BROADENING THE SPECTRUM OF CONTROLS FOR SKIN BIOPSY IN PAINFUL NEUROPATHIES: CERVICAL DEGENERATIVE MYELOPATHY PATIENTS WITH PAINFUL FEET

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BACKGROUND

In recent years, skin biopsy with assessment of intraepidermal nerve fibre density (IENFD) has increasingly been used in the evaluation of small-fibre neuropathy (SFN). An American Academy of Neurology, American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM), and American Academy of Physical Medicine and Rehabilitation evidence-based review issued a Level C recommendation that supports skin biopsy in the diagnostic evaluation of SFN, while the European Federation of Neurological Societies/Peripheral Nerve Society Guideline stated that decreased IENFD reliably indicates the presence of SFN (level A recommendation). Both recommendations, however, emphasize the need to broaden the spectrum of controls for IENFD assessment by the evaluation of skin biopsy in patients whose clinical picture mimics that of SFN.

A similar clinical picture to that exhibited by SFN, with positive sensory symptoms in the lower extremities together with clinical or quantitative sensory testing (QST) sensory abnormities in this distribution may also be found in certain other clinical conditions, particularly those affecting central somatosensory pathways; these may be confused with SFN. Recently, this emerged in a study of patients with multiple sclerosis and restless legs syndrome (*Herrmann et al., 2010*). Another disease that may present with sensory symptoms and signs in the lower legs is cervical degenerative myelopathy (CDM).

AIM

To broaden the spectrum of IENFD controls by the assessment of patients with CDM and painful feet.

METHODS

A large cohort of ***244 CDM patients***, followed by the Department of Neurology of the University Hospital, Brno, was screened for the presence of positive sensory symptoms in the lower legs mimicking SFN. Such symptoms were described by a total of ***42 patients*** from this cohort. These were clinically examined, screened for known risk factors for peripheral neuropathies, and given standard nerve conduction studies. **Nineteen** of these patients **fulfilled the following inclusion criteria**:

(1) pain intensity of at least 3 as assessed by a numerical rating scale ranged from 0 to 10, upon which 0 represented “no pain” and 10 “the worst pain I can imagine”;

(2) no risk factors for peripheral neuropathies in the medical history (in particular, diabetes mellitus, alcohol abuse, uraemia, thyroid disorders, malignancy, or exposure to toxins or medication associated with neuropathy);

(3) normal or increased deep tendon reflexes in the lower legs,

(4) normal nerve conduction studies in the lower extremities, to exclude large nerve-fibre involvement in this distribution;

(5) and absence of other diseases or conditions leading to foot pain (i.e. plantar fasciitis, tarsal tunnel syndrome, osteoarthritis, peripheral vascular disease).

Finally, only ***14 patients fulfilled inclusion criteria*** and were willing to participate (8 men, 6 women, median age: 58; range: 46-63 years – see Table 1).

All the individuals included fulfilled the diagnostic criteria for CDM based on clinical symptoms and signs of cervical cord dysfunction or lesion due to degenerative cervical cord compression, documented by magnetic resonance imaging (MRI).

All patients underwent QST examination in the lower legs following the standardized protocol of the German Research Network on Neuropathic Pain (Deutscher Forschungsverbund Neuropathischer Schmerz, DFNS) (*Rolke, et al., 2006*) and using its standard recommendations.

Skin-punch biopsy samples were taken from the distal calf, approximately 10 cm above the right lateral malleolus. We followed standard recommendations for specimen removal, staining techniques and evaluation (*Lauria, et al., 2010b*). In brief, after fixation in 4% phosphate-buffered paraformaldehyde (pH 7.4) and cryoprotection in 10% sucrose, frozen sections of 50 µm thickness were cut and immunostained with rabbit polyclonal antibodies to human PGP-9.5 (1:200; Ultraclone, Wellow, UK, Ultraclone Cat. RA95101, RRID: AB_2313685) as primary antibody and goat anti-rabbit IgG labelled with fluorescein probe as secondary antibody (1:100; Chemicon, Temecula, USA) (Figure 1). The intraepidermal nerve fibres were counted manually at x630 magnification using a Leica DMLB microscope. The epidermal length of the skin section was measured with calibrated software (ImageJ, 1.42p, RRID: SCR_003070). The average intraepidermal nerve fibre density (IENFD) per millimetre of epidermal length was calculated according to current guidelines (*Lauria, et al., 2010b*). The entire epidermal length of three non-adjacent sections was evaluated in each patient. All the samples were counted by a single observer unaware of the clinical data (IK) and re-evaluated by another (EV) to ensure reliability. The results were evaluated using our own laboratory normative values, obtained from a smaller sample of individuals by immunofluorescence (*Bursova, et al., 2012*) as well as published reference values based on results from a large cohort of normal individuals using bright-field immunohistochemistry (*Lauria, et al., 2010a*).

