Diagnostic and prognostic value of nerve ultrasound in peripheral nerve disease

J.A. Telleman

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Diagnostic and prognostic value of nerve ultrasound in peripheral nerve disease

Diagnostische en prognostische waarde van zenuwechografie bij perifere zenuwziekten

(met een samenvatting in het Nederlands)

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Johannes Arie Telleman
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Promotoren
Prof. dr. L.H. van den Berg
Prof. dr. L.H. Visser

Copromotoren
Dr. W.L. van der Pol
Dr. J.T.H. van Asseldonk

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Chapter 1

General Introduction

Peripheral nerves are the highways that connect our brain to the rest of our body. Through their conduction of nerve impulses they allow us to see, to feel, to talk and to walk. Therefore, it should come as no surprise that peripheral nerve disease can lead to a multitude of symptoms. Sensory functions may become distorted, leading to tingling, numbness or pain, and motor function may become impaired, leading to loss of strength or even paralysis. Just as there is a multitude of symptoms associated with peripheral nerve disease, there is a multitude of causes. Most commonly, a single nerve is impaired (mononeuropathy), e.g. by entrapment of the nerve at the carpal tunnel or ulnar sulcus, or due to the presence of a nerve tumor.[1] On the other hand, there is a broad spectrum of polyneuropathies with axonal, hereditary, inflammatory, and infectious origins that may cause severe impairment of multiple nerves (**Table 1**).[1-3] Discriminating peripheral nerve disorders is of great importance, as treatment options and prognosis can vary markedly. A thorough patient's history and physical examination are invaluable, as peripheral nerve disorders have distinctive features, such as a specific distribution of neurological deficits and a specific type of onset (**Table 2**).[4] However, additional testing is often required to adequately discern different peripheral nerve disorders and their disease mimics (**Figure 1**).

Nerve conduction studies (NCS) are a key instrument in the detection of both mono- and polyneuropathies. Cut-off values and diagnostic protocols have been developed to optimize its diagnostic value, and NCS-abnormalities are the hallmark in most international diagnostic guidelines.[4-6] However, NCS can be cumbersome and technically difficult, and in some cases even extensive NCS may fail to meet diagnostic criteria.[7-10] Other testing modalities, including lab investigation, lumbar puncture, and MRI have been employed to further improve diagnostic yield,[5,6] but even then diagnosing a specific peripheral nerve disorder can be challenging.[8-11] As a result, there is an ongoing need for tools that improve diagnostics in peripheral nerve disease, as well as tools that improve prognostic prediction and monitoring of treatment response.

An emerging player in the field of peripheral neuropathy is nerve ultrasound. It is a tool that allows low-cost, time-efficient imaging of multiple nerves, and it is often readily available. The most commonly identified sonographic feature in peripheral nerve disease is nerve enlargement, but other nerve characteristics, including vascularization and echogenicity, can also be assessed.[12,13] Peripheral nerve disorders have distinct sono-morphological features, including specific patterns of distribution of nerve enlargement, which can help to discriminate these different disorders (**Figure 2**).[12] Nerve enlargement at a solitary entrapment site is suggestive of a mononeuropathy, e.g. enlargement of the median nerve at the carpal tunnel in carpal tunnel syndrome. On the other hand enlargement at multiple entrapment sites can point to a hereditary neuropathy with liability to pressure palsies (HNLPP), and enlargement just proximal of entrapment sites, especially proximal to the ulnar sulcus, to leprosy.[14,15] Severe diffuse enlargement of nerves

Table 1 Causes of peripheral neuropathy

Mononeuropathy	
Nerve entrapment	Carpal tunnel syndrome, ulnar neuropathy (at elbow or Guyon's canal), fibular neuropathy, meralgia paresthetica, tarsal tunnel syndrome
Nerve tumors	Neurofibromas, schwannomas, lymphomas
Traumatic	Fractures (humerus, radius, ulna, fibula, pelvis)
Polyneuropathy	
Carcinoma	Lymphoma
Hereditary	Charcot-Marie-Tooth disease (CMT), Hereditary neuropathy with liability to pressure palsies (HNLPP), neurofibromatosis, porphyria
Idiopathic	Chronic idiopathic axonal polyneuropathy (CIAP)
Infectious	Leprosy, HIV, Lyme's disease
Inflammatory	Guillain-Barré Syndrome (GBS), Chronic inflammatory demyelinating polyneuropathy (CIDP), Lewis-Sumner Syndrome (LSS), Multifocal motor neuropathy (MMN)
Metabolic	Diabetes mellitus, chronic kidney failure, chronic liver failure, hypothyroidism, vitamin deficiencies
Paraneoplastic	Small cell lung cancer
Paraproteinemic	IgM- monoclonal gammopathy of unknown significance (MGUS), Anti-MAG associated polyneuropathy, Waldenström, polyneuropathy organomegaly endocrinopathy M-protein and skin changes (POEMS) syndrome
Systemic disease	Amyloidosis, sarcoidosis, Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis
Toxic	Alcohol abuse, Drug associated (chemotherapy, antimicrobials, immunosuppressants, amiodarone, digoxin),toxins (botulism, lead, mercury)
Vasculitic	Polyarteriitis nodosa, microscopic polyangiitis, Non-systemic vasculitic neuropathy

Table 1 shows an overview of causes of peripheral neuropathy. The list of causes of peripheral neuropathy is extensive and only some (common) examples are shown per type of origin. Small-fiber neuropathy forms a distinct type of peripheral nerve disease, and is therefore not covered in this table.

and nerve fascicles is suggestive of Charcot-Marie-Tooth (CMT) type 1A, a hereditary chronic demyelinating polyneuropathy, while enlargement of more proximal nerve segments is suggestive of an acquired chronic demyelinating polyneuropathy.[14,16] Nerve ultrasound is also able to detect anatomic anomalies, including nerve tumors, and traumatic nerve damage. It can be especially helpful to discriminate axonotmesis, in which axons are damaged but outer nerve structures remain intact, from neurotmesis,

Table 2 Clinical features of acquired chronic demyelinating polyneuropathies and potential disease mimics

Feature	CIDP	MMN	CIAP	LMND
Key neuropathic symptoms	Sensory & motor	Pure motor	Sensory & motor	Pure motor
Disease onset	Subacute / Chronic (> 6 weeks)	Subacute / Chronic (> 6 weeks)	Chronic (>6 weeks)	Chronic (> 6 weeks)
Distribution of neurological deficit	Arms & Legs Symmetric Proximal & distal	Arm predominant Asymmetric Distal	Leg predominant Symmetric Distal	Arm / Leg Asymmetric Distal
Accompanying symptoms	Areflexia Cranial nerve involvement Tremor Ataxia	Areflexia No cranial nerve involvement Cramps / fasciculations	Hyporeflexia	Hyperreflexia Bulbar disfunction Spasticity Fasciculations
Treatment-responsive	Yes	Yes	No	No

Table 2 shows the clinical features associated with acquired chronic demyelinating polyneuropathies. Clinical features of CIDP are shown for a typical phenotype. There are also atypical variants of CIDP, e.g. Lewis-Sumner syndrome, with a large variety in clinical features.
CIAP: chronic idiopathic axonal polyneuropathy. CIDP: chronic inflammatory demyelinating polyneuropathy. LMND: lower motor neuron disease. MMN: multifocal motor neuropathy.

in which the entire nerve is discontinuous, and to detect neuromas, which are important features in determining therapeutic management of peripheral nerve trauma.[17] To discriminate nerve tumors with ultrasound, e.g. neurofibromas and schwannomas, is challenging, though there may be helpful sonographic features.[18,19] Both localized neurofibromas and schwannomas are solitary hypoechoic lesions within a continuous nerve that show posterior acoustic enhancement, but schwannomas are often located eccentric to the nerve, while neurofibromas are most often located centric.[18-20] In addition, neurofibromas may be lobulated or fusiform of shape more often, may have less homogeneous echotexture, and nerve-tumor transition may be less well defined, but results on those features are mixed.[18,19] Plexiform neurofibromas, which have a risk of malignant transformation, are nerve tumors with a diffuse growth pattern within the nerve, and can be recognized by diffusely enlarged, serpentine-like fascicles over a longer tract within the nerve.[21] Still, up till date nerve ultrasound is unable to detect malignant transformation of a plexiform neurofibroma, and in case of suspected malignancy an MRI or PET-CT should be performed.[20-22]

Figure 1 Diagnostic work-up in peripheral nerve disease

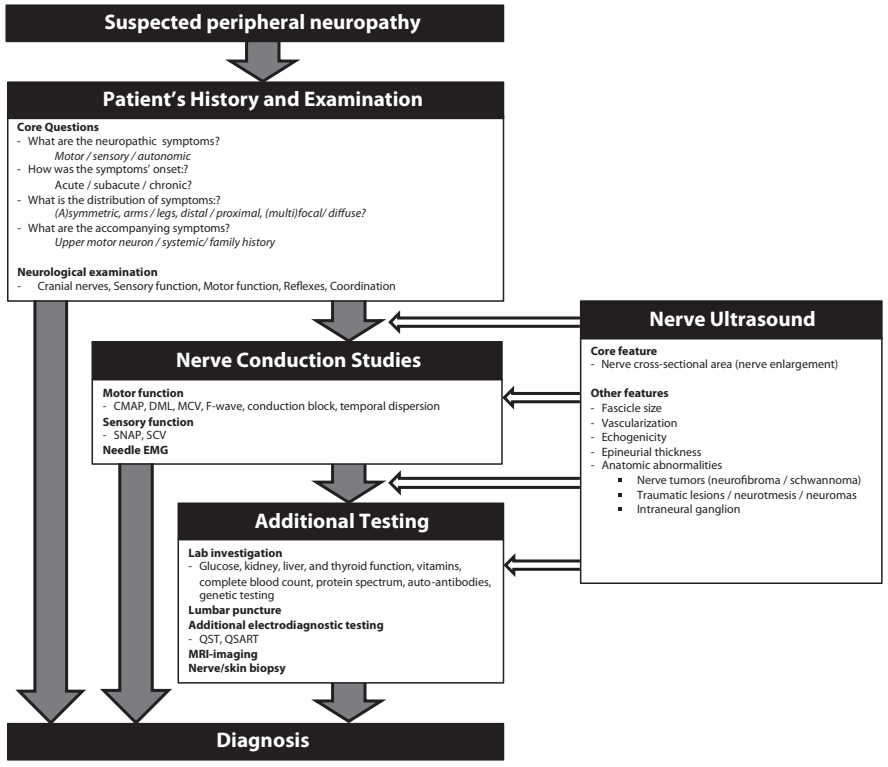

Figure 1 shows the routine diagnostic work-up in patients suspected of peripheral neuropathy (grey arrows). On the right the relatively new diagnostic tool nerve ultrasound is shown. This tool may have different places within diagnostic work-up, depending on the suspected peripheral neuropathy. For instance, in carpal tunnel syndrome nerve ultrasound is complementary to nerve conduction studies (NCS) and could also be performed prior to NCS.[BRON Visser LH] Its role and place in diagnostic strategies in many other peripheral neuropathies, e.g. acquired chronic demyelinating polyneuropathies, has yet to be determined (white arrows).
CMAP: compound muscle action potential. DML: distal motor latency. MCV: motor conduction velocity. QST: quantitative sensory testing. QSART: quantitative sudomotor axon reflex test. SCV: sensory conduction velocity. SNAP: sensory nerve action potential.

Nerve ultrasound is increasingly used in the assessment of peripheral nerve disease. After its first introduction in the 1980s nerve ultrasound has been gradually incorporated into diagnostic guidelines for mononeuropathy, including carpal tunnel syndrome and ulnar neuropathy at the elbow, and in recent years its applicability in the assessment of polyneuropathies is also under investigation.[9,10,12,16,23-28] Studies suggest that nerve ultrasound may be particularly helpful in discriminating acquired chronic demyelinating polyneuro-

Figure 2 Abnormalities identified with nerve ultrasound

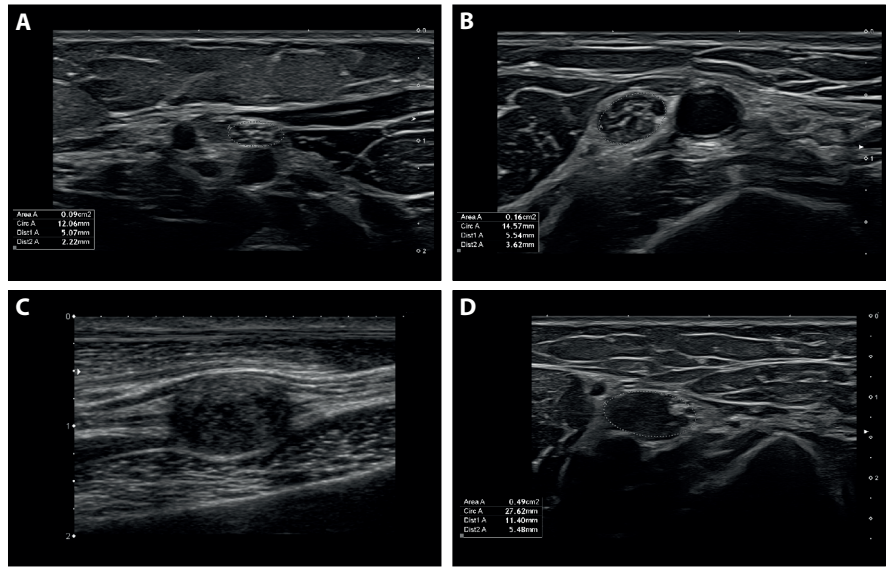

Figure 2 shows images of the median nerve in the arm obtained by nerve ultrasound. 2A Transversal image of a median nerve of a healthy control with a characteristic 'honeycomb' structure (cross-sectional area (CSA) 9mm^2). 2B Transversal image of a median nerve in a patient with multifocal motor neuropathy (MMN) with increased nerve CSA and fascicle size (CSA 16mm^2). 2C Longitudinal image of a median nerve in a patient with neurofibromatosis type 2 showing a schwannoma. 2D Transverse image of a median nerve in a patient with neurofibromatosis type 1 showing a plexiform neurofibroma (CSA 49mm^2).

pathies from disease mimics, which is of importance because of the therapeutic implications.[16,27,28] In addition, there are some studies that suggest a role for nerve ultrasound in prognostic prediction.[29-33] However, most studies were performed in a single center in a relatively small amount of patients, and large multicenter studies investigating the added value of nerve ultrasound have yet to be performed.

The aim of this thesis is to determine the applicability, diagnostic value and prognostic value of nerve ultrasound in peripheral nerve disease, with a focus on acquired chronic polyneuropathies. In **Chapter 2** the literature on nerve ultrasound and its current applications in polyneuropathy is reviewed. **Chapter 3** describes inter-observer variability of nerve ultrasound in a multicenter setting, which is a key element for the applicability of the tool in daily clinical practice in both mono- and polyneuropathies. In **Chapters 4 & 5** the diagnostic value of nerve ultrasound in acquired chronic demyelinating polyneuropathies is investigated. In these chapters both the added value of nerve ultrasound

compared to standard NCS and the validity of a practical ultrasound protocol for establishing a diagnose of an acquired chronic demyelinating polyneuropathy are determined in a multicenter setting. **Chapter 6** describes a multicenter study on the prognostic value of nerve ultrasound in acquired chronic polyneuropathies, while in **Chapters 7, 8 & 9** potential applications of nerve ultrasound in another type of peripheral nerve disease, i.e. neurofibromatosis type 1 and 2, are explored. A **General discussion** concludes this thesis, summarizing main findings of the previous chapters, describing implications of these studies and considering future directions.

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Chapter 2

Review of Literature

Nerve ultrasound in peripheral neuropathies AANEM Monograph

JA Telleman, A Grimm, HS Goedee, LH Visser, CM Zaidman

Muscle Nerve 2018;57:716-728

Abstract

Ultrasound can be used to visualize pathology in peripheral nerves in patients with polyneuropathy. Nerve enlargement is the most frequent pathology, but other abnormalities including abnormal nerve echogenicity and vascularity are also encountered. This monograph presents an overview of the role of nerve ultrasound in the evaluation and management of both inherited and acquired peripheral neuropathies. A description of the sonographic techniques and common abnormalities is provided followed by a presentation of typical findings in different neuropathies. Scoring systems for characterizing the presence and pattern of nerve abnormalities as they relate to different peripheral neuropathies are presented.

Introduction

Ultrasound of nerves is a complimentary technique to electrodiagnostic (EDX) studies that can assist the physician in localization and differential diagnosis of neuropathy. Nerve ultrasound is commonly utilized for mononeuropathies and traumatic neuropathies in which it can directly influence diagnosis and management. Ultrasound is also increasingly being used to assist in the evaluation of peripheral neuropathies and is particularly helpful when a demyelinating neuropathy is suspected. This article will review the technique, current evidence, and applications of nerve ultrasonography in the evaluation of suspected peripheral neuropathies.

Sonographic Technique

In the evaluation of peripheral neuropathy, nerve ultrasound is typically performed on an affected arm and leg, and often includes evaluation of the brachial plexus. A high frequency linear transducer is required, typically >15 MHz Nerves are imaged in the axial plane and should be scanned along their length in order to identify the pattern and distribution of abnormalities. When abnormalities are identified, imaging in the longitudinal plane is often clarifying and can further characterize the suspected abnormality, particularly when small regions of fascicular enlargement are detected.

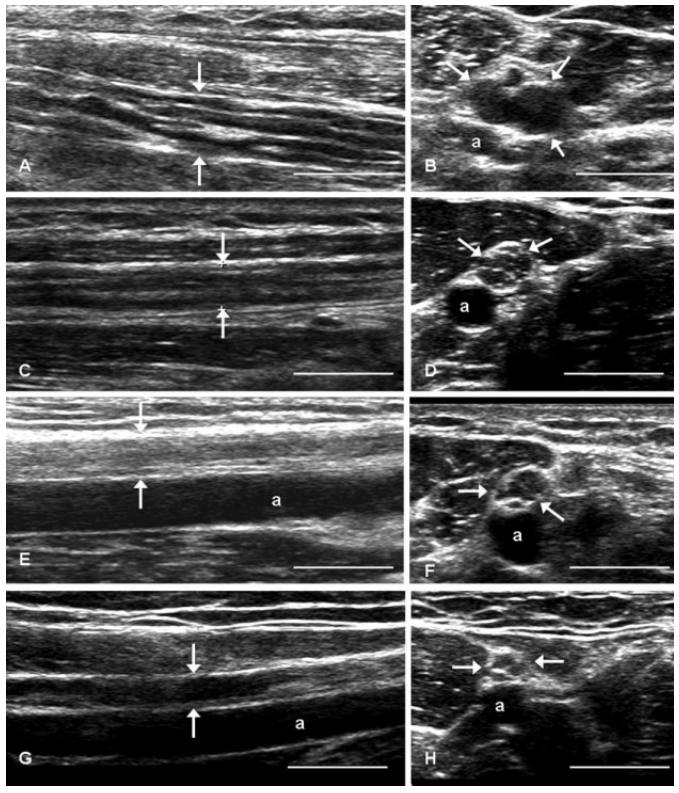
Some experience with normal nerves is essential to accurately identify pathology with nerve ultrasound. The normal nerve epineurium is hyperechoic (bright). The internal components of the nerve appear as a honeycomb structure, with fascicles appearing as hypoechoic (dark) regions outlined by hyperechoic rims. A normal nerve can appear differently along its length. For instance, the nerve roots and trunks of the brachial plexus are typically hypoechoic with one or two large fascicles. The median and ulnar nerves in the forearm, in contrast, show many smaller fascicles. The tibial nerve in the popliteal fossa often appears hyperechoic with indistinct borders, while the peroneal nerve at the fibula can also have indistinct borders and is encapsulated in a fat pad.

Sonographic parameters

Nerve enlargement is the most common abnormality identified using nerve ultrasound (Fig. 1). It is best appreciated qualitatively by imaging along the length of the nerve and then confirming the enlargement quantitatively. Nerve cross-sectional area (CSA) can be determined by tracing the nerve area within the hyperechoic epineurium. The transducer must be angled perpendicularly to the nerve in order to obtain the most accurate (and smallest) measurement of the nerve area. Nerve diameter is less often assessed, measured in the longitudinal plane of the nerve as the distance between the hyperechoic epineurium. Nerve diameter is most often used to quantify the size of the extraforaminal cervical nerve roots as their oblique course can compromise accurate measurement of

the CSA. Individual fascicle size can also be measured. When a nerve CSA is enlarged, the nerve fascicles are also often enlarged, although fascicle enlargement may not be uniform.[1] Normal values of nerve size are widely available for nerves in the neck, arm, and leg but do vary between examiners and laboratories.[2-5] It is therefore recommended that each laboratory establish its own set of normal reference values.

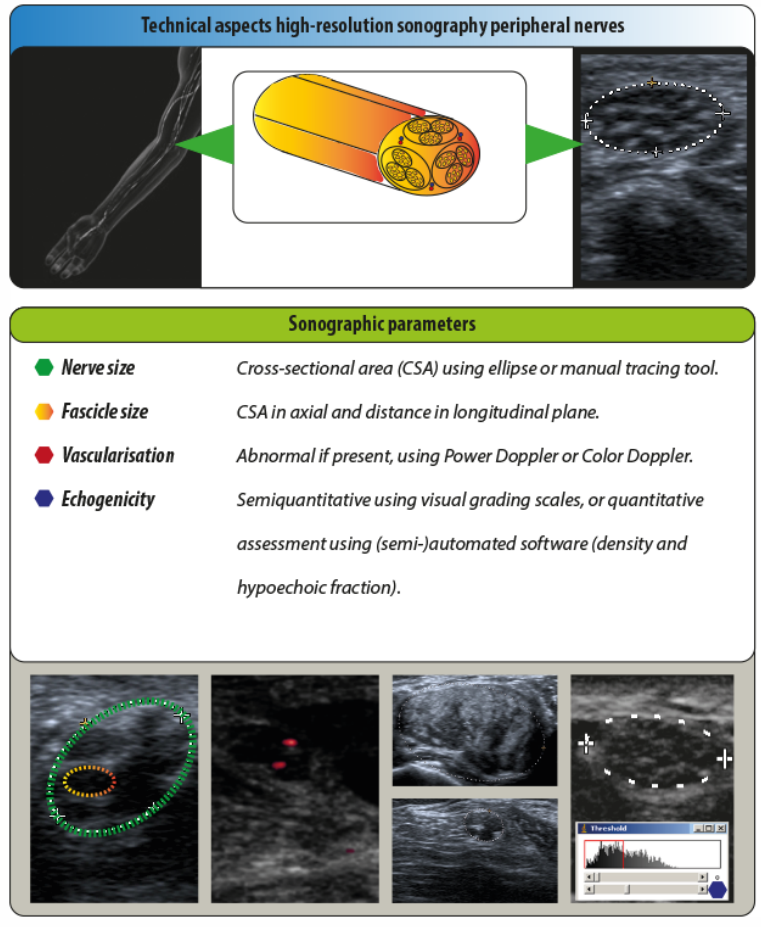
Figure 1 Types of nerve enlargement



Nerves are diffusely enlarged in CMT1A, CIDP, and GBS. The images show nerve size in CMT1A, CIDP, GBS, and a control subject. Longitudinal images are on the left and transverse images on the right. The median nerve (white arrows) is often shown abutting the brachial artery (a). Diffuse enlargement is seen in (A, B) a 55-year-old woman with CMT1A (nerve CSA = 47.8 mm2); (C, D) a 62-year-old man with CIDP (nerve CSA = 18.7 mm2); and (E, F) a 27-year-old man with GBS (nerve CSA = 13.7 mm2). The normal subject (G, H) is a 34-year-old healthy male (nerve CSA = 7.5 mm2) (scale bars = 1 cm).
CIDP: chronic inflammatory demyelinating polyneuropathy, CMT: Charcot–Marie–Tooth disease, CSA: cross-sectional area, GBS: Guillain–Barré syndrome.

Nerve echogenicity and vascularity can also be evaluated and may be abnormal in peripheral neuropathies (Fig. 2). Nerve echogenicity, like the fascicle appearance, varies in normal nerves along their length. It is most often evaluated qualitatively, but can be quantified using imaging processing software. Quantitative measures of echogenicity will vary with ultrasound systems, software, and settings. Automated and semiautomated

Figure 2 Technical aspects of high-resolution sonography in peripheral nerves



Nerve size is most often measured by tracing the nerve within the epineurium (bottom row, first panel, outer circle). Fascicle size is also similarly measured (bottom row, first panel, inner circle). Nerve vascularity is most often qualitatively assessed using power Doppler (bottom row, second panel). Nerve echogenicity can be increased and is most often assessed qualitatively (bottom row, third panel) or it can be quantified (lower right panel).

techniques can be used to calculate ratios of nerve echogenicity to nerve size, termed density and hypoechoic fraction. Nerve vascularity is evaluated using Doppler imaging. A normal nerve has little or no epineurial or intraneural flow on Doppler imaging, while entrapment and acquired neuropathies can show increased vascularity. Note that the increased blood flow is often in very small vessels and may be better detected using power than color Doppler. As with nerve echogenicity, nerve vascularity can also be described qualitatively or quantitatively.

Scoring systems

Nerve enlargement can be further characterized by the degree and pattern of enlargement. Qualitatively, nerve pathology can be spatially classified as focal, regional, or diffuse. Focal enlargement refers to a discrete nerve enlargement as can be seen in nerve tumor, entrapment (carpal tunnel syndrome), or some acquired neuropathies such as multifocal motor neuropathy (MMN). Regional enlargement refers to enlargement in a region of the nerve that is not as discrete or isolated as a focal enlargement, but that also does not extend throughout the course of the nerve. Diffuse enlargement refers to enlargement that involves the nerve along its length, including both proximal and distal segments. A diffusely enlarged nerve is often not uniformly enlarged, as the nerve enlargement is often more pronounced proximally.

Several strategies have been applied to codify and quantify the pattern and extent of nerve enlargement (Table 1). These include intra-nerve variability, a ratio of the largest to smallest CSA within a nerve, as well as inter-nerve variability, a ratio of the intra-nerve variability between 2 nerves. Extensions of this concept can be used to compare limbs from each side and the brachial plexus.[2,6] Other scoring systems codify nerve enlargement using a combination of the presence, location, and degree of nerve enlargement. Examples include the Ultrasound Pattern Score,[7,8] which quantifies nerve enlargement with use of a weighted rating system that scores the presence and degree of nerve enlargement for several nerve sites, as well as the Neuropathy Ultrasound Protocol, which uses a step-wise assessment of coding nerve enlargement in different body regions.[9] Another type of rating system combines nerve enlargement with nerve echointensity.[10] Prospective studies assessing and comparing these scoring systems are needed to better determine how they perform in characterizing nerve pathologies.

Sonographic abnormalities in polyneuropathies

Different types of peripheral neuropathy may show different sonomorphological abnormalities (Fig. 3). In general, nerve enlargement is most often seen in demyelinating neuropathies, both inherited and acquired. Massive nerve enlargement is particularly characteristic of Charcot–Marie–Tooth (CMT) disease type 1A, but can also be seen in chronic inflammatory demyelinating polyneuropathy (CIDP) and leprosy. Nerve enlargement

Table 1 Strategies for identifying specific patterns of sonographic nerve enlargement in demyelinating polyneuropathies

Classes of Sonopathology [10]	
Author	Padua and colleagues 2014
Measurement sites	Variable
Description	Three classes of nerve sonopathology: Class 1: Large nerves with hypoechoic nerves/fascicles Class 2: Large nerves with heterogeneous hypo- and hyperechoic fascicles Class 3: Normal size nerve but abnormal hyperechoic fascicles
Results	In CIDP, nerve class is associated with longer disease duration. Class 3 is the most chronic and occurs in very longstanding disease
Mild/regional/diffuse enlargement [12]	
Author	Zaidman and colleagues 2013
Measurement sites	4: Median and ulnar nerves in the arm and forearm
Description	1. Normal: No nerve enlargement. 2. Mild enlargement: Nerve enlargement at 1 or more anatomical sites, but not more than twice normal average size. 3. Regional enlargement: Nerve enlargement of more than twice normal average size at least at 1 site and normal nerve size at least at 1 other site. 4. Diffuse enlargement: Nerve enlargement at all proximal and distal sites, and more than twice normal size at least at 1 site.
Results	CMT: Diffuse enlargement is most frequently found in CMT1A (89%). CIDP: Diffuse enlargement is found to a lesser degree (37%), mild and regional enlargement are frequently found (20% and 24%, respectively). GBS: Most often normal nerve size or mild enlargement (48% and 38%, respectively). MMN: Most often normal nerve size or mild enlargement (35% and 41%, respectively).

Table 1 Continued

Bochum Ultrasound Score (BUS) [36,82]	
Author	Kerasnoudis and colleagues 2014, Kerasnoudis and colleagues 2015
Measurement sites	4: Ulnar nerve: Guyon's canal, arm; radial nerve: spiral groove; sural nerve: calf
Description	One point is awarded for each site with increased nerve CSA (range: 0-4)
Results	Discrimination of CIDP versus GBS (cut-off value ≥2): sensitivity 90%, specificity 90.4%; discrimination of CIDP versus MMN and MADSAM (cut-off value ≥2): sensitivity 80%, specificity 87.5%.
Ultrasound Pattern Scores (UPS) [8]	
Author	Grimm and colleagues 2015
Measurement sites	12: Median nerve: forearm, elbow, arm; ulnar nerve: forearm, arm; tibial nerve: popliteal fossa, ankle; peroneal nerve: popliteal fossa; vagal nerve; C5 nerve root (longitudinal); C6 nerve root (longitudinal); sural nerve: calf
Description	Calculation of several subscores: UPS-A: Measurement of nerve size of upper and lower extremity nerves. For each site 0 points are awarded if no enlargement is found, 1 if enlargement between 100-150% of normal size is observed, and 2 if enlargement is more than 150% of normal size (range: 0-16). UPS-B: Measurement of nerve size of vagal nerve and C5 and C6 nerve roots. One point is awarded for each enlarged site (range: 0-3). UPS-C: Measurement of nerve size of sural nerve. One point is rewarded in case of enlargement (range: 0-1). UPSS: Sum of UPS-A, UPS-B, and UPS-C scores (range:0-20).
Results	UPSS ≥10 or UPS-A ≥7: Suggestive of CIDP. UPSS ≤10, UPS-A ≤7, and UPS-B ≥1: Suggestive of GBS. UPSS 3-10 and UPS-B ≤1: Suggestive of vasculitic neuropathy. UPSS ≤3: Suggestive of axonal neuropathy.
Neuropathy Ultrasound Protocol (NUP) [9]	
Author	Kerasnoudis and colleagues 2016
Measurement sites	9: Median nerve: wrist, forearm; ulnar nerve: Guyon's canal, forearm, elbow, arm; radial nerve: spiral groove; tibial nerve: ankle; sural nerve: calf
Description	Step 1. Measurement of nerve size of ulnar nerve at Guyon's canal and arm, radial nerve at spiral groove, and sural nerve at calf (the BUS score). Score of 1 point per enlarged site (range: 0-4). Step 2. Measurement of nerve size of median and ulnar nerves at forearm and tibial nerve at ankle. Score of 1 point per enlarged site (range: 0-3). Step 3. Measurement of nerve size of median nerve at wrist and ulnar nerve at elbow. Score of 1 point per enlarged site (range: 0-2).
Results	Score of ≥2 on step 1: Suggestive of CIDP. Score of <2 on step 1 and ≥1 on step 2: Suggestive of MMN. Score of <2 on step 1, 0 on step 2, and ≥1 on step 3: Suggestive of MADSAM. Score of <2 on step 1 and 0 on steps 2 and 3: Suggestive of vasculitic neuropathy or paraproteinemia neuropathy. Correct classification with use of this algorithm: 42/49 CIDP patients (85.7%), 13/15 MMN patients (86.9%), and 5/5 MADSAM patients.
Homogeneity Score [75]	
Author	Grimm and colleagues 2016
Measurement sites	7: Median nerve: forearm, elbow, arm; ulnar nerve: forearm, arm; tibial nerve: popliteal fossa, ankle.
Description	0 points: No or regional enlargement (enlarged and normal values in the same nerve). 1 point: Inhomogeneous enlargement (generalized enlarged CSA in a nerve, but enlargement >150% and <150% above normal limit in the same nerve). 2 points: Mild homogeneity (generalized nerve enlargement in a nerve <150% above normal limit). 3 points: Overt homogeneity (generalized nerve enlargement in a nerve >150% above normal limit). Score (range: 0-3) calculated for each nerve; maximum 9 points.
Results	Significantly higher homogeneity score in CMT patients compared to MMN/MADSAM, CIDP, and control subjects (median nerve 6 [range: 3-9]; compared to 2-3 [range: 0-9] in MMN/MADSAM/CIDP and 0 in control subjects).
Regional Nerve Enlargement Index [75]	
Author	Grimm and colleagues 2016
Measurement sites	7: Median nerve: forearm, elbow, arm; ulnar nerve: forearm, arm; tibial nerve: popliteal fossa, ankle.
Description	1 point: Presence of normal CSA of at least 1 site and enlarged CSA of at least 1 site of the same nerve. 0 points: Not fulfilling the above. Score (range: 0-1) calculated for each nerve, maximum 3 points.
Results	Significantly higher RNEI in MMN patients compared to other neuropathies (median nerve 2 [range: 0-3]; CMT and control subjects: median nerve 0 [range: 0-2]; CIDP: median nerve 1 [range: 0-3]).

