Ultra-high frequency ultrasound imaging of sural nerve: a comparative study with nerve biopsy in progressive neuropathies

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ABSTRACT

Introduction: Nerve ultrasound has been increasingly used in clinical practice as a complementary test for diagnostic assessment of neuropathies, but nerve biopsy remains invaluable in certain cases. The aim of this study was to compare ultra-high frequency ultrasound (UHF-US) to histological findings in progressive polyneuropathies.

Methods: Ten patients with severe, progressive neuropathies underwent ultrasound evaluation of the sural nerve before nerve biopsy. Ultrasound data were compared to histological results in a retrospective manner.

Results: Sural nerves were easily identified on UHF-US. Nerve hyperechogenicity correlated with inflammatory infiltrates on biopsy. Nerve fascicles could be identified and measured on ultrasound in the majority of patients.

Discussion: Hyperechogenicity on UHF-US may be a marker of nerve inflammation in neuropathies. Furthermore, the UHF-US probe allows for the evaluation of sensory nerves in spite of their small size, providing important information on their size and on their internal structure.

Keywords: ultra-high-frequency ultrasound, nerve biopsy, CSA, echogenicity, neuropathy.

INTRODUCTION

Nerve biopsy, in particular of the sural nerve, may be a useful diagnostic tool for the assessment of neuropathies of undetermined etiology[1]. However, it is an invasive procedure and often does not show the full picture in terms of pathological changes (i.e., biopsy might not always be performed on the most appropriate nerve or nerve segment). Magnetic resonance imaging (MRI)[2] and ultrasound (US) [3,4] have limited the indications for diagnostic nerve biopsy toa small number of specific situations. High-frequency US (HF-US) with an 18-20 MHz linear probe has been increasingly used for the evaluation of peripheral nerve injuries and polyneuropathies due to its low cost, wide availability and noninvasive nature[5-8]. However, it does not allow for a good visualization of sensory nerves because of their small diameter.

Recently, an ultra-high frequency US (UHF-US) imaging system using 30 to 70 MHz transducer probes has been developed and approved for human use, allowing for a better visualization of nerve and fascicle anatomy[9-11].

Previous studies involving nerve ultrasound were only performed on mixed nerves, and there are no direct comparisons between US and histological findings on the same nerve.

The aim of this study was to compare sural nerve UHF-US to histopathological findings in patients with severe, progressive polyneuropathies, in order to assess whether this type of US imaging could provide sufficient information to better characterize the disease process.

METHODS

Patients

Patients from the Peripheral Nervous System and Muscle Centre of the Nice University Hospital were included in the present study; all patients gave their informed consent for the collection and use of their data for research purposes. The study was approved by the hospital's research ethics committee. We included patients with severe, progressive neuropathies, which we defined as weakness, ataxic gait or neuropathic pain interfering with

activities of daily life. Diagnostic workup, which included biological (serum, cerebrospinal fluid, and urine) assessment and electrodiagnostic (EDX) evaluation was inconclusive, and this prompted the need for further investigations, with all patients subsequently undergoing sural nerve UHF-US evaluation and nerve biopsy. Consecutive patients fulfilling these criteria were included.

Electrodiagnostic studies

EDX studies were performed on all patients included in the study, using a protocol which examined four motor (tibial, peroneal, median, ulnar) and four sensory (superficial peroneal, sural, median, ulnar) nerves bilaterally. The results were used to divide patients into three patterns: definite chronic inflammatory demyelinating polyneuropathy (defined as per European Federation of Neurological Societies 2010 guideline[12]), axonal polyneuropathy (defined as loss of compound muscle action potential [CMAP] amplitude, with a length-dependent pattern), and mononeuritis multiplex (defined as loss of CMAP amplitude, without a length-dependent pattern and with a normal conduction study contralateral to the affected nerve).

