Eur Radiol (2014) 24:95–101
DOI 10.1007/s00330-013-2981-0

MAGNETIC RESONANCE

Diffusion tensor imaging and tractography to evaluate sacral nerve root abnormalities in endometriosis-related pain: A pilot study

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Received: 11 April 2013 / Revised: 25 June 2013 / Accepted: 24 July 2013 / Published online: 28 August 2013

Abstract
Objective To prospectively evaluate microstructural abnormalities in sacral nerve roots in women affected by chronic pelvic pain associated with endometriosis.
Methods We enrolled 30 women with an ultrasound diagnosis of endometriosis and moderate-severe chronic pelvic pain; 10 age-matched healthy women comprised the control group. All subjects underwent 3 T magnetic resonance imaging (MRI), including diffusion tensor imaging (DTI); the sacral roots were reconstructed by post-processing the DTI data with dedicated software. Mean fractional anisotropy (FA) values in the S1, S2 and S3 roots were quantified. Analysis of FA values was performed by two radiologists in order to evaluate the interobserver agreement.
Results The sacral nerve roots in healthy subjects were clearly visualised. Most of the patients with endometriosis displayed abnormalities of S1, S2 and S3 bilaterally at tractography, including an irregular and disorganised appearance. FA values in the S1, S2 and S3 roots were significantly lower in patients than in controls ($P < 0.0001$, <0.05 and <0.02, respectively) for both observers. No significant difference was found between observers.
Conclusion DTI with tractography is a non-invasive means of detecting changes in the microarchitecture of the sacral nerve roots. It can qualitatively and quantitatively reveal sacral root abnormalities in patients with endometriosis-associated pain.
Key Points
- *MRI is increasingly used for endometriosis and chronic pelvic pain (CPP).*
- *Magnetic resonance tractography can demonstrate microarchitectural abnormalities in sacral nerve roots.*
- *Tractography shows altered microstructure of sacral roots affected by endometriosis and CPP.*
- *S1–S3 fractional anisotropy values are lower in endometriosis than in healthy women.*
- *Sacral nerve root alteration may explain the nature of endometriosis-related CPP.*

Keywords Pelvic MRI · DTI · Tractography · Endometriosis · Chronic pelvic pain

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Introduction

Endometriosis is characterised by the presence of endometriotic-like tissue outside the uterine cavity. Although the prevalence of endometriosis is known to be underestimated owing to the high percentage of asymptomatic women, it is presumed to be 2–10 % [1]. Among women who report chronic pelvic pain and/or infertility, this prevalence can rise to 30–50 % [2]. Endometriosis is a common, chronic and often debilitating disorder. Chronic pelvic pain (CPP) symptoms such as dysmenorrhoea, non-cyclical pain and deep dyspareunia are present in more than 50 % of patients with endometriosis [3].

The pathogenesis of pain in endometriosis is not yet fully understood [4]. Endometriosis may cause pain by compressing or infiltrating nerves near the lesions [5]. Previous studies have shown that pain is associated with the location of endometriotic implants and adhesions, and with the presence of deep endometriosis, although there is no correlation between the stage of endometriosis and the intensity of pain [6, 7]. In women with symptomatic endometriosis, treatment of CPP is complex and in part controversial; current evidence shows some support for the use of non-steroidal anti-inflammatory drugs, suppressive hormonal therapy, laparoscopic surgery for removal of the different foci of endometriosis, hysterectomy, laparoscopic uterosacral nerve ablation or presacral neurectomy [8].

Unfortunately pain tends to recur in up to 50 % of cases after both medical and surgical treatment; risk factors for pain recurrence are poorly understood [7–9]. In 20 % of cases pain is resistant to common treatments.