Table 1 Continued

Intra- and inter-nerve variability and side-to-side difference ratio [2,6]	
Author	Padua and colleagues 2012, Kerasnoudis and colleagues 2013
Measurement sites	2 sites in any nerve
Description	Intra-nerve variability: Highest CSA of nerve/lowest CSA of nerve. Inter-nerve variability: Highest intra-nerve variability/lowest intra-nerve variability. Side-to-side difference ratio: Side of maximal intra-nerve variability/side of minimal intra-nerve variability.
Results	Higher intra- and inter-nerve ratio in MMN (n=2) compared to CIDP (n=2). Higher intra- and inter-nerve ratio in CIDP (n=4) compared to MMN (n=2) but lower side-to-side difference ratio.

BUS: Bochum ultrasound score, CSA: cross-sectional area, CIDP: chronic inflammatory demyelinating polyneuropathy, CMT: Charcot–Marie–Tooth disease, GBS: Guillain–Barré syndrome, MADSAM: multifocal acquired demyelinating sensory motor neuropathy, MMN: multifocal motor neuropathy, RNET: Regional Nerve Enlargement Index, UPS: Ultrasound Pattern Score.

Figure 3 Patterns of sonographic enlargement

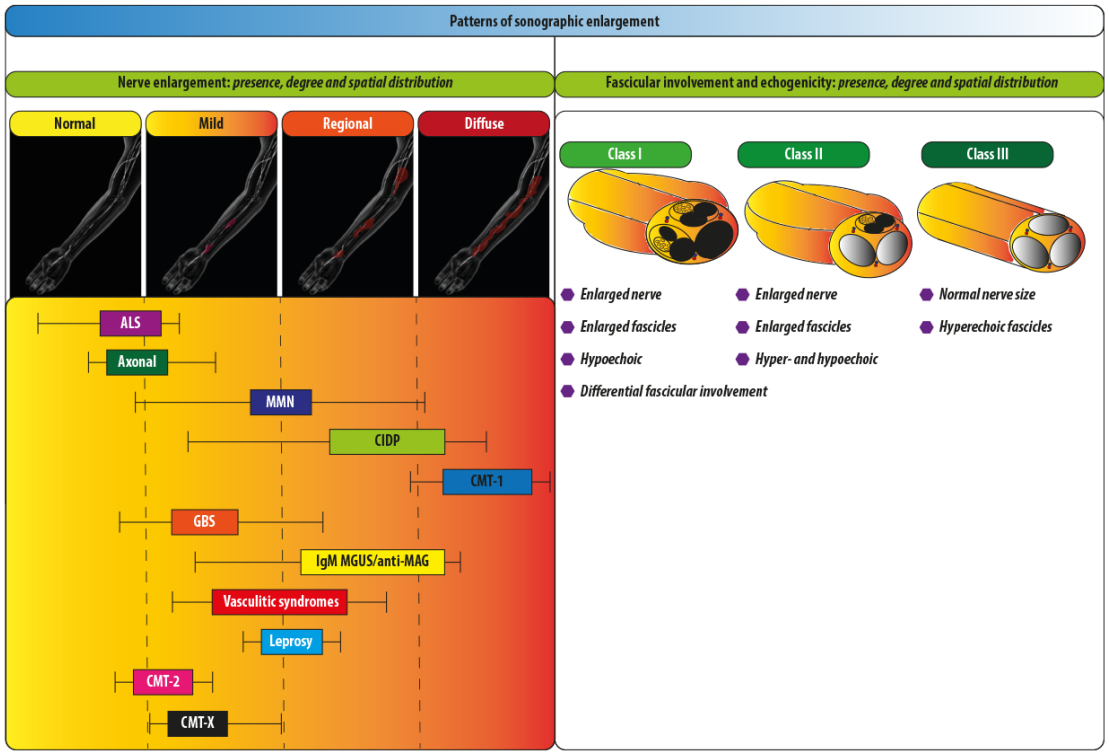


Figure 3: Nerve enlargement varies in degree and distribution in different polyneuropathies. Mild nerve enlargement refers to nerve enlargement not greater than twice normal average size. Regional nerve enlargement refers to nerve enlargement at one portion of the nerve, but not others. Diffuse nerve enlargement is enlargement at both proximal and distal aspects of the nerve. Other classification patterns depend on patterns of nerve enlargement and increased echogenicity.
ALS: amyotrophic lateral sclerosis, CIDP: chronic inflammatory demyelinating polyneuropathy, CMT: Charcot–Marie–Tooth disease, GBS: Guillain–Barré syndrome, IgM: immunoglobulin M, MAG: myelin-associated glycoprotein, MGUS: monoclonal gammopathy of undetermined significance, MMN: multifocal motor neuropathy.

to a lesser degree has been described in a number of other demyelinating or inflammatory neuropathies (Table 2). Other axonal neuropathies typically have either no or very mild nerve enlargement, with rare exceptions including some patients with diabetic neuropathy.[11] The following sections provide descriptions of the specific sonomorphological characteristics of different types of peripheral neuropathy.

Hereditary polyneuropathies

Charcot–Marie–Tooth Disease

CMT is a hereditary neuropathy characterized by inheritance pattern, genetic abnormality, and electrophysiology. CMT1 refers to demyelinating neuropathy with autosomal dominant inheritance. CMT1A is the most common form, caused by a duplication of PMP22 on chromosome 17. CMT2 refers to an axonal neuropathy with autosomal dominant inheritance. CMTX refers to an X-linked inherited neuropathy caused by mutations in the GJB1 gene, and CMT4 refers to autosomal recessive neuropathies, either demyelinating or axonal.

Nerve enlargement is characteristic of CMT1 and in CMT1A is often marked and diffuse. The largest study of nerve sonography in CMT1 reported nerve enlargement in the median and ulnar nerves in all of 35 patients.[12] The majority (89%) had diffuse nerve enlargement, and in 80% nerves were on average more than twice normal size. Other studies have similarly shown high frequencies of nerve enlargement in CMT1 (88-100%).[13-16] Nerve enlargement in CMT1 is often widespread and has been described in the cervical roots and brachial plexus, distal and proximal median, ulnar, and tibial nerve segments, and in small sensory nerves including the greater auricular and sural nerves.

Nerves are enlarged in both children and adults with CMT1A. Yiu and colleagues studied 29 children with CMT1A compared to similar aged control subjects and, as in adults, found increased nerve CSA in the median, ulnar, distal tibial, and sural nerves.[17] Nerve enlargement in CMT1A occurs in the youngest subjects reported, as young as age 19 months[17] and 2 years.[12] Some studies show that in CMT1A nerve size increases with age in children and decreases with age in adults. In children, Yiu and colleagues found strong correlations ($r = 0.68$-0.85) between increased nerve size and age in children with CMT1A compared to similar aged control subjects.[17] In contrast, in adults with CMT1A, 2 studies found decreasing nerve size with age in the sural nerve ($r = -0.6$)[16] and C6 nerve root ($r^2 = 0.36$).[15] Other studies, however, have not shown a correlation between nerve size and age in children or adults with CMT1.[12,13]

In CMT1A, several studies have shown mild-to-moderate correlations between larger nerves and worse disability or EDX abnormalities. Yiu and colleagues[17] found moderate correlations between nerve size and disability in children with CMT1A. Noto and colleagues[15] found a weak ($r^2 = 0.2$) correlation between median nerve size and functional rating and

Table 2 Conditions associated with nerve enlargement

Disease group	Conditions
Hereditary	CMT1 (not specified),CMT1A, CMT1B, CMT1C, CMT2, CMTX, HNPP, Amyloid (familial), Metachromatic leucodystrophy, sarcoidosis, Refsum, POEMS, Anti-MAG
Acquired/immune	CIDP, GBS, MMN, Amyloid (acquired), POEMS, Anti-MAG
Infectious/inflammatory	Leprosy, vasculitis, sarcoidosis
Metabolic	Diabetes mellitus

CIDP: chronic inflammatory demyelinating polyneuropathy, CMT: Charcot–Marie–Tooth disease, GBS: Guillain–Barré syndrome, HNPP: hereditary neuropathy with liability to pressure palsy, MAG: myelin-associated glycoprotein, MMN: multifocal motor neuropathy, POEMS: polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes.

slightly stronger correlations between increased median nerve size and slower conduction velocity ($r^2 = 0.3$-0.4). Schreiber and colleagues[18] also found correlations between larger median nerves in CMT1A with slower conduction velocity ($r_s = -0.69$) and smaller compound motor action potential amplitudes ($r_s = -0.61$). In contrast, Pazzaglia and colleagues[16] found no correlation between ulnar nerve size and function.

In other types of CMT, nerves are also often enlarged, especially when demyelinating features are present, but generally are not as enlarged as in CMT1A. In CMT1B, the few patients described have had enlarged nerves in the arms and legs but generally not to the same extent seen in CMT1A.[7,12,15,19] Similarly, 3 patients with CMT1C (LITAF/SIMPLE) have been described[20] with slight-to-moderate nerve enlargement. Nerves are more enlarged in patients with CMT1A than patients with CMTX or CMT2.[1,18] Two studies have found that median nerve size in CMTX is similar to control subjects, whereas in CMT2 nerves are slightly larger than in the control subjects but not to the same degree as seen in CMT1A.[1,18] One study[7] found increased nerve size in CMTX in the tibial and peroneal nerves in the popliteal fossa and the cervical nerve roots when compared to CMT2.

Hereditary Neuropathy with Liability to Pressure Palsy

Nerve enlargement at multiple common sites of entrapment (median nerve at the wrist or ulnar nerve at the elbow) is a typical characteristic but nonspecific sonographic finding in hereditary neuropathy with liability to pressure palsy (HNPP). Unlike CMT1, nerve enlargement in HNPP is typically limited to common sites of entrapment, although occasionally patients with HNPP also have nerve enlargement outside of common entrapment sites.[14,18,21-23] Nerve enlargement in HNPP is uncommon in the tibial nerve at the ankle,[22,24] even when tibial EDX studies are abnormal.[25]

Acquired Polyneuropathies

Chronic Inflammatory Demyelinating Polyneuropathy

CIDP is an immune-mediated, chronic sensorimotor polyneuropathy typically characterized by proximal predominant weakness with reduced tendon reflexes and distal symmetric sensory symptoms,[26,27] but it also includes several clinical variants including pure sensory, pure motor, and asymmetric CIDP (also known as multifocal acquired demyelinating sensory motor neuropathy, MADSAM, or Lewis–Sumner syndrome).[26,28,29] Nerve conduction studies (NCSs) show demyelination outside of common sites of entrapment.[26,30] Additional supportive diagnostic criteria include gadolinium enhancement and/or hypertrophy of the brachial or lumbosacral plexus on magnetic resonance imaging (MRI), elevated cerebrospinal fluid (CSF) protein level without pleocytosis, and objective clinical improvement after treatment with immunomodulating agents such as steroids or intravenous immunoglobulin (IVIg).[26] Some patients with CIDP do not fulfill these criteria,[31-33] and misdiagnosis is common.[27,31] Ultrasound often detects nerve pathology in CIDP that could aid in the diagnosis and management.[34-36]

Nerve enlargement is common in CIDP (64-89% of patients), typically with a predominance in proximal nerve segments in the upper extremity and the brachial plexus,[8,10,12,13,34-40] and evaluation of these areas has the highest diagnostic yield.[35,40-42] Ultrasound evaluation of the lower extremity is less informative than evaluation of the upper limb in CIDP.[40,42] MRI is required to detect the enlargement in the lumbosacral plexus and very proximal sciatic nerve.[43-46] In addition to nerve enlargement, nerves in CIDP can have increased vascularization, increased echogenicity, and fascicular enlargement or loss of the normal fascicular appearance.[10,34] In contrast, patients with axonal polyneuropathies usually show no or only minimal nerve enlargement outside of common sites of entrapment.[8,35,38,40]

Evaluation of the relationship between nerve size and clinical and EDX findings in CIDP is complicated by study differences in sonographic protocols and heterogeneity of patient characteristics, such as disease duration and prior treatment exposure. Most studies have found a relationship between larger nerve size and slower motor conduction velocities[35,38,41,47] though some others did not.[48,49] One study has suggested that the nerve enlargement in CIDP may reflect underling axonal damage/loss and may not be seen in purely demyelinating lesions,[39] while others have described nerve enlargement at sites of conduction block.[50-52] There appears to be little association between nerve size and the distal compound motor action potential[35,36,38,47,48] or degree of weakness or disability.[10,36,39,41,42,47]

Nerve size in CIDP may vary with disease duration and treatment. Several studies have shown larger or more extensive nerve enlargement in patients with longer disease

duration or longer interval between symptom onset and treatment,[10,12,38,39,47] but others did not find this relationship.[37,40,41] Zaidman and colleagues[12] and Grimm and colleagues[47] found larger nerve size in patients with longer disease duration (>3 months) prior to treatment initiation. Grimm and colleagues reported a predominant proximal involvement in 21 patients with new-onset CIDP, whereas 21 treated patients with longer disease duration showed a more diffuse pattern of nerve enlargement.[47] Padua and colleagues found larger, hypoechoic nerves with loss of fascicular pattern in patients with longer disease duration, but also found that in patients with very long-standing, chronic CIDP nerves had normal size but were hyperechoic with loss of the fascicular pattern, possibly from chronic, severe axon loss.[10]

Two longitudinal studies in CIDP suggest a possible role for nerve ultrasound in assessing treatment responsiveness. A retrospective study by Zaidman and colleagues showed that patients with normal or decreasing nerve size had a favorable treatment response and tolerated medication reductions, while patients with persistently enlarged or increasing nerve size required continuing or escalating medication doses.[53] Another prospective study by Kerasnoudis and colleagues showed that a decrease in intra-nerve variability correlated with favorable outcome following treatment.[54] Additional prospective studies in treatment naïve patients are needed to best determine the role of ultrasound in assessing prognosis and treatment efficacy in CIDP.

Guillain–Barré Syndrome

Guillain–Barré syndrome (GBS) is an acute, immune-mediated polyneuropathy that typically presents with ascending sensory symptoms and flaccid paralysis with reduced or absent reflexes. Disability progresses acutely and reaches a nadir within 4 weeks. Progression can be rapid and severe, and disability may be mitigated by early treatment with plasmapheresis or IVIg. Diagnosis is typically clinical, as laboratory and EDX abnormalities may lag the clinical presentation by several weeks. Supportive diagnostics include EDX findings of demyelination, cytoalbuminologic dissociation in the CSF, and nerve root enhancement on spinal MRI. Ultrasound-identified nerve enlargement may also aid in the diagnosis of GBS.

Nerve enlargement in GBS can be present early in the disease course, but may not be widespread or easily detected. Nerve enlargement has been reported as early as 1-3 days following symptom onset[55] and prior to abnormalities on NCSs.[44] The degree of enlargement in GBS is not as pronounced or as common as in CIDP or CMT1A. One study found nerve enlargement in the median or ulnar nerves in 11 of 21 patients with GBS, most (8) with only mild nerve enlargement.[44] Nerve enlargement can be mild in GBS patients, and an individual with GBS may have many nerves that are at or near the upper limit of normal values. For instance, Gallardo and colleagues found nerve enlargement in

5 of 6 patients with GBS within 10 days of symptom onset but only in 9% of the examined nerve segments, most commonly in the cervical nerve roots and proximal median nerve.[56] Nerve enlargement in GBS may be more common in proximal nerves or spinal nerve roots but has also been described in the large nerves of the arm and more rarely in the leg, the vagus nerve, and, variably, the sural nerve.[12,38,55,57,58] Enlargement of the vagus nerve was predominantly found in patients with autonomic dysregulation.[55] Enlargement of the cervical nerve roots has also been described in some patients with Miller–Fisher syndrome and in acute motor axonal neuropathy.[59-61]

Nerve enlargement in GBS may persist after the acute phase and following resolution of symptoms,[38,62,63] although nerves may become slightly smaller over time.[64] Grimm and colleagues found reductions in nerve size in 21 patients with GBS over 6 months in the cervical nerve roots and vagus nerve, but not in the nerves in the limbs.[55] Similarly, Razali and colleagues[57] demonstrated only slight, mostly non-significant reductions in nerve size in the arms and legs of 17 patients with GBS imaged repeatedly over 12 weeks from symptom onset.

Multifocal Motor Neuropathy

MMN is an immune-mediated, pure motor neuropathy characterized by slowly progressive, typically asymmetric weakness of the limbs.[65-68] The characteristic EDX criteria for MMN are conduction block outside common sites of entrapment and normal sensory studies.[26,65,66] Extensive NCSs are sometimes needed to identify the conduction block or other features of demyelination.[65,69] Additional supportive criteria may help to identify patients with MMN and include the presence of anti-GM1 antibodies,[60] increased CSF protein level, abnormal brachial plexus MRI demonstrating T2-hyperintensity or enlargement, and responsiveness to IVIg.[26] Nerve ultrasound could aid in the identification of patients with MMN and distinguish MMN from amyotrophic lateral sclerosis (ALS).

Most studies of nerve ultrasound in MMN assessed nerve size, with virtually no published data on nerve vascularization, echogenicity, or epineurial thickness. Nerve enlargement in MMN, when present, is often mild and multifocal and found in the brachial plexus and large peripheral nerves of upper and lower extremities.[12,61,70-72] The presence of multifocal nerve enlargement differentiates MMN from ALS and healthy control subjects with sensitivities of 87-100% and specificities of 94-100%.[71-73] Nerve enlargement in MMN, in contrast to CIDP, is often less pronounced and is typically asymmetric.[12,61,70,71,74,75] The intra- and inter-nerve variability and side-to-side difference ratio is also increased in MMN.[6,61,76] Individual fascicles within the nerve may also be differentially enlarged, with sparing of neighboring fascicles.[77] Thus asymmetric, multifocal nerve enlargement may suggest MMN, but further studies are needed to determine the specificity of this finding compared to other polyneuropathies such as asymmetric variants of CIDP.

Most studies have found little or no association between nerve size and the degree of weakness, clinical disability, or EDX findings in MMN.[61,70-72] Beekman and colleagues reported similar frequencies of nerve enlargement in limbs and nerve segments with and without clinical and NCS abnormalities.[70] Grimm and colleagues found no relation between nerve size and NCSs.[71] Kerasnoudis and colleagues found little association between nerve size and EDX results, with correlations only between the median nerve and its compound muscle action potential amplitude.[61] In contrast, Loewenbrück and colleagues found some relation between the size of cervical nerve roots and the superior trunk of the brachial plexus to measures of strength and disability,[72] and a recent longitudinal study by Rattay and colleagues found a correlation between nerve and fascicle size and therapeutic response.[77] As with many studies of nerve ultrasound in polyneuropathies, interpretation of these studies for characterizing MMN is limited by their use of heterogeneous, partially-treated patient populations and different imaging and clinical and EDX protocols.

Distinguishing demyelinating polyneuropathies with ultrasound

Several strategies have been developed to describe and quantify the different sonographic patterns of nerve enlargement in CMT, CIDP, and other inflammatory/immune-mediated polyneuropathies. Generally, nerve enlargement in CMT1A is the largest and most extensive, and typically all nerve segments outside of common entrapment sites are enlarged, although the degree of enlargement can be heterogeneous.[12,75] In CIDP, the presence, pattern, and degree of nerve enlargement are more variable than in CMT1. In CIDP, nerves are generally smaller than in CMT1A, although both can show diffuse, widespread nerve enlargement.[12,13,38-41,47] New onset, short duration CIDP can show normal nerve size, regional nerve enlargement, and only rarely homogenous nerve enlargement similar to CMT1A. Longer standing CIDP tends to show more frequent and more homogenous nerve enlargement.[47] Marked nerve asymmetry may support an atypical variant of CIDP or MMN. Nerve enlargement in GBS, MMN, and MADSAM is either smaller than in CMT1 and CIDP or more regional, inhomogeneous, or asymmetric. These patterns are mostly derived from reports of relatively small numbers of patients and from different imaging protocols.[10,34,36,41,78-81] Standardized ultrasound protocols might better characterize the patterns of nerve enlargement and could clarify the role of nerve ultrasound in differentiating between the acquired demyelinating neuropathies. Currently, only one scoring system, the Bochum Ultrasound Score, has been prospectively evaluated in a single center cohort study by the same authors that introduced it.[82] Larger prospective studies are required to determine the diagnostic performance of these pattern scores and ratios in differentiating between the demyelinating polyneuropathies.

Nerve sonography in other polyneuropathies

Leprosy

Leprosy is infrequent in the Western world, but remains a serious health problem in Asia, Africa, and South America.[83] It is caused by infection with *Mycobacterium leprae* and results in skin and peripheral nerves lesions. Diagnosis is based on identification of the skin lesions, skin smear tests, palpation of peripheral nerves, and NCSs. Early detection is often difficult, especially in the case of primary neuritic leprosy, a variant without skin lesions. Patients with leprosy suffer from recurrent acute immunological reactions. These reactions can be difficult to identify using current diagnostic techniques, and this results in delayed treatment and increased morbidity. Ultrasound may improve the diagnosis and management of these reactions.[84]

Nerve ultrasound is superior to the clinical examination for identifying nerve enlargement in leprosy.[85] Several nerve ultrasound studies have documented enlargement of multiple nerves and thickening of the epineurium in leprosy patients.[85-90] Nerve enlargement in leprosy is most common in the ulnar nerve, followed by the median and peroneal nerves.[90] A feature that may be specific to leprosy is pronounced nerve enlargement only a few centimeters proximal to common entrapment sites (e.g., ulnar nerve at the elbow, median nerve at carpal tunnel).[85-87,89]

Ultrasound findings may inform treatment decisions in patients with leprosy. Nerves are larger in patients with leprosy reactions than those without.[86,91,92] Nerves with active leprosy reactions are hypervascular, and this may resolve with effective treatment. Martinoli and colleagues found hypervascularization in 71% of nerves of patients with active leprosy reactions compared to only 2 nerves (5.9%) of patients without. The hypervascularization decreased after treatment in 6 of 14 patients, most of whom (5 of 6) had a good clinical response.[86] Two other studies found hypervascularity only in patients with active reactions.[85,92] Chaduvula and colleagues conducted a prospective cohort study on 57 patients with leprosy monitoring disease activity for 2 years and found that the hypervascularization present in 20 of 36 patients with active leprosy reactions (55%) at baseline resolved in all but 1 patient (2.7%) following treatment.[92] Based on these findings nerve ultrasound is a promising tool for monitoring disease activity and treatment response in leprosy.

Other peripheral neuropathies

Most acquired or idiopathic axonal neuropathies show either no nerve enlargement or only mild nerve enlargement on average, with considerable overlap in nerve size between patients and control subjects. In diabetes mellitus, for instance, nerves are on average slightly enlarged compared to control subjects, with more prominently enlarged nerves in those with more poorly controlled diabetes and in those with diabetic peripheral

neuropathy.[11,93-95] In sarcoidosis, one study of 13 patients showed only slightly larger tibial, peroneal, and sural nerves compared to control subjects.[96] Nerve enlargement has also been reported in small series of patients with neuropathy associated with other systemic diseases, including neuropathy associated with paraproteins and vasculitis.

Nerve enlargement in neuropathies associated with elevated paraproteins varies depending on the disorder. In patients with neuropathy and monoclonal gammopathy of undetermined significance (MGUS), mild, regional nerve enlargement may be present, particularly if their NCS has demyelinating features.[97] Similarly, most (23 of 28) patients with neuropathy and anti-MAG (myelin-associated glycoprotein) antibodies have mild, regional nerve enlargement.[98] In POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) syndrome, the nerve enlargement is variable. One study found nerve enlargement primarily at common sites of entrapment in 8 of 8 patients studied.[99] Another found slightly larger median nerve size in the wrist, forearm, and elbow of 31 patients with POEMs when compared to control subjects (n=85) and that nerve size in POEMs decreased following treatment.[100] In axonal neuropathy associated with multiple myeloma[97] and in neuropathy in acquired amyloidosis,[101] abnormal nerve morphology is uncommon. In contrast, in transthyretin-related familial amyloidosis nerve enlargement could be widespread or only at common entrapment sites.[102] Nerve enlargement in vasculitic neuropathy is also often mild, regional, and sometimes affects only single fascicles. In 14 patients with systemic vasculitis, 70% (22 of 31) of clinically-involved nerves showed focal nerve enlargement.[103] Nerve enlargement in vasculitis may preferentially involve the sural and superficial peroneal nerves,[104] may occur in nerve regions just proximal to common sites of entrapment, typically does not involve the brachial plexus, and only rarely shows hypervascularization.[105]

Future Directions

Ultrasound of nerves in polyneuropathy is a rapidly expanding field with many opportunities for additional study. Changes in nerve morphometry in many common conditions has not been well characterized. For instance, nerve ultrasound in many toxic and parainfectious neuropathies (i.e., chemotherapy, shingles) is relatively unexplored. Longitudinal studies are required to determine how nerve morphology changes with treatment. Other avenues for study include exploration and expansion of sonographic techniques beyond measuring nerve size, including analysis of nerve fascicle size, nerve movement and gliding, elastography, and quantitative nerve echo intensity and blood flow. Finally, comparative effectiveness studies with ultrasound and electrophysiology are needed to clarify how to best incorporate nerve ultrasound into the EDX laboratory.

Conclusion

Multiple studies have shown a prominent role for nerve ultrasound in the evaluation of polyneuropathies. Generally, demyelinating neuropathies display nerve enlargement, while most axonal neuropathies do not. Thus, if nerve enlargement is found, heredity or immune-mediated demyelinating neuropathy should be considered. One exception is axonal neuropathy from vasculitis, which often shows nerve enlargement in clinically-affected nerves. Nerve ultrasound also detects abnormal fascicle size, nerve echogenicity, and nerve vascularity, which is particularly important in the evaluation of management of leprosy. Quantified scoring systems—taking into account the location, degree, and homogeneity of nerve enlargement—are promising tools to better classify abnormalities of nerve morphology, thereby aiding in the distinction between neuropathies. Prospective studies are ongoing and anticipated to help better define the role of ultrasound in the evaluation and treatment of polyneuropathies.

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Chapter 3

Inter-observer Variability

Nerve ultrasound: a reproducible diagnostic tool in peripheral neuropathy

JA Telleman*, IJT Herraets*, HS Goedee, C Verhamme, S Nikolakopoulos,
JT van Asseldonk, WL van der Pol, LH van den Berg, LH Visser

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Abstract

Objective: To determine interobserver variability of nerve ultrasound in peripheral neuropathy in a prospective, systematic, multicenter study.

Methods: We enrolled 20 patients with an acquired chronic demyelinating or axonal polyneuropathy and 10 healthy controls in 3 different centers. All participants underwent an extensive nerve ultrasound protocol, including cross-sectional area measurements of median, ulnar, fibular, tibial, and sural nerves, and brachial plexus. Real-time image acquisition was performed blind by a local and a visiting investigator (reference). Five patients were investigated using different types of sonographic devices. Intraclass correlation coefficients were calculated, and a random effects model was fitted to identify factors with significant effect on interobserver variability.

Results: Systematic differences between measurements made by different investigators were small (mean difference 0.11 mm^2 (95%-CI 0.00 – 0.23 mm^2)). Intraclass correlation coefficients were generally higher in arm nerves (0.48 – 0.96) than leg nerves (0.46 – 0.61). The hospital site and sonographic device did not contribute significantly to interobserver variability in the random effects model.

Conclusions: Interobserver variability of nerve ultrasound in peripheral neuropathy is generally limited, especially in arm nerves. Different devices and a multicenter setting have no effect on interobserver variability. Therefore, nerve ultrasound is a reproducible tool for diagnostics in routine clinical practice and (multicenter) research.

Introduction

Nerve ultrasound is a valuable and increasingly used diagnostic tool for entrapment neuropathies, traumatic neuropathies, and more recently inflammatory polyneuropathies.[1-8] Interobserver variability of nerve ultrasound has not been studied in detail in patients with mono- or polyneuropathy. This hampers the applicability of ultrasound for diagnostic work-up of peripheral neuropathy in routine clinical practice.

Previous studies that addressed interobserver variability of nerve ultrasound generally found high intraclass correlation coefficients (ICCs)[9-17] but had important limitations, including data acquisition in healthy controls only, the use of still images rather than real-time image acquisition, and the assessment of a limited number of nerves and nerve sites. Furthermore, few studies addressed the possibility of variation introduced by differences between sonographic devices,[10] and none looked at interobserver variability in a multicenter setting.

The main objective of this study was to determine reproducibility of nerve ultrasound in the assessment of peripheral neuropathy. We therefore performed a prospective, multicenter cohort study in patients and controls. We used a standardized extensive sonographic protocol to analyze interobserver variability and its determinants systematically.

Methods

Standard protocol approvals, registrations, and patient consents

This prospective multicenter cohort study was performed between May 2016 and May 2017 at the Neurology outpatient clinics of the Elisabeth-Tweesteden Hospital Tilburg, a large general teaching hospital, and two tertiary referral centers for neuromuscular disorders, i.e. the University Medical Center Utrecht and Academic Medical Center Amsterdam. Thirty participants were included in this study: 10 healthy controls and 20 patients. Patients with chronic inflammatory demyelinating polyneuropathy (CIDP), multifocal motor neuropathy (MMN), and chronic idiopathic axonal polyneuropathy (CIAP), known at the outpatient clinics of the participating hospitals, were eligible for inclusion. Controls were recruited from the hospital staff. Inclusion criteria for patients were 1) age older than 18 years and 2a) a diagnosis of possible, probable, or definite CIDP or MMN according to the international consensus criteria, or 2b) a diagnosis of CIAP based on the criteria of clinical examination, nerve conduction studies and laboratory testing included in the Dutch guideline of polyneuropathies.[18,19] Inclusion criteria for controls were 1) age older than 18 years and 2) absence of symptoms compatible with neuropathy. Exclusion criteria for this study were 1) history of polyneuropathy other than CIDP, MMN,

or CIAP and 2) physical inability to undergo the nerve ultrasound protocol. The Brabant Regional Ethics Committee (NL50375.028.14) and the boards of all participating hospitals approved this study. All participants gave written informed consent.

Study design

Nerve ultrasound protocol

We used a previously described sonography protocol that includes brachial plexus, median, ulnar, fibular, tibial, and sural nerves (figure 1).[20] We investigated arm nerves bilaterally and leg nerves unilaterally, because we have shown previously that investigation of both legs has limited added diagnostic value.[2,5] Measurement of nerve size (cross-sectional area (CSA in mm^2)) was performed perpendicular to the nerve and within the hyperechoic rim.

Multicenter protocol and ultrasound equipment

Participants were investigated on the same day by a local investigator from one of the three participating hospitals (JT (Elisabeth-Tweesteden Hospital), SG (UMC Utrecht), CV (AMC Amsterdam)) and a visiting investigator (reference) (IH).

In the Elisabeth-Tweesteden Hospital, 10 participants (5 healthy controls, 3 patients with CIDP, and 2 with MMN) underwent nerve ultrasound on a Toshiba Xario XG (Toshiba, Tokyo, Japan) with a 7- to 18-MHz linear-array transducer (PLT-1204BT). To determine variability introduced by the use of sonographic devices of different brands, two investigators (IH and JT) evaluated another 5 participants (2 patients with CIDP, 1 with MMN, and 2 with CIAP) using both the Toshiba machine and an Esaote MyLab Class C (Esaote Benelux BV, Maastricht, The Netherlands; 6- to 18-MHz linear-array transducer (LA435)). They changed devices at random.

In the UMC Utrecht, 10 participants (5 healthy controls, 3 patients with CIDP, and 2 with MMN) underwent nerve ultrasound on a Philips EPIQ7 (Philips Medical Instruments, Bothell, WA) with a 5- to 18-MHz linear-array transducer (L18-5).

