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Pelvic floor muscle electrical coupling in chronic pelvic pain: Insights into pathophysiology and botulinum toxin treatment effects

Monica Albaladejo-Belmonte [a], Marta Tarazona-Motes [b], Francisco Jose Nohales-Alfonso [b], Maria De-Arriba [b], Jose Alberola-Rubio [c], Javier Garcia-Casado [a,*]

[a] Centro de Investigación e Innovación en Bioingeniería, Universitat Politècnica de València, Edif. 8B, Camino de Vera SN, Valencia 46022, Spain
[b] Servicio de Obstetricia y Ginecología, Hospital Universitario y Politécnico La Fe de Valencia, Valencia, Spain
[c] Sonda Devices S.L., Calle Colon, 1 - 4, Valencia 46004, Spain

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ABSTRACT

This study aimed to assess the electrical coupling between both pelvic floor muscle (PFM) sides (two-sided coupling) and within individual PFM sides (one-sided coupling) in chronic pelvic pain (CPP) before and after botulinum neurotoxin type A (BoNT/A) treatment. Surface electromyographic (sEMG) signals were recorded from the left and right PFM of 24 patients (P) with CPP before and after being treated with BoNT/A (Weeks 0,8,12,24). Recordings were also made in 24 healthy women (H). PFM two-sided and one-sided coupling was evaluated during contractions by the cross-correlation (*CC*) and the imaginary part of coherency (*iCOH*) of their sEMG signals. Significant differences between their values were assessed comparing P(0) vs. P(8,12,24) and H vs. P(0,8,12,24). This study showed that PFM two-sided coupling is similar across groups before treatment, while PFM one-sided coupling on the patients' most painful side is deranged before and also after BoNT/A treatment: amplitude coupling is lower (<*CC*) and phase difference is greater (>*iCOH*) than healthy women's. This could be justified by altered neuromotor control strategies developed as an adaptation to muscle pain, structural and electrical changes in PFM, and alterations in their innervation pattern, which may influence the onset, perpetuation, or recurrence of CPP after treatment.

1. Introduction

Chronic pelvic pain (CPP) is a common gynaecological disorder with a broad impact on the lives of women that suffer from it (Grinberg et al., 2020). It relates to a chronic or persistent pain in the pelvic region with no proven well-defined local pathology (Cerruto, 2021) and it is usually treated by a combination of therapies, according to the patient's clinical phenotype (Parsons et al., 2022). In cases refractory to conventional therapies, botulinum neurotoxin type A (BoNT/A) can be injected into the pelvic floor muscles (PFM), as it modulates pain by inhibiting the release of different neurotransmitters and disrupts the transfer of pain receptors (Kumar, 2018). It also leads to the chemical denervation of the muscle fibres (Choudhury et al., 2021).

In a high number of cases CPP is associated with the PFM, their connecting fascia and connective tissues and receives the name of myofascial pelvic pain syndrome (Lin et al., 2022). This syndrome is usually related to a PFM overactive dysfunction, which can be a cause or a consequence and a perpetuating factor of CPP. As a cause, PFM overactivity may lead to a persistent PFM contraction that can cause local ischemia, subsequent hypoxia in the tissue and the release of sensitizing substances that may be precursors to CPP (Jantos, 2020). For its part, CPP is associated with disturbances in pain processing that can promote protective, defensive, or withdrawal responses in the PFM, which may unleash or perpetuate PFM overactivity in patients, thus supporting a feedback cycle in which pain and muscle dysfunction are mutually maintained (Padoa et al., 2021).

The association between CPP and overactive PFM dysfunction has been confirmed by several studies that have reported significantly higher amplitude or power of the patient's PFM electrical activity by surface electromyography (sEMG) when compared to healthy women (Albaladejo-Belmonte et al., 2021b) as well as significant reductions in them after treatment (Albaladejo-Belmonte et al., 2021a). On the other hand, little is known about the impact that CPP may have on PFM electrical coupling or synchronization, i.e., the relationship between the

* Corresponding author.
E-mail addresses: moalbel@ci2b.upv.es (M. Albaladejo-Belmonte), tarazona_marmot@gva.es (M. Tarazona-Motes), dearriba_margar@gva.es (M. De-Arriba), jgarciac@ci2b.upv.es (J. Garcia-Casado).

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electrical activity patterns of different PFM areas (Zhang et al., 2023), which could be significant considering that previous studies have reported altered coupling in other muscles in pain conditions (Sanderson et al., 2021). This question was addressed in two previous studies, although their analyses did not include robust metrics against effects of volume conduction and they did not evaluate the effect of therapies, such as BoNT/A injection, on PFM electrical coupling (Albaladejo-Belmonte et al., 2021b; Houston et al., 2023).While another previous study characterized the effect of BoNT/A treatment on the PFM electrical activity, it assessed changes in its power, spectral content and complexity, but not in the electrical coupling between different regions (Albaladejo-Belmonte et al., 2021a). We hypothesized that the impact/effect of CPP and its treatment with BoNT/A on the PFM electrical coupling could be different from what has been previously observed in the power or amplitude of the PFM activity, as it may be influenced by aspects or physiological processes other than the higher neural drive to the PFM that underlies overactive dysfunctions. Hence, its assessment could bring more insights into the coordination and propagation of PFM electrical activity, as well as into the pathophysiology of CPP and the role that PFM may have on it, and a more thorough and detailed knowledge on the effect of therapies currently used to treat CPP.