Recently, it has been suggested that endometriotic lesions can develop their own nerve supply, thereby creating a direct, two-way interaction between lesions and the central nervous system [10]. There is a body of evidence pointing to a correlation between neurotropism and pain: ectopic (i.e., outside the uterine cavity) implants develop a sensory and sympathetic nerve supply in both mouse models [11] and women [12]; bioptic (i.e. examined under the microscope) endometriotic lesions display increased expression of neural biomarkers such as neurofilaments and nerve growth factor [13]; in eutopic (i.e. normally positioned) endometrium, there is an increased density of fibres associated with the heterogeneous expression of nociceptive receptors [14]. Furthermore, surgical interruption of pelvic nerve pathways, i.e. presacral neurectomy, can result in long-term relief of pelvic pain [15, 16].

As sensory and sympathetic fibres innervating lesions are connected to the spinal cord through the sacral nerves of the pelvic region, we decided to evaluate microstructural sacral nerve abnormalities in endometriosis by means of diffusion tensor imaging. Diffusion tensor imaging (DTI) is an MRI technique used mainly to assess the integrity of fibre tracts and, in particular, to measure fractional anisotropy (FA), which is an index of tissue organisation at the subvoxel level [17]. In addition, tractography can be used to visualise the neural tracts on three-dimensional images [18, 19].

Although DTI and tractography have so far been used mainly to investigate the central nervous system, these techniques have also been applied to the study of the uterus and prostate [20]. Furthermore, DTI and tractography can be used to study lumbar and sacral nerve roots in humans and to assess the architectural configuration of the peripheral nerves [21, 22]. The aim of our study was to evaluate tractography as a means of studying sacral nerves in women affected by endometriosis and CPP in order to investigate the association between endometriosis and sacral nerve abnormalities.

Materials and methods

Subjects

This pilot study was approved by the ethics Committee at our institution. Every woman signed a written informed consent form before inclusion in the study. Between November 2010 and May 2011, we enrolled 30 patients (mean age: 26± 3 years) who had clinical and ultrasound evidence of pelvic endometriosis associated with debilitating CPP (dysmenorrhoea, non-cyclic pain and/or deep dyspareunia), defined as persistent pain for more than 6 months, located in the pelvic region and of medium to high intensity. All patients were scheduled for laparoscopic treatment of endometriosis. Inclusion criteria were age between 18 and 40 years; moderate to severe pain symptoms, defined by a score higher than 7 on the visual analogue scale (VAS), which ranges from 0 (no pain) to 10 (worst pain); clinical suggestion of endometriosis (dysmenorrhoea, dyspareunia and non-cyclic pelvic pain) and ultrasound diagnosis of ovarian cystic endometriomas and/or deep endometriotic nodules. Exclusion criteria were previous pelvic surgery; current pregnancy; cancer and/or major medical illnesses; spine diseases and neuropathies; contraindications to MRI. The control group consisted of ten age-matched healthy volunteers (mean age: 25±2 years) with clinical and ultrasound examinations excluding the presence of endometriosis. All subjects underwent an accurate anamnesis, gynaecological and transvaginal ultrasound examinations before MRI.

MRI acquisition

Patients underwent a standard MR examination of the female pelvis; both patients and healthy women underwent the DTI study of the sacral nerves. All subjects underwent imaging using a 3-T (GE Discovery MR750 GE Healthcare, Milwaukee, WI, USA) magnet with one multichannel phased-array surface body coil (eight channels, 127.73 MHz).

No preparation other than a 3-h fasting period before the MR examination was required; a negative superparamagnetic oral contrast agent (Ferumoxil, Lumirem 175 mg/l, Guerbet) was administered to symptomatic patients in order to reduce the signal intensity of the bowels. Hyoscine N-butylbromide (Buscopan 20 mg/ml, Boehringer Ingelheim) was administered by either intravenous or muscular injection to reduce normal bowel peristaltic artefacts a few minutes before the beginning of the MR examination to both patients and healthy subjects.

The DTI sequence was acquired at the beginning of the examination when women were more relaxed and motionless. DTI was performed with diffusion-weighted single-shot and spin echo planar imaging (EPI) with 15 gradient directions, spectral fat suppression and parallel imaging (matrix 96×96,

FOV 360×360 mm, FA 90°, TR 5,000 ms, minimum TE of 71, $b=0$ and 1,000 s/mm^2, EPI factor 2) with 50 contiguous slices, each 4 mm thick, orientated on the paracoronal (to the sacrum) plane. The acquisition time for DTI was 15 min.

