like SP and their receptors, are involved in mechanisms of neurogenic

inflammation, which are relevant for pain initiation in women affected by this chronic disease. Taking together, these findings seem to indicate that in both groups (EM with/without hormonal treatment), neurogenic inflammation is present and responsible for acyclical painful symptoms.

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Author contributions Conceptualization, RVV and SM; experiments, RVV and SM; writing—original draft preparation, RVV; writing—review and editing, RVV, SM and JS.

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Data availability Data available on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval The study was approved by the Institutional Review Board of the Charité University Medical Centre (Ethic vote EA4/036/12). All subjects provided written informed consent and the studies were conducted in accordance with the Declaration of Helsinki.

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References

1. Gruber TM, Mechsner S (2021) Pathogenesis of endometriosis: the origin of pain and subfertility. *Cells* 10(6):1381
2. Velho RV, Halben N, Chekerov R, Keye J, Plendl J, Sehoul J, Mechsner S (2021) Functional changes of immune cells: signal of immune tolerance of the ectopic lesions in endometriosis? *Reprod Biomed Online* 43(2):319–328
3. Laganà AS, Salmeri FM, Ban Frangež H, Ghezzi F, Vrtačnik-Bokal E, Granese R (2020) Evaluation of M1 and M2 macrophages in ovarian endometriomas from women affected by endometriosis at different stages of the disease. *Gynecol Endocrinol* 36(5):441–444
4. La Rosa VL, Barra F, Chiofalo B, Platania A, Di Guardo F, Conway F, Di Angelo Antonio S, Lin LT (2020) An overview on the relationship between endometriosis and infertility: the impact on sexuality and psychological well-being. *J Psychosom Obstet Gynaecol* 41(2):93–97