The objective of the present study was thus to characterize alterations in the PFM two-sided and one-sided myoelectrical couplings in patients with chronic myofascial pelvic pain both before and after treatment with BoNT/A. This was accomplished by comparing them with those of women with no pelvic conditions.

2. Materials and methods

2.1. Database composition and treatment protocol

Twenty-five patients with CPP with the main symptom of deep dyspareunia associated with a myofascial pelvic pain syndrome were recruited in this prospective, minimally invasive, non-masked and non-randomized Phase III clinical trial by a physician of the Obstetrics and Gynecology Service of the Hospital Universitari i Politècnic La Fe (Valencia, Spain). Inclusion criteria were to have reached the age of majority (18 years old), absence of active pelvic infections or a general malignant, pelvic or psychiatric disease, to show no contraindication to BoNT/A and not to have participated at any clinical trial testing an experimental drug at least 30 days before the onset of the present study. The study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital Universitari i Politècnic La Fe (protocol code: SEMG, date of approval: 25th July 2018). All the patients provided their written informed consent to participate in the project and one patient was lost to follow-up.

Patients were treated with a single injection of 90 U.I. of incobotulinumtoxinA (Xeomin®, Merz Pharmaceuticals GmbH, Frankfurt, Germany) diluted in 2 ml of lidocaine at 2 % into the most painful side of their pubococcygeus muscle under digital guidance (left side was injected in 18 patients and right in the remaining 6). To identify the most painful side the physician performed a digital palpation of each PFM side and asked the patient to rate the pain experienced during it according to the Visual Analogue Scale of 11 points.

Twenty-four women with no pelvic conditions and with a similar age (40.9 ± 7.2 years, p-value = 0.21, two-tailed two-sample *t*-test) and parity (1.5 ± 0.8, p-value = 0.99, two-tailed Mann-Whitney *U* test) to those of the patients also voluntarily enrolled in the study. Table 1 gives a summary of their most relevant clinical characteristics. Neither patients nor healthy women had endometriosis, as it was an exclusion criterion of the study.

2.2. Acquisition and preprocessing of sEMG signals

The patients' and healthy women's PFM activity was assessed by sEMG. In patients, the recording was performed before the injection of BoNT/A (Week 0) and repeated 8, 12, and 24 weeks after it.

Table 1
Demographic and clinical characteristics of patients and healthy women.

		Mean ± SD or Number of participants (%)	
		Patients	Healthy women
Age (years)		43.8 ± 8.8	40.9 ± 7.2
BMI (kg/m^2)		24.9 ± 3.7	22.1 ± 3.1
No. deliveries		1.5 ± 0.7	1.5 ± 0.8
Vaginal delivery		18/24 (75.0)	17/24 (70.8)
Episiotomy		16/24 (66.7)	16/24 (66.7)
Assisted vaginal delivery		7/24 (29.2)	6/24 (25.0)
Menopause		8/24 (33.3)	3/24 (12.5)
Abdominal or pelvic surgeries	Pelvic floor reconstructive surgery	1/24 (4.2)	0/24 (0.0)
	Appendicectomy	3/24 (12.5)	3/24 (12.5)
	Cesarean section	6/24 (25.0)	7/24 (29.2)
	Vaginal hysterectomy	2/24 (8.3)	1/24 (4.2)
	Anexectomy	1/24 (4.2)	0/24 (0.0)
	Cystectomy	1/24 (4.2)	0/24 (0.0)
	Salpingectomy	1/24 (4.2)	0/24 (0.0)
	Myomectomy	1/24 (4.2)	
		1/24 (4.2)	
Duration of pain (years)		4.8 ± 4.9	–
Provoked vulvodynia		18/24 (75.0)	–
Concurrent treatments for CPP	Benzodiazepines	11/24 (45.8)	–
	Nonsteroidal anti-inflammatory medication	8/24 (33.3)	
	Amitriptyline	1/24 (4.2)	
	Pregabalin	3/24 (12.5)	
	Physical therapy	2/24 (8.3)	

SD: standard deviation.

sEMG recordings were carried out using self-adhesive electrodes. To this purpose, the skin of the perineum, which did not show excessive hair in any participant, was gently rubbed with an abrasive gel (Nuprep 114 g, Weaver and Company, Aurora, CO, USA) and cleaned with isopropyl alcohol to minimize skin-electrode impedance and noise. Four monopolar signals were obtained from its surface with Ag/AgCl electrodes (Red Dot 2660–5, 3 M, St. Paul, MN, USA) with a contact surface of 3.2 cm x 3.2 cm: two from the upper and lower areas of the right side (R_u and R_l, respectively); and two from the upper and lower areas of the left side (L_u and L_l, respectively), which were referenced to an electrode attached to the right ischial tuberosity. The interelectrode distance between the electrodes on the same side was 4 cm. An additional ground electrode was placed on the left ischial tuberosity, as depicted in Fig. 1.