A standard study protocol of the female pelvis was then performed according to a previously described procedure [23, 24]. Briefly, it included single-shot fast spin echo (FSE) sequences (matrix 384×224, FOV 360×360, FA 90°, TR 2,000, TE 102, slice thickness 6 mm) orientated on the sagittal plane; T2-weighted fast recovery (FR) FSE high-resolution (HR) sequences (matrix 448×256, FOV 230×230 mm, FA 90°, TR 6,279 ms, TE 1,322 ms, slice thickness 3 mm) orientated on the axial, coronal and parasagittal (to the uterus) planes; T2-weighted FR FSE HR CUBE 3D sequences (Matrix 256×256, FOV 300×300 mm, FA 90°, TR 2540 ms, TE 162 ms, slice thickness 3 mm) orientated on the axial plane; T1-weighted FSE sequences (matrix 320×192, FOV 240×240 mm, FA 90°, TR 586 ms, TE 8 ms, slice thickness 3 mm) orientated on the axial plane. Moreover, LAVA-flex sequences with spectral fat suppression and parallel imaging reconstruction, (matrix 288×224, FOV 310×310 mm, FA 12°, TR 4 ms, TE 2 ms, slice thickness 4 mm) were orientated on the axial plane. Total acquisition time for the standard MR examination was 40 min.

Image analysis

Standard MR images were evaluated by two radiologists (LM, 10 years' experience; VV, 3 years' experience), who were required to reach a consensus, using an LMD Sony 2451-MD monitor (resolution of 1,220×1,920 pixels), according to previously described criteria. The two radiologists (LM and VV) analysed the images, blinded to the clinical information, i.e. they were unaware whether the images belonged to patients or healthy women, in order to avoid potential bias due to preconditioning.

Image analysis was performed twice. In a first analysis radiologists evaluated the presence of endometriosis in consensus according to previously published criteria [24]. Briefly, radiologists considered macroscopic ovarian endometriomas and implants of fibrotic tissue, widely located in different pelvic sites, including small foci of glandular tissue (hyperintense on T2 WI) and/or haemorrhagic foci (hyperintense on T1 WI).

The second time, 3D reconstruction of the sacral roots was done separately by the two radiologists in order to assess interobserver variability. The DTI images were analysed by means of dedicated vendor-specific software (Function 6.3.1) on a dedicated console. Current distortion and motion artefacts were reduced during postprocessing analysis before fibre reconstruction. Chemical shift artefacts were reduced by applying spectral fat suppression. A three-dimensional reconstruction of the first three sacral nerve roots (S1, S2 and S3) on both sides was achieved by positioning circular ROIs at their emergencies in the nerve foramina on b0 images (Fig. 1). T2 FSE FR sequences were considered for anatomical correlation.

The ROI sizes ranged from 25 to 50 mm^2 to fit the size of the respective nerve roots and minimise partial volume effects when the mean FA values were calculated within the ROI. Statistical analysis was performed using the Statistical Package for Social Sciences, version 16.0 (SPSS, Chicago, IL, USA). Radiologists achieved FA values separately, and a two-tailed unpaired t-test was used to compare FA in the S1–S3 nerve roots on both sizes of patients and healthy women for both observers. Values of $P<0.05$ were considered significant. Moreover, in order to analyse the interobserver variability, we compared the FA values obtained from each observer using Bland-Altman plots on MedCalc® version 12.4.0.0 (MedCalc Software, Ostend, Belgium). Values of $P<0.05$ were considered significant.