In the AMC Amsterdam an additional 5 participants (4 patients with CIDP, 1 with MMN) underwent nerve ultrasound on an Esaote MyLabTwice (Esaote, Genoa, Italy) with a 6- to 18-MHz linear-array transducer (LA435, for upper and lower extremity nerves) and a 3- to 13-MHz linear-array transducer (LA533, for brachial plexus).

Investigators (all of whom had at least one year's experience of performing nerve ultrasound measurements) were free to position participants in line with their own routine practice, and were allowed to apply their preferred window of depth and measurement tools (all investigators used the ellipse tool except for the local investigator of the AMC

who used the tracked trace tool) to determine nerve CSA. This ensured that investigators performed their examination under circumstances that closely resembled their normal routine, and studies to date have not shown that depth and measurement tools increase interobserver variability.[21] However, investigators were not allowed to use a zoom function, as a previous study has already shown that this may increase interobserver variability.[22] All investigators were blinded to results of clinical examination, as well to all previously performed and one another's nerve ultrasound investigations.

Figure 1 Sonographic protocol

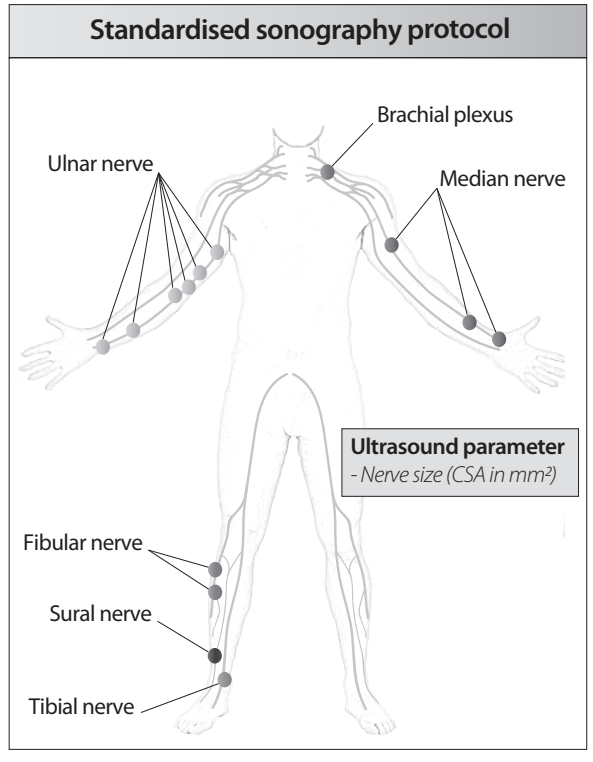

Figure 1 shows the sonographic protocol applied in this study. Arm nerves were investigated bilaterally, leg nerves unilaterally. Standardized sites of measurement were applied: The median nerve was measured at the wrist, forearm (at 1/3 of the distance between wrist and elbow crease) and arm (at 1/2 of the distance between elbow crease and anterior axillary fold). The ulnar nerve was measured at the wrist, forearm (at 1/3 of the distance between wrist and medial epicondyle), 2.5 cm distal to the medial epicondyle, at the ulnar sulcus (at the level of the medial epicondyle), 2.5 cm proximal to the medial epicondyle, and at the arm (at 1/2 of the distance between medial epicondyle and anterior axillary fold). At the brachial plexus, nerve roots C5, C6, and C7 were measured at the inter-scalene level. The fibular nerve was measured at the fibular head and popliteal fossa, the posterior tibial nerve at the medial malleolus, and the sural nerve 14 cm proximal to the lateral malleolus.

Statistics

Statistical analyses were performed with IBM SPSS 22 (IBM Corp., Armonk, NY) and MLwiN 2.36 (CMM, Bristol, UK). We focused on nerve CSA, as this is the most relevant parameter in distinguishing neuropathies.[2] To determine the reliability of nerve ultrasound, several aspects were investigated.

1. Presence of systematic differences: Systematic differences between measurements made by different investigators may affect the reliability of sonography for establishing a diagnosis. Bland-Altman Plot analysis was performed and the mean difference between investigators and 95%-confidence interval (CI) were calculated to determine if there were systematic differences in nerve size.
2. Variability of differences: If there are no systematic differences, a higher variability of the difference between investigators may still cause a lower reliability of sonography because diagnosis in the individual patient is often based on a single measurement and a fixed cut-off value. SD of the difference between investigators was calculated per nerve site to determine if the variability of the difference at those sites was comparable. SDs were also calculated for the different hospitals, sonographic devices, patients and controls, and for groups of nerves with different amounts of mean nerve size.
3. Correlation of nerve size measurements: To determine the correlation of CSA measurements of 2 investigators, ICCs were calculated per nerve site. One-way analysis of variance (ANOVA) with patient as factor was applied to determine the variability between groups and within groups. ICCs were calculated with the following formula: (variability between groups - variability within groups) / (variability between groups + variability within groups).
4. Correlation of the classification of measurements as abnormal: Previously published reference values were used to classify measurements as 'not enlarged' or 'enlarged'.[20] To determine the level of agreement between the 2 investigators in the classification of 'not enlarged' or 'enlarged' with a single cut-off value, Fleiss' kappa values were calculated.
5. Mixed model analysis: A random effects model with the mean difference in CSA between investigators as outcome measure was fitted to quantify the effect of multiple determinants (that are commonly encountered in routine clinical practice) on variability in nerve size measurements. Nerve site was entered as second-, participant as third-, and hospital of investigation as fourth-level random effect (individual measurements nested in nerve sites nested in participants nested in hospitals). The use of different sonographic devices, measurement of either patients or controls, and of either right or left side were entered as fixed effects. Markov chain Monte Carlo algorithms were used to calculate the Bayesian Deviance Information Criterion (DIC) (the employed method in MLwiN for cross-classified factors such as participants and nerve sites).[23]

Data availability

Anonymized data will be shared by request from any qualified investigator.

Results

Patients and measurements

Baseline characteristics of participants are shown in table 1. Comparison of ultrasound results from different investigators was possible in 829 out of a total of 840 (98.7%) measurements. Comparison was not possible because of storage problems (1 measurement), the presence of a porth-a-cath system in 1 patient (3 measurements), or problems with identifying the C7 nerve root (7 measurements).

Table 1 Baseline characteristics

	Patients	Controls
Total number of participants	20	10
Sex, male / female	15 / 5	5 / 5
Age in years, median (range)	60.5 (37 – 77)	27.5 (25 – 36)
Diagnosis CIDP (definite / probable / possible)	12 (10 / 1 / 1)	-
Diagnosis MMN (definite / probable / possible)	6 (5 / 0 / 1)	-
Diagnosis CIAP	2	-
Disease duration in months, median (range)	42 (2 – 264)	-
Treatment duration in months, median (range)	15 (0 – 121)	-

Mean difference, variability of the difference, and ICCs

Figure 2 summarizes nerve size measurements by 2 investigators. The mean difference between investigators was 0.11 mm^2 (95%-CI 0.00 – 0.23 mm^2). The mean difference between investigators and ICCs are shown per nerve site in table 2.

Overall, the variability of the difference (SD) between investigators was 1.7 mm^2 but it varied substantially per nerve site (table 2). SD of arm nerves varied from 1.0 – 1.7 mm^2. SD of large leg nerves and brachial plexus nerve roots was much higher (1.5 – 3.1 mm^2), while SD of the sural nerve was lowest (0.9 mm^2). SD also increased in larger nerves: SD 1.0 in nerves with a mean size <5mm^2 (n=179), 1.6 in nerves with a mean size ≥5 and <10mm^2 (n=485), 2.3 in nerves with a mean size ≥10 and <15mm^2 (n=134), and 3.3 in nerves with a mean size ≥15mm^2 (n=31).

Figure 2 Nerve Size

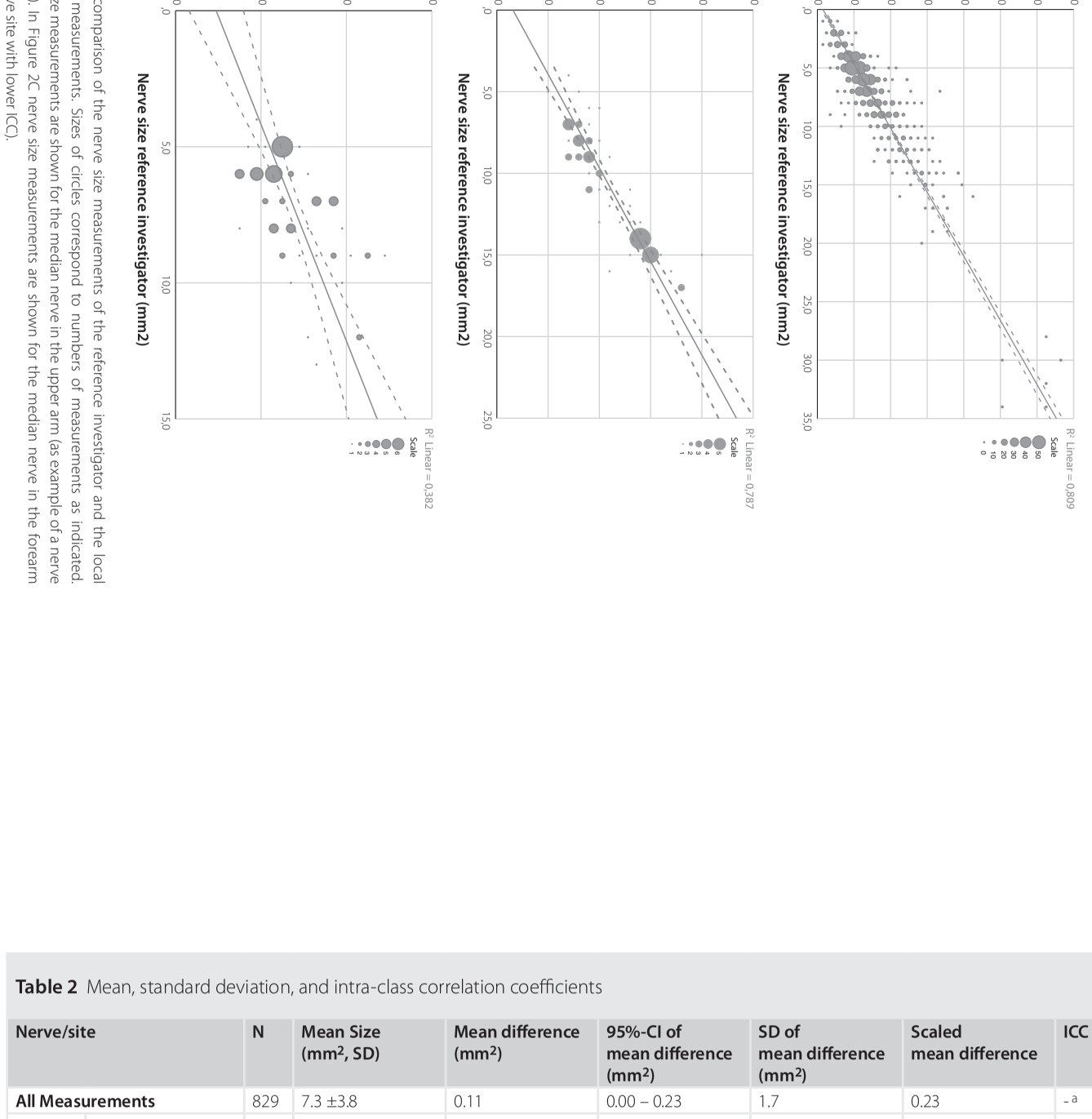

Figure 2A shows a comparison of the nerve size measurements of the reference investigator and the local investigators for all measurements. Sizes of circles correspond to numbers of measurements as indicated. In Figure 2B nerve size measurements are shown for the median nerve in the upper arm (as example of a nerve site with a high ICC). In Figure 2C nerve size measurements are shown for the median nerve in the forearm (as example of a nerve site with lower ICC).

Table 2 Mean, standard deviation, and intra-class correlation coefficients

Nerve/site		N	Mean Size (mm^2, SD)	Mean difference (mm^2)	95%-CI of mean difference (mm^2)	SD of mean difference (mm^2)	Scaled mean difference	ICC
All Measurements		829	7.3 ±3.8	0.11	0.00 – 0.23	1.7	0.23	- [a]
Median	**Wrist**	60	10.1 ±2.6	0.22	0.00 – 0.59	1.5	0.15	0.84
	Forearm	60	7.2 ±1.8	0.28	0.00 – 0.65	1.8	0.25	0.61
	Arm	60	10.8 ±3.3	0.06	0.00 – 0.41	1.6	0.15	0.89
Ulnar	**Wrist**	60	5.0 ±1.1	0.14	0.00 – 0.49	1.0	0.20	0.65
	Forearm	59	5.6 ±1.4	0.18	0.00 – 0.54	1.3	0.23	0.60
	Distal to ME	60	6.1 ±1.4	0.18	0.00 – 0.53	1.2	0.20	0.67
	Sulcus (at ME)	60	7.4 ±1.6	0.46	0.04 – 0.88	1.7	0.23	0.48
	Proximal to ME	60	7.4 ±2.2	0.09	0.00 – 0.45	1.2	0.16	0.86
	Arm	60	6.9 ±2.3	0.24	0.00 – 0.61	1.3	0.19	0.85
Plexus	**C5**	59	7.4 ±6.0	0.08	0.00– 0.46	1.7	0.23	0.96
	C6	59	6.7 ±5.9	0.08	0.00 – 0.46	2.2	0.33	0.95
	C7	52	6.9 ±5.6	0.07	0.00 – 0.44	3.1	0.45	0.86
Fibular	**Popliteal Fossa**	30	6.7 ±1.7	0.41	0.00 – 0.92	1.5	0.22	0.60
	Fibular Head	30	9.1 ±1.8	0.01	0.00 – 0.42	1.9	0.21	0.61
Tibial	**Medial Malleolus**	30	11.9 ±2.2	0.02	0.00 – 0.42	2.7	0.23	0.46
Sural	**Calf**	30	2.0 ±0.5	0.05	0.00 – 0.47	0.9	0.45	0.10

Table 2 shows the mean nerve size and mean difference between investigators with its 95%-CI for all measurements and per nerve site. Results of mean difference between investigators presented are after correction by multilevel mixed modelling. In addition, SD of the difference between investigators and ICCs for measurements of nerve size are shown. A scaled mean difference was calculated for each nerve site as: (SD of mean difference / Mean nerve size). a. not calculable due to the multilevel mixed structure of data.
95%-CI: 95% confidence interval. ICC: intra-class correlation coefficient. ME: medial epicondyle. N: number of valid measurements. SD: Standard deviation.

SD ranged from 1.6 – 1.9 mm^2 in the three hospitals, indicating a relatively small influence of different hospitals on overall variability. SD was 1.8 mm^2 in participants investigated twice on the same sonographic device compared to 1.4 mm^2 in participants investigated on two different sonographic devices, indicating that different devices have no influence on overall variability.

Kappa values

Kappa values for the classification of nerve enlargement are shown in table 3. Values ranged from -0.13 – 1.00. Frequencies of discrepancies between investigators ranged from 0.0 – 28.8% of measurements, depending on the nerve site.

Table 3 Kappa values for presence of nerve enlargement

Nerve/Site		Cut-off (mm^2)	Kappa	95%-CI of Kappa	Mismatch
Overall		-	0.66	0.59 – 0.73	10.1%
Median	**Wrist**	≤11	0.78	0.53 – 1.00	8.3%
	Forearm	≤9	0.35	0.10 – 0.60	13.3%
	Arm	≤9	0.80	0.54 – 1.00	10.0%
Ulnar	**Wrist**	≤7	1.00	0.75 – 1.00	0.0%
	Forearm	≤6	0.18	-0.07 – 0.43	28.8%
	Distal to ME	≤9	0.48	0.23 – 0.74	3.3%
	Sulcus (at ME)	≤9	-0.10	-0.35 – 0.15	18.3%
	Proximal to ME	≤9	0.71	0.46 – 0.96	8.3%
	Arm	≤9	0.66	0.40 – 0.91	8.3%
Plexus	**C5**	≤8	0.82	0.56 – 1.00	6.8%
	C6	≤8	0.96	0.70 – 1.00	1.7%
	C7	≤8	0.65	0.38 – 0.92	13.5%
Fibular	**Popliteal Fossa**	≤9	0.46	0.11 – 0.82	6.7%
	Fibular Head	≤11	-0.13	-0.48 – 0.23	23.3%
Tibial	**Medial Malleolus**	≤14	0.26	-0.09 – 0.62	13.3%
Sural	**Calf**	≤3	NA		0.0%

Table 3 shows kappa values and 95%-confidence intervals for the correlation of classification of nerve enlargement by investigators, as well as the percentage of measurements in which there is a mismatch between the investigators in the classification 'not enlarged' or 'enlarged'.
95%-CI: 95% confidence interval. ME: medial epicondyle.

Kappa value for our recently published protocol to determine the presence of an acquired chronic demyelinating polyneuropathy (enlargement of the median nerve at the forearm or arm or at the C5, C6, or C7 nerve roots) was 0.72 (95%-CI 0.37 – 1.00), and with exclusion of the C6 and C7 nerve roots 0.86 (95%-CI 0.51 – 1.00).[2]

Mixed model analysis

Multilevel modeling showed that, compared to the baseline model (DIC 3264.801), a 3-level model fitted the data best (DIC 3195.163), with an estimated overall mean difference of 0.102 mm^2 and significant random effects for 'nerve site' (SD 0.30 mm^2), and 'participant' (SD 0.43 mm^2) and a residual variance (SD 1.66mm^2). Neither the addition of 'hospital' as a fourth-level random effect (DIC 3196.095) nor the addition of fixed factors 'mean nerve size', 'different devices', 'right or left side', and 'patients or controls' improved the model significantly. It should be noted that the residual variance was considerably larger than the random effects of 'nerve site' and 'participant', thus the effect of those factors on reliability seems relatively minor.

Discussion

This study shows that inter-observer variability of sonographic assessment of nerve size is generally limited, and that a multicenter setting and the use of different brands of ultrasound devices do not increase this variability. For defined cut-offs for nerve enlargement, kappa values were in the range of good to excellent for most nerve sites in the arms, and poor to moderate for leg nerve sites. This indicates that nerve ultrasound is reproducible when a clearly defined protocol of arm nerves is used.

The multilevel model indicated that a large part of the observed variation remains to be explained. Significant contributing factors may be partially addressed in future multicenter studies, in particular the selection of nerves of interest (i.e. arms more than legs), but others, such as individual patient characteristics (e.g. less contrast in echogenicity between nerves and surrounding tissues due to the presence of fibrosis), can probably not be anticipated.

As most sonographic devices record nerve size in whole mm^2 or tenths of mm^2, there were no relevant systematic differences between investigators at most nerve sites. Our findings at the wrist and arm level (high ICC), and forearm and leg nerves (low ICC) were in line with previous findings.[10,11,15-17,24-27] Also, ICCs of nerve root measurements were comparable to 2 previous studies.[13,28] One other study that assessed nerve root size at intrascalene level found far lower ICCs, but this study assessed nerve size on still images, which might have hindered correct identification of the nerve roots.[29] The ICC at the ulnar

sulcus was relatively low, and – comparable to a previous study – we found a systematic difference between investigators.[15] Assessing nerve diameter instead of CSA at this site may lead to less interobserver variability,[9] but further study is required. Investigators were free to position the participants during ultrasound assessment, and as a result the amount of flexion in the elbow differed to some degree. Standardized positioning of the arm when assessing the ulnar nerve at the sulcus could possibly decrease interobserver variability. For the sural nerve, we observed a low ICC, most likely due to its small size in combination with rounded measurements on whole mm^2. In future studies, therefore, measurements at this site will have to be performed at a level of precision of at least 0.1 mm^2 to prove any diagnostic value of the assessment of this nerve.

Variability of the difference between investigators varied considerably between nerve sites and increased for nerves with a higher mean nerve size (SD 1.0 for nerves $<5mm^2$ compared to 3.3 for nerves $\geq 15mm^2$). SDs were highest at the brachial plexus (1.7 – 3.1 mm^2) and the tibial nerve (2.7 mm^2). The technical issues of ultrasound measurements at these sites are well known (i.e. difficulty to determine the exact site of splitting of the tibial nerve, and the considerable anatomic variation and depth of the brachial plexus and nerve roots). Although these sites may have diagnostic value in specific types of nerve pathology, the high variability makes these sites less suitable as part of diagnostic protocols or multicenter studies.

This study documented interobserver variability between physicians, hospitals, and different brands of sonographic devices; healthy controls as well as patients with CIAP, CIDP and MMN were investigated. We think that the wide range of abnormalities and the corresponding range in CSA-values at both entrapment and non-entrapment sites support the robustness of our findings and their relevance for other mono- and polyneuropathies, including carpal tunnel syndrome, and hereditary neuropathies. In contrast to previous studies, which investigated only one parameter with regard to inter-observer variability (e.g. ICC), we investigated multiple parameters, including mean differences, SDs, kappa values, and a random effects model, thus providing very important additional information on the reproducibility of nerve ultrasound, as this is determined by a combination of multiple aspects.

A limitation of this study is the relatively small sample size of patients and the fact that not all participants were investigated by all 4 investigators. However, we found small mean differences between investigators at all nerve sites, with relatively small 95% Cis of this mean difference. It would, therefore, be unlikely that we would have found large systematic differences between investigators if we would have used a larger sample size. Another limitation is that there was some variation in experience with nerve ultrasound between investigators which may, to some degree, have affected results, but all investigators had at least 1 year of experience with sonographic investigation of the nerves included in our protocol.[11,30]

Our study shows that interobserver variability of nerve ultrasound in peripheral neuropathy is limited, especially in arm nerves. Different devices and a multicenter setting have no significant influence on this interobserver variability. Therefore, nerve ultrasound is a reproducible tool for diagnostics in peripheral neuropathy in routine clinical practice and (multicenter) research.

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Chapter 4

Diagnostic Value

Nerve ultrasound improves detection of treatment-responsive chronic inflammatory neuropathy: a prospective cohort study

IJT Herraets*, HS Goedee*, JA Telleman*, RPA van Eijk, JTH van Asseldonk, LH Visser, LH van den Berg, WL van der Pol

Submitted

Abstract

Objective: To determine the diagnostic value of a practical sonographic protocol in detection of chronic inflammatory demyelinating polyneuropathy (CIDP), and multifocal motor neuropathy (MMN) and to determine the added value of nerve ultrasound in the detection of treatment-responsive patients.

Methods: Consecutive patients who fulfilled predefined criteria of clinical suspicion of chronic inflammatory neuropathy were included in this prospective cohort study. All patients underwent nerve ultrasound and nerve conduction studies (NCS). A decision to treat patients was made based on these results. Objective treatment response was evaluated according to predefined stringent criteria. A diagnosis of CIDP/MMN was established if NCS were abnormal (fulfilling criteria of demyeliniation of the EFNS/PNS) or if nerve ultrasound was abnormal (fitting CIDP/MMN according to our previously described protocol) in combination with an objective treatment response.

Results: We included 100 incident patients with clinical suspicion of chronic inflammatory neuropathy. A diagnosis of CIDP or MMN was established in 38 patients. Sensitivity and specificity of nerve ultrasound were 97.4% and 69.4%, respectively, and of NCS 78.9% and 93.5%, respectively. Added value of nerve ultrasound in the detection of CIDP/MMN was 21.1%.

Conclusions: Nerve ultrasound and NCS are complementary techniques with superior sensitivity in the former and specificity in the latter. Addition of nerve ultrasound significantly improves the detection of treatment-responsive chronic inflammatory neuropathy compared to routine diagnostic tests. Therefore, it should be included in future revisions of diagnostic consensus criteria.

Introduction

Polyneuropathy is one of the most common disorders in neurological practice.[1] Distinction of chronic inflammatory neuropathies as chronic inflammatory demyelinating polyneuropathy (CIDP), Lewis-Sumner Syndrome (LSS) and multifocal motor neuropathy (MMN) is important as these disease are treatable and significant disability can occur over time.

Nerve ultrasound is an emerging tool for the diagnostic work-up of polyneuropathy.[2-4] Although nerve enlargement is more generally associated with neuropathy, specific patterns of nerve enlargement are associated with rare variants, in particular those with an inflammatory etiology. We recently showed that enlargement of the brachial plexus and median nerve in the forearm and upper arm reliably distinguishes patients with CIDP, MMN and LSS from more common axonal neuropathies and motor neuron disease.[2] Moreover, results from this study suggested that this short and reproducible sonographic protocol could facilitate early and accurate identification of patients with potentially treatable neuropathy.[2,4,5]

Current diagnostic criteria depend primarily on results from nerve conduction studies (NCS). Consequently, NCS protocols often need to be extensive and time-consuming. NCS require specific infrastructure and trained personnel which are not always available. Moreover, NCS have high specificity but lack sensitivity and are, therefore, sometimes insufficient to diagnose treatment-responsive chronic inflammatory neuropathies.[6-9]

Nerve ultrasound could shorten the time to diagnosis and improve identification of patients with chronic inflammatory neuropathy.[5] However, diagnostic performance of nerve ultrasound has not been studied in an unbiased approach. In this stuy, we aimed to establish the clinical value of our previously published nerve ultrasound protocol in a cohort of consecutive incident patients clinically suspected of an chronic inflammatory neuropathy. Moreover, we systematically assessed whether addition of nerve ultrasound to routine NCS improves identification of patients who may benefit from treatment.

Methods

Study design and patients

This prospective cohort study was performed between February 2015 and July 2018 in the UMC Utrecht, a large tertiary referral center for neuromuscular disorders in The Netherlands. Our study protocol was approved by the medical ethics committee Brabant (NL42895.008.12). All patients gave written informed consent.

We included consecutive patients at our outpatient clinic with a strong clinical suspicion of a chronic inflammatory neuropathy. We defined 'strong clinical suspicion' as a subacute or chronic sensorimotor polyneuropathy (complaints ≥6 weeks) and ≥2 out of the following criteria: 1) asymmetric involvement, 2) proximal weakness, 3) areflexia 4) sensory ataxia, 5) rapid progression of complaints, 6) postural tremor, and 7) pain in a symmetric or multifocal distribution; OR a subacute or chronic pure motor or pure sensory neuropathy with ≥1 of the above-mentioned criteria (Supplemental figure 1).[8,10-13] This definition covered asymmetric variants (i.e. MMN and LSS) as well as classical, pure motor and pure sensory variants of CIDP. Exclusion criteria for this study were: 1) previous diagnosis (and treatment) of polyneuropathy, 2) age <18 or >80, and 3) physical inability to undergo nerve ultrasound investigations.

Routine diagnostic work-up

Diagnostic work-up of all patients consisted of a standardized interview using questionnaires, clinical examination, appropriate laboratory investigations and nerve conduction studies (Supplemental Table 1). In addition, treating physicians could request any additional tests (e.g. MRI, lumbar puncture) they deemed necessary to establish a diagnosis.

Standardized clinical examination consisted of bilateral grading of motor function of 14 muscle groups in arms and legs using the Medical Research Council (MRC) scale; bilateral measurement of grip strength in Kilopascals (kPa) with the Martin Vigorimeter (Martin Medizintechnik, Tuttlingen, Germany) and testing of sensory function with the modified INCAT Sensory Sum score (ISS).[14] Questionnaires included the INCAT Overall Disability Sum Score (ODSS) and Rasch-built Overall Disability Scale (RODS; for CIDP or MMN depending on the clinical phenotype).[15-17]

Nerve conduction studies (NCS) were performed according to a previously described protocol by experienced clinical neurophysiologists who were blinded for nerve ultrasound results and additional diagnostic investigations. Limbs were warmed in water at 37 °C for 45 minutes prior to examination with a Nicolet VIKING IV EMG machine (CareFusion Japan).[3] All NCS were graded following the EFNS/PNS-guidelines for CIDP (definite, probable or possible) or MMN (definite conduction block, probable conduction block, no conduction block).[12,13] For the purpose of this study, we categorized NCS that met 'definite/probable/possible' criteria for CIDP and for MMN the presence of at least one definite or probable conduction block as 'abnormal' and other outcomes as 'normal'.

Nerve ultrasound

Central to this study was nerve ultrasound following a protocol described previously.[3] Nerve ultrasound was performed by an experienced ultrasonographer, blinded for the results of NCS and additional diagnostic investigations. Investigations were performed on

a Philips Epiq 7 (Philips Medical Instruments) with a 5-18 MHz linear array transducer. We used a previously published protocol.[3] In short, this protocol consists of nerve size (cross sectional area (CSA)) measurement at standardized sites: the median nerve at 1/3 of the forearm, at 1/2 of the upper arm, and the C5, C6, and C7 nerve roots. Nerve ultrasound was regarded as abnormal if uni- or bilateral nerve enlargement was found at ≥1 of the measured sites.[3]

Treatment strategies

Patients with both NCS and nerve ultrasound results compatible with chronic inflammatory neuropathy (group 1) and patients with abnormal NCS, but a normal nerve ultrasound (group 2) were treated for a chronic inflammatory neuropathy with intravenous immunoglobulins (IVIg) and/or corticosteroids in the case of a suspicion of CIDP (Figure 1: Flowchart). Patients with normal results for both NCS and nerve ultrasound (group 3) did not receive treatment, and were excluded from further follow-up (Figure 1). Patients with normal NCS, but abnormal nerve ultrasound results (group 4) which allowed another diagnosis to be established, based on additional investigations, received no treatment for CIDP or MMN, and were excluded from further follow-up. On the other hand, patients in group 4 for whom no other diagnosis could be established were either directly offered trial treatment with IVIg and/or corticosteroids by their treating physicians, or were invited for a second clinical evaluation (Figure 1). An independent specialist in polyneuropathies and neuromuscular disorders performed this second evaluation and ordered additional ancillary investigations if deemed necessary. If the clinical phenotype of CIDP/MMN, according to the EFNS/PNS criteria, was still present at second evaluation and no other diagnosis could be established, patients were also offered trial treatment with IVIg. Clinical course and treatment effect were evaluated during a 1-year follow-up period in all patients.

Evaluation of Treatment effect

We defined improvement as: 1) MRC sum score: an increase ≥1 point, 2) Hand Held Dynamometry (HHD, in Newton): an increase in strength of ≥10% in two muscle groups in the same region (proximal arm, distal arm, proximal leg, distal leg) or an increase in strength of ≥25% in one muscle group, 3) Vigorimetry: an increase of ≥8 kPa in one or both hands,[18] 4) RODS; a minimal clinically important difference (MCID) score (calculated for each patient using individually obtained standard errors) >1.96 for CIDP and > 1.00 for MMN,[17,19] 5) ODSS: a decrease of ≥1 point,[20] and 6) ISS: a decrease of ≥1 point.[20] 'Objective treatment effect' was defined as improvement in MRC sum score (modality 1) in combination with improvement in ≥1 of the other modalities (2-6).

Diagnostic classification

We defined the diagnostic criteria for CIDP/MMN as follows: 1) a clinical phenotype fitting the EFNS/PNS clinical criteria for CIDP/MMN in combination with 2) a clinical course fitting CIDP/MMN during a 1-year follow-up period, and with either 3a) NCS in accordance with the respective EFNS/PNS criteria,[12,13] or 3b) abnormal nerve ultrasound as defined previously in combination with 'objective treatment effect'.

Statistical analysis

All data were summarized as mean (standard deviation (SD)) for normally distributed variables, median (range) for non-normal distributed variables and n (%) for categorical variables. Depending on the distribution of the variable, we compared results of groups of patients using the independent t-test (continuous, normal), Wilcoxon test (continuous, non-normal) or chi-square test (categorical). Results were considered significant when alpha was below 0·05. Both NCS and ultrasound were scored as abnormal (1) or normal (0); a similar approach was undertaken for patients either having CIDP/MMN (1) or not (0). Subsequently we calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) from 2x2 tables. All analyses were conducted in SPSS 22 (SPSS Inc., Chicago IL, USA).

Results

Baseline Characteristics

Baseline characteristics of the 100 patients initially suspected of a chronic inflammatory neuropathy are shown in Table 1. We obtained a diagnosis of CIDP in 20, of LSS in 4 and of MMN in 14 patients. All diagnoses are listed in Supplemental Table 2.

Diagnostic classification

Based on the findings of NCS and nerve ultrasound, 31 patients were included in group 1 (diagnosis CIDP/LSS/MMN n = 29), 3 patients in group 2 (diagnosis CIDP/LSS/MMN n = 1), and 41 patients in group 3 (diagnosis CIDP/LSS/MMN n = 0). Group 4 consisted of 25 patients, of whom 15 were treated despite normal NCS results (Figure 1 and Supplemental Table 3-4). Of the 15 treated patients, 8 had an 'objective treatment effect' (53.3%) (Table 2). Therefore, the defined diagnostic criteria of CIDP//LSS/MMN used in this study were fulfilled in these patients (4 CIDP, 4 MMN).