The patients remained in the lithotomy position and performed a protocol of voluntary PFM activation while their sEMG signals were being recorded. The protocol consisted of a resting period of 3 min followed by five maximum voluntary contractions of 5 s each with 10 s intervening relaxation breaks. The patients were asked to carry out at least one maximum voluntary test contraction before starting the protocol to confirm their understanding of the process. To ensure that contractions were performed at maximum level, a technician monitored the sEMG signals' amplitude on a screen that displayed them in real-time and provided corrective feedback to the participant during the protocol when a notable decrease in the intensity of a given contraction was noticed. Furthermore, to reduce crosstalk in the sEMG recordings, the clinician ensured by visual inspection the patients did not contract other nearby muscles, such as the abdominal, gluteal, or lower limb muscles,

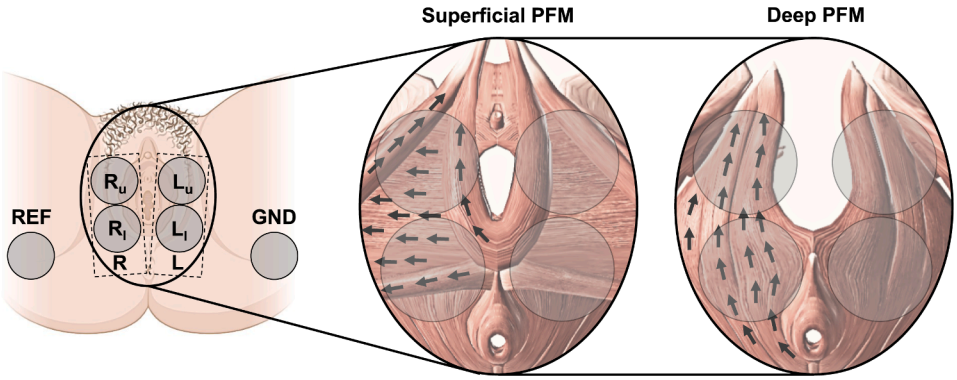

Fig. 1. Arrangement of electrodes used to record the sEMG signals of the pelvic floor muscles (PFM). The fiber direction of their different muscle bundles is shown with black arrows. Pictures modified from app.biorender.com (perineum) and www.biodigital.com (Superficial PFM, Deep PFM).

while contracting PFM and also provided corrective feedback when they did so.

Signals were filtered (band-pass bandwidth: [3, 1000] Hz) and digitalized (sampling rate: 10 kHz; 16 bits) by a multipurpose biosignal amplifier (Grass 15LT + 4 Grass 15A94, Grass Instruments, West Warwick, RI, USA) with a gain of x20,000, input impedance of 20 MΩ, 35 pF (16.4 MΩ @50 Hz), input noise < 3 μV peak to peak and CMRR 90 dB.

The amplifier was A/C-powered and signals were recorded in a regular hospital room so that raw recordings contained strong power line interference. Furthermore, preliminary analysis showed their most relevant spectral content was within the [30, 450] Hz bandwidth. Therefore, signals were digitally filtered to remove the power line interference (comb filter at 50 Hz and a quality factor of 30 designed with the *iirnotch* function from MATLAB), and other unwanted signal sources (17th-order Butterworth high-pass filter at 30 Hz and 8th-order Butterworth low-pass filter at 450 Hz) with zero-lag filters. Butterworth high-pass and low-pass filters were used as they are a common choice in sEMG studies (del Olmo and Domingo, 2020), and their orders were maximized as much as possible without distorting the signal. One bipolar signal from each PFM side was also obtained from the two monopolar recordings from that side (right PFM side: $R = R_u - R_l$; left PFM side: $L = L_u - L_l$).

The signals' envelopes were obtained for further analysis. They were extracted by means of a rectification and the use of a fourth-order Butterworth low-pass filter with a cut-off frequency at 10 Hz (Turpin et al., 2021; Zeng et al., 2018). The five contractions performed by the patients were visually identified and their onset and offset times were manually delimited in each of the envelopes obtained, as can be seen in Fig. 2.

2.3. Computation of coupling parameters

The amplitude and phase coupling between the myoelectrical activities of two different PFM areas were assessed during each contraction by two parameters:

2.3.1. Cross-correlation (CC)

Given two discrete-time signals $x[n]$ and $y[n]$ with zero mean, their normalized cross-correlation at zero lag is computed as follows:

$$CC = \left(\sum_i x(i)\cdot y(i)\right) \Big/ \sqrt{\sum_i x(i)^2 \cdot \sum_i y(i)^2} \quad (1)$$

CC ranges from −1 to 1 and quantifies the linear relationship between the amplitudes of $x[n]$ and $y[n]$, i.e., their amplitude coupling. The greater the association between the fluctuations of the two signals, the greater the CC value.

2.3.2. Imaginary part of coherency (iCOH)

Given two discrete-time signals $x[n]$ and $y[n]$, *iCOH* is the imaginary part ($\Im$) of their complex coherency, i.e. their cross-power spectrum at a given frequency ($S_{xy}(f)$) normalized by the root square of the product of their auto-spectrums at that frequency ($S_{xx}(f)$, $S_{yy}(f)$) (Nolte et al., 2004):

$$iCOH(f) = \Im(S_{xy}(f) / \sqrt{(S_{xx}(f)S_{yy}(f))}) \quad (2)$$

In this study, $S_{xy}(f)$, $S_{xx}(f)$, and $S_{yy}(f)$ were computed using the *cpsd* function from MATLAB, dividing each contraction into ten overlapped signal segments of 4 s so that the frequency resolution was 0.25 Hz. Furthermore, *iCOH* value was summarized for each subject as the mean of its absolute value across frequencies within a bandwidth of [0, 10] Hz, as in other studies (Basti et al., 2022), since maximum peaks were found at highly variable frequency points across subjects in both patients and healthy women and, in most cases, *iCOH* showed several local maximum values across the same coherency bandwidth that were very close to its maximum value. This parameter was used as a metric of phase coupling between two signals. It assesses their phase relationship without the