Results

The VAS scores in the 30 patients ranged from 7 to 10, the mean value being 8.5±1. Fourteen of the 30 patients were found to have endometriosis in the posterior compartments, which involved the following structures: uterosacral ligaments, rectosigmoid colon, vagina, uterus and Douglas pouch. Nine patients had endometriotic implants or endometriomas in the middle compartments, confined mainly to the ovaries. In

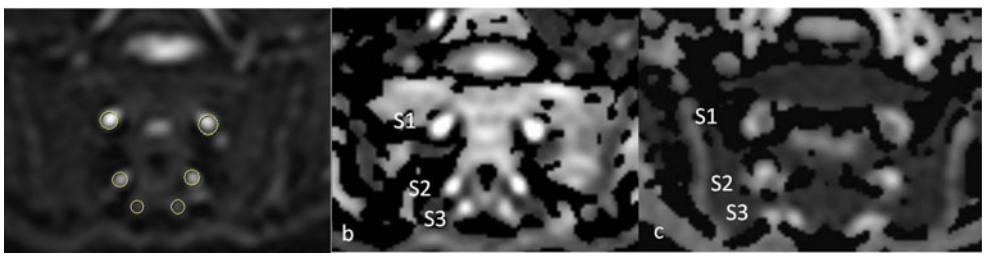

Fig. 1 Paracoronal diffusion tensor imaging (DTI): **a** DTI b0 image; **b** apparent diffusion coefficient (ADC) map; **c** fractional anisotropy (FA) map. Regions of interest (ROIs) for S1, S2 and S3 root reconstruction were drawn on b0 images and then automatically copied onto FA images

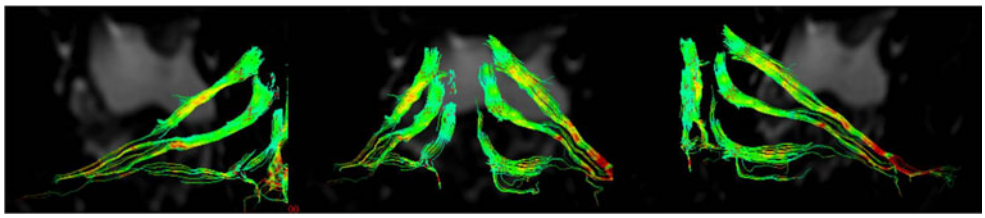

Fig 2 Example of fibre tracking reconstruction in a healthy woman showing S1, S2 and S3 nerve roots. Images are displayed in the coronal and sagittal planes (radiological convention). Fibre bundles S1 to S3 display a homogeneous appearance and regular course bilaterally

only one patient MRI revealed endometriosis in the anterior compartments, i.e. in the bladder. The remaining six patients were found to have implants in both the medium and posterior compartments. Tractography tracked spinal roots in all the healthy women, revealing a regular and homogeneous appearance of the sacral roots (Fig. 2).

All but two patients with endometriosis showed abnormalities in the sacral root microstructure, reconstructions, consisting mainly of fibre irregularities and disorganisation combined with the loss of the simple unidirectional course (Fig. 3), which was shown in healthy subjects. In the two patients with no sacral root abnormalities, implants were confined to the bladder in one and to the ovary in the other.

According to both radiologists, FA values were significantly lower in patients than in healthy women in all three sacral roots examined, as shown in Table 1. As there were no significant differences in FA between the left and the right side in either group, and for both radiologists, the left and right sides were pooled for each root and averaged. Moreover, no significant interobserver variability was found in the comparison of FA values between the two observers as is shown on the Bland–Altman plots calculated for each side of sacral roots for both observers (Fig. 4).

Discussion

Although laparoscopy is the gold standard for the diagnosis of endometriosis, MRI has also proved to be an effective and highly accurate means of diagnosing this condition [23].