Ultrasound evaluation

UHF-US was performed on the sural nerve to be biopsied using a 50-70 MHz linear array transducer (Vevo MD, FUJIFILM Visualsonics, Toronto, Ontario, Canada). Ultrasound was performed on the posterolateral side of the calf, on a 10cm segment starting from posterior to the lateral malleolus and extending proximally towards the mid-calf. Nerve and fascicle cross-sectional area (CSA), fascicle count, and visual assessment of echogenicity were carried out on transverse nerve images; the image that best permitted identification of all nerve fascicles was selected for measurements. . CSA measurements were performed within the hyperechoic rim using the manual tracing method. As there are no reference values for sural nerve CSA using UHF-US, the values proposed by the Goedee group using HF-US [5]were used. The fascicle with the largest CSA was selected for analysis. Nerves were

defined as mainly hypoechoic if their echogenicity was similar to that of blood vessels and as hyperechoic if comparable to subcutaneous fat echotexture.

Sural nerve biopsy

Sural nerve biopsy was performed on the side with the lowest sural sensory nerve potential amplitude on nerve conduction studies; if no potential could be elicited on either side, the left sural nerve was chosen. The procedure was performed under local anesthetic (Xylocaine 400mg/20ml). No landmark with regard to the site of nerve ultrasound was used when performing sural nerve biopsy. 3-5 cm of the sural nerve were excised and the specimen was divided into three fragments as per general recommendations[13] ; no other intraoperative measurements were performed. Nerve and fascicle CSA were calculated on 2µm semithin sections on transverse nerve images; measurements were performed perpendicular to the nerve and along the length of epineurium (nerve CSA) and perineurium (fascicle CSA) using a manual tracing method and the laboratory's available software (Leica, Nanterre, France). Sural nerve CSA values of 1.4 ± 0.7 mm^2 [14] and fascicle CSA values of 0.1 ± 0.06 mm^2 [15] were considered as normal. The largest fascicle CSA was considered for analysis.

Statistical analysis

For statistical analysis, SciPy library from Python 3.7 was used. As the distribution of values was not normal, Spearman's test was used for correlation assessment between nerve and fascicle CSA values, with or without taking into consideration the inflammatory status of the nerve biopsy, obtained with the two techniques. Significance level was set at $p < 0.05$. Pearson's test was used to evaluate correlations between echogenicity and inflammation.

RESULTS

Patients

10 patients (6 men and 4 women, mean age at first examination: 61.7 years, range: 36-75 years) with severe, progressive neuropathies, who had UHF-US and sural nerve biopsy performed between January 2013 and December 2017 were enrolled. Their demographic, clinical and paraclinical data are presented in Table 1.

US evaluation

Overall sural nerve CSA values ranged between 2.8 and 6.9 mm^2 (mean 4.74 mm^2); an enlargement of nerve CSA was found in 5 patients. Fascicle CSA values ranged between 0.2 and 0.7 mm^2 (mean 0.32 mm^2). The UHF-US evaluation allowed for the identification of between 4 and 12 fascicles per nerve (Figure 1). Seven nerves were hyperechoic on US evaluation (Table 1).

Nerve biopsy

An inflammatory infiltrate was found in 5 patients. Patient 2 had an epineural lymphocytic inflammatory infiltrate, adjacent to an arteriole and a capillary vessel. Patient 3 demonstrated findings suggestive of periarteritis nodosa, with lymphocytes, numerous neutrophils, and rare plasmocytes and histiocytes in the vessel wall; furthermore, several vessels showed thromboses. Patient 4 showed several perivascular, peri- and epineural lymphocytic infiltrates. Patient 7 had a discrete epineural lymphocytic infiltrate. Patient 8 presented several epineural lymphocytic infiltrates. Patients 9 and 10 had predominantly axonal degeneration on biopsy; patient 6 had a near-normal nerve biopsy, with only a few lymphocytes on the periphery of the epineural capillaries and no axonal loss or myelin damage. Endoneurial connective tissue was increased in all patients. Nerve CSA values ranged between 1.26 and 6.24 mm^2 (mean 3.12 mm^2); enlargement of nerve CSA was found in 7 patients. Fascicle CSA values ranged between 0.13 and 0.40 mm2 (mean 0.28 mm^2); enlargement of fascicle

CSA was found in all except for patient 4 (Table 1). Fascicles could be adequately counted in 8 patients, with overall numbers ranging from 5 to 10 (Table 1).