Apart from the 8 patients with the diagnosis of CIDP/MMN with normal NCS and abnormal nerve ultrasound, an additional 3 patients in group 4 had some degree of treatment effect that did not meet the predefined criteria for objective treatment effect (improvement of MRC score with 10 points and improvement of the RODS score but without MCID (n=2);

Table 1 Baseline characteristics

	Inclusions (n=100)
Age in years (mean, SD)	58.0 (13.5)
Sex, male / female	78 / 22
Duration of symptoms in months (median, range)	24 (1-264)
Clinical criteria set A	
Sensorimotor	31
Motor > sensory	6
Pure motor	46
Pure sensory	17
Clinical criteria set B	
Asymmetrical complaints	54
Proximal weakness	33
Areflexia	36
Sensory ataxia	14
Rapid progression	13
Postural tremor	9
Pain (symmetric/multifocal)	24
Clinical suspicion of	
CIDP	
Classical	30
Pure motor	9
Pure sensory	17
LSS	7
MMN	37
Final diagnosis	
CIDP	
Classical	14
Pure motor	2
Pure sensory	4
MMN	14
LSS	4
Various	62

Table 1 shows the baseline characteristics of 100 patients in whom there is a strong clinical suspicion of a chronic inflammatory neuropathy; data are shown as number of patients unless stated otherwise.
CIDP: chronic inflammatory demyelinating polyneuropathy, Classical: classical phenotype of CIDP, pure motor: pure motor phenotype of CIDP, pure sensory: pure sensory phenotype of CIDP. LSS: Lewis Sumner Syndrome, MMN: multifocal motor neuropathy, Various: all other diagnosis

Figure 1 Flowchart

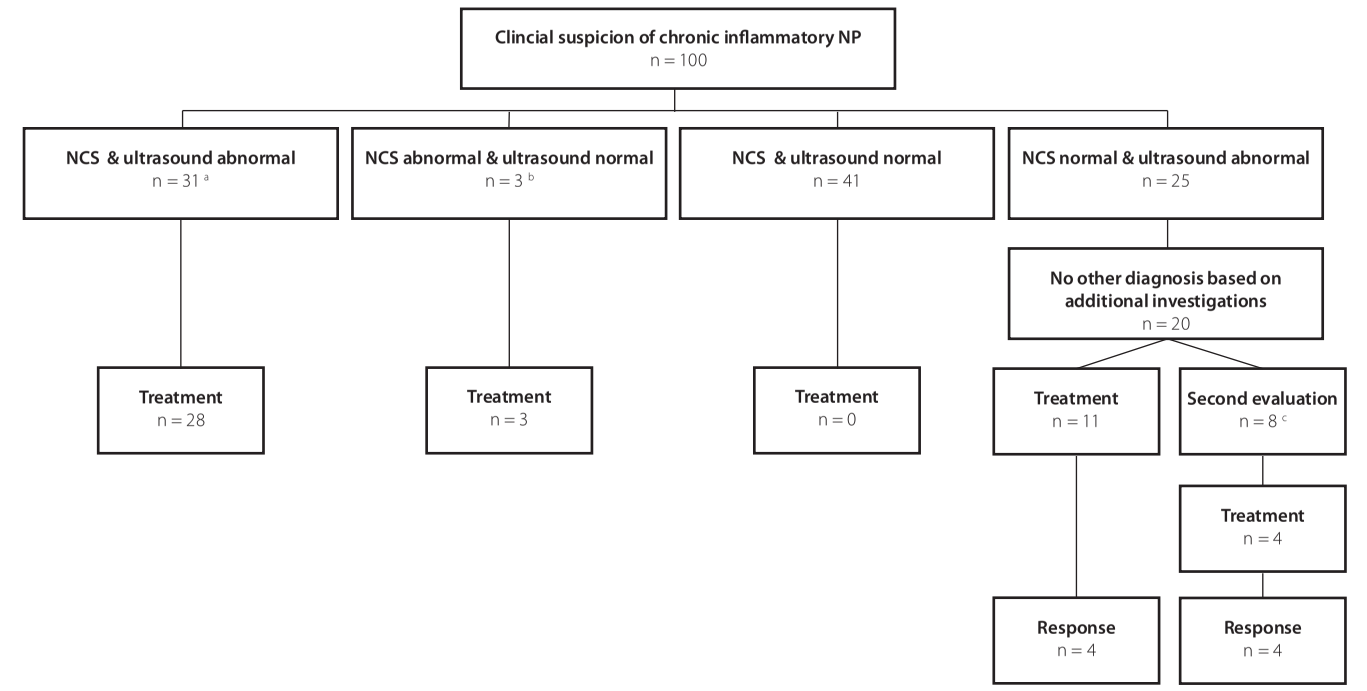


Figure 1: This flowchart shows the diagnostic work-up, follow-up and diagnoses established in the 4 groups based on NCS and nerve ultrasound results. The diagnosis of CIDP/MMN is base on the predefined diagnostic criteria as described in the methods section of this article. a and b : other diagnosis established, c: no treatment received, d: no second evaluation performed

IgM MGUS: IgM monoclonal gammopathy of undetermined significance. NCS: Nerve Conduction Studies, NP: neuropathy, PSMA: Progressive Spinal Muscular Atrophy

Table 2 Treatment response

Patient	Diagnosis	Follow-up (months)	MRC sum score	RODS MCID	Vigorimetry Right	Vigorimetry Left	ODSS	ISS	HHD	Objective treatment effect
1	CIDP	12	+1	+	+5	+7	-2	-17	NP	+
2	CIDP	12	+14	+	+70	+58	0	0	NP	+
3	MMN	12	+1	+	-14	-5	-1	NA	+	+
4	CIDP	16	+6	-	+3	+30	-3	-4	+	+
5	MMN	16	+3	-	-22	-20	0	NA	+	+
6	MMN	16	+1	-	+13	+10	0	NA	+	+
7	MMN	15	+3	+	-10	+11	0	NA	+	+
8	CIDP	19	+8	-	+20	+24	0	NP	+	+

Table 2 shows specifics on treatment response of the 8 patients with normal NCS and abnormal nerve ultrasound with the consensus diagnosis of CIDP/MMN. NA: not applicable, NP: not performed; "-" no improvement, "+" improvement
CIDP: Chronic Inflammatory Demyelinating Polyneuropathy, HHD: Hand Held dynamometry, ISS: INCAT Sensory Sum score, MCID: minimal clinically important difference, MMN: Multifocal Motor Neuropathy, MRC: Medical research council, ODSS: Overall Disability Sum Score, RODS: Rasch-built Overall Disability Scale

improvement of MRC sum score could not be reached because of maximum baselinescore but improvement in ≥1 of the other modalities was fulfilled (n=1)) (Supplemental Table 4) As these patient showed treatment effect and no alternative diagnosis could be made a working diagnosis of CIDP/MMN was established in these patients. However, results of ultrasound were regarded as false positive in the analyses, as these patients did not fulfil the stringent criteria for 'objective treatment effect'.

Added value of nerve ultrasound

A diagnosis of chronic inflammatory neuropathy was established in 38 of 100 patients. In 29 of these 38 patients (76.3%), NCS and nerve ultrasound were both abnormal (group 1), in 1 (2.6%) only NCS (group 2) and in 8 (21.0%) only nerve ultrasound (group 4) (Figure 2). There were no significant differences in clinical characteristics between patients with normal NCS and abnormal NCS (Supplemental Table 5). An 'objective treatment effect'

Figure 2 Added value of nerve ultrasound

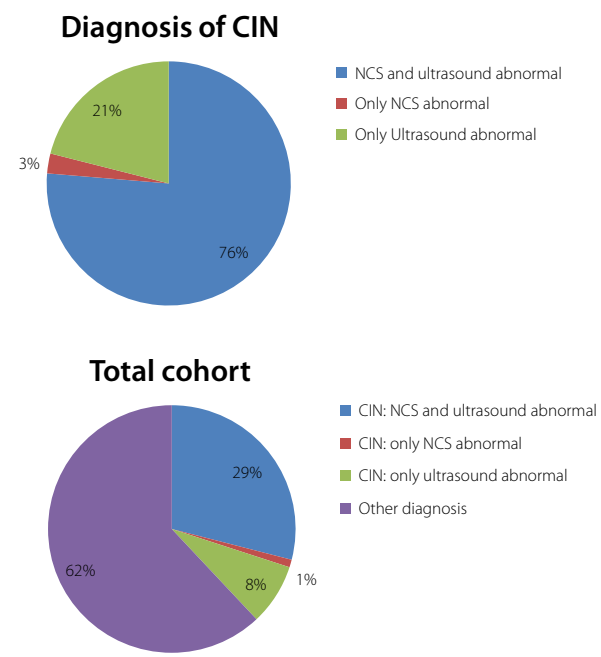


Figure 2 shows the distribution of the diagnoses of CIDP/LSS/MMN established with abnormal NCS, abnormal nerve ultrasound or both in the total cohort (n=100) (A) and in the group of patients with a diagnosis of CIDP/LSS/MMN (n=38) (B).
CIDP: Chronic Inflammatory Demyelinating Polyneuropathy, LSS: Lewis-Sum ner Syndrome, MMN: Multifocal Motor Neuropathy, NCS: Nerve Conduction Studies

was found in 14 of 28 patients (50.0%) with both abnormal NCS and ultrasound (group 1), 1 of 3 (33.3%) with only abnormal NCS (group 2) and 8 of 15 (53.3%) with only abnormal nerve ultrasound (group 4).

Diagnostic accuracy of nerve ultrasound and NCS

Sensitivity and specificity for the diagnosis of chronic inflammatory neuropathy were 76.9% and 93.5%, respectively for NCS and 97.4% and 69.4% for nerve ultrasound (Table 3). Based on the results of this study we deviced two potential strategies to diagnose chronic inflammatory neuropathy (Figure 3).

Table 3 Diagnostic value of nerve ultrasound and NCS

	Ultrasound	NCS
Test positive / total positive Sensitivity (%)	37 / 38 97·4	30 / 38 78.9
Test negative / total negative Specificity (%)	43 / 62 69.4	58 / 62 93.5
NPV (%)	97.7	87.9
PPV (%)	66.1	88.2

Table 3 shows the diagnostic value of nerve ultrasound and NCS for the diagnosis of chronic inlammatory neuropathy according to our predefined consensus criteria
NCS: Nerve Conduction Studies, NPV: Negative Predictive Value, PPV: Positive Predictive Value

Supportive criteria

Results from ancillary investigations are summarized in Supplemental Table 3.

Figure 3 Potential diagnostic strategies in suspected chronic inflammatory neuropathy

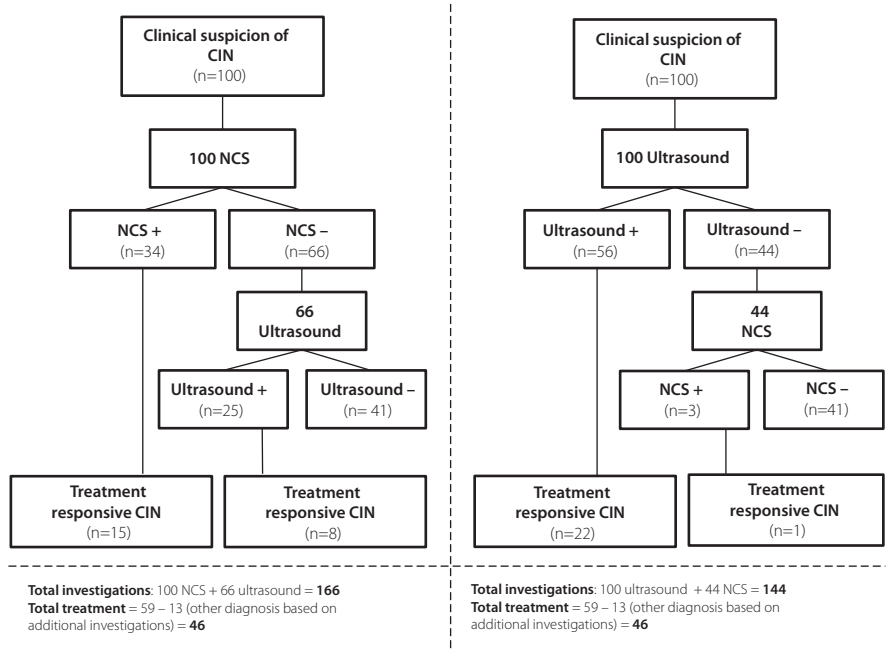


Figure 3 shows two potential diagnostic strategies in patients suspected of chronic inflammatory neuropathy and the number of investigations needed to identify patients with treatment-responsive CIDP, LSS, and MMN. In strategy A NCS are performed as primary investigation, followed by nerve ultrasound. In strategy B nerve ultrasound is performed prior to NCS.
CIDP: chronic inflammatory demyelinating polyneuropathy, CIN: chronic inflammatory neuropathy, LSS: Lewis-Sumner syndrome, MMN: multifocal motor neuropathy, NCS: nerve conduction studies, Ultrasound: nerve ultrasound

Discussion

In this study, we show that nerve ultrasound is a useful tool for the diagnosis of chronic inflammatory neuropathy. It has high sensitivity and acceptable specificity in a cohort of consecutive patients with a clinical suspicion of CIDP, LSS and MMN, and facilitates identification of additional patients with treatment response. Characteristics of nerve ultrasound and NCS differ, with superior sensitivity in the former and specificity in the latter. Therefore, these investigations are most likely complementary techniques in the diagnostic work-up of polyneuropathy in general and chronic inflammatory neuropathy in particular.

In contrast to previous studies, we obtained our results by applying an unbiased approach. Previous studies suggested both high sensitivity (61-90%) and specificity (72-100%) of nerve ultrasound for the identification of patients with chronic inflammatory neuropathies,[3,21,22] but the inclusion of patients with a diagnosis according to the EFNS/PNS or AAN consensus criteria was a source of potential bias. Although we found comparable high levels of sensitivity, specificity of ultrasound may be slightly lower than previously reported. This implies that ultrasound and NCS can best be used as complementary techniques.

The use of ultrasound allows the detection of additional patients who will respond to treatment at the expense of some false-positives. We identified 8 patients (21%) with normal NCS but with abnormal nerve ultrasound and objective treatment response. All these 8 patients fulfilled the predefined and stringent criteria for objective treatment response. Three more patients had some degree of treatment effect but did not meet these stringent criteria of objective treatment effect. Moreover, in contrast to current diagnostic criteria based primarily on NCS (only positive NCS may suffice), we used more stringent diagnostic consensus criteria for nerve ultrasound to establish a diagnosis of CIDP/LSS/MMN (i.e. in case of abnormal nerve ultrasound results only, an objective treatment response had to be present). Therefore, our estimate of the added value of nerve ultrasound in identifying treatment-responsive patients with chronic inflammatory neuropathy may be relatively conservative.

Ultrasound study results are as yet not incorporated in the diagnostic consensus criteria for CIDP and MMN.[12,13,23] These criteria currently rely mostly on NCS study results, although a diagnosis of 'possible' MMN can be made in the absence of conduction block or other demyelinating features.[12,13] Even if the presence of other ancillary abnormalities is required, the rate of treatment response may be disappointing.[11,24] The findings in our study that addition of a short and practical nerve ultrasound protocol significantly improves detection of patients with (objective) treatment response. We recently showed that this diagnostic nerve ultrasound protocol, has low inter-rater and inter-hospital variability.[4] In contrast, reliability of extensive NCS protocols, needed to establish the diagnose of a chronic inflammatory neuropathy according to the currently applied diagnostic consensus criteria, has not been evaluated prospectively. Nerve ultrasound lacks some of the disadvantages of NCS and other ancillary investigations, including burden to the patient, and the possibility of adverse events, cost and limitations in availability, and in addition its diagnostic performance is superior to techniques that are part of current consensus criteria, including MRI (sensitivity in qualitative studies approximately 40%) of the brachial plexus and elevated CSF protein.[12,13,23] Therefore, nerve ultrasound should be included as diagnostic tool in future revisions of diagnostic criteria, in our opinion on par with NCS. As sensitivity is high, nerve ultrasound could serve as a first screening tool (Figure 3, strategy

B) for patients suspected of chronic inflammatory neuropathy. NCS could be used to confirm the diagnosis of chronic inflammatory neuropathy in patients with abnormal ultrasound results, to detect CIDP, LSS and MMN in cases with normal ultrasound but with strong clinical suspicion, or to further predict response to treatment with immunoglobulins. This approach could decrease the demand for labour intensive NCS and burden to patients, reduce the number of treatment-trials needed in patients suspected of potentially treatable neuropathies and could thus improve cost-effectiveness.

Our study also has some limitations. A limitation of the study design is that not all 100 patients with suspected chronic inflammatory neuropathy received treatment. In theory, treatment-responsive patients without NCS and ultrasound abnormalities could have been missed and diagnostic accuracy of both NCS and nerve ultrasound could thus be overestimated. However, immunoglobulin treatment carries the risk of potentially severe adverse events and treatment of all 100 patients with a clinical suspicion of CIDP/MMN would not have been ethical. Another limitation was the difference in follow-up duration. Nevertheless, we followed all patients for one year minimal. Lastly, the treating physician was free in his/her treatment decisions and, therefore, small differences in treatment protocol between patients were present, though all patients received immunoglobulins (and in case of CIDP also corticosteroids) if necessary.

In conclusion, nerve ultrasound and NCS are complementary techniques with superior sensitivity in the former and specificity in the latter. Addition of nerve ultrasound significantly improves the detection of treatment-responsive chronic inflammatory neuropathy compared to routine diagnostic tests. Therefore, it should be included in future revions of diagnostic consensus criteria.

Supplemental Table 1 Specifications of diagnostic work-up

Modality	Description of performed work-up
MRC score	Bilateral measurement of motor function of: - Abduction of the arm - Flexion and extension of the forearm and wrist - Spreading of the fingers - Abduction of the thumb - Flexion of the hip - Flexion and extension of the ankle and foot - Eversion of the foot - Extension of the hallux MRC sum score: 0-140 points
ISS	INCAT Sensory Sum Score Measurement of gnostic and vital sensibility in arms and legs
Vigorimetry	Bilateral measurement of grip strength in Kilopascals (kPa) with the Martin Vigorimeter (Martin Medizintechnik, Tuttlingen, Germany)
RODS	Rasch-built Overall Disability Scale Standardized questionnaire for CIDP or MMN (depending on clinical phenotype)[16,17]
INCAT ODSS	INCAT Overall Disability Sum Score Standardized questionnaire[15]
Laboratory investigations	To exclude other causes of polyneuropathy: - Renal, liver, and thyroid function - Glucose - Vitamins - Complete blood count - Protein spectrum
Nerve Conduction Studies	Bilateral evaluation of demyelination and axonal loss in: - Median and ulnar nerves (recordings from hand muscles) - Fibular and tibial nerves (recordings from foot muscles) - Sural nerve Extended with (in case of suspicion of MMN): - Musculocutaneous nerve (recordings from the biceps muscle) - Median and radial nerves (recordings from forearm muscles)
Nerve ultrasound	Bilateral measurement of cross- sectional area (CSA) of: - Median nerve at the forearm and arm - Nerve roots C5,C6, and C7 at the interscalene level Cut-off values for nerve enlargement: - Median nerve at the forearm >10 mm^2 - Median nerve at the arm >13 mm^2 - Nerve roots C5,C6, or C7 >8 mm^2

Supplemental Table 2 Diagnoses established in the cohort of 100 patients with a clinical suspicion of a chronic inflammatory neuropathy

Diagnoses	Patients (n=100)
Adult polyglucosan body disease	1
ALS	1
Axonal neuropathy, not CIAP	3
Benign muscle cramp fasciculation syndrome	1
Cervical radiculopathy	2
CIAP	12
CIAP in combination with mitochondrial neuromyopathy	1
CIDP	
Classical	14
Pure motor	2
Pure sensory	4
Working diagnosis; definition of 'objective treatment effect' not fulfilled[a]	3
Distal myopathy	1
Functional disorder	1
Hirayama Syndrome	4
HNLPP	1
IgM-MGUS polyneuropathy	1
Immune-mediated polyradiculitis associated with Sjögren syndrome	1
LSS	4
Lumbar spinal stenosis	2
MMN	14
Multifocal axonal neuropathy associated with Crohn's disease	1
Neuralgic amyotrophy	1
Neurolymphomatosis	1
Post-infectious axonal polyneuropathy	1
PSMA	15
Post-Guillain Barre Syndrome	3
Ulnaropathy	1
Vasculitis	4

a. Regarded as false positive during the analyses as criteria of objective treatment effect were not fulfilled
ALS: Amyotrophic Lateral Sclerosis, CIAP: Chronic Idiopathic Axonal Polyneuropathy, CIDP: Chronic Inflammatory Demyelinating Polyneuropathy, GBS: Guillain-Barré Syndrome, HNLPP: Hereditary Neuropathy with Liability to Pressure Palsies, MGUS: Monoclonal Gammopathy of Undetermined Significance, MMN: Multifocal Motor Neuropathy, LSS: Lewis Sumner Syndrome, PSMA: Progressive Spinal Muscular Atrophy. Classical: classical phenotype of CIDP, pure motor: pure motor phenotype of CIDP, pure sensory: pure sensory phenotype of CIDP

Supplemental Table 3 Patient characteristics per sub group

Patient	Diagnosis	Clinical Phenotype	EFNS-classification	EFNS NCS-classification	Sonography Score	Supportive Criteria		
						Elevated CSF Protein	Abnormal MRI	Anti-GM1
Group 1								
1	CIDP	Classical	Definite	Definite	10	+	NP	NP
2	CIDP	Pure sensory	Definite	Definite	5	NP	NP	NP
3	LSS		Probable	Possible	3	NP	-	NP
4	MMN		Definite	Definite	3	+	+	-
5	LSS		Possible	Possible	1	NP	-	NP
6	LSS		Probable	Possible	4	NP	-	NP
7	CIDP	Classical	Definite	Definite	9	+	NP	NP
8	MMN		Definite	Definite	4	-	NP	-
9	CIDP	Classical	Probable	Possible	4	NP	NP	NP
10	MMN		Definite	Definite	2	+	NP	-
11	MMN		Definite	Definite	3	NP	NP	-
12	CIDP	Classical	Definite	Definite	6	NP	NP	NP
13	CIDP	Classical	Definite	Definite	1	+	NP	NP
14	LSS		Definite	Probable	2	-	-	NP
15	CIDP	Pure sensory	Definite	Possible	1	+	-	NP
16	CIDP	Classical	Definite	Definite	7	+	NP	NP
17	MMN		Definite	Definite	4	NP	NP	-
18	CIDP	Classical	Possible	Possible	5	NP	NP	NP
19	CIDP	Pure sensory	Definite	Definite	5	NP	NP	NP
20	MMN		Definite	Definite	2	NP	+	-
21	CIDP	Pure motor	Definite	Definite	8	NP	+	-
22	CIDP	Classical	Definite	Definite	3	+	+	NP
23	MMN		Definite	Definite	2	+	-	-
24	CIDP	Classical	Definite	Definite	3	NP	NP	NP
25	MMN		Probable	Probable	4	NP	NP	+
26	MMN		Probable	Probable	4	NP	+	-
27	CIDP	Classical	Definite	Definite	3	NP	NP	NP
28	CIDP	Pure motor	Definite	Definite	2	NP	NP	NP
29	CIDP	Classical	Probable	Possible	1	-	-	NP
30 [a]	PSMA		Probable	Possible	1	+	-	NP
31 [b]	IgM-MGUS polyneuropathy		Negative	Definite	3	NP	NP	NP
Group 2								
1	MMN		Probable	Probable	0	+	+	-
2 [a]	PSMA		Probable	Probable	0	+	NP	-
3 [a]	PSMA		Definite	Possible	0	+	+	NP
Group 4								
1	CIDP	Pure sensory	Negative	Negative	3	NP	-	NP
2	CIDP	Classical	Negative	Negative	3	NP	+	NP
3	MMN		Possible	Negative	3	+	+	-
4	CIDP	Classical	Negative	Negative	5	+	+	NP
5	MMN		Possible	Negative	7	+	+	-
6	MMN		Possible	Negative	2	NP	-	+
7	MMN		Possible	Negative	3	NP	-	-
8	CIDP	Classical	Negative	Negative	5	NP	+	NP
9 [c]	Working diagnosis CIDP	Pure sensory	Negative	Negative	3	+	+	NP
10 [d]	Working diagnosis CIDP	Classical	Negative	Negative	3	+	NP	NP
11 [e]	Working diagnosis CIDP	Classical	Negative	Negative	4	+	+	NP
12 [a]	PSMA		Negative	Negative	4	NP	+	NP
13 [f]	CIAP		Negative	Negative	4	NP	NP	NP
14 [a]	PSMA		Negative	Negative	2	NP	+	-

Supplemental Table 3 Continued

Patient	Diagnosis	Clinical Phenotype	EFNS-classification	EFNS NCS-classification	Sonography Score	Supportive Criteria		
						Elevated CSF Protein	Abnormal MRI	Anti-GM1
Group 4								
15 [g]	Functional disorder		Negative	Negative	2	NP	+	-
16 [h]	Status after GBS		Negative	Negative	3	+	-	NP
17 [i]	Neuralgic amyotrophy	I	Negative	Negative	6	+	+	NP
18 [i]	Post-infectious axonal polyneuropathy		Negative	Negative	1	+	NP	
19 [i]	CIAP		Negative	Negative	5	NP	NP	
20 [j]	CIAP		Negative	Negative	1	+	-	
21 [k]	Vasculitis		Negative	Negative	3	NP	NP	NP
22 [k]	Cervical radiculopathy		Negative	Negative	1	NP	NP	NP
23 [k]	Mononeuropathy		Negative	Negative	7	+	NP	-
24 [k]	Vasculitis		Negative	Negative	1	+	NP	NP
25 [k]	Lumbar radiculopathy		Negative	Negative	4	+	-	NP

NA: not applicable, NP: not performed; "-" = no improvement, "+" = improvement. a. Respiratory failure and no treatment effect. b. Diagnosis based on additional investigations. c. Loss to follow-up due to depression; 2nd NCS 5 months after primary work-up: definite according to EFNS/PNS NCS criteria. d. Exacerbation COPD; deceased. e. Decline due to cerebral infarction. f. No treatment effect; no progression without treatment. g. No treatment effect and no objective weakness with repeated neurological examination. h. Symptoms resolved without treatment. i. Symptoms stable without treatment. j. No treatment; loss to follow-up. k. Diagnosis based on additional investigations Anti-GM1: anti-GM1 antibodies, CIAP: chronic idiopathic axonal polyneuropathy, CIDP: chronic inflammatory demyelinating polyneuropathy, GBS: Guillain-Barré Syndrome , MGUS: Monoclonal Gammopathy of Undetermined Significance , MMN: multifocal motor neuropathy, NCS: nerve conduction studies, PSMA: progressive spinal muscular atrophy

Supplemental Table 4 Specifics on treatment effect per sub group

	Diagnosis	MRC sum score	RODS	RODS MCID	Vigorimetry Right	Vigorimetry Left	ODSS	ISS	HHD	Objective treatment effect
Group 1										
1	CIDP	+9	+4	-	+2	-24	-1	-3	-	+
2	CIDP	-2	-4	-	-20	-5	-1	+3	NP	-
3	CIDP	-6	-2	-	+15	+19	+4	+11	NP	-
4	MMN	-1	+1	-	-12	-13	-3	NA	+	-
5	CIDP	-6	0	-	-7	+16	0	+5	NP	-
6	CIDP	-17	-6	-	-82	-35	+1	NA	+	-
7	CIDP	+4	NP	NP	+15	+50	NP	NA	NP	+
8	MMN	0	+3	+	+32	+5	-1	NA	+	-
9	CIDP	+7	+10	+	-2	-4	-3	-9	NP	+
10	MMN	-3	-5	-	+8	+9	0	NA	+	-
11	MMN	+4	0	-	-9	-5	0	NA	NP	-
12	CIDP	+11	+13	+	+57	+38	-3	0	NP	+
13	CIDP	+8	0	-	+12	+12	0	-4	NP	+
14	CIDP	+6	+21	+	-25	+35	0	+1	+	+
15	CIDP	0	+10	+	+8	+3	-1	-7	NP	-
16	CIDP	+69	+35	+	+47	+46	-5	-10	NP	+
17	MMN	-1	-4	-	-23	-5	-1	NA	+	-
18	CIDP	+6	0	-	0	-15	0	-8	NP	-

Supplemental Table 4 Continued

	Diagnosis	MRC sum score	RODS	RODS MCID	Vigorimetry Right	Vigorimetry Left	ODSS	ISS	HHD	Objective treatment effect
Group 1										
19	CIDP	NA	NA	NA	NA	NA	NA	NA	NA	Loss to follow-up
20	MMN	+4	+1	-	+80	+5	-1	NA	NP	+
21	CIDP	+50	+17	+	+10	+10	-3	+1	NP	+
22	CIDP	+6	+19	+	+25	+20	-6	-5	NP	+
23	MMN	+1	-9	-	+8	+7	+1	NA	-	+
24	CIDP	+3	-4	-	-12	- 24	+1	-4	NP	+
25	MMN	0	+8	+	NP	NP	0	NA	NP	-
26	MMN	+13	+3	+	+6	+6	-3	NA	+	+
2	CIDP	NT	NT	NT	NT	NT	NT	NT	NT	NT
28	CIDP	NT	NT	NT	NT	NT	NT	NT	NT	NT
29	CIDP	+21	+2	-	+102	+94	-3	-17	NP	+
30	PSMA	-16	NA	NA	NA	NA	NA	NA	-	-
Group 2										
1	MMN	+5	+3	+	+7	+11	-1	NA	+	+
2	PSMA	-11	NA	NA	NA	NA	NA	NA	-	-
3	PSMA	-4	NA	NA	NA	NA	NA	NA	-	-
Group 4										
1	Working diagnosis CIDP	Max	NP	NP	NP	NP	+1	0	-	-
2	Working diagnosis CIDP	+10	+1	-	-11	0	0	NP	NP	-
3	Working diagnosis CIDP	+10	+2	-	-15	-32	+1	+6	NP	-
4	PSMA	+1	NP	NP	NP	NP	+1	NP	NP	-
5	CIAP	0	NP	NP	-15	-10	0	+2	-	-
6	PSMA	-1	NP	NP	NP	NP	NP	NP	-	-
7	Functional disorder	0	NP	NP	NP	NP	NP	NP	-	-

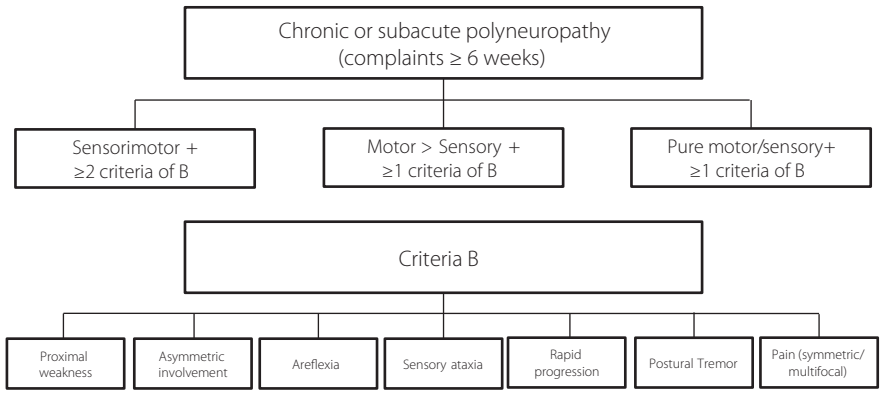
NA: not applicable; NP: not performed; NT; not treated; "-" no improvement, "+" improvement
CIDP: Chronic Inflammatory Demyelinating Polyneuropathy, HHD: Hand Held dynamometry, ISS: INCAT Sensory Sum score, MCID: Minimal Clinically Important Difference, MMN: Multifocal Motor Neuropathy, ODSS: Overall Disability Sum Score, PSMA: progressive spinal muscular atrophy, RODS: Rasch-built Overall Disability Scale

Supplemental Table 5 Clinical characteristics of patients with CIDP/LSS/MMN with and without NCS abnormalities

	CIDP/LSS/MMN Abnormal NCS (n=30)	CIDP/LSS/MMN Normal NCS (n=8)	P-value
Age in years (mean, SD)	56.5 (12.6)	58.6 (12.7)	0.67
Sex Male / Female	23 / 7	8 / 0	0.31
Duration of symptoms in months (median, range)	33 (3-264)	24·0 (9-144)	0.74
Number of sites with nerve enlargement (median, range)	3 (0-10)	3 (2-7)	0.77
CSF protein Norma / Abnormal	3 / 10	0 / 3	1.0-
MRI brachial plexus Normal / Abnormal	7 / 6	3 / 5	0.66
Clinical phenotype CIDP Classical Pure motor Pure Sensory LSS MMN	 11 2 3 4 10	 3 0 1 0 4	0.72
Anti-GM1 autoantibodies Absent / Present	12 / 1	5 / 1	1.00

Clinical characteristics of the 38 patients with the consensus diagnosis of CIDP/LSS/MMN. Data are shown in number of patients unless stated otherwise.
CIDP: Chronic Inflammatory Demyelinating Polyneuropathy, LSS: Lewis Sumner Syndrome, MMN: Multifocal Motor Neuropathy, CSF: Cerebral Spinal fluid, , NCS: Nerve Conduction Studies. Classical: classical phenotype of CIDP, pure motor: pure motor phenotype of CIDP, pure sensory: pure sensory phenotype of CIDP

Supplemental Figure 1 Predefined criteria of strong clinical suspicion of a chronic inflammatory neuropathy



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Chapter 5

Diagnostic Value

Validation of a practical nerve ultrasound protocol for the diagnosis of CIDP and MMN: a prospective multicenter cohort study

IJT Herraets*, JA Telleman*, HS Goedee*, C Verhamme, F Eftimov, C Saris, N van Alfen, RPA van Eijk, JTH van Asseldonk, LH Visser, LH van den Berg, WL van der Pol

In preparation

Abstract

Objective: To validate a practical nerve ultrasound protocol for the detection of chronic inflammatory neuropathy, i.e. chronic inflammatory demyelinating polyneuropathy (CIDP), Lewis-Sumner Syndrome (LSS) and multifocal motor neuropathy (MMN) in a multicentre setting, and to determine its added value in the detection of treatment-responsive patients compared to conventional nerve conduction studies (NCS).