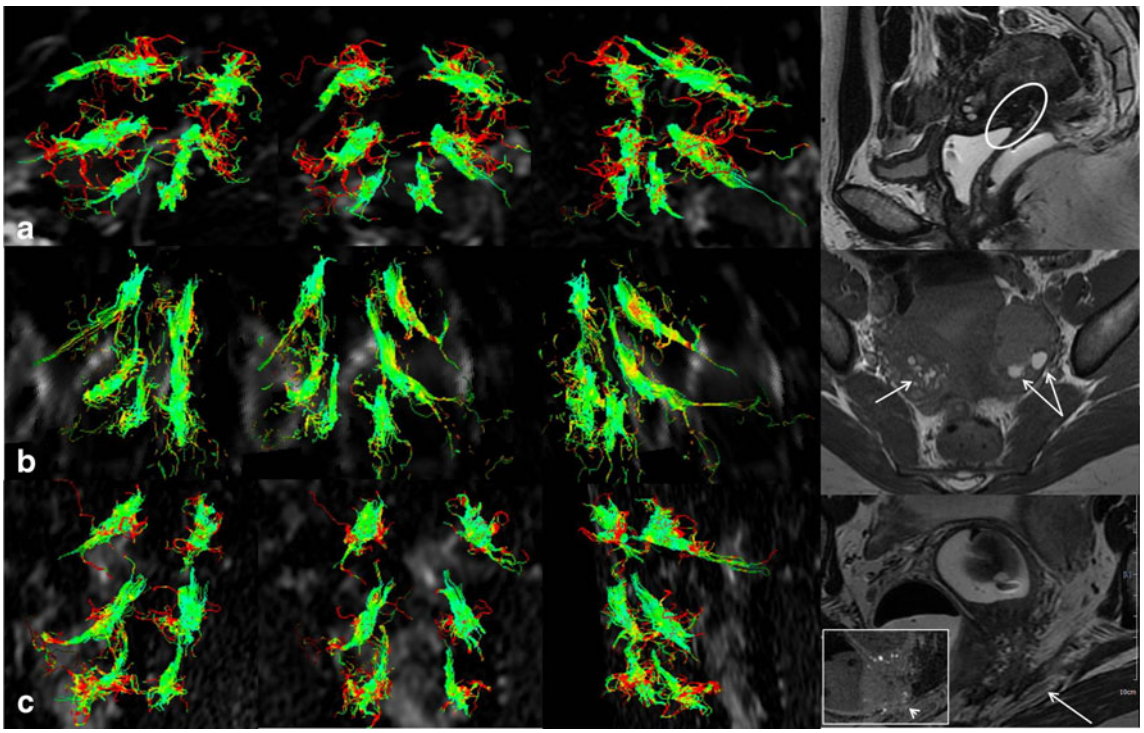

Fig. 3 Fibre tracking reconstruction in three women affected by endometriosis of the posterior compartment (**A**), of the medium (**B**) and of both (**C**). The fibre bundles in all three cases are short, stubby and have lots of branches. In **A**, T2-weighted fast recovery fast spin echo (FSE) image (sagittal plane) shows an endometriosis implant located in the posterior vaginal fornix (*circle*), which is elevated and associated with a retroverse uterus. In **B**, T1-w FSE image (*axial plane*) shows multiple implants in both ovaries (*arrows*). In **C**, T2-weighted fast recovery FSE image (*axial plane*) shows widespread endometriosis tissue infiltrating the rectum, peri-rectal space and piriformis muscle (*arrow*). The same area is shown on the T1 FSE fat-saturated (FS) image in the box below

Table 1 Mean fractional anisotropy (FA) of sacral nerve roots in 30 patients with endometriosis and 10 healthy women controls. As there were no significant differences between the left and the right side, both sides were pooled for each root (*S1*, *S2* and *S3*) and averaged

	Observer 1		Observer 2	
Sacral roots	Patients	Controls	Patients	Controls
S1	0.2963 ***	0.3521	0.2852 ***	0.3501
S2	0.2667 *	0.2924	0.2726 *	0.2952
S3	0.2404 **	0.2670	0.2535 **	0.2583

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.0001$ (by two tailed t-test)

However, despite the excellent ability of both laparoscopy and MRI to reveal the macroscopic characteristics of endometriosis, findings do not correlate with the occurrence and intensity of pain. Indeed, there is a discrepancy between the severity of the disease detected by laparoscopy and the degree of pain experienced by the patients [6, 7].