Comparison between UHF-US and histopathology findings

Four patients demonstrated increases in nerve CSA on both UHF-US and nerve biopsy(Table 1). No statistical correlations were found between the 2 techniques for nerve or fascicle CSA, regardless of whether inflammatory changes on nerve biopsy were considered or not. All nerves with an inflammatory infiltrate on biopsy had a hyperechoic appearance on US (Figure 2), and this reached statistical significance ($p< 0.03$).

DISCUSSION

The UHF-US probe allowed for a good visualization of the overall sural nerve and of its internal structure in all patients. The number of fascicles identified was in agreement with previous histological studies [14,16] ; in contrast, the HF probe visualizes only one third of the fascicles in mixed nerves [11].

Overall nerve CSA values were higher by US evaluation than biopsy. In contrast, the literature shows that biopsy specimens have larger nerve CSA than HF ultrasound examination in cadavers with no known ante-mortem nerve pathology[17]. This discrepancy may be due to procedural artifacts second to histological slide preparation, nerve edema, and to the higher resolution of the UHF-US probe. In this regard, the US probe permits evaluation of a longer nerve segment and allows for selection of an optimal segment for evaluation,. This may lead to more precise CSA values compared to biopsy, where only a fixed and limited nerve segment is available for study. The same considerations could possibly explain the absence of a correlation between nerve or fascicle CSA values when comparing ultrasound imaging and histopathology in our patients. However, no definitive conclusion can be drawn as normative values for the UHF-US probe are lacking.

All patients with inflammatory infiltrates on nerve biopsy had hyperechoic nerve ultrasound studies. On the other hand, there were 2 patients who had hyperechoic nerves and no inflammatory infiltrate. Although this suggests that UHF-US has a high sensitivity but rather low specificity in identifying nerve inflammation, the limited number of patients does not permit a definitive conclusion.

Previous studies describe a hypoechogenicity as being related to inflammatory changes in the nerve (possibly predominant edema and scarce inflammatory infiltrate), whereas hyperechogenicity suggests chronic irreversible changes, such as predominantly axonal damage, increased connective tissue and fibrosis [6,18] . While at first glance our data may disagree with those studies, there are several factors to note. First, previous studies examined exclusively mixed nerves, while the present study focused on a purely sensory one. Second, previous studies have not assessed correlations between ultrasound and histology findings from the same nerve in neuropathy patients using the UHF probe. Furthermore, the fact that there was still inflammation in the biopsy specimens of patients with longstanding disease suggests that hyperechogenicity may reflect not simply fibrosis, but also ongoing inflammation [6,19-21]. Another explanation for this discrepancy is that the hyperechogenicity may reflect increased visualization of interfaces between different nerve structures, previously not visualized due to the poorer spatial resolution of HF probes; working with very high frequencies could lead to the identification of very small interfaces, changing the hypoechogenicity encountered with the HFUS into hyperechogenicity seen with the UHF probe.

Most of the patients with nerve CSAs higher than reference values on US had an inflammatory infiltrate on nerve biopsy, suggesting the possibility that this might be a marker for inflammation; however, this did not reach statistical significance, possibly because of the small number of patients.

Our study has several limitations. First, the small number of patients and their heterogeneity have underpowered the statistical analysis. Second, seeing that previous data regarding the evaluation of sensory nerves and the use of a HFUS probe is limited no direct comparisons can be made to pre-existing literature. Third, the normal for nerve CSA were those described for the HF probe, which has a lower resolution; furthermore, as there are no

normative data for US fascicle CSA values or fascicle number for sensory nerves, we used data from anatomical studies as reference. Finally, because nerve biopsy was performed in the standard method employed in our department, without taking into consideration the site of ultrasound measurements, different sites within the same nerve may have been examined using the two methods, thereby possibly explaining the discrepancies between US and histologic results.