Methods: We included 100 consecutive patients clinically suspected of chronic inflammatory neuropathy in three participating centers. The study protocol consisted of standardized interviews, neurological examination, nerve ultrasound and NCS. We validated 2 nerve ultrasound protocols: a protocol including median nerve at the forearm and arm, and the C5, C6, and C7 nerve roots (Protocol A) and a protocol without assessment of the C6 and C7 nerve roots (Protocol B). Sensitivity and specificity were determined for establishing a diagnosis of CIDP, LSS, or MMN according to the EFNS/PNS. In addition, added value of both protocols in detecting treatment-responsive patients was determined.

Results: Protocol B showed a sensitivity of 87.4% and a specificity of 67.3% respectively for a diagnosis of chronic inflammatory neuropathy according to the EFNS/PNS criteria. Its sensitivity and specificity for the detection of treatment-responsive chronic inflammatory neuropathy were 84.6% and 72.8% respectively. The added value of nerve ultrasound in the detection of treatment-responsive chronic inflammatory neuropathy, i.e. patients with normal NCS but with abnormal nerve ultrasound and treatment response, was 25%.

Conclusions: A short ultrasound protocol including the median nerve at forearm and arm and C5 nerve root shows high diagnostic accuracy and is able to detect up to 25% additional patients with a treatment-responsive polyneuropathy compared to conventional NCS.

Introduction

Polyneuropathy is a common disorder with an incidence of 77/100.000 persons-years.[1] Inflammatory neuropathies are rare and their spectrum includes chronic inflammatory demyelinating polyneuropathy (CIDP), Lewis-Sumner Syndrome (LSS) and multifocal motor neuropathy (MMN), which often respond to immunomodulatory treatment. Distinction of CIDP, LSS and MMN from the more common, predominantly axonal polyneuropathies presently depends primarily on nerve conduction study (NCS) results.[2,3] These NCS protocols designed to detect conduction blocks or other demyelinating features are time and labour intensive and their execution requires specific expertise.

Nerve ultrasound is a relatively new diagnostic tool to identify patients with CIDP, LSS and MMN.[4-6] In a cross-sectional study, we showed that a short sonographic protocol of five nerve points of the median nerve and brachial plexus bilaterally has high sensitivity and specificity in discriminating CIDP, LSS and MMN from disease mimics.[5] This protocol recently also showed good to excellent test characteristics in a prospective single center cohort study of patients suspected of chronic inflammatory neuropathy and good reproducibility between observers and hospitals.[7,8] To validate this sonographic protocol for multicenter use, we tested its performance in consecutive patients clinically suspected of CIDP, LSS and MMN enrolled in three hospitals in The Netherlands and also compared these results with our previously described reference cohort.[7] Moreover, we determined the added value of nerve ultrasound in the detection of treatment-responsive patients with normal NCS.

Methods

Study design and patients

This prospective cohort study was performed between January 2014 and January 2018 in 3 tertiary neuromuscular centers, i.e. the University Medical Center Utrecht (UMCU), the Amsterdam UMC (location Amsterdam Medical Center) and the Radboudumc Nijmegen, and one large teaching hospital, i.e. the Elisabeth-Tweesteden Hospital Tilburg (ETZ). Results from the UMCU were published in a previous study and were used for comparison with results of the other hospitals (reference cohort).[7] The study was approved by the METC Brabant (NL42895.008.12) and all patients gave written informed consent.

In- and exclusion criteria have been published previously.[7] In summary, consecutive patients presenting at any of the (neuromuscular) outpatient clinics of the participating hospitals with a 'high clinical suspicion of an acquired chronic demyelinating polyneuropathy' according to predefined criteria were eligible for inclusion (Supplemental

Figure 1). Exclusion criteria were: 1) a previous diagnosis of (and treatment for) polyneuropathy; 2) age <18 or >80; and 3) physical inability to undergo nerve ultrasound investigation. Diagnostic work-up consisted of a standardized interview using questionnaires and neurological examination, appropriate laboratory investigations, NCS and nerve ultrasound as described previously (Supplemental Table 1).[7] Further investigations (e.g. MRI of the brachial plexus, antibody testing or lumbar puncture, as outlined in diagnostics standards or to the discretion of the treating physician) could be performed if thought necessary. We used the EFNS/PNS criteria to interpret NCS results for CIDP and MMN (definite conduction block, probable conduction block, no conduction block).

Nerve ultrasound was performed by experienced ultrasonographers blinded for the results of NCS. Investigations were performed on an Esaote MyLabTwice (Esaote, Genoa, Italy) with a 6 – 18 MHz linear-array transducer (LA435, for upper and lower extremity nerves, Amsterdam UMC and Radboudumc) and a 3 – 13 MHz linear-array transducer (LA533, for brachial plexus, Amsterdam UMC), and a Toshiba Xario XG (Toshiba, Tokyo, Japan) with a 7 – 18 MHz linear-array transducer (PLT-1204BT, ETZ). We used a previously published protocol in which cross-sectional areas (CSA) of all nerves were determined. Presence of nerve enlargement was determined based on previously described cut-off values.[5] Uni- or bilateral nerve enlargement at ≥1 of the measured sites (median nerve at the forearm and arm, and C5, C6, and C7 nerve roots) was considered abnormal.[5] Because measurement of the C6 and C7 nerve roots is more complex, and inter-observer variability is higher at these sites (which may affect performance of the diagnostic protocol in a multicentre setting),[8] we validated the protocol both with and without the inclusion of these nerve roots (protocol A (with inclusion of C6 and C7) and protocol B (without C6 and C7) respectively).

Primary outcome of this study was sensitivity and specificity of the ultrasound protocols to identify patients with CIDP/MMN according to the EFNS/PNS criteria. A secondary goal was to assess if nerve ultrasound could also identify treatment-responsive patients without characteristic NCS abnormalities in this multicentre cohort.[7] In addition, we performed a combined analysis of our current cohort and the previously described UMCU cohort (n=100; Supplemental Table 2).

Statistics

Statistics were performed with SPSS 25 (SPSS Inc., Chicago IL, USA), and the R-library metafor version 1.9-9, Viechtbauer W, 2016). We used mean (standard deviation (SD)) for normally distributed variables, median (range) for non-normal distributed variables and n (%) for categorical variables to summarize data. We compared results from participating hospitals using One-way ANOVA (Tukey HSD post hoc test) for normally distributed continuous variables, Kruskal Wallis test (Mann-Whitney U post hoc test) for non-normal

distributed continuous variables and Chi-square test or Fisher's-exact for categorical variables. Results were considered significant when alpha was below 0.05.

Both NCS and ultrasound were coded as abnormal (1) or not (0) and a similar approach was used for patients with CIDP/MMN according to the EFNS/PNS criteria (primary aim) (1) or not (0), and patients with CIDP/MMN based on treatment response (secondary aim) (1) or not (0). We calculated the sensitivity, and specificity from 2x2 tables. Results across centres were pooled using a random-effects meta-analysis. In case one of the cells contained a zero, a small constant (0.5) was added to each cell.

Results

Baseline characteristics of the multicenter cohort

We included 100 patients with a 'high clinical suspicion of an acquired chronic demyelinating polyneuropathy' in three of the participating hospitals (Amsterdam UMC, Radboudumc and ETZ). Baseline characteristics of these patients are shown in Table 1. The specification of all diagnoses established in this cohort can be found in Supplemental Table 2. Another 100 patients, whose characteristics have been published previously, were included at the UMCU (Supplemental table 2).[7]

The number of included patients was evenly distributed among hospitals (Amsterdam UMC n=35, ETZ n=31, Radboudumc n=34). Of these patients, 11 were diagnosed with MMN, 24 with CIDP and 4 with LSS according to the EFNS/PNS criteria. The distribution of patients diagnosed with CIDP/LSS/MMN was 21/35 (53.8%) at the Amsterdam UMC, 10/31 (25.6%) at the ETZ and 8/34 (20.5%) at the Radboudumc (Table 1).

CSA of nerves

Mean nerve CSAs at the sites included in the protocol are shown stratified per hospital in Table 2. Mean CSA of the C5, C6, and C7 nerve roots was higher in the Amsterdam UMC compared to the other hospitals (all $p < 0.001$), while no other significant differences were found.

Diagnostic value of nerve ultrasound (EFNS/PNS-criteria)

The pooled sensitivity and specificity of ultrasound for CIDP/MMN defined as patients who fulfilled the EFNS/PNS diagnostic criteria were 96.4% and 40.0% respectively for sonographic protocol A, and 87.4% and 67.3% for sonographic protocol B (Table 3).

Table 1 Baseline characteristics

	Total cohort (n=100)	Amsterdam UMC (n=35)	ETZ (n=31)	Radboudumc (n=34)	P-value
Sex Male / Female	73 / 27	23 / 12	22 / 9	28 / 6	0.28
Age in years (mean, SD)	60.7 (12.6)	58.7 (13.5)	61.9 (10.9)	61.5 (13.1)	0.53
Disease duration in months (median, range)	15 (1-720)	18.0 (1-240)	6.0 (1-120)	28.5 (2-720)	<0.01
Diagnosis					0.10
CIDP					
Classical	17	9	5	3	
Pure motor	5	1	2	2	
Pure sensory	2	1	1	0	
LSS	4	2	1	1	
MMN	11	8	1	2	
Various	61	14	21	26	
Clinical criteria set A					0.02
Sensorimotor	42	14	16	11	
Motor > sensory	14	1	6	7	
Pure motor	31	17	7	8	
Pure sensory	13	3	2	8	
Clinical criteria set B					
Asymmetrical complaints	55	26	15	14	0.02
Proximal weakness	33	18	11	4	<0.01
Areflexia	40	9	15	16	0.10
Sensory ataxia	7	1	2	4	0.34
Rapid progression	28	9	14	5	0.02
Postural tremor	6	3	0	3	0.27
Pain	30	7	11	12	0.28

Table 1 shows the baseline characteristics of 100 patients with a high clinical suspicion of an inflammatory demyelinating polyneuropathy. Data are stratified per hospital. Data represent number of patients unless stated otherwise.
CIDP: chronic inflammatory demyelinating polyneuropathy (CIDP), LSS: Lewis-Sumner syndrome, MMN: Multifocal motor neuropathy

Added value of nerve ultrasound in detection of treatment-responsive CIDP and MMN

Apart from the 33 patients with a diagnosis of CIDP/MMN based on an abnormal NCS according to the EFNS/PNS criteria, we identified 11 additional patients with normal NCS findings, but abnormal nerve ultrasound who responded to treatment (CIDP n=5, MMN n=6). The added value of nerve ultrasound in identifying treatment-responsive chronic inflammatory neuropathy was therefore 25% (11/44). Patients with normal NCS but abnormal ultrasound results who responded to treatment were found in all hospitals,

Table 2 Nerve cross sectional area (CSA) stratified per hospital

	Total cohort (n=100)	Amsterdam UMC (n=35)	ETZ (n=31)	Radboudumc (n=34)	P-value
Median nerve at forearm	7.0 (4-33)	8.0 (5-33)	8.0 (4-13)	7.0 (5-21)	0.11
Median nerve at upper arm	12.0 (6-44)	12.0 (9-42)	12.0 (6-19)	11.0 (6-44)	0.80
Nerve root C5	7.0 (1-33)	9.0 (5-33)	5.0 (2-14)	6.0 (1-20)	<0.01
Nerve root C6	7.0 (2-35)	13.0 (8-35)	4.0 (2-14)	5.0 (3-24)	<0.01
Nerve root C7	6.0 (1-44)	17.0 (11-44)	4.0 (2-14)	5.0 (1-24)	<0.01

Table 2 shows the median (range) nerve size per center for the investigated nerve sites; data are shown in median.

Table 3 Diagnostic accuracy of nerve ultrasound for CIDP/MMN according to the EFNS/PNS criteria

Center	Protocol	Sensitivity (Test positive / total positive)	Specificity (Test negative / total negative)
Amsterdam UMC	**A**	100.0% (21/21)	0.0% (0/14)
	B	100.0% (21/21)	64.3% (9/14)
ETZ	**A**	90.0% (9/10	61.9% (13/21)
	B	90.0% (9/10	66.7% (14/21)
Radboudumc	**A**	87.5% (7/8)	57.5% (15/26)
	B	87.5% (7/8)	69.2% (18/26)
Total	**A**	96.4%	87.4%
	B	40.0%	67.3%

Table 3 shows sensitivity and specificity of nerve ultrasound protocol A and B per center for establishing a diagnose of chronic inflammatory neuropathy according to the EFNS/PNS criteria as well as the pooled results (Total).
NPV:negative predicitive value, PPV: positive predictive value

making the possibility of physician or center bias less likely. The added value of ultrasound varied from 22.0% to 27.0% among hospitals (Figure 1).

Diagnostic value of nerve ultrasound (consensus criteria)

The pooled sensitivity and specificity of ultrasound to detect chronic inflammatory neuropathy according to our previously published consensus criteria (NCS fulfilling the EFNS/PNS criteria or normal NCS in combination with abnormal ultrasound and treatment

Figure 1 Additional value of nerve ultrasound per center

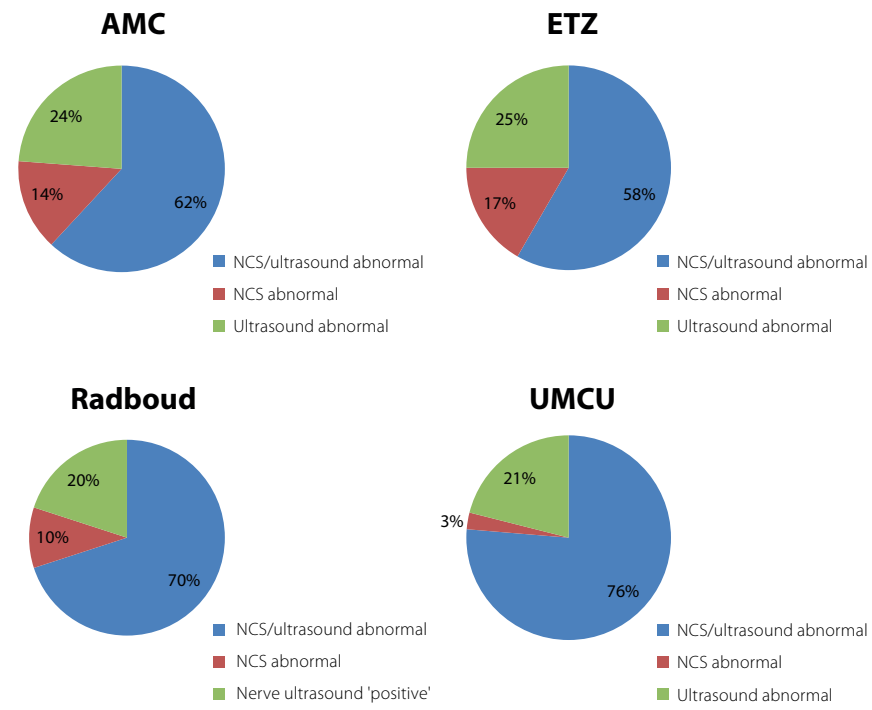


Figure 1 shows the distribution of the diagnoses of chronic inflammatory neuropathy established with abnormal NCS, abnormal nerve ultrasound or both in the three participating centers in this multicenter cohort (n=100), and the previously published UMCU cohort (n=100).
CIDP: Chronic Inflammatory Demyelinating Polyneuropathy, LSS: Lewis-Sum ner Syndrome, MMN: Multifocal Motor Neuropathy; NCS: Nerve Conduction Studies

response)[7] was 96.4% and 44.9% respectively for sonographic protocol A, and 84.6% and 72.8% for sonographic protocol B (Table 4).

Diagnostic value of nerve ultrasound (pooled analysis of multicenter and UMCU cohort)

When analysing the results of this multicenter cohort in combination with our previously published UMCU cohort[7] (Supplemental Table 2) the pooled sensitivity and specificity for the diagnosis of CIDP/MMN were 96.9% and 51.0% when applying sonographic protocol A and 90.5% and 71.9% when applying protocol B (Table 4). The pooled sensitivity and specificity for NCS were 76.1% and 93.4% respectively.

Table 4 Diagnostic accuracy of nerve ultrasound for CIDP/MMN according to the predefined consensus criteria

Center	Protocol	Sensitivity (Test positive / total positive)	Specificity (Test negative / total negative)
Amsterdam UMC	**A**	100.0% (22/22)	0.0% (0/13)
	B	86.4% (19/22)	69.2% (9/13)
ETZ	**A**	91.7% (11/12)	68.4% (13/19)
	B	83.3% (10/12)	73.7% (14/19)
Radboudumc	**A**	90.9% (10/11)	65.2% (15/23)
	B	81.8% (9/11)	73.9% (17/23)
UMCU	**A**	97.4% (37/38)	69.4% (43/62)
	B	94.9% (36/38)	71.0% (44/62)
Total I	**A**	96.4%	44.9%
	B	84.6%	72.8%
Total II	**A**	96.9%	51.0%
	B	90.5%	71.9%

Table 4 shows sensitivity and specificity of nerve ultrasound protocol A and B per center for establishing a diagnose of chronic inflammatory neuropathy according to our predefined consensus criteria as well as the pooled results for the 3 participating hospitals in this study (Total I) and for the 3 hospitals and the previously published UMCU cohort (Total II).
NPV: negative predictive value, PPV: positive predictive value.

Analysis of the UMCU cohort showed that the specificity of nerve ultrasound protocol B was slightly higher than protocol A and the sensitivity slightly lower (Table 4), although all patients diagnosed with CIDP and MMN according to the previously described diagnostic criteria with normal NCS and abnormal nerve ultrasound results (n=8) were also diagnosed with protocol B.

Discussion

This multicenter study shows that a short sonographic protocol has high sensitivity and moderate specificity to identify patients with chronic inflammatory neuropathy. This sonographic protocol includes the median nerve in arm and forearm and the C5 nerve root bilaterally and is a slightly modified version of a previously published protocol. Ultrasound did not only allow reliable identification of patients with an inflammatory neuropathy according to EFNS/PNS criteria, but also allowed identification of an additional 25% of patients with normal NCS results who nevertheless responded to treatment.

Previous studies exploring the usefulness of different ultrasound protocols for the diagnosis of CIDP and MMN reported high sensitivity and specificity.[4,9,10] Prior to this study we systematically assessed optimal ultrasound protocol characteristics by ROC analysis in a cross-sectional study.[5] This protocol, encompassing the median nerve at arm and forearm and brachial plexus nerve roots bilaterally, is shorter than the more extensive protocols suggested by other authors which may be less applicable in routine clinical practice. Moreover, we determined inter-observer variability,[8] and performance of this protocol in consecutive patients suspected of chronic inflammatory neuropathy in a single center[7] and in this study in a multicentre setting. Results from our studies suggest that slight modifications to the initial ultrasound protocol, i.e. exclusion of C6 and C7 nerve roots, improves diagnostic accuracy, most likely due to elimination of the sites with highest inter-observer variability.[8] Retrospective analysis of our previously published UMCU cohort revealed that the slight modification of the initially investigated protocol did not significantly reduce diagnostic accuracy, confirming that the bilateral measurement of 3 nerves points has high diagnostic accuracy in a multicenter setting. This protocol is short, easy to perform and has all characteristics needed for routine clinical practice.

In accordance with two previous studies, we found that nerve ultrasound improves detection of patients with a treatment responsive inflammatory neuropathy but without characteristic NCS abnormalities.[5,7] The additional yield was approximately 25% across centres. The available evidence strongly suggests that sensitivity of nerve ultrasound exceeds that of NCS, whilst for specificity the opposite is true.[7] Therefore, in future diagnostic strategies nerve ultrasound may be applied first to exclude CIDP and MMN, followed by NCS to confirm the diagnosis, to explore alternative diagnoses, or to estimate the odds of response to treatment. This approach would have the benefit of decreasing the number of patients that need to undergo painful and time and labour intensive NCS and would aid in detecting treatment-responsive patients. We believe that ultrasound and NCS should become complementary techniques and do not favour scenarios in which ultrasound would become the dominant diagnostic technique or would even fully replace NCS. If clinicians would prefer the use of ultrasound only for practical reasons, they would need to remain vigilant and carefully monitor treatment effect (to reduce the risk of a false positive diagnosis and continued treatment), and disease course to avoid misdiagnosis of (for example) motor neuron disease.

A limitation of this study is the heterogeneity in included patient characteristics in the participating hospitals, which is most likely caused by differences in the patient population referred to these hospitals. Pooling of the data will likely have limited the effects of this heterogeneity. NCS protocols were slightly different between hospitals, but were all evaluated following the EFNS/PNS NCS criteria. Nerve ultrasound was performed on different sonography devices but we previously found that this did not cause significant

variability.[8] Treatment response was defined based on the discretion of the treating physician in this multicenter study, which may have led to some overestimation of treatment effect.

This multicenter study validated a short sonographic protocol of the median nerve at the forearm, upper arm and C5 bilaterally as a diagnostic tool to detect chronic inflammatory neuropathy in a multicentre cohort of consecutive incident patients clinically suspected of this disease. Nerve ultrasound is a reliable diagnostic tool, complementary to NCS. These data support the inclusion of nerve ultrasound in future updates of consensus diagnostic criteria for CIDP and MMN.

Supplemental Table 1 Specifications of diagnostic work-up

Modality	Description of performed work-up
MRC score	Bilateral measurement of motor function of: - Abduction of the arm - Flexion and extension of the forearm and wrist - Spreading of the fingers - Abduction of the thumb - Flexion of the hip - Flexion and extension of the ankle and foot - Eversion of the foot - Extension of the hallux MRC sum score: 0-140 points
ISS	INCAT Sensory Sum Score Measurement of gnostic and vital sensibility in arms and legs
Vigorimetry	Bilateral measurement of grip strength in Kilopascals (kPa) with the Martin Vigorimeter (Martin Medizintechnik, Tuttlingen, Germany)
RODS	Rasch-built Overall Disability Scale Standardized questionnaire for CIDP or MMN (depending on clinical phenotype)
INCAT ODSS	INCAT Overall Disability Sum Score Standardized questionnaire
Laboratory investigations	To exclude other causes of polyneuropathy: - Renal, liver, and thyroid function - Glucose - Vitamins - Complete blood count - Protein spectrum
Nerve Conduction Studies	Amsterdam UMC Bilateral evaluation of demyelination and axonal loss in: - Median and ulnar nerves (recordings from hand muscles) - Musculocutaneous nerve (recordings from biceps) - Radial nerve (recordings from forearm) - Fibular and tibial nerves (recordings from foot muscles) - Sural nerve If not yet fulfilling the criteria and negative peak CMAP amplitude < 1 mV: - Median nerve (recordings from forearm muscles) ETZ/Radboudumc Bilateral evaluation of demyelination and axonal loss in: - Median and ulnar nerves (recordings from hand muscles) - Fibular and tibial nerves (recordings from foot muscles) - Sural nerve

Supplemental Table 1 Continued

Modality	Description of performed work-up
Nerve Conduction Studies	UMCU Bilateral evaluation of demyelination and axonal loss in: - Median and ulnar nerves (recordings from hand muscles) - Fibular and tibial nerves (recordings from foot muscles) - Sural nerve Extended with (in case of suspicion of MMN): - Musculocutaneous nerve (recordings from the biceps muscle) - Median and radial nerves (recordings from forearm muscles)
Nerve ultrasound	Bilateral measurement of cross- sectional area (CSA) of: - Median nerve at the forearm and arm - Nerve roots C5,C6, and C7 at the interscalene level Cut-off values for nerve enlargement: - Median nerve at the forearm >10 mm^2 - Median nerve at the arm >13 mm^2 - Nerve roots C5,C6, or C7 >8 mm^2

Supplemental Table 2 Established diagnoses

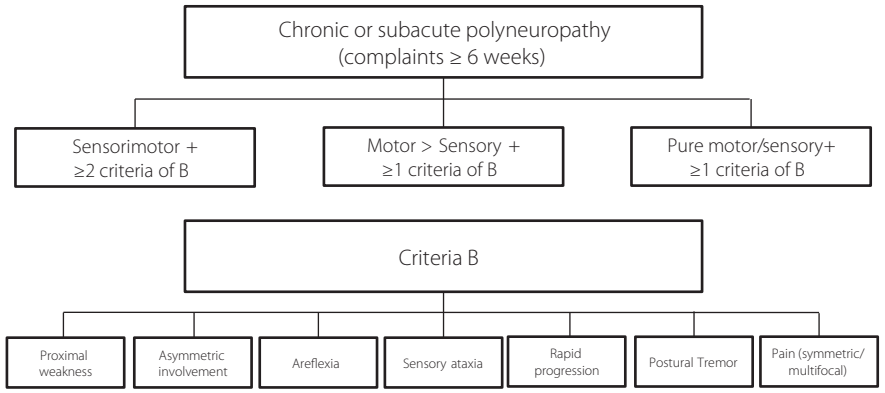
Diagnoses	Multicenter cohort (n=100)	UMCU Cohort (n=100)
Adult polyglucosan body disease	0	1
ALS	3	1
Axonal neuropathy, not CIAP	10	3
Benign muscle cramp fasciculation syndrome	0	1
BSCL2 mutation associated peripheral nerve demyelination in MS; Silver syndrome	1	0
Cervical radiculopathy	1	2
CIAP	8	12
CIAP in combination with mitochondrial neuromyopathy	0	1
CIDP: EFNS/PNS criteria fulfilled	24	16
CIDP: EFNS/PNS criteria not fulfilled	6	7
Critical illness polyneuropathy	1	0
Distal myopathy	0	1
Functional disorder	0	1
Hirayama Syndrome	0	4

Supplemental Table 2 Continued

Diagnoses	Multicenter cohort (n=100)	UMCU Cohort (n=100)
HMSN type 1	1	0
HMSN type 2	4	0
HNLPP	1	1
IgM-MGUS polyneuropathy	2	1
Immune mediated polyradiculitis associated with Sjögren syndrome	1	1
LSS	4	4
Lumbar spinal stenosis	0	2
MMN: EFNS/PNS criteria fulfilled with abnormal NCS	5	10
MMN: EFNS/PNS criteria fulfilled with normal NCS	6	4
Mononeuritis multiplex	1	
Multifocal axonal neuropathy associated with Crohn's disease	0	1
Multiple compression neuropathies, no genetic diagnosis	2	0
Neuralgic amyotrophy	5	1
Neurolymphomatosis	0	1
Neurosarcoidosis	1	0
Paraneoplastic demyelinating polyneuropathy	1	0
Peripheral nerve demyelination in multiple sclerosis	1	0
PNP of unknown origin (no CIDP/MMN); loss to follow-up	1	0
Post-infectious axonal polyneuropathy	0	1
PSMA	5	15
Small fiber neuropathy	1	0
SSpinal muscular atrophy	2	0
Status after GBS	0	3
Ulnaropathy	0	1
Vasculitis	2	4

ALS: Amyotrophic Lateral Sclerosis, CIAP: Chronic Idiopathic Axonal Polyneuropathy, CIDP: Chronic Inflammatory Demyelinating Polyneuropathy, HMSN: Hereditary Motor and Sensory Neuropathy, HNLPP: Hereditary Neuropathy with Liability to Pressure Palsies, MGUS: Monoclonal Gammopathy of Undetermined Significance, MMN: Multifocal Motor Neuropathy, MS: Multiple Sclerosis, PSMA: Progressive Spinal Muscular Atrophy

Supplemental Figure 1 Predefined criteria of strong clinical suspicion of a chronic inflammatory neuropathy



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Chapter 6

Prognostic Value

Prognostic value of nerve ultrasound: a prospective multicenter study on the natural history of polyneuropathy

JA Telleman*, IJT Herraets*, HS Goedee, C Verhamme, F Eftimov, D Lieba-Samal, RPA van Eijck, JTH van Asseldonk, WL van der Pol, LH van den Berg, LH Visser

In preparation

Abstract

Objective: To determine prognostic value of ultrasonographic nerve size development in chronic inflammatory demyelinating polyneuropathy (CIDP), and multifocal motor neuropathy (MMN).

Methods: In this prospective multicenter cohort study, we enrolled patients with CIDP (typical n=52, atypical n=74), and MMN (n=72), of which 71 were treatment-naive. Patients with chronic idiopathic axonal polyneuropathy (CIAP, n=35) were disease controls. Questionnaires, standardized neurological examination (including grip strength), and nerve ultrasound were obtained at inclusion and 1 year of follow-up. Correlation between nerve size and clinical outcome measures, and nerve size development over time were determined using linear mixed effects models.

Results: Nerve size development over time was heterogeneous in both CIDP and MMN. In MMN, there was a negative correlation between the size of the C5 nerve root and grip strength (-1.3 kPa / mm^2 (95%-CI -2.3 – -0.2 kPa / mm^2). No other significant correlations between nerve size and clinical outcome measures were found. Presence of nerve enlargement at inclusion predicted development of grip strength in MMN (an increase of 27.6 kPa in 1 year in patients without enlargement compared to 10.0 kPa with enlargement), and MMN patients with enlargement confined to the brachial plexus seemed to have more favourable outcome. No other predictive effects of ultrasonographic nerve size were found.

Conclusions: Prognostic value of nerve ultrasound is limited. It does not predict treatment response. In MMN, degree and distribution of nerve enlargement found during the diagnostic phase may have some prognostic value. Performance of nerve ultrasound after the diagnostic phase should not be encouraged.

Introduction

Nerve ultrasound is emerging as a low-cost, widely available tool for the investigation of peripheral nerves. Its diagnostic value has been established for mononeuropathies,[1,2] and more recently to distinguish inflammatory and potentially treatment-responsive polyneuropathies from more common forms.[3,4] We found that nerve ultrasound has low interobserver variability and can be used in a multicenter setting even if different types of sonographic devices are used.[5]

The prognostic value of nerve ultrasound, i.e. its value in predicting disease course or the effects of immune-modulatory treatment in inflammatory neuropathies such as chronic inflammatory demyelinating polyneuropathy (CIDP) and multifocal motor neuropathy (MMN), has been suggested but not been investigated in detail.[6-10] In CIDP and MMN only few predictors of treatment-response have been identified, including axonal damage, presence of conduction blocks and prolonged disease duration before start of treatment.[11-14] New and sensitive prognostic tools would be helpful to predict disease course, treatment efficacy and particularly remission, since patients with CIDP and MMN often require long term and expensive treatment with subcutaneous or intravenous immunoglobulins (IVIg). We performed a prospective multicenter cohort study in both treatment-naive and treated patients with CIDP, and MMN. We determined nerve size development and its potential prognostic value over time in these diseases. We also included patients with chronic idiopathic axonal polyneuropathy (CIAP) as a control group, as we hypothesized that this disease generally shows no nerve enlargement and that nerves would therefore not alter over time.