Although the pathophysiological mechanisms of CPP in endometriosis are not yet fully understood, according to a recent report on the relationship between endometriosis and pain, endometriosis-related pain should not only be ascribed to the presence of ectopic lesions, but rather to how spinal cord and brain neuron activity is affected by sensory and autonomic nerve activity from nerves that have sprouted from nearby tissues to innervate such growths [7]. Two-way connection (sensory and sympathetic nerve fibres) between innervated lesions and the spinal cord is concentrated within the sacral segments of the pelvic region [19]. It is conceivable that sprouted and disorganised nerve fibres are not confined to the endometriotic lesions but that they are even present in more proximal segments, i.e. in the sacral roots. This hypothesis could explain the high percentage of pain recurrence/persistence after surgical removal of endometriotic lesions or hormonal suppressive therapy.

An altered fibre tracking in patients with endometriosis could indicate a loss of unidirectionality of the nerve fibres, which correlates well with the decreased FA values found in these patients. In fact, quantitative measurements revealed significantly lower FA values in all three sacral roots in the patient group than in controls. The alignment of axons with myelin sheaths and the compartmentalisation of fibre bundles contribute to anisotropic diffusion in peripheral nerves. The decreased anisotropy we observed in the sacral roots of our patients may be due to a range of factors, including sprouting. Our findings point to an ultrastructural nervous injury that is not confined to the endometriosis implants but extended to the sacral nerve roots. Recent studies have demonstrated that DTI and tractography can be used to visualise peripheral nerves and spinal roots in both normal and pathological conditions [21, 25, 26].