In conclusion, UHF-US can be used as a non-invasive, rapid and reproducible tool to evaluate nerve pathology. Hyperechogenicity of sensory nerves seems to correlate with nerve inflammation, thus offering insights into the pathophysiology of the disease process. Although nerve US will probably not replace nerve biopsy for the forseeable future, it could aid in the diagnostic process and possibly in the follow-up of patients. Further studies are needed to establish normal reference values for sensory nerves and validate our findings in larger cohorts.

ABBREVIATIONS

CSA – cross-sectional area; EDX – electrodiagnostic studies; HF-US – high-frequency ultrasound; UHF-US – ultra-high-frequency ultrasound; US – ultrasound

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Figure 1. Fascicle number evaluation. Image A shows the hypoechoic sural nerve on nerve cross-sections. Image B highlights the fascicles. Image C shows the histological image of the same nerve.

Figure 2. Comparison between ultrasound and histological images. Image A shows hyperechogenicity of the sural nerve obtained with a 50MHz probe. Image B shows perivascular inflammatory infiltrate consisting mainly of mononuclear cells in the same nerve.

Table 1. Demographic, EDX, ultrasound, and biopsy data

Patient number	Sex	Age at diagnosis (years)	Duration of disease (years)	EDX data		Ultrasound data				Biopsy data									Final diagnosis
				EDX pattern	Sural SNAP amplitude (μV)	N-CSA (mm^2)	F-CSA (mm^2)	Fascicle count	Echogenicity	N-CSA (mm^2)	F-CSA (mm^2)	Fascicle count	Inflammatory infiltrate	Schwann cell hyperplasia with/without onion bulb formation	Thin myelin	Large myelinated fiber loss	Unmyelinated fiber loss	Axonal degeneration	
1	M	60	5	Definite CIDP	0	4.1	0.7	12	Hypo	2	0.19*	10	-	+	+	+	-	-	CIDP
2	F	75	10	Axonal	0	6.9*	0.2	NA	Hyper	4.36*	0.3*	8	+	+	+	+	-	+	CIDP
3	M	53	0.5	Definite CIDP	0	5.4*	0.3	10	Hyper	3.92*	0.3*	6	+	+	-	+	-	+	PNS vasculitis
4	F	36	21	Axonal	5	5.7*	0.2	NA	Hyper	2	0.13	10	+	+	+	-	-	-	CIDP
5	M	59	1	Definite CIDP	7	6*	0.3	10	Hypo	3.59*	0.38*	8	-	+	+	+	+	+	CIDP
6	F	53	1	Definite CIDP	9	2.8	0.3	8	Hypo	1.26	0.19*	6†	-	-	-	-	-	-	DADS
7	M	69	4	Axonal	1.45	3.7	0.3	7	Hyper	2.84*	0.4*	10	+	+	-	+	+	-	PNS vasculitis
8	M	73	0.5	MNM	0	5.8*	0.37	8	Hyper	2.49*	0.36*	6	+	-	-	+	-	+	CIDP
9	M	73	0.5	MNM	6.7	3.7	0.2	4	Hyper	2.55*	0.23*	5	-	-	+	+	-	-	PNS vasculitis
10	M	66	11	Axonal	4	4.31	0.37	9	Hyper	6.24*	0.35*	6†	-	+	-	+	+	-	NDIN

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* abnormal values compared to reference values (see Methods)

† artifacts related to biopsy sample preparation prevented adequate fascicle count

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Definite CIDP pattern on EDX was established as per 2010 EFNS diagnostic criteria.

Abbreviations: EDX – electrodiagnostic studies; SNAP – sensory nerve action potential; N-CSA – nerve cross-sectional area; F-CSA – fascicle cross-sectional area; CIDP – chronic inflammatory demyelinating polyneuropathy; MNM – mononeuritis multiplex; Hypo – hypoechogenicity; Hyper – hyperechogenicity; NDIN – non-demyelinating inflammatory neuropathy; PNS – peripheral nervous system.