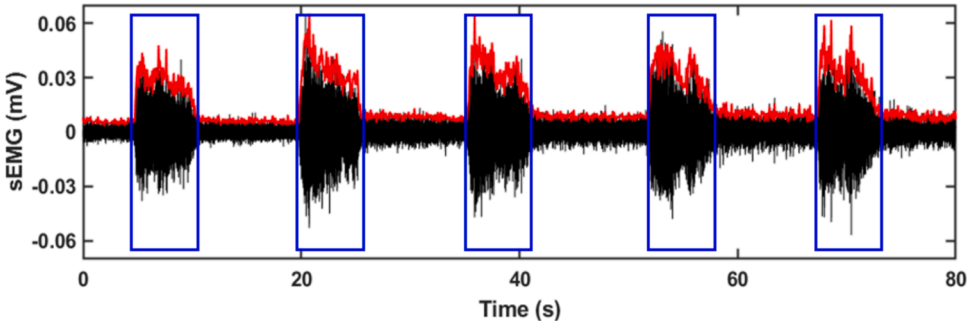

Fig. 2. sEMG signal annotation. Black line: monopolar sEMG signal recorded from the upper area of a patient's left PFM (L_U); red line: 4x-scaled envelope extracted; blue bloxes: annotated contractions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effect of any linear transfer function between the two signals, associated with near zero phase coherence (Korhonen et al., 2021) so that it is a robust measure to identify true interactions against the effects of volume conduction.

CC and *iCOH* were computed between the envelopes of the time-matching contractile segments annotated in (1) the bipolar signals of the left and right PFM sides (PFM two-sided coupling), (2) the monopolar signals of the most painful PFM side's upper and lower channels (one-sided coupling on the most painful PFM side) and (3) the monopolar signals of the least painful PFM side's upper and lower channels (one-sided coupling on the least painful PFM side). It should be noted that the term "two-sided coupling" is used to refer to the coupling between the electrical activities recorded of two different PFM sides (one on the left side and the other on the right side), while the term "one-sided coupling" is used for that between the activities of two areas located on the same PFM side (left or right). In healthy women the coupling measures of approaches (2) and (3) were computed between the monopolar signals of the left and right PFM sides, respectively, since the left PFM side received the BoNT/A injection in most patients.

The median of the five values of each parameter (one value per contraction) was calculated for each participant, coupling approach (1, 2, 3) and recording session, and considered for further analysis. This way, the impact of occasional contractions performed at submaximal levels on the final results was limited.

2.4. Statistical analysis

The statistically significant differences between the patients' *CC* and *iCOH* values at Week 0 vs. Week 8, 12, or 24 were assessed by the two-tailed Wilcoxon signed-rank test, while the statistically significant differences between the values of each parameter in healthy women vs. patients at Week 0, 8, 12 or 24 were studied by the two-tailed Mann-Whitney *U* test. A 5 % significance level was assumed in all the statistical tests carried out.

3. Results

Figs. 3-4 contain the box-whisker plots of the parameters computed from the signals of healthy subjects (H, red boxes) and patients (P) at Weeks 0 (dark blue boxes), 8, 12 and 24 of the study (light blue boxes). They show the values related to the PFM two-sided coupling and one-sided coupling on the most painful side (left side in healthy women) and the least painful side (right side in healthy women), respectively. They also indicate when the distributions statistically compared in P(0) vs. P(8), P(12) or P(24) and in H vs. P(0), P(8), P(12) or P(24) showed significantly different values by blue and red asterisks, respectively, placed under the second box-whisker plot of the comparison in question. Table 2 shows a summary of the statistically significant differences obtained.

The following subsections provide a more detailed description of the results shown, according to the different areas of the PFM involved in the computed coupling.

3.1. PFM two-sided coupling

As can be seen in Fig. 3, neither *CC* nor *iCOH* showed significantly different values between healthy subjects and patients before treatment. After BoNT/A, the patients' *CC* values dropped (Fig. 3, Table 2), with statistically significant differences at Weeks 12 (p-value < 0.05) and 24p-value < 0.05) with respect to Week 0. *iCOH* also dropped slightly at the follow-up visits and whereas there were no statistically significant differences between these and values at P(0), its value was significantly lower than the healthy women's *iCOH* at Week 24.