Methods

Standard protocol approvals, registrations and patient consents

This international prospective longitudinal multicenter cohort study was conducted between May 2015 and May 2018 at the Neurology outpatient clinics of two tertiary referral centers in the Netherlands, i.e. the University Medical Center Utrecht and the Amsterdam University Medical Center, a large general teaching hospital in The Netherlands, i.e. the Elisabeth-Tweesteden Hospital in Tilburg and a tertiary referral center in Austria, i.e. the Allgemeines Krankenhaus in Vienna. The study was approved by the Brabant Regional Ethics Committee (NL50375.028.14) and the boards of all participating hospitals. All participants gave written informed consent.

In- and Exclusion criteria

Patients with CIDP (both typical and atypical), MMN, and CIAP were eligible for inclusion. Patients with CIDP and MMN could be incident (newly diagnosed) or prevalent (diagnosed earlier), be treatment-naïve or use (maintenance) therapy with IVIg, corticosteroids or plasmapheresis (in CIDP only). Only newly diagnosed CIAP patients were eligible for inclusion. Both newly diagnosed and prevalent patients with CIDP and MMN were included consecutively. Inclusion criteria were:

1) age ≥18

2a) a diagnosis of possible, probable or definite CIDP or MMN according to the EFNS/PNS criteria,[15,16]

2b) a strong suspicion of CIDP or MMN based on previously described consensus criteria (i.e. patients with a clinical phenotype of CIDP or MMN according to the EFNS/PNS criteria (typical/atypical), ultrasound results compatible with a diagnosis of CIDP or MMN and objective treatment effect but without characteristic nerve conduction abnormalities),[4,17] or

2c) a diagnosis of CIAP according to previously published clinical criteria, nerve conduction studies (NCS) results and laboratory testing.[18]

Exclusion criteria for this study were:

1) prior history of polyneuropathy other than CIDP, or MMN, and

2) physical inability to undergo nerve ultrasound.

Study Design

We used questionnaires, standardized neurological examination and nerve ultrasound at inclusion and after 1 year of follow-up (Figure 1). An extra follow-up visit at 6 months could be performed in treatment-naive CIDP and MMN patients in order to document potential early nerve size changes after start of treatment. Neurological examination consisted of testing of muscle strength and sensory functions. Muscle strength of 14 muscle groups was graded bilaterally with the Medical Research Council (MRC) scale, and grip strength was determined in Kilopascal (kPa) with Martin Vigorimetry (Martin Medizintechnik, Tuttlingen, Germany). Sensory functions were tested bilaterally with the modified INCAT Sensory Sum score (mISS). In addition, the INCAT Overall Disability Sum Score (ODSS), Rasch-built Overall Disability Scale (RODS; for CIDP),[19] and Modified Rankin Score (MRS; for CIAP) were obtained.

Figure 1 Flowchart

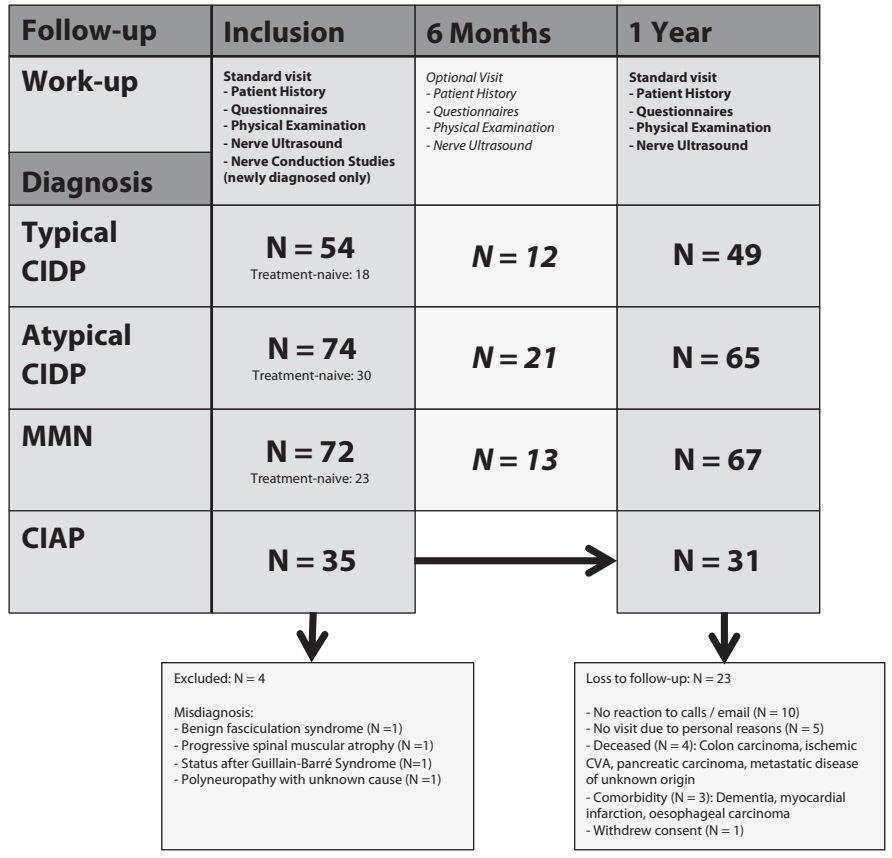

Figure 1: Flowchart. This figure shows the standardized work-up applied in this study, including optional pre-study visits (patients already under treatment that underwent nerve ultrasound in diagnostic work-up prior to study inclusion) and optional 6 month follow-up visit for newly diagnosed patients with CIDP and MMN. The figure additionally shows the number of in- and excluded patients, and loss to follow-up.
CIAP: chronic idiopathic axonal polyneuropathy, CIDP: chronic inflammatory demyelinating polyneuropathy, MMN: multifocal motor neuropathy.

Nerve ultrasound was performed by investigators with ≥1 year of experience with nerve ultrasound, who were blinded for results of previous ultrasound investigations. Ultrasound was performed with a Philips EPIQ7 (Philips Medical Instruments, Bothell, WA) at the UMC Utrecht, an Esaote MyLabTwice (Esaote, Genoa, Italy) at the Amsterdam UMC, a Toshiba Xario XG (Toshiba, Tokyo, Japan) at the ETZ Tilburg, and a GE Logiq E9 Platform (GE Healthcare, Chicago, USA) at the Algemeine Krankenhaus in Vienna. All investigators used

a high-frequency probe of ≥18 MHz. Nerve cross-sectional area (CSA) was measured bilaterally within the hyperechoic rim at standardized sites in upper extremity nerves: the median nerve (at wrist, forearm, and upper arm), ulnar nerve (at wrist, forearm, distal to the ulnar sulcus, at the ulnar sulcus (at the medial epicondyle), proximal to the ulnar sulcus, and at the upper arm), and the brachial plexus (C5 and C6 nerve roots).

In patients with newly diagnosed CIDP, MMN, and CIAP, nerve conduction studies (NCS) were performed following the centers' standardized protocols and graded according to the criteria of the EFNS/PNS.[15,16] In all centers NCS included at least of median, ulnar, fibular, tibial, and sural nerves. NCS were evaluated for presence of axonal loss (present if distal compound muscle action potential (CMAP) was: <3.5 mV for the median nerve, <2.8 mV for the ulnar nerve, <2.5 mV for the fibular nerve, or <2.9 mV for the tibial nerve), and presence of possible or definite conduction blocks in the median nerve.

Statistics

We used SPSS version 25 (SPSS Inc., Chicago IL, USA) for statistical analysis. Data were analyzed for four different disease groups: typical CIDP (according to the clinical criteria of the EFNS/PNS), atypical CIDP (according to the clinical criteria of the EFNS/PNS, including pure motor CIDP, pure sensory CIDP, Lewis-Sumner syndrome, and distal predominant CIDP), MMN, and CIAP. Data were summarized per disease group as mean (standard deviation (SD)) for normally distributed variables, median (range) for non-normal distributed variables, and n (%) for categorical variables. We compared mean nerve CSA at all investigated nerve sites between disease groups with Kruskal-Wallis test and post-hoc Mann-Whitney U tests based on non-normal distribution of the data. The average nerve CSA of the right and left side was used in all analyses.

We used CSA of the median nerve at the forearm, upper arm and the C5 nerve root for additional analyses. These sites show relatively low inter-observer variability,[5,17] and were used to study associations with vigorimetry and ODSS (for all disease groups), RODS CIDP (for typical and atypical CIDP), MRS (for CIAP) and mISS (for typical and atypical CIDP and CIAP). The MRC-sum score was omitted from these analyses because of extreme skewness of the data.

The relationship between each outcome and nerve CSA was assessed using linear mixed effects models (LME). Each model contained a random intercept per individual and nerve CSA of the investigated nerve site as fixed effect. Models were fitted with restricted maximum likelihood (REML) based on an unstructured covariance matrix.

To determine nerve size development over time, a similar approach was used, with LMEs where nerve CSA served as outcome measure, and study duration (in months) as fixed effect. The random part contained a random intercept and slope (for study duration) per individual in order to correct for variability in nerve size development due to individual patient characteristics.

To determine the prognostic value of nerve ultrasound each patient was assigned a code of either enlargement (1) or no enlargement (0) for the investigated non-entrapment nerve site at the inclusion visit.[17,20] These results were entered in an LME as fixed effect. Study duration (in months), and the interaction between study duration and presence of enlargement at inclusion were also entered as fixed effects to determine whether the development over time depended on the presence of nerve enlargement. A random intercept and slope for study duration were entered for patients to correct for variability due to individual patient characteristics. CIAP was excluded from these analyses due to the limited number of patients with nerve enlargement (median nerve at the forearm n = 2, 5.7%; median nerve at the upper arm n = 1, 2.9%; C5 nerve root n = 0, 0%).

To evaluate presence of other potential prognostic factors in CIDP and MMN, patients were dichotomized as either having decreased or increased (i.e. change larger than 0) in vigorimetry or ODSS at 1 year of follow-up. Differences in clinical, NCS, and sonographic parameters between these groups were tested using the independent t-test (continuous, normal), Mann-Whitney U test (continuous, non-normal), chi-square test (categorical) or Fishers' exact test (categorical, small sample size).

RESULTS

Study population

A total of 237 patients were included in this study (Figure 1): 129 patients with CIDP (52 typical and 74 atypical), 72 with MMN, and 35 with CIAP. Of the patients with chronic inflammatory neuropathy, 71 were treatment-naive at inclusion (18 typical CIDP, 30 atypical CIDP, 23 MMN). Baseline characteristics of 233 patients are shown in Table 1; 4 patients were excluded from the final analysis because of a changed diagnosis during follow-up.

The 1-year follow-up visit was completed by 210 patients (90.1%) and 23 patients were lost to follow-up (9.9%). There were no significant differences in age, sex, disease type, disease duration, or treatment status between the groups that completed 1 year of follow-up or were lost to follow-up, although mean age was 5.3 years higher and median disease duration 17.6 months shorter in patients lost to follow-up.

Table 1 Baseline Characteristics

		CIDP Typical (n=52)	CIDP Atypical (n=74)	MMN (n=72)	CIAP (n=35)
Hospitals	**AMC Amsterdam**	12 (23%)	6 (8%)	6 (8%)	0 (0%)
	ETZ Tilburg	8 (15%)	12 (16%)	4 (6%)	19 (54%)
	UMC Utrecht	31 (60%)	53 (72%)	61 (85%)	16 (46%)
	AKW Vienna	1 (2%)	3 (4%)	1 (1%)	0 (0%)
Age (years) Mean (SD)		60.3 ±14.0	59.0 ±13.0	53.6 ±10.7	63.5 ±8.9
Sex (M/F)		35 (67.3%) / 17 (32.7%)	52 (70.3%) / 22 (29.7%)	57 (79.2%) / 15 (20.8%)	20 (57.1%) / 15 (42.9%)
Disease duration (months) Median (range)		29 (1-360)	50 (2-312)	72 (3-550)	60 (10-240)
EFNS/PNS Criteria	**Definite**	39 (75%)	64 (86%)	44 (61%)	-
	Probable	3 (6%)	2 (3%)	12 (17%)	-
	Possible	1 (2%)	0 (0%)	16 (22%)	-
	Not fulfilled	9 (17%)	8 (11%)	0 (0%)	-
Follow-up 1 year completed (N/Y)		5 (11%) / 47 (89%)	9 (12%) / 65 (88%)	5 (7%) / 67 (93%)	4 (11%) / 31 (89%)
Treatment received (during 1 year follow-up period)	**IVIg**	22 (47%)	40 (62%)	66 (99%)	-
	Corticosteriods	5 (11%)	5 (8%)	-	-
	IVIg + Corticosteroids	10 (21%)	9 (14%)	-	-
	Plasmaferesis	1 (2%)	3 (5%)	0 (0%)	-
	No treatment: in remission	6 (13%)	10 (15%)	1 (1%)	-
	No treatment: no remission	4 (9%)	1 (2%)	0 (0%)	-
In remission at 1 year of follow-up (N/Y)		35 (74%) / 12 (26%)	49 (75%) / 16 (25%)	65 (97%) / 2 (3%)	-

Table 1 shows the baseline characteristics of 233 included patients per disease group. In addition, details on the treatment received by patients completing 1 year follow-up during this year are shown.
CIAP: chronic idiopathic axonal polyneuropathy, CIDP: chronic inflammatory demyelinating polyneuropathy, IVIg: intravenous immunoglobulins, MMN: multifocal motor neuropathy.

Correlation between nerve size and clinical outcome measures

No correlation between nerve size and grip strength in (a)typical CIDP and CIAP was found (Table 2). We observed a negative correlation between grip strength and CSA of the C5 nerve root (slope -1.3 kPa; 95%-CI -2.3 – -0.2 kPa), p = 0.02) in patients with MMN. This indicates that grip strength decreases with 1.3 kPa for each mm^2 increase in CSA at the C5

Table 2 Correlation of vigorimetry and Nerve Size

CIDP Typical			
Nerve Site	***Mean grip strength (kPa)***	***Correlation of grip strength and nerve size in kPa/mm2 (95%-CI)***	***P-value of correlation***
MFA	70.2	-0.4 (-1.6 – 0.9)	0.57
MUA	66.6	0.0 (-0.9 – 0.9)	0.99
C5	70.8	-0.5 (-1.5 – 0.6)	0.38
CIDP Atypical			
Nerve Site	***Mean grip strength (kPa)***	***Correlation of grip strength and nerve size in kPa/mm2 (95%-CI)***	***P-value of correlation***
MFA	60.1	0.4 (-0.5 – 1.4)	0.35
MUA	57.1	0.4 (-0.1 – 1.0)	0.10
C5	65.2	-0.1 (-0.8 – 0.7)	0.87
MMN			
Nerve Site	***Mean grip strength (kPa)***	***Correlation of grip strength and nerve size in kPa/mm2 (95%-CI)***	***P-value of correlation***
MFA	74.9	-0.1 (-1.6 – 1.4)	0.92
MUA	86.2	-0.8 (-1.9 – 0.2)	0.10
C5	**85.0**	**-1.3 (-2.3 – -0.2)**	**0.02**
CIAP			
Nerve Site	***Mean grip strength (kPa)***	***Correlation of grip strength and nerve size in kPa/mm2 (95%-CI)***	***P-value of correlation***
MFA	81.4	-0.1 (-2.8 – 2.7)	0.95
MUA	52.9	2.9 (-0.6 – 6.4)	0.10
C5	83.2	-0.5 (-4.1 – 3.1)	0.79

Table 2 shows the correlation of grip strength and nerve size of the median nerve at forearm and upper arm and the C5 nerve root (in mm^2) per disease group. Results obtained by the fitted LME's are shown, including the mean grip strength (intercept) and average increase/decrease in grip strength per mm^2 in nerve size (slope) including a 95%-CI and p-value of the slope.
95%-CI: 95%-confidence interval, CIDP: chronic inflammatory demyelinating polyneuropathy, LME: linear mixed model, MFA: median nerve at the forearm, MMN: multifocal motor neuropathy, MUA: median nerve at the upper arm, kPa: kilopascal.

nerve root. This negative correlation was also present at the median nerve at the upper arm, although not significant (p = 0.10). The negative correlation with the C5 nerve root size was more pronounced (slope -3.8 kPa; 95%-CI -6.7 – -0.9 kPa) in treatment-naive patients with MMN and also present in patients with pure motor CIDP (n = 11, slope -4.8 kPa (95%-CI -8.3 – -1.4 kPa), p = 0.01). There was no significant correlation of nerve CSA with other outcome measures (ODSS, RODS, MRS and mISS).

Nerve size development over time

Nerve CSA of the median nerve at the forearm, upper arm and at the C5 nerve root was significantly higher in CIDP and MMN than in CIAP, both at inclusion and at 1 year follow-up (Figure 2). We observed a decrease in nerve size over time of the median nerve at the forearm in atypical CIDP (slope – 0.067 mm^2 / month; 95%-CI -0.121 – -0.013 mm^2 / month) and MMN (slope -0.056 mm^2 / month; 95%-CI -0.099 – -0.013 mm^2). This corresponds with an average decrease of nerve CSA of 0.804 mm^2 and 0.672 mm^2 per year at these sites (-7.8% and -7.7% of the baseline mean nerve size per year, respectively). Nerves size in patients with typical CIDP and CIAP did not change over time (Figure 3). Further analysis of patients with atypical CIDP showed that the decrease of nerve size over time was attributable to distal predominant CIDP (n=35; slope -0.107 mm^2 / month; 95%-CI -0.195 – -0.018 mm^2 / month) but not to pure motor CIDP (n = 11), pure sensory CIDP (n = 11) and Lewis-Sumner Syndrome (n = 21). Among treatment-naive patients, a reduction in size of the median nerve at the forearm was observed only in MMN (slope -0.114 mm^2 / month; 95%-CI -0.178 – -0.054 mm^2). Patients that did not use maintenance therapy with IVIg after 1 year of follow-up (n = 30; 12 typical CIDP, 16 atypical CIDP, 2 MMN) also showed large heterogeneity in nerve size development, and no significant development of nerve size was observed in this group of patients.

Prognostic value of nerve CSA on development of clinical outcome measures

Presence of enlargement of the median nerve at the upper arm predicted deterioration of grip strength in patients with typical CIDP and MMN (Table 3). This predictive effect was more pronounced in treatment-naive MMN patients (slope 1.13 kPa / month (95%-CI 0.13 – 2.13 kPa / month) without enlargement versus -0.82 kPa / month (95%-CI -1.67 – 0.03 kPa / month) with enlargement, p = 0.006). This indicates that patients without nerve enlargement of the median nerve at the upper arm have higher grip strength after 1 year of follow-up than patients with nerve enlargement (an increase of 13.56 kPa / year compared to a decrease of 9.84 kPa / year). No significant effect of the presence of nerve enlargement on grip strength was observed at other nerve sites (Figure 4).

Presence of enlargement of the C5 nerve root at inclusion predicted a significantly improved ODSS over time in treatment-naïve MMN patients (slope 0.00 points per month (95%-CI -0.06 – 0.06 per month) without enlargement versus -0.12 points per month (95%-CI -0.19 – -0.04 per month) with enlargement, p = 0.02). It also predicted significantly better RODS over time in typical CIDP (slope 0.28% (95%-CI -0.23 – 0.79%) without enlargement versus 1.03% (95%-CI 0.52 – 1.55%) with enlargement, p = 0.04). This positive effect of presence of enlargement at the C5 nerve root was also observed for vigorimetry, ODSS and mISS in typical CIDP, and for vigorimetry and ODSS in the complete group of MMN patients, though these results were not significant (Table 4).

Figure 2 Nerve size at inclusion and follow-up

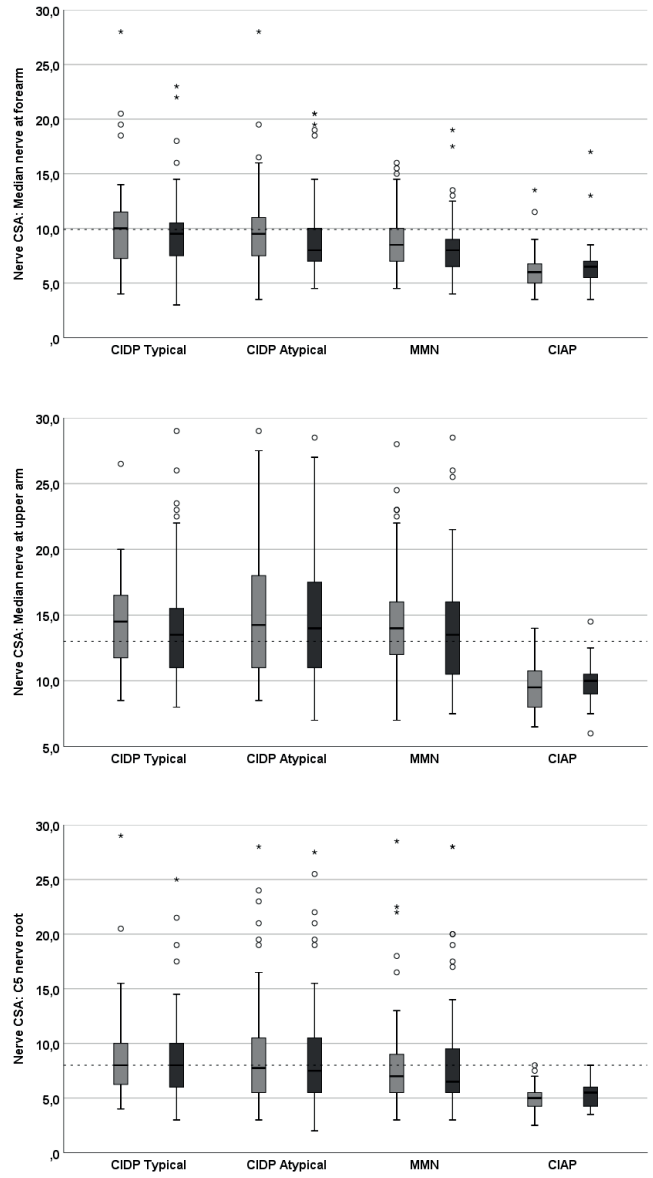

Figure 2 shows boxplots of median and C5 nerve size in mm^2 per disease group at inclusion and 1 year of follow-up. Nerve size at inclusion is shown in light grey, nerve size at 1 year of follow-up in dark grey. The dotted lines represent the cut-off value for demyelination established in our previously published diagnostic cohort study.[20]

Figure 3 Development of nerve size over time in individual patients

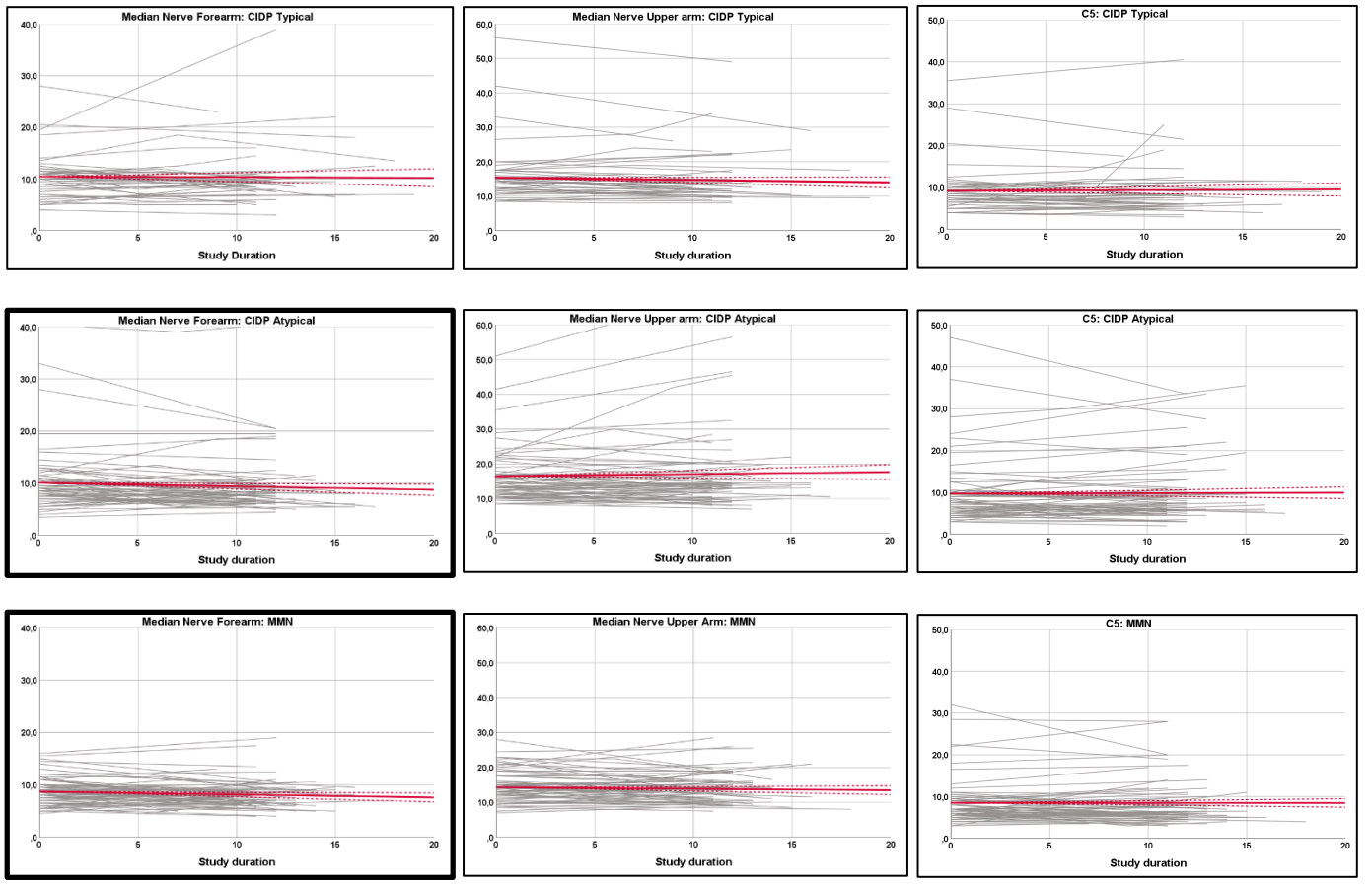

Figure 3 shows development of nerve size of the median nerve (at forearm level and arm level) and the C5 nerve root over time in CIDP (typical), CIDP (atypical), MMN, and CIAP for individual patients and an estimated overall nerve size development. After mixed model analysis, no significant correlation between time and nerve size was found, except for the median nerve at the forearm in atypical CIDP (slope – 0.067 mm^2 / month; 95%-CI -0.121 – -0.013 mm^2 / month) and MMN (slope -0.056 mm^2 / month; 95%-CI -0.099 – -0.013 mm^2). Grey lines represent individual patients, red lines represent overall nerve size development over time, red dotted lines represent 95%-confidence interval of the nerve size development over time.

95%-CI: 95%-confidence interval, CIAP: chronic idiopathic axonal polyneuropathy, CIDP: chronic inflammatory demyelinating polyneuropathy, MMN: multifocal motor neuropathy.

Figure 4 Prognostic value of nerve enlargement

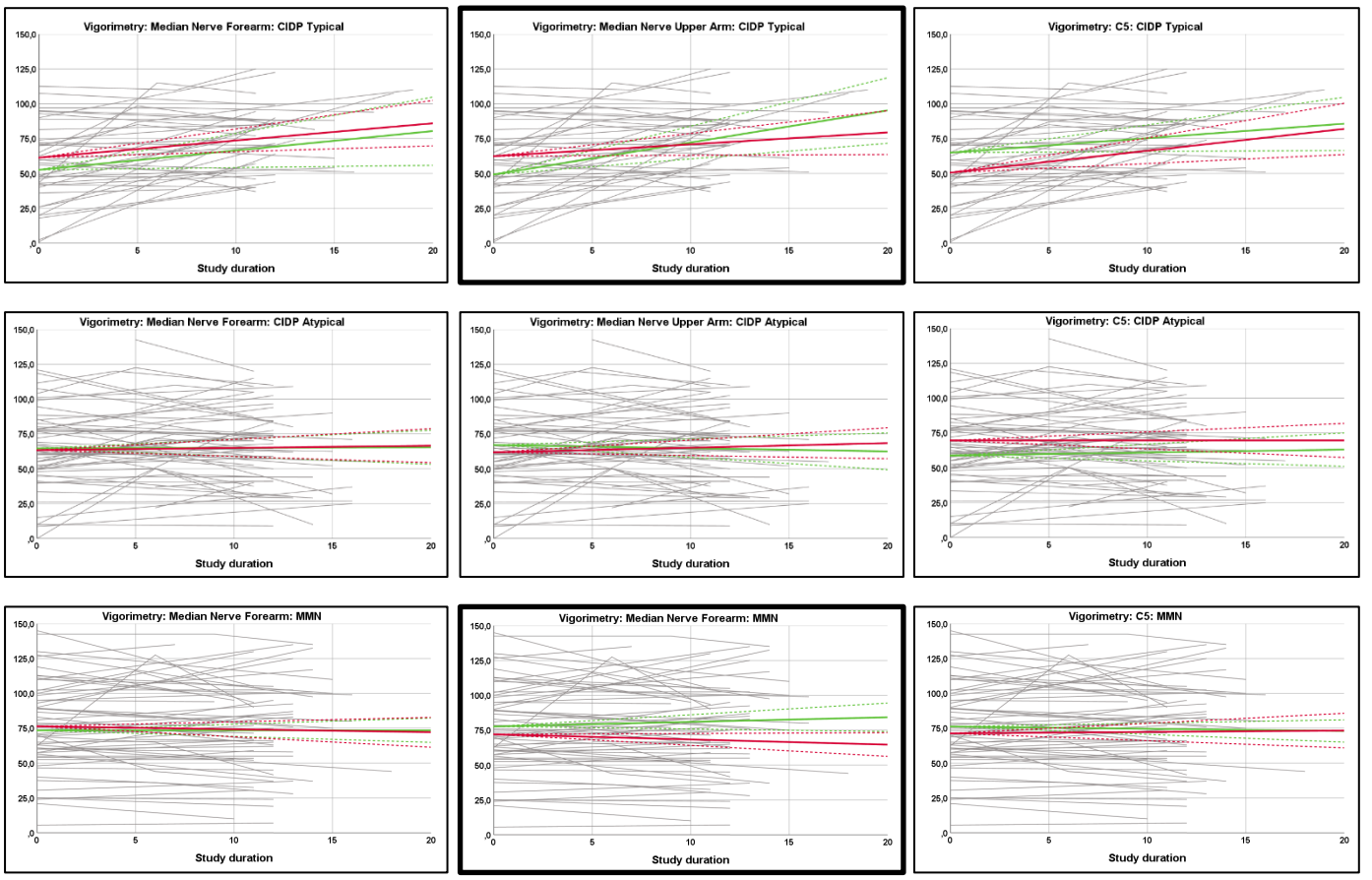

Figure 4 shows the effect of presence of nerve enlargement at inclusion at given sites (i.e. median nerve at forearm and arm, and C5 nerve root) on the development of vigorimetry over time in typical CIDP, atypical CIDP, MMN. Estimated slopes obtained from LME's for patients without enlargement (green) and with enlargement (red) are shown with 95%-CI (dotted lines). Grey lines represent individual patients. Only a significant effect for the median nerve at arm level in typical CIDP (slope 2.30 kPa / month (95% CI 1.12 – 3.47 kPa / month) without enlargement versus 0.85 kPa / month (95% CI 0.06 – 1.63 kPa / month) with enlargement, p = 0.04), and MMN (slope 0.33 kPa / month (95%-CI -0.18 – 0.84 kPa / month) without enlargement versus -0.37 kPa / month (95%-CI -0.79 – 0.06 kPa / month) with enlargement, p = 0.04). The lowest box shows plots for treatment-naive patients with MMN, though only significant at upper arm (slope 1.13 kPa / month (95%-CI 0.13 – 2.13 kPa / month) without enlargement versus -0.82 kPa / month (95%-CI -1.67 – 0.03 kPa / month) with enlargement, p = 0.02) , increase in grip strength also tended to be lower in case of enlargement at the forearm or C5 nerve root, though not significant. 95%-CI:"95%-confidence interval, CIDP: chronic inflammatory demyelinating polyneuropathy, kPa: kilopascal, LME: linear mixed model, MMN: multifocal motor neuropathy.

