Clinical Neurophysiology 128 (2017) 505–511

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Peripheral nerve ultrasonography in patients with transthyretin amyloidosis ☆

Simon Podnar [a,*], Stayko Sarafov [b], Ivailo Tournev [b,c], Gregor Omejec [a], Janez Zidar [a]

[a] Institute of Clinical Neurophysiology, Division of Neurology, University Medical Centre Ljubljana, Slovenia
[b] Department of Neurology, Alexandrovska University Hospital, Sofia Medical University, Sofia, Bulgaria
[c] Department of Cognitive Science and Psychology, New Bulgarian University, Bulgaria

ARTICLE INFO

Article history:
Accepted 15 January 2017
Available online 29 January 2017

Keywords:
Familial amyloid polyneuropathy
Nerve cross-sectional area
Peripheral nerves
Polyneuropathy
Transthyretin
Transthyretin-related amyloidosis
Ultrasonography

HIGHLIGHTS

- In patients with transthyretin (TTR) amyloidosis we found peripheral nerve thickening using nerve ultrasonography.
- Nerves were thickened particularly at common entrapment sites and in the proximal limb segments.
- Our findings may point to ischaemic nerve damage in the watershed zones in TTR amyloidosis.

ABSTRACT

Objective: To systematically study peripheral nerve morphology in patients with transthyretin (TTR) amyloidosis and TTR gene mutation carriers using high-resolution ultrasonography (US).
Methods: In this prospective cross-sectional study we took a structured history, performed neurological examination, and measured peripheral nerve cross-sectional areas (CSAs) bilaterally at 28 standard locations using US. Demographic and US findings were compared to controls.
Results: Peripheral nerve CSAs were significantly larger in 33 patients with familial amyloid polyneuropathy (FAP) compared to 50 controls, most dramatically at the common entrapment sites (median nerve at the wrist, ulnar nerve at the elbow), and in the proximal nerve segments (median nerve in the upper arm, sciatic nerve in the thigh). Findings in 21 asymptomatic TTR gene mutation carriers were less marked compared to controls, with CSAs being larger only in the median nerve in the upper arm. Nerve CSAs correlated with abnormalities on nerve conduction studies.
Conclusion: Using US, we confirmed previous pathohistological and imaging reports in FAP of the most pronounced peripheral nerve thickening in the proximal limb segments.
Significance: Similar to US findings in diabetic and vasculitic neuropathies these predominantly proximal locations of nerve thickening may be attributed to ischaemic nerve damage caused by poor perfusion in the watershed zones along proximal limb segments.

1. Introduction

Transthyretin (TTR) amyloidosis is an autosomal dominant inherited disorder caused by around 130 different pathogenic amino acid substitutions in the TTR protein (Plante-Bordeneuve and Said, 2011; Sekijima, 2015). To become functional, four TTR molecules need to assemble into a tetramer. Changes in the primary protein structure lead to TTR tetramer instability, allowing it to dissociate into monomer molecules. Monomers misfold and align into amyloid fibrils, which accumulate extracellularly and cause damage to various target tissues (Sekijima, 2015). Peripheral nerves are the commonest target, presenting clinically as the so-called "familial amyloid polyneuropathy" (FAP) (Plante-Bordeneuve and Said, 2011), a condition first described in Portugal (Andrade, 1952).

☆ Presented in part at the 2nd Congress of the European Academy of Neurology, Copenhagen, May 2016.

* Corresponding author at: Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, SI-1525 Ljubljana, Slovenia. Fax: +386 1 522 1533.
E-mail addresses: simon.podnar@kclj.si (S. Podnar), stayko_sarafov@abv.bg (S. Sarafov), itournev@emhpf.org (I. Tournev), gregor.omejec@gmail.com (G. Omejec), janez.zidar@kclj.si (J. Zidar).

http://dx.doi.org/10.1016/j.clinph.2017.01.013
1388-2457/

As FAP is caused by intraneural amyloid accumulation, peripheral nerves would be expected to be thickened, a finding confirmed by two imaging studies (Granata et al., 2014; Kollmer et al., 2015). Recently, a high-quality prospective cross-sectional study of 20 patients with TTR amyloidosis demonstrated increased thickness and MR signal changes in the sciatic, tibial and fibular nerves (Kollmer et al., 2015). Another study, using high-resolution ultrasonography (US), reported peripheral nerve findings in five gene carriers and seven patients with TTR amyloidosis (Granata et al., 2014). However, the first study was limited to the sciatic nerve and its branches (Kollmer et al., 2015), and the second enrolled only a small number of subjects (Granata et al., 2014). Therefore, the complete pattern of nerve changes in FAP could not be determined from these studies.

In order to obtain more data on the extent and distribution of peripheral nerve alterations in TTR amyloidosis, we designed a systematic study of the upper and lower limb peripheral nerve morphology using high-resolution US. We specifically aimed to look at the utility of US to diagnose TTR amyloidosis, to study demographic, clinical and electrophysiological factors possibly related to nerve enlargement in this disorder, and to consider if the specific pattern of nerve involvement can suggest information about the pathogenic mechanisms of nerve damage in FAP.