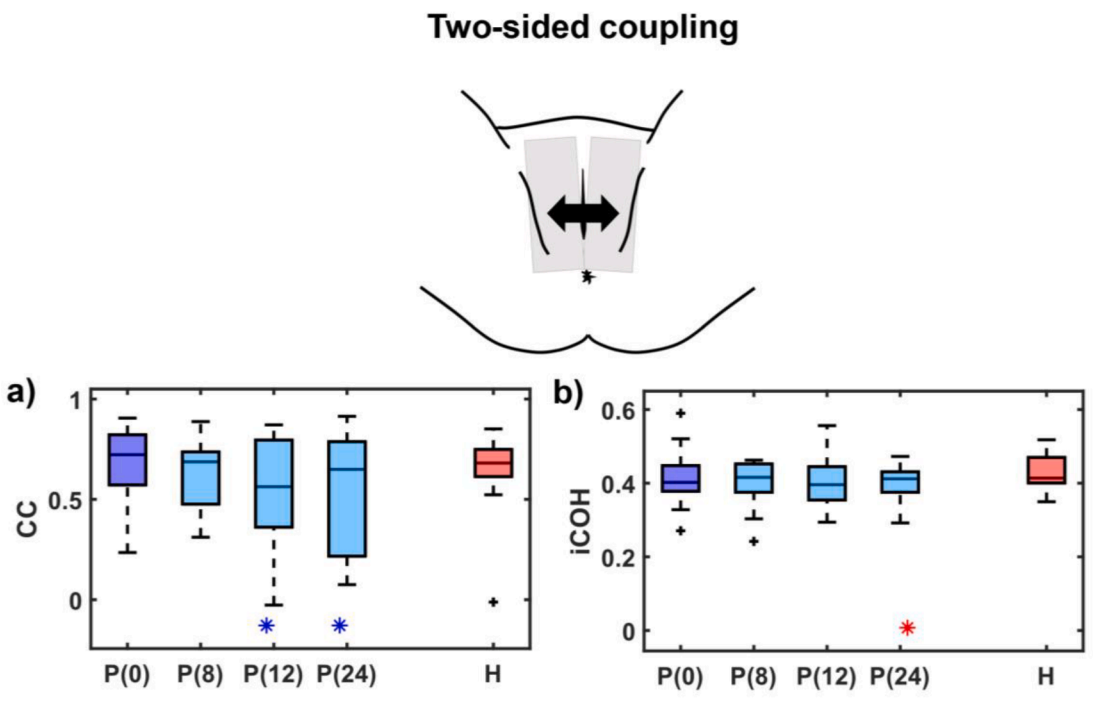

Fig. 3. PFM two-sided coupling assessed from bipolar signals in healthy subjects (H, red boxes) and patients (P, blue boxes) according to their cross-correlation (CC; a) and imaginary part of coherency (iCOH; b). Blue asterisk at Week i: Wilcoxon signed-rank test's p-value [P(0) vs. P(i)] < 0.05. Red asterisk at Week i: Mann-Whitney U test's p-value [H vs. P(i)] < 0.05. N $= 24$ in all P and H boxes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