5. Vitale SG, La Rosa VL, Rapisarda AMC, Laganà AS (2017) Impact of endometriosis on quality of life and psychological well-being. *J Psychosom Obstet Gynaecol* 38(4):317–319
6. Mechsner S (2016) Endometriose. *Der Schmerz* 30(5):477–490
7. Dückelmann AM, Taube E, Abesadze E, Chiantera V, Sehouli J, Mechsner S (2021) When and how should peritoneal endometriosis be operated on in order to improve fertility rates and symptoms? The experience and outcomes of nearly 100 cases. *Arch Gynecol Obstet* 304(1):143–155
8. Mechsner S (2022) Endometriosis, an ongoing pain—step-by-step treatment. *J Clin Med* 11(2):467
9. Arnold J, Vercellino GF, Chiantera V, Schneider A, Mechsner S, de Arellano MLB (2013) Neuroimmunomodulatory alterations in non-lesional peritoneum close to peritoneal endometriosis. *NeuroImmunoModulation* 20(1):9–18
10. Maddern J, Grundy L, Castro J, Brierley SM (2020) Pain in endometriosis. *Front Cell Neurosci* 14:1–16
11. Brandes I, Neuser M, Kopf A, Chiantera V, Sehouli J, Mechsner S (2017) Endometriosis-associated pain in patients with and without hormone therapy. *J Endometr Pelvic Pain Disord.* 9(3):200–205
12. Donnez J, Dolmans MM (2021) Endometriosis and medical therapy: from progestogens to progesterone resistance to GnRH antagonists: a review. *J Clin Med* 10(5):1–21
13. Carlyle D, Khader T, Lam D, Vadivelu N, Shiwlochan D, Yonghee C (2020) Endometriosis pain management: a review. *Curr Pain Headache Rep* 24(9):49
14. Weidner N, Semple JP, Welch WR, Folkman J (1991) Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. *N Engl J Med* 324(1):1–8
15. Velho RV, Taube E, Sehouli J, Mechsner S (2021) Neurogenic inflammation in the context of endometriosis—what do we know? *Int J Mol Sci* 22(23):13102
16. Vercellini P, Fedele L, Aimi G, Pietropaolo G, Consonni D, Crosignani PG (2007) Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: a multivariate analysis of over 1000 patients. *Hum Reprod* 22(1):266–271
17. Tokushige N, Markham R, Russell P, Fraser IS (2006) High density of small nerve fibres in the functional layer of the endometrium in women with endometriosis. *Hum Reprod* 21(3):782–787
18. Tokushige N, Markham R, Russell P, Fraser IS (2006) Nerve fibres in peritoneal endometriosis. *Hum Reprod* 21(11):3001–3007
19. Yan D, Liu X, Guo SW (2019) Neuropeptides substance P and calcitonin gene related peptide accelerate the development and fibrogenesis of endometriosis. *Sci Rep* 9(1):1–22
20. Arnold J, de Arellano MLB, Rüster C, Vercellino GF, Chiantera V, Schneider A, Mechsner S (2012) Imbalance between sympathetic and sensory innervation in peritoneal endometriosis. *Brain Behav Immun* 26(1):132–141
21. Wang G, Tokushige N, Markham R, Fraser IS (2009) Rich innervation of deep infiltrating endometriosis. *Hum Reprod* 1(1):1–8
22. Wei Y, Liang Y, Lin H, Dai Y, Yao S (2020) Autonomic nervous system and inflammation interaction in endometriosis-associated pain. *J Neuroinflammation* 17(1):80
23. McKinnon BD, Evers J, Bersinger NA, Mueller MD (2013) Induction of the neurokinin 1 receptor by TNF α in endometriotic tissue provides the potential for neurogenic control over endometriotic lesion growth. *J Clin Endocrinol Metab* 98(6):2469–2477
24. Garcia-Recio S, Gascón P (2015) Biological and pharmacological aspects of the NK1-receptor. *Biomed Res Int* 2015:495704
25. Tokushige N, Markham R, Russell P, Fraser IS (2009) Effect of progestogens and combined oral contraceptives on nerve fibers in peritoneal endometriosis. *Fertil Steril* 92(4):1234–1239
26. Anaf V, Simon P, El Nakadi I, Fayt I, Simonart T, Buxant F, Noel JC (2002) Hyperalgesia, nerve infiltration and nerve growth factor expression in deep adenomyotic nodules, peritoneal and ovarian endometriosis. *Hum Reprod* 17(7):1895–1900
27. Chao MV (2003) Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci* 4(4):299–309
28. Bjorling D, Beckman M, Clayton M, Wang ZY (2002) Modulation of nerve growth factor in peripheral organs by estrogen and progesterone. *Neuroscience* 110(1):155–167
29. Bohonyi N, Pohóczky K, Szalontai B, Perkecz A, Kovács K, Kajtár B, Orbán L, Varga T, Szegedi S, Bódis J, Helyes Z, Koppán M (2017) Local upregulation of transient receptor potential ankyrin 1 and transient receptor potential vanilloid 1 ion channels in rectosigmoid deep infiltrating endometriosis. *Mol Pain*. <https://doi.org/10.1177/1744806917705564>
30. Wang Y (2008) The functional regulation of TRPV1 and its role in pain sensitization. *Neurochem Res* 33(10):2008–2012
31. Yong PJ (2017) Deep dyspareunia in endometriosis: a proposed framework based on pain mechanisms and genito-pelvic pain penetration disorder. *Sex Med Rev.* 5(4):495–507
32. Peng B, Alotaibi FT, Sediqi S, Bedaiwy MA, Yong PJ (2020) Role of interleukin-1 β in nerve growth factor expression, neurogenesis and deep dyspareunia in endometriosis. *Hum Reprod* 35(4):901–912
33. Tokushige N, Markham R, Russell P, Fraser IS (2008) Effects of hormonal treatment on nerve fibers in endometrium and myometrium in women with endometriosis. *Fertil Steril* 90(5):1589–1598
34. McKinnon BD, Bertschi D, Bersinger NA, Mueller MD (2015) Inflammation and nerve fiber interaction in endometriotic pain. *Trends Endocrinol Metab* 26(1):1–10

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