Table 3 Prognostic value of nerve enlargement on development of grip strength

CIDP Typical			
Nerve Site	**No nerve enlargement at inclusion**	**Nerve enlargement at inclusion**	**P-value**
	Slope (95%-CI) in kPa/month	**Slope (95%-CI) in kPa/month**	
MFA	1.39 (016 – 2.61)	1.23 (0.42 – 2.05)	0.84
MUA	2.30 (1.12 – 3.47)	0.85 (0.06 – 163)	0.04
C5	1.04 (0.08 – 1.99)	1.57 (0.65 – 2.49)	0.43
CIDP Atypical			
Nerve Site	**No nerve enlargement at inclusion**	**Nerve enlargement at inclusion**	**P-value**
	Slope (95%-CI) in kPa/month	**Slope (95%-CI) in kPa/month**	
MFA	0.04 (-0.57 – 0.65)	0.16 (-0.45 – 0.77)	0.79
MUA	-0.23 (-0.89 – 0.43)	0.33 (-0.23 – 0.88)	0.21
C5	0.21 (-0.39 – 0.81)	0.00 (-0.62 – 0.61)	0.63
MMN			
Nerve Site	**No nerve enlargement at inclusion**	**Nerve enlargement at inclusion**	**P-value**
	Slope (95%-CI) in kPa/month	**Slope (95%-CI) in kPa/month**	
MFA	0.00 (-0.43 – 0.44)	-0.21 (-0.75 – 0.32)	0.52
MUA	0.33 (0.18 – 0.84)	-0.37 (-0.079 – 0.06)	0.04
C5	-0.16 (-0.56 – 0.24)	0.10 (-0.52 – 0.72)	0.50

Table 3 shows the effect of presence of nerve enlargement at inclusion on development of grip strength over time (in kPa/month).
CIDP: chronic inflammatory demyelinating polyneuropathy, MFA: median nerve at foream, MMN: multifocal motor neuropathy, MUA: median nerve at upper arm.

Additional analyses showed that MMN patients with nerve enlargement confined to the brachial plexus had a more favorable outcome than MMN patients with more generalized enlargement.

Other prognostic factors in CIDP and MMN

Prognostic effects of previously identified clinical and NCS factors were tested in our multicenter cohort.[11-14] Shorter disease duration to treatment, a subacute start of complaints (nadir ≤6 weeks), lower age, absence of conduction block in the median nerve, and absence of axonal loss were all associated with improved vigorimetry and/or ODSS in both typical CIDP and MMN (p-value all <0.05).

Table 4 Effect of presence of nerve enlargement on outcome measures

Nerve Site	**CIDP Typical**				**MMN**		**MMN Treatment-naive**	
	Vigorimetry	**ODSS**	**RODS**	**mISS**	**Vigorimetry**	**ODSS**	**Vigorimetry**	**ODSS**
MFA	-3.3%	0.0%	+5.9%	-16.1%	-3.4%	0.0%	-12.0%	-8.8%
MUA	**-30.1%**	-17.5%	+0.8%	+4.6%	**-11.3%**	0.0%	**-26.6%**	-8.8%
C5	+11.0%	-11.7%	**+15.3%**	-8.0%	+4.2%	-16.2%	-0.8%	**-52.6%**

Table 4: Table 4 shows the estimated effects of presence of enlargement of the median nerve at forearm and upper arm and C5 nerve root at inclusion on several outcome measures. Dark red indicates significant worsening of an outcome measure in case of enlargement, dark green significant improvement. Light red and green also indicate worsening/improvement, though results of the LME were not significant in that case. A percentual difference in change per year between patients with and without enlargement at inclusion is shown, in which the mean value of the outcome measure, obtained with LME, is used as starting value.
CIDP: chronic inflammatory demyelinating polyneuropathy, LME: linear mixed model, MFA: median nerve at the forearm, mISS: modified INCAT Sensory Sum score, MMN: multifocal motor neuropathy, MUA: median nerve at the upper arm, ODSS: Overall Disability Sum Score, R-ODS: Rasch-Built Overall Disability Scale.

Discussion

This study showed that nerve ultrasound has limited prognostic value in patients with inflammatory neuropathies. MMN is a possible exception, since larger nerve size at inclusion was associated with lower grip strength after one-year follow-up. Moreover, patients with MMN who had brachial plexus enlargement only fared better than patients with more generalized nerve enlargement. Nevertheless, ultrasonographic nerve abnormalities were very heterogeneous, which limits its prognostic value in individual patients.

Previous studies on the prognostic value of nerve ultrasound showed promising results by suggesting a correlation between decreasing nerve size and better outcome. In the study of Zaidman et al improved grip strength also showed normalization of nerve size.[10] In other studies a decrease in a sonographic score for nerve enlargement (UPSS) and in intra-nerve variability ratio was associated with an improved clinical outcome.[6-8] However, we could not replicate these findings that were obtained in studies mostly retrospective in design, with small sample size and with predominantly treated patients included. It is less likely that this prospective study of a large group of untreated patients suffers from comparable inclusion bias.

Nerve size development in CIDP and MMN was very heterogeneous. This heterogeneity may be explained by the assumption that despite the fact that enlargement is the final common pathway of pathophysiological processes underlying CIDP and MMN, its reversal is not crucial for nerve function improvement. Onion bulb formation, inflammatory cell infiltrates and endoneurial edema, interstitial accumulation of amorphous substances or fibrosis can all cause nerve enlargement,[21-23] but their relation with the development of clinical symptoms may differ. It remains to be shown whether other ultrasound parameters than CSA are better predictors of outcome. Some small studies found that differences in echogenicity correlated with clinical outcome in CIDP patients, with patients showing hyperechoic nerves having a worse outcome.[7,21,24] The value of additional sonographic parameters may thus deserve further attention.[7,21,24-27]

Despite the limited level of correlation of nerve size with clinical outcome measures, a few of our observations may be helpful in clinical practice. Patients showing only nerve enlargement of the brachial plexus on average had a better therapeutic prognosis than patients with more generalized peripheral nerve enlargement. This pattern of distribution may therefore have some prognostic value. Differences in patterns may reflect variation in underlying pathophysiological processes or represent different stages in the disease. Although additional studies are needed, involvement of the brachial plexus only may be a prognostically beneficial factor in addition to previously identified clinical and NCS prognostic factors.[11-14]

This study had several limitations. First, the follow-up duration of 1 year was relatively short, and though we included a large group of patients with CIDP, subgroups of patients with clinical subtypes of CIDP were small. Another limitation is that we only included data on nerve size in analyses. It was not possible to perform reliable post-hoc classification of nerves based on nerve echogenicity, among other things, due to the use of different sonographic devices in this multicenter study. In our study, follow-up visits were planned irrespective of the time interval between the last course of IVIg treatment. As clinical complaints may vary markedly, this may have affected results on correlation between nerve size and outcome measures, though the results on prognostic value of nerve enlargement at inclusion are likely less biased, as these represent long term effects. Treatment of inflammatory neuropathies is often required for longer periods of time. To ensure that we will not miss prognostic effects after 1-year follow up, this study will continue another year.

Nerve ultrasound becomes increasingly important for the diagnosis of CIDP and MMN. A short ultrasound protocol allows reliable identification of these patients.[3,4,17] The current study shows that initial sonographic abnormalities remain present over time, which suggests that nerve ultrasound is a useful diagnostic tool even in case of diagnostic delay.

The usefulness of nerve ultrasound as a follow-up tool seems, based on the results of this study, relatively limited. Nerve ultrasound does not detect changes in nerve sizes that reflect treatment efficacy, remission or exacerbations, and its use after the initial diagnostic phase should not be encouraged.

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Chapter 7

Neurofibromatosis Type 1

Nerve ultrasound: a useful screening tool for peripheral nerve sheath tumors in NF1?

JA Telleman, MD Stellingwerff, GJ Brekelmans, LH Visser

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Abstract

Objective: To determine ultrasonographic peripheral nerve involvement in patients with asymptomatic neurofibromatosis type 1 (NF1).

Methods: Thirteen asymptomatic and four minimally symptomatic patients with neurofibromatosis type 1 (NF1) were included in this cross-sectional pilot-study to detect asymptomatic abnormalities of the brachial plexus, and upper and lower extremity nerves. Patients underwent clinical examination, nerve conduction studies (NCS) and high-resolution ultrasonography (HRUS).

Results: HRUS showed abnormalities in 16 patients (94.1%). Neurofibromas were identified in 10 patients (58.8%): Localized neurofibromas were found in 3 patients (17.6%), plexiform neurofibromas in 3 (17.6%) and both in 4 (23.5%). In 6 patients (35.3%) only nerve enlargement without an abnormal fascicular pattern was observed. Severe involvement of the peripheral nervous system with multiple plexiform neurofibromas was observed in 7 patients (41.2%), while 4 patients (23.5%) had no or only minor involvement. Both NCS and HRUS were performed on 73 individual nerve segments. In 5.5% abnormalities were found with both tests, in 50.7% only with HRUS and in 1.4% only with NCS.

Conclusions: HRUS frequently showed subclinical involvement of the peripheral nerves in NF1, also when NCS were normal. HRUS findings ranged from normal to widespread peripheral nerve involvement. Since the presence of plexiform neurofibromas and the benign tumor load are risk factors for the development of a malignant peripheral nerve sheath tumor (MPNST), HRUS may be a useful tool to identify a subgroup of patients that could benefit from regular follow-up.

Introduction

Neurofibromatosis type 1 (NF1) is the most prevalent type of neurofibromatosis and is characterised by café au lait macules (CALMS) and neurofibromas.[1] In case of peripheral nerve involvement serious complications may ensue. Benign peripheral nerve sheath tumors (PNSTs) can cause neuropathic complaints, and polyneuropathy can be present.[2-5] Furthermore, development of a malignant PNST (MPNST) is a leading cause of mortality.[1,6]

A PET/CT and MRI scan of a specific region can identify the malignant transformation of a PNST, but PET/CT should only be used if it is highly clinically suspicious, since radiation may increase the likelihood of a malignant transformation.[7,8] Also, both techniques are relatively expensive and time-consuming. Whole-body MRI (WB-MRI) has been suggested as a screening technique for PNSTs, but this technique has the same limitations.[9] Due to these limitations and because the risk of malignant transformation is relatively low, there is no consensus about screening all NF1 patients for MPNST. However, MPNST has a high mortality, and there may be other techniques that could improve the screening process.

High-resolution ultrasound (HRUS) is a quick and cost-effective technique to study the morphology of multiple nerves that is increasingly being used as a diagnostic tool in polyneuropathy.[10] Sonographic characteristics of symptomatic PNSTs and MPNSTs have been described, but the existence of sonomorphological abnormalities in asymptomatic patients has not yet been investigated.[11-14] This pilot study was performed to determine the subclinical sonographic nerve involvement in neurofibromatosis and to explore the role of HRUS as a tool to screen for MPNST.

Methods

Standard protocol approvals, registrations, and patient consents

We performed a cross-sectional pilot study between December 2015 and June 2016 in the Elisabeth-Tweesteden Hospital, a large general teaching hospital in the Netherlands. The study was approved by the Brabant Regional Ethics Committee (no NL54951.028.15). We recruited asymptomatic adult patients with known NF1 at our outpatient clinic. All patients gave written informed consent. Inclusion criteria were: 1) diagnosis of NF1 based on NIH Diagnostic Criteria[15] and/or positive genetic testing, and 2) age 18-80. Exclusion criteria were: 1) comorbidity associated with (poly)neuropathy (e.g. diabetes, alcoholism), 2) comorbidity mimicking neuropathic complaints (e.g. myelopathy), and 3) inability to undergo HRUS. Patients underwent clinical examination, NCS and HRUS, and data were compared.

Clinical examination

Clinical examination was performed prior to NCS and HRUS by one of the investigators (MS). Details about the patient's symptoms of neuropathy (e.g. subjective sensory changes, loss of strength) and their potential risk factors for developing polyneuropathy were recorded. In addition, a neurological examination was performed, in which the number of CALMS, cutaneous and subcutaneous neurofibromas was determined, and in which sensory function and deep tendon reflexes were tested. The number of CALMS in patients was graded: grade 0: 0 CALMS, grade 1: 1-5, grade 2: 6-10, grade 3: 11-15, grade 4: 16-20, grade 5: >20. The motor function of 14 muscle groups (deltoid, elbow flexors and extensors, wrist flexors and extensors, dorsal interossei, abductor pollicis brevis, iliopsoas, quadriceps, hamstrings, ankle flexors and extensors, peroneus longus and extensor hallucis longus) was graded using the Medical Research Council (MRC) scale. Grip strength was determined with the CITEC hand held dynamometer (C.I.T. Technics, Haren, The Netherlands).

Nerve conduction studies

NCS were performed by our lab technicians and residents in clinical neurophysiology and analyzed by one of the investigators (GB) who was blinded for the results of the clinical examination and HRUS. A reduced, one-sided NCS protocol (without testing the F-waves, H-reflexes or needle electromyography) was applied to limit the burden for the participating patients. Sensory nerve action potentials (SNAPs) and sensory conduction velocity (SCV) of the median, ulnar and sural nerves were recorded. Compound muscle action potentials (CMAPs), motor conduction velocity (MCV) and the distal motor latency (DML) of the median, ulnar, fibular and tibial nerves were also registered. Recording sites were the abductor pollicis brevis (APB), abductor digiti minimi (ADM), extensor digitorum brevis (EDB) and abductor hallucis brevis (AHB). The median nerve was stimulated at the wrist and elbow, the ulnar nerve at the wrist, distal to the cubital tunnel and proximal to the cubital tunnel, the fibular nerve at the ankle, fibular head and popliteal fossa, and the tibial nerve at the ankle and popliteal fossa. A decreased or absent CMAP or SNAP amplitude and reduced MCV or SCV were regarded as a peripheral nerve being neurophysiologically involved. Details about the normative values of NCS are shown in Table E-1.

High-resolution ultrasonography

One of the investigators (JT), blinded to the results of the clinical examination and the NCS, performed HRUS on a Toshiba ultrasound machine (Xario XG; Toshiba, Tokyo, Japan) with a 7-18 MHz linear-array transducer (PLT-1204BT). The investigator assessed the median, ulnar, tibial, fibular, and sural nerves, and brachial plexus bilaterally (12 nerve segments in total, with the brachial plexus regarded as an independent nerve segment) following a previously published protocol.[16] If possible, the complete trajectory of each nerve was visualized, as well as the ischiadic nerve in the distal and proximal thigh. However, standardized analysis of the proximal part of the ischiadic nerve, as well as the pelvic and

lumbosacral plexus was not performed, as these nerve structures are often not assessable by HRUS due to their deep lying trajectory. Nerve cross-sectional area (CSA) was recorded at predetermined anatomical sites along each nerve segment (Figure E-1): 1) the median nerve at the wrist, forearm and arm, 2) the ulnar nerve at the wrist, forearm, distal to the sulcus, at the sulcus, proximal to the sulcus and at the arm, 3) the brachial plexus at the truncal level: the superior, medial, and inferior trunk, 4) the fibular nerve at the fibular head and popliteal fossa, 5) the tibial nerve at the ankle, and 6) the sural nerve 14 cm proximal to the lateral malleolus. Nerve CSA and vascularization (assessed with Power Doppler) were determined in case there were any abnormalities along the tract of a nerve segment. The probe was held perpendicular to the nerve and measurements were performed within the hyperechoic rim.

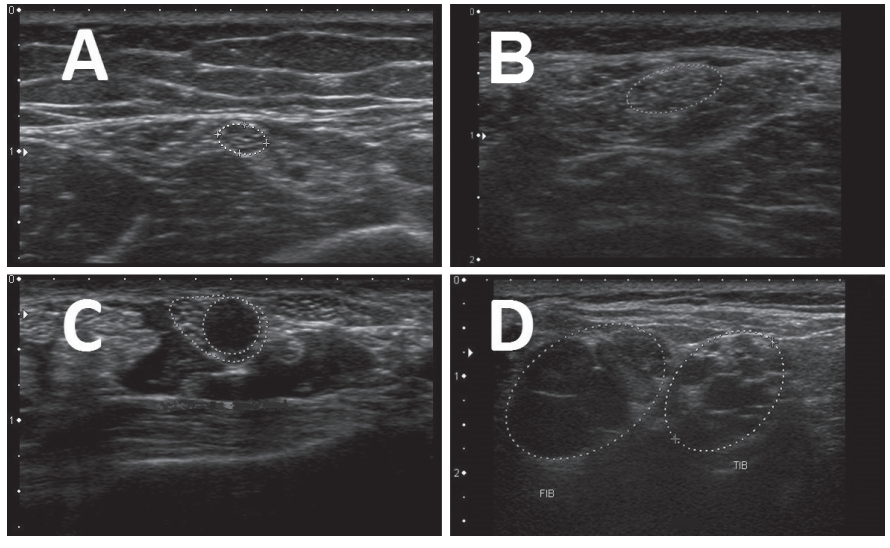
Definitions of HRUS abnormalities

The presence of PNSTs, nerve enlargement and the degree of sonographic peripheral nerve involvement were determined in all patients. A PNST was defined as a hypoechoic mass identified along the tract of a nerve segment. If a solitary hypoechoic mass was found, this was classified as a localized neurofibroma, and if multiple hypoechoic serpentine-like fascicles (hypoechoic fascicles showing a varying degree of enlargement and a tortuous course along the tract of the nerve) were found along the tract of a nerve, this was classified as a plexiform neurofibroma (Figure 1). Nerve enlargement was defined as an increased CSA compared to previously published reference values.[16] A CSA 100-150% above the reference value was defined as mild nerve enlargement, and a CSA >150% above the reference value was defined as severe nerve enlargement. Both an increased CSA due to the presence of a localized or plexiform neurofibroma and an increased CSA due to general swelling of the nerve without an abnormal fascicular pattern or a hypoechoic mass were regarded as nerve enlargement. Sonographic peripheral nerve involvement in patients was graded as: 1) no or minor nerve involvement, 2) moderate nerve involvement, and 3) severe nerve involvement. No or minor involvement was defined as the presence of nerve enlargement or a localized neurofibroma in ≤3 nerve segments (≤25% of total nerve segments), without the presence of severe nerve enlargement or a plexiform neurofibroma. Severe nerve involvement was defined as the presence of severe nerve enlargement or a localized neurofibroma in ≥6 nerve segments (≥50% of total nerve segments) or the presence of a plexiform neurofibroma. Patients who did not fulfill either of these criteria were graded as having moderate nerve involvement.

Statistics

We used IBM SPSS 19 (SPSS Inc., Chicago, IL) for statistical analyses. Data were visually inspected for normality and are presented as mean ± standard deviation (SD) for parametric data or median (range) for non-parametric data. Pearson's correlation

Figure 1 Sonographic findings in asymptomatic NF1



Sonography revealed a wide variety of sonomorphological abnormalities in asymptomatic patients with NF1. A. Normal ulnar nerve at the arm with an intact honeycomb structure in patient 9 (CSA 5 mm^2). B. Severe enlargement of the fibular nerve at the popliteal fossa without the presence of a PNST or an abnormal fascicular pattern in patient 5 (CSA 37 mm^2). C. Localized neurofibroma of the median nerve at the wrist in patient 10 (CSA of entire nerve 23 mm^2, CSA of PNST 13 mm^2). D. Plexiform neurofibroma of the fibular and tibial nerves in patient 17 (CSA of fibular nerve 172 mm^2, CSA of tibial nerve 149 mm^2).
CSA: cross-sectional area, PNST: peripheral nerve sheath tumor.

coefficient or Spearman's rank correlation coefficient were determined where appropriate. The correlation between HRUS and NCS was determined with the Chi-square or Fisher's exact test. The level of significance was set at 0.05.

Results

Clinical characteristics

We enrolled 17 patients with a diagnosis of NF1 based on NIH diagnostic criteria and/or positive genetic testing. Detailed results of the clinical examinations can be found in Table 1. In our cohort there were two pairs of first degree relatives (patients 1 and 9; patients 10 and 12). The median age of patients was 42 years (range 19-69). None of the patients reported risk factors for developing polyneuropathy (e.g. diabetes, alcoholism) or a history

of MPNST. Though all patients claimed to be asymptomatic upon entering the study, 4 patients (23.5%) complained of mild numbness or weakness, which came to light during the patient history. Mild loss of strength was reported by 3 of these patients, and mild sensory loss by all 4. Thirteen patients (76.5%) reported no neuropathic complaints. Clinical examination revealed a reduced MRC -sum score in 2 patients (11.8%), but no hypesthesia.

Nerve conduction studies

NCS were performed in all but two patients who refused to undergo them. Signs of axonal neuropathy were found in 3 patients (20.0%), and signs of demyelination were found in none of the patients. In patient 7 all the lower extremity nerves were affected, while in patient 11 only the sural nerve showed signs of axonal damage and in patient 17 only the tibial nerve.

High-resolution ultrasonography

HRUS showed multiple morphological abnormalities of the peripheral nerve, including PNSTs (localized neurofibromas and plexiform neurofibromas) and peripheral nerve enlargement (Table 2). Morphological abnormalities were found in both the upper and lower extremity nerves, and no clear predilection of proximal or distal nerve segments was present. Extensive sonographic data per patient, including CSA -measurements of PNSTs and all predetermined nerve sites can be found in Table E-2.

PNSTs were found in 10 of 17 patients (58.8%). Localized neurofibromas were found in 7 patients (41.2%, 1-4 nerve segments affected), and plexiform neurofibromas were also found in 7 patients (41.2%, 2-11 nerve segments affected). In 4 patients both localized and plexiform neurofibromas were encountered. Localized neurofibromas showed a hypoechoic aspect, clearly defined borders and no vascularization, while plexiform neurofibromas showed serpentine-like hypoechoic fascicles, clearly defined borders and no vascularization. Nerve enlargement was encountered in ≥1 nerve segments in 16 patients (94.1%), and severe nerve enlargement in 12 patients (70.6%). Apart from nerve enlargement due to the presence of a localized or plexiform neurofibroma, we encountered nerve enlargement in nerve segments with a normal fascicular pattern and without a hypoechoic mass (Figure 2). Focal nerve enlargement at an entrapment site or non-entrapment site was found in 9 patients (52.9%). Diffusely enlarged nerve segments with a normal fascicular pattern were found in 8 patients (47.1%, 1-9 nerve segment affected). In 5 of those patients a plexiform neurofibroma with serpentine-like hypoechoic fascicles was found in at least one other nerve segment.

There was a broad range in the sonographic findings, with 4 patients (23.5%) having no or only minor peripheral nerve involvement, while 6 (35.3%) had moderate and 7 (41.2%) severe involvement (Table 2, Table E-2).

Table 1 Patient characteristics

Patient	General Characteristics				Patient History		Clinical examination		
	Sex	*Age*	*Diagnostic criteria*	*Genetic testing*	*Sensory complaints*	*Loss of strength*	*CALMS*	*MRC sum score*	*Sensory Loss*
1 [a]	F	52	+	n.p.	Numbness: radial side of left hand	Distal arms	9	140	-
2	F	42	+	Positive; unknown	-	-	19	140	-
3	F	46	+	n.p.	-	-	9	140	-
4	F	61	+	n.p.	-	-	5	140	-
5	M	46	+	n.p.	Numbness: both hands, dig 2-5	Right distal arm	5	120/120 [c]	-
6	F	36	+	R304X	Numbness: right hand, dig 2-3	-	12	140	-
7	M	64	-	c2409+1G>T	-	-	3	140	-
8	M	43	+	n.p.	-	-	1	140	-
9 [a]	F	26	+	n.p.	-	-	>20	132	-
10 [b]	F	59	+	n.p.	Tingling: left hand, Pain: right lower leg	Distal arms	10	140	-
11	M	44	+	n.p.	-	-	3	140	-
12 [b]	M	30	+	c.4110+1G>C	-	-	10	140	-
13	M	38	+	UV 3315-3C>G	-	-	13	119/120 [d]	-
14	F	25	+	Negative	-	-	10	140	-
15	M	69	+	22delG exon 4	-	-	7	140	-
16	F	22	+	Positive; unknown	-	-	9	140	-
17	M	19	+	6037insA	-	-	13	140	-

General characteristics and findings on patient history and clinical examination are shown for individual patients.
CALMS: café au lait macules, MRC: medical research council, n.p.: not performed. a. first degree relatives. b. first degree relatives c. no complete physical examination due to vagal reaction. d. amputation of left lower leg.

Table 2 Sonographic abnormalities in NF1 patients

Patients		Amount of nerve segments with:					
		Nerve Enlargement	*Severe Nerve Enlargement*	*PNST*	*LNF*	*PNF*	*Diffuse Enlargement with normal fascicles*
No or Minor Nerve Involvement	3	1	0	0	0	0	0
	9[b]	0	0	0	0	0	0
	14	1	0	1	1	0	0
	16	1	0	0	0	0	0
Moderate Nerve Involvement	1[a, b]	4	1	0	0	0	0
	2	7	1	0	0	0	0
	6[a]	6	0	0	0	0	0
	10[a, c]	6	3	3	3	0	1
	11	5	3	0	0	0	3
	12[c]	7	2	4	4	0	2
Severe Nerve Involvement	4	6	6	4	2	2	3
	5[a]	11	9	2	0	2	9
	7	11	10	7	1	6	5
	8	12	12	12	1	11	0
	13[d]	9	9	9	0	9	0
	15	11	6	4	2	2	3
	17	11	10	10	0	10	1

Data are presented as the number of abnormal nerve segments per patient. A total of 12 nerve segments were investigated with sonography: the bilateral median, ulnar, fibular, tibial and sural nerves and the brachial plexus. Patients are grouped according to the degree of peripheral nerve involvement. Enlargement was defined as a CSA 100-150% above the normal limit. Severe enlargement was defined as a CSA >150% above the normal limit.
CSA: cross-sectional area. LNF: localized neurofibroma. PNF: plexiform neurofibroma. PNST: peripheral nerve sheath tumor. a. reports minor neuropathic complaints b. first degree relatives. c. first degree relatives. d. 3 nerve segments not measurable due to a lower leg amputation.

Correlation of HRUS, clinical examination and NCS

No significant relation was found between the degree of peripheral nerve involvement and the patients age (p=0.128) or number of CALMS (p=0.337). Of the 13 clinically affected nerve segments, 9 showed abnormalities on HRUS (5 PNST(s), 4 enlargement without PNSTs), and of the 4 clinically affected nerve segments investigated with NCS none

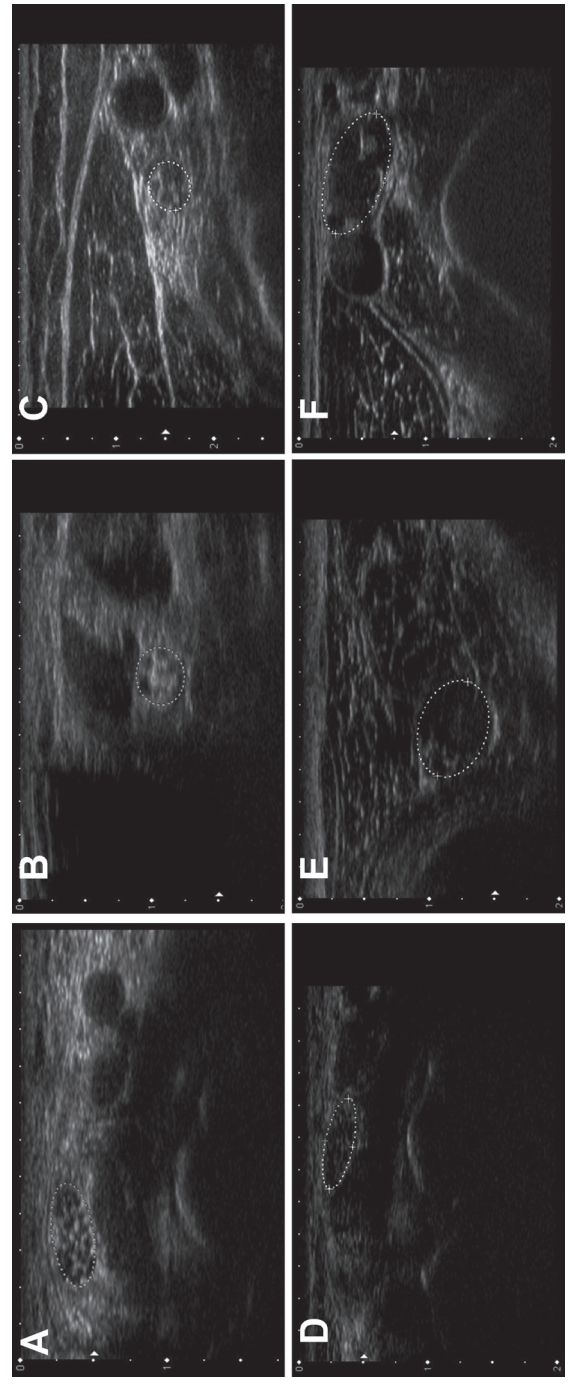


Figure 2 Diffusely enlarged nerve segments in NF1

Different patterns of diffuse nerve enlargement were found in NF1 patients. Images A-C show a diffusely enlarged median nerve with a normal fascicular pattern (patient 5), while images D-F show a median nerve that is enlarged due to a plexiform neurofibroma (patient 17). A Median nerve at wrist (CSA 16 mm^2). B. Median nerve at forearm (18 mm^2). C Median nerve at arm (17 mm^2). D Median nerve at wrist (12 mm^2). E Median nerve at forearm (32 mm^2). F Median nerve at arm (35 mm^2).

showed abnormal conduction velocities (Table 3). Of the nerve segments with normal findings upon clinical examination, 90 (57.7%) showed abnormalities on HRUS. (PNST(s) in 47 (30.1%), enlargement without PNST in 43 (27.6%)).

Table 3 Correlation of clinically affected nerves, NCS, and sonography

		HRUS					NCS (N=73)	
		Normal	***Abnormal***	***PNST***	***Nerve Enlargement***	***Severe Nerve Enlargement***	***Normal***	***Abnormal***
Clinically affected	***No (N=154)***	64 (42%)	90 (58%)	47 (31%)	90 (58%)	59 (38%)	64 (93%)	5 (7%)
	Yes (N=13)	4 (31%)	9 (69%)	5 (38%)	8 (62%)	6 (46%)	4 (100%)	0 (0%)
NCS	***Normal (N=68)***	31 (46%)	37 (54%)	20 (29%)	37 (54%)	22 (32%)		
	Abnormal (N=5)	1 (20%)	4 (80%)	3 (60%)	4 (80%)	4 (80%)		

Data are presented as the number of nerve segments (%). The brachial plexus was excluded from analysis. A total of 167 nerve segments (median, ulnar, fibular, tibial and sural nerves) were investigated with HRUS. A total of 73 segments were investigated with NCS.
HRUS: high-resolution ultrasonography of the nerves, NCS: nerve conduction studies, PNST: peripheral nerve sheath tumor.

A total of 73 nerve segments were investigated with both NCS and HRUS. In 4 segments (5.5%), the findings were abnormal with both tests, in 37 segments (50.7%) only with HRUS, and in 1 (1.4%) only with NCS. When PNSTs were identified sonographically, the NCS were abnormal in 13.0%. There was no significant correlation between the presence of NCS abnormalities and PNSTs (p=0.317) or presence of NCS abnormalities and any abnormality on HRUS (p=0.377).

Discussion

This study investigated sonomorphological abnormalities in 13 asymptomatic and 4 minimally symptomatic NF1 patients. PNSTs, and nerve enlargement without apparent PNSTs were frequently observed when the clinical history and examination did not reveal any signs of neuropathy. The sonomorphological characteristics of PNSTs in our study were comparable to those described in previous studies in symptomatic patients, with localized neurofibromas posing as hypoechoic lesions with a well-defined margin and plexiform neurofibromas posing as diffuse lesions with serpentine-like hypoechoic fascicles.[17-20] Interestingly, we also observed diffusely enlarged nerve segments with a normal fascicular pattern. The diffuse enlargement we encountered may point to the presence of a plexiform neurofibroma in those segments, as most of those patients also had plexiform neurofibromas in other nerve segments. On the other hand, this type of nerve enlargement is frequently encountered in demyelinating neuropathies, and not all patients had a plexiform neurofibroma as well. It may therefore also indicate another type of nerve pathology in NF1. Because we investigated asymptomatic nerve segments, we could not perform histopathological analysis to determine the pathophysiology of nerve enlargement in those segments. However, the distinction between the presence of a plexiform neurofibroma and other possible nerve pathology would be of importance when evaluating the risk of developing an MPNST in NF1 patients. Further research, e.g. follow-up of such abnormalities over time in a larger cohort, is therefore necessary to establish the cause of sonographic nerve enlargement without an abnormal fascicular pattern in NF1.