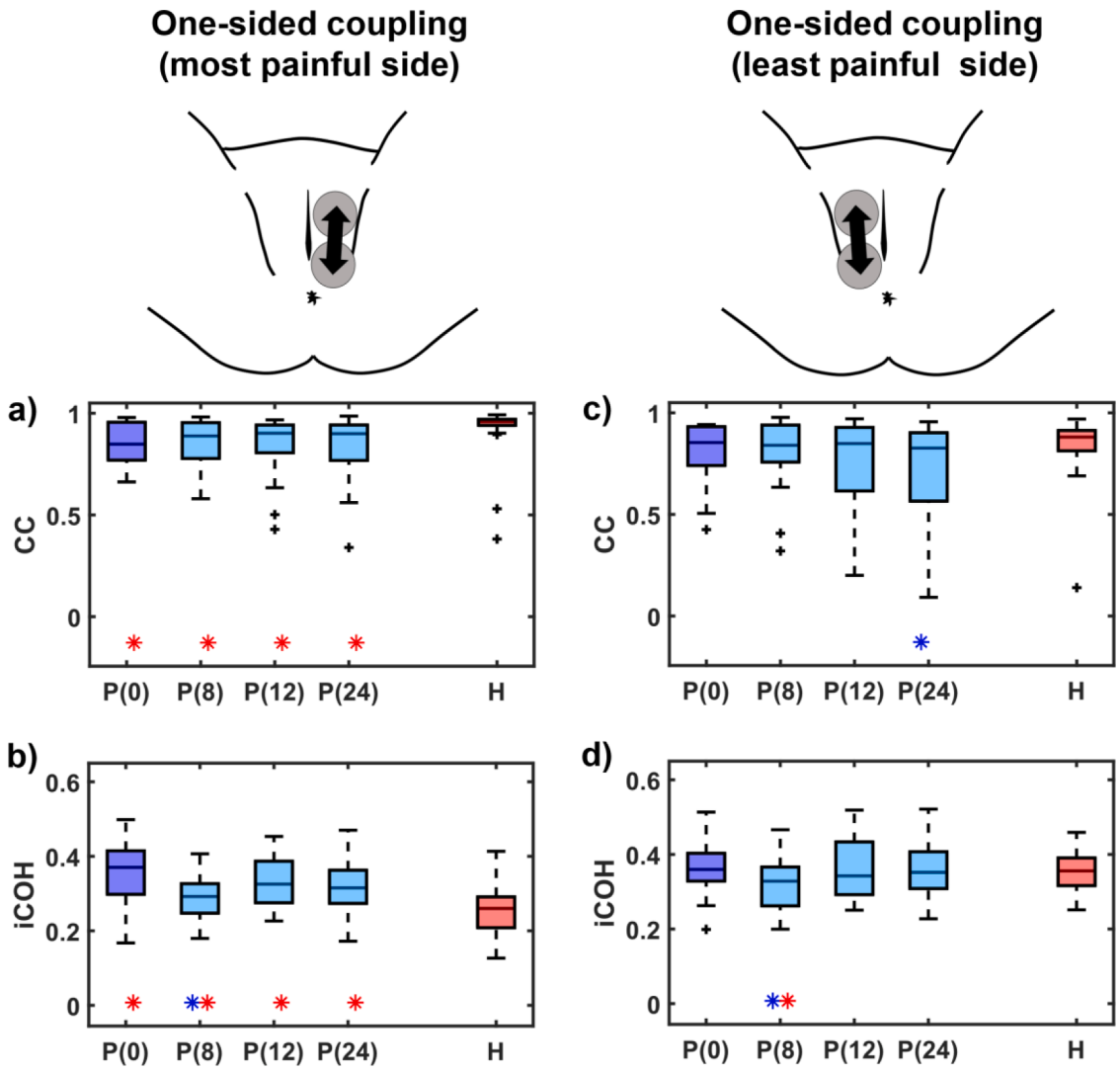

Fig. 4. PFM one-sided coupling assessed from monopolar signals in healthy subjects (H, red boxes) and patients (P, blue boxes) according to their cross-correlation (CC; a,c) and imaginary part of coherency (iCOH, b,d). Blue asterisk at Week i: Wilcoxon signed-rank test's p-value [P(0) vs. P(i)] < 0.05. Red asterisk at Week i: Mann-Whitney U test's p-value [H vs. P(i)] < 0.05. N = 24 in all P and H boxes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Significant differences between patients' coupling before vs. after BoNT/A, and between patients (P) vs. healthy women (H)'s coupling, assessed from bipolar signals (two-sided coupling) and monopolar signals (one-sided coupling).

		P (0) vs.			H vs.			
		P (8)	P(12)	P(24)	P (0)	P (8)	P(12)	P(24)
Two-sided coupling	*CC*		↓	↓				
	iCOH							↓
One-sided coupling	*CC*				↓↓	↓↓	↓↓↓	↓↓↓
(most painful side)	*iCOH*	↓↓			↑↑↑	↑	↑↑↑	↑↑
One-sided coupling	*CC*			↓				
(least painful side)	*iCOH*	↓				↓		

3.2. PFM one-sided coupling (most painful side)

It can be seen in the left column of Fig. 4 that the patients' one-sided *CC* and *iCOH* on the most painful side at Weeks 0, 8, 12 and 24 were significantly different from those of the healthy women (p-values < 0.01 in almost all cases). Fig. 4 and Table 2 show that *CC* was significantly lower in patients than in healthy women, whereas *iCOH* was significantly higher. However, the magnitude of these differences changed throughout the study, which was especially remarkable in the case of the difference across both groups' *iCOH* distributions at Week 0 (p-value $<$ 0.001) vs. Week 8 (p-value $= 0.04$).

As Table 2 (P(0) vs. P(i)) shows, only one significant difference was obtained on the possible effect of BoNT/A treatment when the patients' parameters at follow-up visits were compared with their values at Week 0: *iCOH* at Week 8 (p-value < 0.01).

3.3. PFM one-sided coupling (least painful side)

As can be seen in the right column of Fig. 4, *iCOH* at Week 8 was the only parameter from the least painful PFM side that showed significantly different values between healthy subjects and patients, which was significantly lower in patients. As in the most painful side, Table 2 shows few significant differences between the patients' pre-treatment vs. post-treatment parameters: *CC* at Week 24 and *iCOH* at Week 8 (p-value $<$ 0.05 in both cases).

4. Discussion

4.1. PFM two-sided coupling

The two parameters computed showed that PFM two-sided coupling was not significantly different between patients and healthy subjects before treatment. This finding is consistent with the only study that had previously evaluated PFM two-sided coupling in patients with PFM pain, although interstitial cystitis/bladder pain syndrome was their main symptom rather than deep dyspareunia, and robust metrics against volume conduction effects were not assessed (Houston et al., 2023).

After BoNT/A, the *CC* between both sides showed significantly lower values from those at the beginning of the study (Table 2, P(0) vs. P(i)), indicating a significant drop in the linear relationship between the amplitudes of the patients' left and right PFM activities with respect to the pre-treatment situation. This was an expected outcome, considering that the toxin was injected into only one side of the PFM. Lower *CC* values after treatment could be related to greater irregularity of PFM activity subsequent to the action of the toxin (Albaladejo-Belmonte, Nohales-Alfonso, et al., 2021), since it has been reported that the *CC* between the spike trains of neurons that receive common excitatory input is lower when they fire irregularly than when they do so regularly (Ostojic et al., 2009).

4.2. PFM one-sided coupling (most painful side)

The results obtained showed deranged PFM one-sided coupling on the patients' most painful side (the one that received the BoNT/A injection) (Table 2, H vs. P(0)). The left PFM side was injected in 75 % of the patients, so that the lower *CC* values obtained in this group before BoNT/A treatment are in agreement with the results reported by the only previous study that assessed PFM one-sided coupling on the left side in patients with untreated CPP. As in the present study, it showed a lower amplitude coupling of the activity within this side in patients than in their healthy counterparts (Albaladejo-Belmonte et al., 2021b).