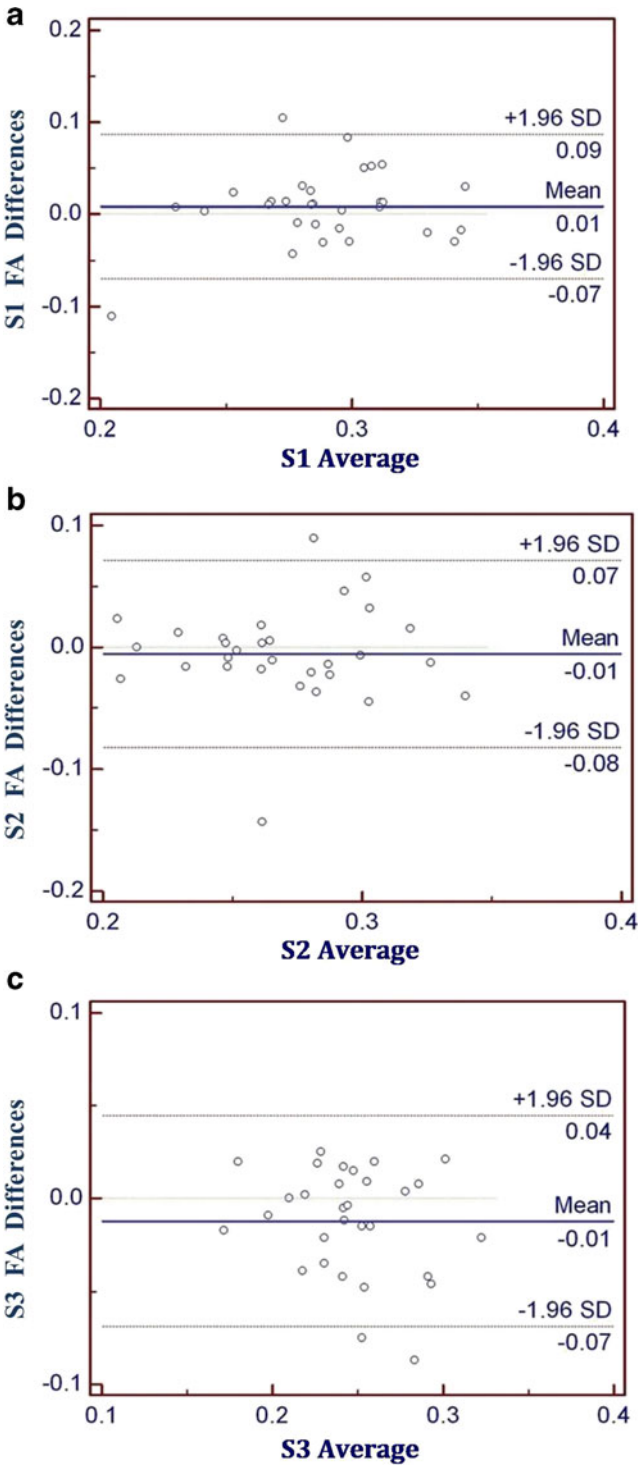

Fig. 4 Bland–Altman plots of FA values calculated by two independent observers. Y-axis represents differences in FA values; X-axis represents average FA values calculated by the two observers. Horizontal lines: the *middle line* indicates the mean of the FA differences between the two observers; *dashed lines* indicate the upper and bottom limits of the 95 % confidential interval. **b** S1 roots ($P<0.05$). **b** S2 roots ($P<0.05$). **c** S3 roots ($P<0.05$)

When studying the peripheral nerves, DTI with tractography has been recommended to visualise the proximal sciatic nerve

using a 1.5-T MRI [27, 28] and the distal median nerve using a 3 T MRI [29, 30]. When studying the spinal roots, Eguchia and colleagues [25] used tractography to study the lumbar nerve roots and quantitatively evaluate lumbar nerve entrapment with foraminal stenosis. Van der Jagt and colleagues [21] used tractography to investigate changes in the microstructural properties of sacral plexus nerves in healthy subjects, concluding that this new methodology may provide a new, more effective means of analysing and diagnosing neurogenic bladder dysfunctions.

In our study, we provide further evidence of the possibilities offered by tractography to study the sacral nerve roots and its potential role in the evaluation of symptomatic patients with endometriosis.

We are aware that our study has certain limitations, which mainly consist of partial volume artefacts.

We used a slice thickness of 4 mm with voxel size of 3.75× 3.75×4 mm, which is not ideal to study thin structures as the sacral roots [31]. A thinner slice thickness would improve the spatial resolution, but at the expense of the acquisition time. However, the issue of the relationship between the DTI spatial resolution and the size of the sacral roots is difficult to solve with the actual technology.

To minimise artefacts that could affect our data we applied parallel imaging, minimum TE, fat suppression and post-processing automatic correction of eddy distortion. We are aware that the partial volume effect is one of the most important limitations of our study, but it should have affected data from both symptomatic and healthy subjects to the same extent. Moreover, because of partial volume effects, differences between groups could have been underestimated rather than overestimated.

Partial volume effects should have mainly affected smaller fibres, and this may explain some discrepancy in our results. In fact, the lowest mean value of disagreement between the two observers emerged when the S1 and S2 FA values were compared, whereas the highest, albeit not significant, disagreement emerged when the S3 FA values were compared. Furthermore, the sacral root reconstruction allows S1 and S2 to be traced more easily than S3, which often appears thinner and shorter. For the same reason, we decided not to extend this study to the analysis of the S4 root, also bearing in mind that the pudendal plexus originates in S1, S2 and S3. A larger number of gradient directions would be more appropriate for tract reconstruction, though this would markedly increase acquisition time.

Lastly, a control group of patients with endometriosis but not CPP would have strengthened our findings; this point, however, will be addressed in an ongoing study on a large number of women with symptomatic and asymptomatic endometriosis. It would also be interesting to compare sacral root architecture between patients with CPP affected by endometriosis and patients with CPP related to other causes.

Despite these limitations, this is to the best of our knowledge the first noninvasive study of the neural anatomy in patients with endometriosis-associated CPP using DTI and tractography. It sheds light on the pathophysiology of the pain associated with this condition and may help both clinicians and surgeons to select a better and more personalised therapeutic approach. Identification of sacral nerve microstructural abnormalities may be the basis for understanding the reason why pain does not improve after traditional treatments of the disease and for selecting those patients who might benefit from alternative treatments, such as presacral neurectomy, anaesthetic nerve infiltration, neurostimulation or different types of pain killers that target neuropathic pain, e.g. antidepressants, anticonvulsants and minor opioids.

Acknowledgements Parts of this article were presented in electronic poster form at ECR 2013: “Fibre tracking evaluation of sacral nervous pathways 3D architecture in women affected by endometriosis”.

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