RESULTS

All the patients included into the study showed only mild and predominantly sensory clinical impairment (mainly sensory loss, in upper and lower extremities, but without pronounced lack of stability when walking) which corresponds with high values of mJOA score in all the cases (Table 1). Clinical presentation of CSM thus corresponds with the “pseudopolyneuropathic pattern” of distal sensory involvement in lower extremities, which mimics the SFN (in part of the in combination with plurisegmental sensory loss in one or both upper extremities probably due to lesion of dorsal horns in the cervical level).

QST abnormalities were found in all but two of the CSM patients (86%). Most frequently, a combination of both positive and negative sensory signs emerged upon QST examination (7 patients, i.e. 50%) (Table 1).

The IENFD values for all 14 patients were within the range of age- and sex-related reference values when using the reference data from the worldwide normative study (*Lauria, et al., 2010a*). When using our own normal data, IENFD values were borderline in two of the patients, ranging between x-2 SD and x-2.5 SD of the reference data set, while all the other values did not exceed normal range (Table 1). On a group basis, however, mean IENFD values in the CSM group were not significantly different (6.87 ± 2.78 fibres/ mm of epidermal length) from the cohort of healthy volunteers matched for age and gender with the current study (7.97 ± 2.21 fibres/ mm, p >0.05) (*Vlckova-Moravcova, et al., 2008; Bursova, et al., 2012*).

CONCLUSIONS

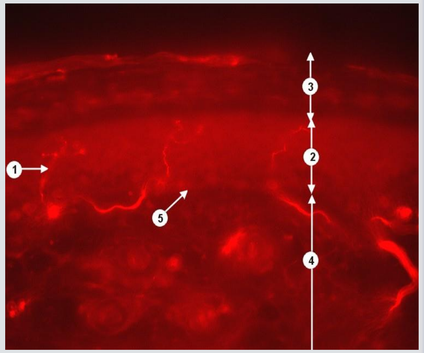
The study confirmed normal skin biopsy findings in patients with CDM as one of the clinical conditions mimicking SFN and provided further support for the use of IENFD assessment in case of suspicion of SFN.

Table 1: Demographic data, mJOA score, evoked potentials, skin biopsy findings and quantitative sensory testing in CSM patients

Patient Nr.	Sex	Age	mJOA	MEP	SEP	IENFD	QST abnormality	
							Gain	Loss
1	M	46	17	N	N	5.9†‡	MPT, MPS	MDT, VDT
2	M	55	16	N	N	4.3†	PHS	CDT, TSL, MDT
3	F	60	14	C	N	7.4†‡	0	0
4	F	59	17	N	C	14.7†‡	0	0
5	M	61	17	N	N	5.0†‡	PHS, MPT, MPS	CDT, TSL
6	F	55	13	C	N	4.3†	PHS	MPT
7	M	62	16	N	N	7.5†‡	PHS, MPT, HPT	0
8	M	51	17	N	N	6.3†‡	PHS, WUR	CDT, TSL, MDT
9	F	61	15	N	N	4.7†‡	0	WDT, MPT
10	M	53	17	N	C	6.4†‡	0	WDT, TSL
11	F	63	16	N	N	5.0†‡	PPT	CDT
12	F	57	16	N	C	7.2†‡	0	CDT, WDT, TSL, MDT
13	M	62	14	N	C	6.4†‡	0	PPT, MDT
14	M	50	17	N	N	11.2†‡	PHS	CDT, WDT, TSL

Legend to Table 1:
CSM – cervical spondylotic myelopathy
mJOA - modified Japanese Orthopaedic Association (JOA) scale (*Benzel, et al., 1991*)
MEP – motor evoked potentials
SEP – somatosensory evoked potentials
N – normal finding of MEP or SEP
C – central conduction abnormality in MEP/ SEP attributed to possible cervical spinal cord lesion
IENFD – intraepidermal nerve fibre density
QST – quantitative sensory testing
† IENFD value within normal range, after *Lauria, et al., 2010a*
‡ IENFD value within normal range, after *Bursova, et al., 2012*
CDT - cold detection threshold
WDT - warm detection threshold
TSL - thermal sensory limen
HPT - heat pain threshold
PPT - pressure pain threshold
MPT - mechanical pain threshold
MPS - mechanical pain sensitivity
WUR - wind-up ratio
MDT - mechanical detection threshold
VDT - vibration detection threshold
PHS - paradoxical heat sensation

Figure 1: Skin biopsy: PGP-9.5–immunoreacted 40-µm cryosections of skin



Legend to Figure 1:
1: intraepidermal nerve fibers
2: epidermis
3: stratum corneum
4: dermis
5: dermo-epidermal junction

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