A previous study reported that PFM activity power, spectral content and complexity became more similar to those of healthy subjects after BoNT/A treatment (Albaladejo-Belmonte et al., 2021a). Conversely, in the present study, the alterations found in the electrical coupling with respect to healthy women remained after toxin infiltration (Table 2, H vs. P(j)). As described by Hodges et al., muscle pain acts as a stimulus to the nervous system that motivates it to modify its motor control strategies with respect to the painful muscle, inducing a redistribution of its activity and changes in its loading (Hodges and Smeets, 2015). This motor adaptation may persist even after pain resolution as it occurs progressively and pain cessation may not provide an impulse that motivates or leads the nervous system to restore the original motor strategy (Hodges and Smeets, 2015), thus justifying why one-sided coupling on the PFM injected side was still altered after treatment, even when most patients reported a significant pain relief.

Other factors could also have influenced the persistence of an altered one-sided coupling on the most painful side after treatment: 1) permanent structural and electrical changes in the PFM associated with CPP pathophysiology when myofascial pelvic pain is the main component, 2) PFM denervation and its subsequent altered reinnervation after a pelvic mechanical injury, and 3) external musculoskeletal factors. As for reason 1), a longstanding muscle contraction associated with PFM overactivity may spark off a local ischemia (Ross et al., 2021) that can eventually unleash a structural and electrical remodeling of the tissue, and thus biomechanical or neurophysiological changes that may promote the persistence of a redistributed activity within the muscle even after pain resolution (Hodges and Smeets, 2015). As for reason 2), vaginal deliveries or sustained efforts to defecate, which are a potential cause of CPP as they may lead to a chronic sensitization of the injured area, are associated with alterations in PFM innervation patterns (Min et al., 2022). Hence, PFM denervation and subsequent reinnervation would have caused long-term changes in the number of muscle fibres innervated by each motoneuron and their spatial territory, thus leading to altered motor unit action potential waveforms (Li et al., 2019). This could have induced a greater waveform variability across the motor units within the PFM side, and thus decreased their amplitude and phase coupling. As regards 3), external musculoskeletal factors related to the lumbo-pelvic-hip complex such as hip dysfunctions (Dune et al., 2022; Proulx et al., 2023), poor posture (Proulx et al., 2023), sacroiliac joint disorders or lumbar spine disorders (Dune et al., 2022), are commonly present in patients with CPP. The pathological mechanical processes associated with these (for example, abnormal transfer of loads (Morrison and Parrotte, 2020)) could also have promoted a redistribution of activity within PFM and thus have influenced the anomalous PFM coupling found in patients both before and after treatment.

Regardless of the reason underlying the altered coupling on the most painful PFM side, our results imply that while BoNT/A would mitigate the overactivation of motor units (Albaladejo-Belmonte et al., 2021a), it would not revert the alleged adapted motor control strategies, the permanent structural and electrical changes in the tissue or the altered innervation pattern in the PFM that could explain the deranged coupling of its electrical activity. However, the transient chemical denervation of motor units provoked by the toxin's mechanism of action implies an alteration of the PFM's motor unit recruitment patterns, which could justify why the magnitude of the differences across both groups' PFM electrical couplings changed throughout the visits of the study.

4.3. PFM one-sided coupling (least painful side)

Unlike the most painful side, the coupling of the electrical activity within the patients' least painful PFM side generally displayed similar features to those of the healthy subjects' right side, both before and after BoNT/A injection (Table 2, H vs. P(j)). This result did not agree with the only previous study that also assessed the coupling of electrical activity on individual PFM sides and which reported a lower amplitude coupling of the activity within both sides (Albaladejo-Belmonte et al., 2021b), which could be justified by the signal preprocessing steps performed in both studies. The previous study assessed raw sEMG signals that contained both PFM contractions and the intervening resting periods, while we obtained the envelope of the signals to reduce the amount of noise (Turpin et al., 2021) and only analysed segments of PFM contractions. We thus believe that the lower *CC* values in (Albaladejo-Belmonte et al., 2021b) could be mostly related to differences in the onset and offset times of the contractions rather than in their amplitude coupling throughout them.

It should also be noted that, while both *CC* and *iCOH* values were not significantly different across groups before treatment, they experienced different changes throughout the visits of the study. The patients' *iCOH* significantly decreased at Week 8 with respect to their values at Week 0, which was also found on the most painful side, while *CC* did not diminish significantly until the end of the study (Week 24). Both parameters were computed to assess PFM electrical coupling; however, they characterize different dimensions, as *CC* quantifies the linear relationship between the amplitudes of the two signals compared, while *iCOH* measures the magnitude of their phase difference. The fact that both showed different results implies that they are complementary metrics that would characterize or be affected by different aspects of the

neuromotor control of the PFM, which could be altered differently by CPP pathophysiology.

5. Limitations

The sEMG recordings were performed with self-adhesive electrodes attached to the skin of the perineum, which implies that the resulting sEMG signals contained information on the activity of the deepest muscles (with which myofascial pelvic pain is usually related), as shown by a previous study of our group (Albaladejo-Belmonte et al., 2021a), and also of the bundles nearest the surface such as the bulbospongiosus or the ischiocavernosus. Furthermore, while the clinician gave detailed instructions and feedback to the patients to prevent them from contracting nearby muscles, there is a chance that their activity was also recorded as crosstalk in the signals, which would have biased the results related to *CC* in the case of monopolar recordings. On the other hand, this would not have affected *iCOH* values since the cross-spectrum of signals coupled with no phase difference, as is the case with crosstalk and other common mode signals, is real (Nolte et al., 2004). It would thus be advisable to further validate the results obtained in this study with those from sEMG signals recorded by intravaginal probes, which are less sensitive to crosstalk. However, commercial probes usually have only one recording pole, and when multipole they do not allow to study PFM two-sided and one-sided coupling at the same time. Their insertion and placement can also be quite uncomfortable and even painful, especially when the woman suffers from sexual pain, as in the case of our patients. Crosstalk could also be effectively reduced in future studies by means of perineal self-adhesive electrodes by recording the activity of accessory muscles that may contaminate PFM sEMG recordings, such as the gluteal and hip adductor muscles) and applying additional processing steps (Flury et al., 2017). This was not performed in the present study as it was carried out in a hospital setting during time-restrained visits in which, apart from an exhaustive clinical evaluation, a treatment procedure was executed.