2. Materials and methods

2.1. Patients and controls

To determine the US reference values of the peripheral nerve cross-sectional areas (CSA), we prospectively examined a group of healthy adult subjects. The exclusion criteria for this control group were: (1) skin numbness or paraesthesia; (2) muscle atrophy or weakness; (3) polyneuropathy or other disorders of the peripheral nervous system; and (4) chronic diseases of other organs (e.g., heart, brain, eye and kidney). Control subjects were recruited and evaluated at the Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Slovenia by one of the authors (GO).

Between 15 September and 15 October 2015, we prospectively enrolled two groups of subjects with genetically confirmed mutations in the *TTR* gene: (1) patients with symptoms and clinical signs of FAP, and (2) carriers without symptoms of FAP. The vast majority of patients were either treated with the TTR tetramer stabiliser tafamidis (Said et al., 2012) or were included in phase 3 of the randomised, double-blind, placebo-controlled clinical trial of Patisiran (“Apollo”, TTR02-004, AlnilamPharm) (Suhr et al., 2015). Subjects with *TTR* mutations were evaluated by one of the authors (SP), either in the Department of Neurology, Alexandrovska University Hospital, Sofia, Bulgaria, or at homes or in their neighbourhood. The study was approved by the National Medical Ethics Committee, and written informed consent was signed by all subjects prior to the investigation.

2.2. History and clinical neurologic examination

The investigator evaluating subjects with mutations in the *TTR* gene took a short history of the disease and performed a focused neurological examination designed for these patients (Ando et al., 2013). Disease severity was estimated using Peripheral nerve disease (PND) staging, clinical staging of TTR-FAP (Coutinho et al., 1980), and the SAMV (sensory, autonomic, motor and visceral organ) impairment score (Ando et al., 2013). In seven patients with FAP, the same examiner also performed electrodiagnostic (EDx) studies using a commercial EMG system (Keypoint; Natus, Alpine Biomed, Skovlunde, Denmark). Nerve conduction studies (NCSs) were performed according to the standard protocol using surface stimulation and recording. In motor NCSs of the median, ulnar, fibular and tibial nerves, distal distances of 8 cm were used. In sensory NCSs of the median, ulnar, and sural nerves, there was a distance of 14 cm between the recording and stimulation sites. Concentric needle electromyography (EMG) of the right anterior tibial, vastus lateralis, the first dorsal interosseous, and biceps brachii muscles was also performed. Patient data on orthostatic hypotension, cardiac conduction defects, and kidney involvement, missing information on patients’ and carriers’ EDx studies, and body mass index (BMI) were obtained from patients’ and carriers’ medical records compiled by two other authors (SS and IT). From available EDx data for each FAP patient and carrier, the mean compound motor action potential (CMAP) amplitude was calculated. In addition, EDx abnormalities were staged as 0 – normal EDx studies, 1 – pathological lower limb and normal upper limb studies, 2 – pathological upper and lower limb studies, 3 – all NCSs responses virtually absent.

2.3. Ultrasonography (US)

All control subjects were Slovenian, examined using the same US device (ProSound Alpha 7, Hitachi Aloka Medical, Ltd, Tokyo, Japan) with a 4–13 MHz linear array transducer. All patients with FAP and *TTR* gene carriers were Bulgarian, and all underwent US examination performed using a portable device (MyLabGamma, Esaote, Spa, Florence, Italy) with a 6–18 MHz linear array transducer. Both US investigators measured the CSAs of the median and ulnar nerves at the wrist, forearm, elbow, and in the upper arm, of the fibular nerve at the caput fibulae and in the popliteal fossa, of the tibial nerve at the ankle and in the popliteal fossa, of the sciatic nerve in the lower thigh, and of the sural nerve approximately 10 cm proximal to the ankle. Both investigators were careful to lay the transducer lightly perpendicular to the nerve, and both used a trace method of CSA measurement with exclusion of the hyperechoic rim.

2.4. Statistics

For categorical variables, frequencies and percentages were calculated. As the distribution of numerical variables in at least one study group was not symmetrical, median (range) values were calculated. The Chi-square test was used to assess differences in categorical variables between the groups. Differences in numerical variables between the groups were tested by the Kruskal–Wallis test. In addition, the Mann–Whitney *U*-test with Bonferroni correction for multiple comparisons was used as a post hoc test.

US data were analysed from the right side in patients and carriers and from the side of the dominant arm in controls. In patients and carriers with missing data on the right side, data from the left side were included in the analysis. In each evaluated nerve segment, reference limits for CSAs were calculated from data obtained in control subjects. We first tested the data distributions using the Kolmogorov–Smirnov test, and for normally distributed CSAs, we set the upper reference limit (URL) at the mean +1.65 SD (exclusion of upper 5% data, i.e., single tail). For asymmetrically distributed CSAs, we set the URL at the 95th percentile (exclusion of upper 5% data, i.e., single tail). In patients and carriers, we used CSA reference values to calculate the sensitivities of these measures in each of the examined nerve segments. In the same two groups, the mean value, SD and standard error of absolute differences between the nerves on the right and left side, and a paired *t*-test were calculated.

We built a multivariate linear regression model, to determine which of the studied characteristics are associated with nerve CSAs. In addition, multinomial logistic regression was used to determine which characteristics best differentiated the study