The dimensions of the electrodes used to perform sEMG recordings and their arrangement on the perineum were decided considering previous studies in the field (Macêdo et al., 2018; Moretti et al., 2017), before tutorials that standardized electrode geometry for sEMG recordings were published (Merletti and Muceli, 2019). This selection implied some limitations that need to be considered when interpreting the results of the present study: the electrodes had a relatively large contact area, suggesting that signals suffered from a spatial low-pass filtering before their detection in both monopolar and bipolar configurations; and the interelectrode distance of those placed on the same side was greater than 10 mm, which probably led to additional spatial filtering which would have introduced spectral dips within the sEMG bandwidth (Merletti and Muceli, 2019). In addition, the sEMG potential associated with the extinction of the action potentials at the end of the muscle fibers could have also contributed significantly to the monopolar signals as common mode components and thus have biased the results related to one-sided *CC* (*iCOH* is robust against coupling between common mode signals, as explained above). All these phenomena would have affected both healthy women's and patients' signals similarly, as the same electrode arrangement was used to record them, which implies that the statistical differences obtained between their PFM electrical coupling were probably unrelated to the limitations above. However, the spatial filtering associated with the electrode contact surface and interelectrode distance could have removed or modified the spectral content of the signals at frequency components that could be significantly related or affected by CPP pathophysiology. This hampers comparisons between the present results and those of other studies using electrodes with smaller contact surfaces and inter electrode distances, and it should be considered when defining the methodology of future studies. Given that the PFM electrical activity is heterogeneous across their different regions, recording protocols using multiple electrodes should be implemented to achieve a complete representation of it.

It is important to acknowledge the limitations of the sEMG recording protocol used for the patients' sEMG signals at Week 0. These signals were recorded on the same visit at which the patients were also physically examined and treated with BoNT/A, both of which are time-consuming procedures. This time constraint led to the design of a simple protocol of PFM voluntary contractions that could be easily and quickly explained to the patient and monitored by the clinician, and that allowed the assessment of both PFM resting and contractile activity. However, it did not include endurance nor quick flick contractions like the Glazer protocol (Hacad and Glazer, 2012), which could be involved with other neuromotor control strategies than those in the 5 s-tonic contractions performed by the participants in the study. These additional types of contractions could have provided further insights into CPP pathophysiology. Therefore, it is recommended that future studies make efforts to use this or another simplified protocol that also includes these two other types of contractions to enhance the understanding of CPP pathophysiology.

There are other factors that could also have biased the signals recorded in the present study, such as the arrangement of the electrodes irrespective of the PFM innervation zones, whose location within the muscle varies across participants, possible small changes in the electrode positioning in patients throughout the visits of the study, the performance of contractions of variable intensity levels during the protocol and across different visits, and differences in the ability to voluntarily contract the PFM across patients and healthy women due to the clinical condition of the formers. These should be considered when interpreting the results of the present study and comparing them with other studies.

6. Conclusions

The present study characterized PFM two-sided and one-sided electrical coupling in CPP associated with myofascial pelvic pain, providing more reliable results than previous studies with similar purpose, and evaluated the impact of BoNT/A treatment on it for the first time. While PFM two-sided coupling was not deranged with respect to healthy subjects in the initial pathological situation, the patients' PFM one-sided coupling on the most painful side was significantly altered. In particular, our results showed that their one-sided amplitude coupling (*CC*) was significantly lower than that of healthy women, while their phase coupling (*iCOH*) was significantly higher. This could be justified by altered neural control strategies, permanent structural and electrical changes and/or variations in the PFM innervation pattern associated with CPP pathophysiology. Furthermore, while perturbed coupling experienced some changes after BoNT/A treatment, probably associated with the toxin's motor mechanism of action, it did not become more similar to that of healthy subjects after treatment. This suggests that, unlike its positive impact on mitigating PFM overactivity, BoNT/A would not have a significant effect on the permanent structural/electrical/neural changes present in the patients' PFM in the pre-treatment situation. These changes could play a role in the onset and/or perpetuation of PFM overactivity and thus of CPP, or even in the recurrence of painful symptoms months after BoNT/A treatment, as has been reported by some patients. These findings provide new insights into the altered PFM electrophysiological condition in CPP associated with myofascial pelvic pain syndrome and the BoNT/A effect on PFM activity.

CRediT authorship contribution statement

Monica Albaladejo-Belmonte: Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Marta Tarazona-Motes:** Writing – review & editing, Resources, Investigation, Data curation. **Francisco Jose Nohales-Alfonso:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Maria De-Arriba:** Writing – review & editing, Resources, Investigation. **Jose Alberola-Rubio:** Writing – review & editing, Validation, Supervision, Software,

Resources, Project administration, Methodology, Data curation, Conceptualization. **Javier Garcia-Casado:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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