In our study, NCS revealed signs of neuropathy in 20.0% of patients, which is much higher than the 1.3-2.3% reported in larger databases.[2, 3] This difference may be due to the small number of patients investigated in our study. On the other hand, the larger databases may have investigated patients with neuropathic complaints with NCS only. Subclinical neurophysiological nerve involvement in those cohorts may therefore be more prevalent as well.

Previous studies have described both axonal and demyelinating neuropathy in neurofibromatosis, while the NCS in our study did not reveal any demyelinating signs, but this may be due to the limited NCS protocol and small sample size. NCS were often normal when HRUS findings were abnormal, and no clear correlation between the techniques was found. This discrepancy in nerve morphology and nerve function is also observed in other peripheral nerve diseases, including acquired inflammatory neuropathies.[21] Our understanding of the correlation of nerve morphology and nerve function in those diseases is still limited, but it seems that nerves can show multiple morphological abnormalities without the nerve function being impaired.[21] Additional research needs to be performed to further explore this correlation.

MPNST is a leading cause of morbidity and mortality in NF1, with a lifetime risk of developing MPNST of 8-13% and a 5-year survival rate of 16-54%.[1, 6] It often presents as a painful, rapidly growing mass causing neurological deficit. [22] Several risk factors for developing MPNST have been described, including NF1 gene microdeletions, increased benign tumor load and the presence of plexiform neurofibromas. [23-25] Still, it is not well known which patients are prone to malignant transformation, and the risk of this transformation has been deemed relatively low. Therefore, there is no consensus about screening all NF1 patients, because the presently available screening techniques, including WB-MRI, are relatively expensive and time-consuming. In our study we found a broad spectrum of sonographic peripheral nerve involvement in NF1, with 23.5% of patients showing no or only minor peripheral nerve involvement, while 41.2% showed severe involvement of the peripheral nerves with presence of plexiform neurofibromas. As the benign tumor load and the presence of plexiform neurofibromas have been identified as risk factors for developing MPNST, HRUS may be able to identify a specific subgroup of NF1 patients at higher risk for developing MPNST. It could identify patients with severe peripheral nerve involvement, which can possibly benefit from regular follow-up, while patients showing no or only minor involvement could potentially be excluded from this regular follow-up.

HRUS is inexpensive and allows multiple nerves to be assessed quickly. Therefore, it may also be useful as a screening tool during follow-up in the group of patients with severe nerve involvement. However, one has to be aware that PNSTs can have a thoracic, abdominal or pelvic localization, and will not be detected by sonography because of their deeper lying position.[26,27] Moreover, currently, it is not possible to determine tumor volume with HRUS, and tumor volumetry is an important tool in WB-MRI to detect the growth and malignant transformation of a plexiform neurofibroma.[9] Still, with HRUS it could be possible to detect the growth of a PNST by measuring changes in CSA or changes in vascularization. Though the measurement of changes over time can be influenced by factors leading to intra- and inter-observer variability, studies investigating HRUS in leprosy found that changes in CSA and vascularization during follow-up were correlated with treatment response.[28,29] In NF1 such changes may also be correlated with the growth of a PNST, but further research in larger cohorts is necessary to determine if HRUS can reliably detect growth. Also, additional research is necessary to determine if the detection of such growth by HRUS is useful to preselect specific PNSTs that warrant further investigation with MRI or PET/CT to determine malignant transformation.

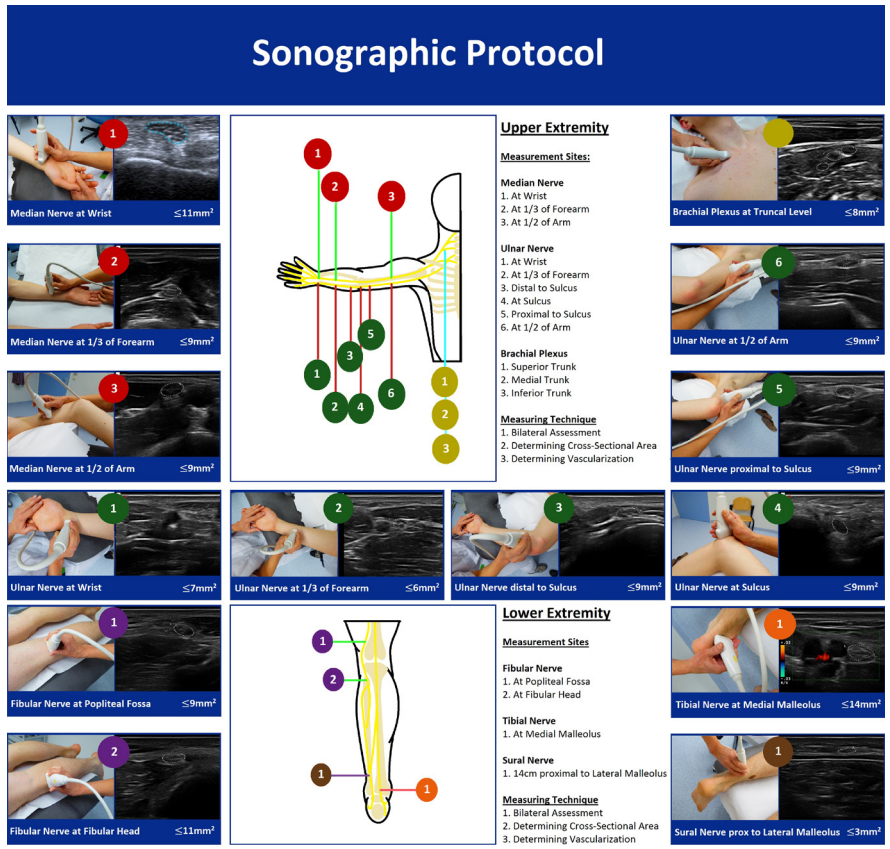
This study provided some interesting findings. Frequent subclinical sonomorphological involvement of peripheral nerves can be found in NF1. HRUS may have applications as a screening tool in NF1, but the current study was a cross-sectional study in only 17 patients and it is not known how sonographic abnormalities change over time. Therefore, further research should be performed to investigate the applicability of HRUS as a screening tool in a larger group of NF1 patients and to determine the development of peripheral nerve involvement in NF1 over time.

Table E2 Detailed sonographic findings in asymptomatic NF1 patients

		Cut-off	Minor involvement				Moderate involvement						Severe involvement						
Patient number		-	*3*	*9[a]*	*14*	*16*	*1[a]*	*2*	*6*	*10[b]*	*11*	*12[b]*	*4*	*5*	*7*	*8*	*13*	*15*	*17*
Median R	***PNST***	-	-	-	-	-	-	-	-	-	-	**L(13)**	-	-	**P(30)**	**P(52)**	**P(16)**	**P(17)**	**P(66)**
	WR	11	9	9	10	10	11	8	10	9	11	9	12	16	14	18	11	12	12
	FA	9	5	5	5	4	7	6	8	5	8	6	5	18	18	7	8	8	32
	UA	9	9	6	8	5	8	12	8	9	13[c]	13	14	17	27	50	16	17	35
Median L	***PNST***	-	-	-	**L(12)**	-	-	-	-	**L(23)**	-	-	-	-	**P(36)**	**P(38)**	**P(23)**	**L(14)**	**P(64)**
	WR	11	9	9	9	8	11	9	12	11	11	12	13	16	15	17	10	10	11
	FA	9	6	5	6	6	8	5	9	10	8	5	9	8	18	5	16	6	52
	UA	9	8	7	9	7	9	14	11	10	10[c]	14	16	18	29	23	13	14	38
Ulnar R	***PNST***	-	-	-	-	-	-	-	-	**L(9)**	-	-	**P(14)**	**P(16)**	**P(21)**	**P(67)**	**P(24)**	-	**P(100)**
	WR	7	4	4	5	4	5	6	7	4	7	4	7	9	9	15	7	8	5
	FA	6	5	5	5	4	7	3	4	5	8	5	12	8	13	23	10	8	17
	DS	9	8	9	7	3	7	8	9	7	13	10	15	11	16	19	6	8	7
	SU	9	8	5	6	13	16	8	9	7	9	8	16	16	14	14	11	13	8
	PS	9	6	7	8	5	8	7	10	6	9	7	15	15	14	20	11	11	31
	UA	9	5	5	7	5	7	6	6	7	10	6	18	15	21	14	13	10	100
Ulnar L	***PNST***	-	-	-	-	-	-	-	-	**L(10)**	-	-	**L(11)**	**P(27)**	**P(23)**	**P(43)**	**P(18)**	-	**P(55)**
	WR	7	4	4	5	4	6	5	8	5	6	5	9	8	14	10	5	5	6
	FA	6	7	5	6	4	5	4	6	4	7	6	12	13	12	38	8	6	16
	DS	9	6	6	7	4	7	5	7	7	9	7	11	15	16	15	8	11	25
	SU	9	8	6	8	7	9	7	8	7	5	7	17	12	16	14	13	13	15
	PS	9	8	6	7	5	10	7	11	8	9	11	15	27	18	16	8	10	26
	UA	9	7	5	7	4	6	4	8	5	7	7	10	15	23	23	14	13	55
Plexus R	***PNST***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**P(21)**	**P(59)**	-	-
	ST	8	5	5	3	5	6	2	4	12	12	6	3	10	7	19	16	21	10
	MT	8	4	4	5	3	4	3	6	4	12	5	3	11	8	21	19	15	9
	IT	8	3	4	4	3	3	2	6	4	8	3	4	8	6	12	22	9	8
Plexus L	***PNST***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**L(29)**	**P(37)**	-	-
	ST	8	6	4	4	4	6	3	5	8	7	8	7	8	9	9	37	8	7
	MT	8	5	3	2	7	5	4	3	3	4	8	4	8	10	6	33	9	5
	IT	8	5	3	4	4	4	3	3	4	2	7	8	6	6	5	29	7	7
Fibular R	***PNST***	-	-	-	-	-	-	-	-	-	-	**L(13)**	**P(21)**	-	**P(18)**	**P(32)**	**P(129)**	**L(21)**	**P(125)**
	FH	11	11	11	11	7	11	11	10	18	8	6	17	20	15	32	10	13	14
	PF	9	7	8	9	4	7	12	11	22	8	13	15	35	18	13	45	21	125
Fibular L	***PNST***	-	-	-	-	-	-	-	-	-	-	**L(13)**	**L(14)**	-	**P(26)**	**P(32)**	-	**P(19)**	**P(173)**
	FH	11	10	9	11	9	13	13	12	11	9	8	12	21	15	13	-[d]	10	16
	PF	9	9	7	9	5	9	11	12	11	9	13	14	37	26	32	-[d]	19	173
Tibial R	***PNST***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**P(26)**	**P(26)**	-	**P(50)**
	MM	14	9	10	11	11	15	12	10	12	9	14	11	20	21	26	26	12	50
Tibial L	***PNST***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**P(58)**	-	-	**P(56)**
	MM	14	9	9	12	12	12	16	17	15	10	9	12	27	22	58	-[d]	16	56
Sural R	***PNST***	-	-	-	-	-	-	-	-	-	-	**L(5)**	-	-	**L(9)**	**P(10)**	**P(14)**	-	**P(33)**
	PM	3	2	3	2	2	3	4	3	2	3	5	3	8	9	10	3	5	6
Sural L	***PNST***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**P(10)**	-	-	**P(22)**
	PM	3	2	3	2	2	3	4	2	3	3	3	2	7	5	10	-[d]	4	5
Enlarged segments			1	0	1	1	4	7	6	6	5	7	6	11	11	12	9	11	11
Severely enlarged segments			0	0	0	0	1	1	0	3	3	2	6	9	10	12	9	6	10
Segments with PNSTs			0	0	1	0	0	0	0	3	0	4	4	2	7	12	9	4	10

The cut-off values and cross-sectional area (CSA) measurements of the 17 individual NF1 patients are shown in mm^2 for the standardized measurement points along the tract of the 12 investigated nerve segments (the bilateral median , ulnar, fibular, tibial and sural nerves and the brachial plexus). CSA measurements 100-150% above reference value are marked light grey, CSA measurements >150% above reference value (severe enlargement) are marked dark grey. The presence of PNSTs is also shown for each nerve segment (bold). Localized neurofibromas are marked with 'L'(CSA of localized neurofibroma), and plexiform neurofibromas are marked with 'P'(maximum CSA of plexiform neurofibroma along the investigated nerve segment). Data of the patients are grouped according to the degree of peripheral nerve involvement that patients showed (1. Minor (or no) involvement, 2. Moderate involvement, 3. severe involvement).
WR=wrist, FA=Forearm, UA=Upper arm, DS=Distal of sulcus, SU=In sulcus, PS=Proximal of sulcus, ST=Superior trunk, MT=Medial trunk, IT=Inferior trunk, FH=Fibular head, PF=Popliteal fossa, MM=Medial malleolus, PM=Proximal of malleolus. PNST: peripheral nerve sheath tumor. a. First degree relatives. b. First degree relatives. c. severe enlargement at elbow. d. Not measurable due to lower leg amputation.

Figure E1 Sonographic Protocol



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Chapter 8

Neurofibromatosis Type 1

Nerve Ultrasound in Neurofibromatosis type 1: a Follow-up Study

JA Telleman, MD Stellingwerff, GJ Brekelmans, LH Visser

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Abstract

Objective: To investigate development of sonographic abnormalities and applications of high-resolution ultrasonography (HRUS) in neurofibromatosis type 1 (NF1).
Methods: Sixteen asymptomatic or minimally symptomatic NF1 patients underwent HRUS at inclusion and 1 year follow-up. Upper and lower extremity nerves were investigated. Peripheral nerve involvement was graded.
Results: Plexiform neurofibromas (PNFs) were found in 7 patients (43.8%) at inclusion and 10 (62.5%) at follow-up. All initially identified PNFs were also found at follow-up; additional PNFs were found by extended longitudinal assessment at follow-up. All 3 patients with minor and 7 patients with severe peripheral nerve involvement had similar involvement at follow-up. Mean nerve size change was -0.2mm^2 (±1.6) and 0.3mm^2 (±6.2) in patients with minor and severe involvement. Mean PNF size change was -0.1mm^2 (±9.9).
Conclusions: HRUS allows qualitative assessment of peripheral nerves, which makes it advantageous as initial imaging technique in suspected neuropathy. Patients with minimal nerve involvement remained so, and might therefore require less follow-up for malignant peripheral nerve sheath tumor (MPNSTs) development. Measured change in PNF size was highly variable. Repeating an extensive standardized HRUS protocol during follow-up thus seems less useful to screen for MPNSTs.

Introduction

Peripheral nerve sheath tumors (PNSTs) are one of the main characteristics of neurofibromatosis type 1 (NF1). They can undergo malignant transformation, which is a leading cause of mortality.[1,2]

Though the development of a malignant PNST (MPNST) has serious consequences for patients, there is no clear consensus on screening patients. Whole-body MRI is a technique that could be applied to screen patients, but it is relatively expensive and time-consuming.[3,4] An emerging technique is high-resolution ultrasonography (HRUS), which allows quick investigation of multiple nerves, and this technique may have applications as a screening tool in NF1. A study by Winter et al. showed frequent presence of plexiform neurofibromas in NF1 patients, and in a recent cross-sectional pilot study in asymptomatic and minimally symptomatic NF1 patients we found a high variability in sonographic peripheral nerve involvement.[5,6] Some patients showed almost no abnormalities, while others showed widespread peripheral nerve involvement with multiple plexiform neurofibromas. As a high benign tumor load and the presence of plexiform neurofibromas are risk factors for malignant transformation patients with multiple sonographically identified plexiform neurofibromas may benefit from more frequent follow-up, while patients without abnormalities may be excluded from this.[7,8] However, the development of sonographic abnormalities over time in NF1 is still unknown. We performed the current follow-up study to gain additional insight in this development. Thereby we aimed to explore the role of HRUS as a screening tool for MPNST in NF1 further.

Methods

We performed this prospective study between January 2016 and May 2017 in the Elisabeth-Tweesteden Hospital, a large general teaching hospital in the Netherlands. NF1 patients without any or with minimal neuropathic complaints that participated in our earlier cross-sectional pilot study were approached.[6] The Brabant Regional Ethics Committee approved this study (NL54951.028.15) and all patients gave written informed consent.

Patients underwent clinical examination and HRUS 1 year after their primary visit. The study procedures at follow-up were comparable to those in our previous pilot study, but nerve conduction studies were not repeated.[6] In summary, we obtained a detailed patient history and performed a neurological examination in which we determined muscle strength and sensory function. One of the investigators (JT) performed an extensive HRUS protocol on a Toshiba ultrasound machine (Xario XG; Toshiba, Tokyo, Japan) with a 7-18 MHz linear-array transducer (PLT-1204BT). The investigator was blinded to the results of

previous HRUS examinations and clinical examination at follow-up was performed after HRUS. Nerve cross-sectional area (CSA) was determined at predetermined sites along the median, ulnar, fibular, tibial, and sural nerves, and brachial plexus (Telleman 2017: supplementary material: figure e-1,) and findings were compared to previously published reference values.[6,9] The presence of PNSTs (localized or plexiform neurofibromas) and nerve enlargement was determined. A PNST was defined as a hypoechoic mass identified along the tract of a nerve segment. If a solitary hypoechoic mass was found, this was classified as a localized neurofibroma, and if multiple hypoechoic serpentine-like fascicles (hypoechoic fascicles showing a varying degree of enlargement and a tortuous course along the tract of the nerve) were found along the tract of a nerve, this was classified as a plexiform neurofibroma. Nerve CSA 100-150% above the reference value was graded as mild enlargement, and nerve CSA >150% above the reference value was graded as severe enlargement. Also, overall sonographic peripheral nerve involvement was graded as 1) minor, 2) moderate, or 3) severe.[6] Minor involvement was defined as the presence of nerve enlargement or a localized neurofibroma in ≤3 nerve segments (≤25% of total nerve segments), without the presence of severe nerve enlargement or a plexiform neurofibroma. Severe nerve involvement was defined as the presence of severe nerve enlargement or a localized neurofibroma in ≥6 nerve segments (≥50% of total nerve segments) or the presence of a plexiform neurofibroma. Patients who did not fulfill either of these criteria were graded as having moderate nerve involvement.

In our previous study we encountered diffusely enlarged nerve segments without the hypoechoic serpentine-like fascicles characteristic for a plexiform neurofibroma. We hypothesized that these changes also represent the presence of a neurofibroma in those nerve segments. However, diffuse nerve enlargement is a feature that can also be found in other types of nerve pathology, e.g. hereditary and inflammatory neuropathies,[10] and we could not perform biopsies to confirm the origin of this diffuse nerve enlargement. At follow-up, we expanded our HRUS protocol with longitudinal assessment over a longer tract in case of a diffusely enlarged nerve segment or in case of a nerve segment without nerve enlargement, but with abnormal fascicles on transversal images. Thereby, we aimed to improve characterization of abnormalities in those nerve segments and to increase detection of neurofibromas.

Results

Clinical Characteristics

We included 16 of the 17 NF1 patients that participated in our previous study. One patient that had minor peripheral nerve involvement on her primary visit didn't participate because of her busy schedule. Of the 12 patients that were asymptomatic at inclusion 1 reported some difficulty with the lifting of heavy objects. Of the 4 patients that reported some aspecific neuropathic complaints at inclusion only 1 reported complaints at follow-up (tingling in both hands consistent with carpal tunnel syndrome). Clinical examination at inclusion revealed mild hypesthesia and loss of strength in hands and distal arms that were not attributable to a specific neuropathy in 4 patients, but those abnormalities were not encountered at follow-up in any of those patients. At follow-up only mild weakness in 1 other patient was found (left deltoid muscle MRC grade 4 in patient 4) and hypesthesia was found in none of the patients.

High-resolution ultrasonography at inclusion

We found sonographic abnormalities at inclusion in all 16 participating patients. PNSTs were found in 10 patients (62.5%): only localized neurofibromas in 3 (18.8%), plexiform neurofibromas in 3 (18.8%), and both in 4 (25.0%). In 8 patients (50.0%) we found diffusely enlarged nerve segments without the presence of a PNST. In 5 of those patients (62.5%) we found a plexiform neurofibroma in at least one other nerve segment. Peripheral nerve involvement was graded as minor in 3 patients (18.8%), moderate in 6 patients (37.5%), and severe in 7 patients (43.8%). More details on HRUS findings at inclusion can be found in our previously published article.[6]

High-resolution ultrasonography at 1 year follow-up

We also found sonographic abnormalities at follow-up in all 16 patients (table 1, detailed results in table A.1). Only localized neurofibromas were found in 2 patients (12.5%), only plexiform neurofibromas in 7 (43.8%), and both in 3 (18.8%). As at inclusion, all encountered PNSTs had clearly defined borders and didn't show any vascularization. All PNSTs found at inclusion were also identified at follow-up, except for one, which was a small (3mm^2), eccentrically positioned localized neurofibroma of the right median nerve in patient 12. In 2 patients new localized neurofibromas were identified.

Due to the extension of our sonographic protocol with longitudinal assessment of diffusely enlarged nerve segments over a longer tract we were able to improve characterization of abnormalities in these nerve segments. In 9 segments with PNSTs originally classified as localized neurofibroma and 13 nerve segments originally classified as diffusely enlarged without PNST we found hypoechoic serpentine-like fascicles compatible with a plexiform neurofibroma during the extensive longitudinal assessment, and those

Table 1 Sonographic abnormalities at inclusion and follow-up

Patients		Peripheral Nerve Involvement at follow-up	Amount of nerve segments					
			Nerve Enlargement		Localized neurofibromas		Plexiform neurofibromas	
			Inclusion (Severe Enlargement)	*Follow-up (Severe Enlargement)*	*Inclu-sion*	*Follow-up (New)*	*Inclu-sion*	*Follow-up (Reclassified /New)*
Minor Nerve Involvement at inclusion	***3***	Minor	1 (0)	1 (0)	0	**2 (2)**	0	0
	9[a]	-	0 (0)	-	0	-	0	-
	14	Minor	1 (0)	2 (0)	1	1 (0)	0	0
	16	Minor	1 (0)	1 (0)	0	0	0	0
Moderate Nerve Involvement at inclusion	***1***[a]	Minor	4 (1)	3 (0)	0	0	0	0
	2	Minor	7 (1)	1 (0)	0	0	0	0
	6	Moderate	6 (0)	6 (0)	0	0	0	0
	10[b, c]	Severe	6 (3)	6 (5)	3	1 (0)	0	**5 (5/0)**
	11[b]	Severe	5 (3)	5 (1)	0	0	0	**2 (2/0)**
	12[c]	Severe	7 (2)	5 (1)	4	1 (0)	0	**2 (2/0)**
Severe Nerve Involvement at inclusion	***4***	Severe	6 (6)	7 (4)	2	**3 (2)**	2	**3 (1/0)**
	5	Severe	11 (9)	11 (10)	0	0	2	**7 (5/0)**
	7	Severe	11 (10)	12 (10)	1	0	6	**10 (4/0)**
	8	Severe	12 (12)	12 (11)	1	0	11	**12 (1/0)**
	13[d]	Severe	9 (9)	9 (9)	0	0	9	9 (0/0)
	15	Severe	11 (6)	10 (7)	2	0	2	**6 (4/0)**
	17	Severe	11 (10)	11 (10)	0	0	10	10 (0/0)

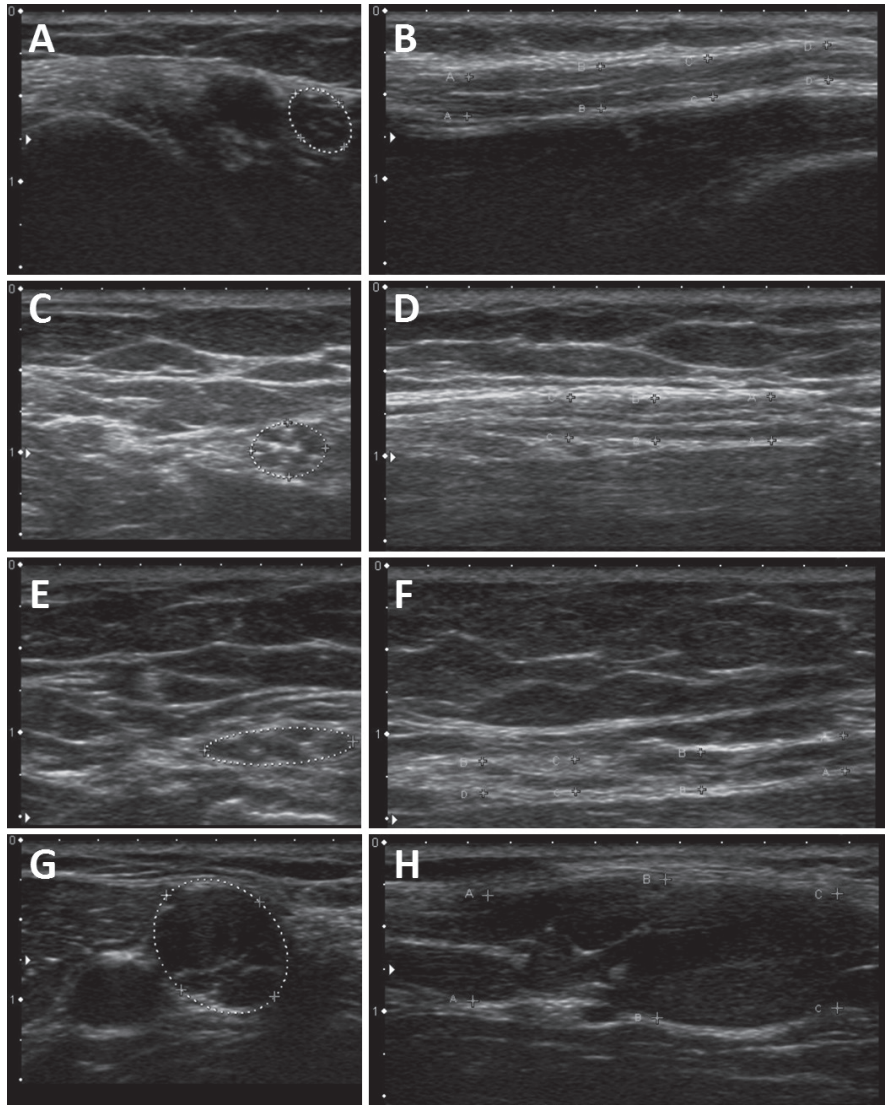
Data are presented as the number of abnormal nerve segments encountered at inclusion and follow-up. Twelve nerve segments were investigated with sonography: the bilateral median, ulnar, fibular, tibial and sural nerves and the brachial plexus. Patients are grouped according to the degree of peripheral nerve involvement at inclusion. Segments with newly identified neurofibromas at follow-up or segments that were reclassified as containing a plexiform neurofibroma based on the more extensive longitudinal evaluation at follow-up are shown in bold.
a. first degree relatives. b. reports minor neuropathic complaints during follow-up visit c. first degree relatives. d. 3 nerve segments not measurable due to a lower leg amputation.

segments were therefore reclassified as segments with a plexiform neurofibroma (Figure 1E-F). Also, 2 segments that had normal nerve size at inclusion but contained an abnormal fascicular pattern showed serpentine-like characteristics on longitudinal images, and were therefore reclassified as segments with a plexiform neurofibromas as well. All of the segments with a plexiform neurofibroma at inclusion showed identical characteristics at follow-up, and none of these segments was reclassified. Although we expanded our sonographic protocol, in 8 patients (50.0%) we still encountered nerve segments that were diffusely enlarged but did not show hypoechoic serpentine-like fascicles compatible with a plexiform neurofibromas even with extensive longitudinal imaging (Figure 1C-D). However, in all these patients at least one other nerve segment contained a plexiform neurofibroma.

All 3 patients with minor and all 7 patients with severe peripheral nerve involvement at inclusion remained in the same category. Of the 6 patients with moderate peripheral nerve involvement at inclusion 1 remained in the same category, while 2 had minor peripheral nerve involvement at follow-up. Three patients were reclassified as having severe peripheral nerve involvement, because one or more nerve segments were reclassified as containing a plexiform neurofibroma based on the findings of more extensive longitudinal imaging.

Nerve size measurements at inclusion and follow-up were compared (figure 2, detailed results in table A.2). We measured a mean change in nerve size at predetermined measurement sites of -0.2mm^2 (±1.6), -0.2mm^2 (±2.0), and 0.3mm^2 (±6.2) respectively in patients with minor, moderate, and severe peripheral nerve involvement. Median change in size of PNSTs was 0.5mm^2 (range -1 to 4mm^2) for localized neurofibromas and 0.5mm^2 (range -35 to 28mm^2) for plexiform neurofibromas (mean -0.1mm^2 (±9.9)). An increase in size was measured in 50.0% of plexiform neurofibromas, while a decrease was measured in 39.4%.

Figure 1 Classification of nerve segments in NF1



Transversal (A,C,E,G) and longitudinal (B,D,F,H) images obtained at follow-up are shown for different patients with NF1. A+B: Median nerve in the upper arm with a normal CSA (9mm^2) and a normal lamellar structure on longitudinal images (Patient 14), nerve segment was classified as normal. C+D: Enlarged median nerve in the upper arm (CSA 12mm^2) with a normal lamellar structure on longitudinal images (Patient 12), nerve segment was classified as diffusely enlarged without PNST. E+F: Enlarged median nerve in the upper arm (CSA 15mm^2) with an abnormal, hypoechoic, serpentine-like structure on longitudinal images (Patient 15), nerve segment was reclassified as plexiform neurofibroma. G+H: Severely enlarged median nerve in the upper arm (CSA 55mm^2) with an abnormal, hypoechoic, serpentine-like structure on longitudinal images (Patient 17), nerve segment was classified as plexiform neurofibroma.

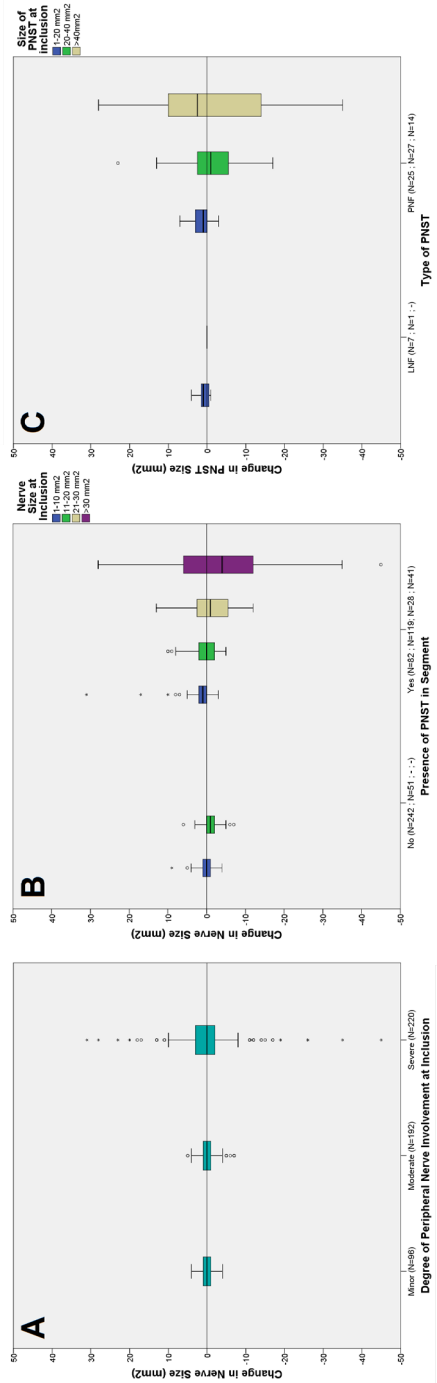


Figure 2 Change in Nerve and PNST Size

The boxplots represent the change in nerve and PNST size after 1 year of follow-up. The PNST classification of the follow-up visit was used for these boxplots. Amount of measurements per investigated group is shown on the x-axis. A: Change in nerve size at predetermined measurement sites for patients with different amounts of peripheral nerve involvement. B. Change in nerve size at predetermined measurement sites for segments with and without the presence of a PNST, grouped by nerve size measured at inclusion. C. Change in PNST size for different types of PNST, grouped by PNST size at inclusion. If a PNST was not present at inclusion or a segment was not classified as containing a PNST at inclusion, the size of the nerve at the predetermined nerve site most close to the PNST was used to determine the group of "PNST size" at inclusion.
LNF: localized neurofibroma. PNF: plexiform neurofibroma. PNST: peripheral nerve sheath tumor.

Discussion

HRUS may have applications as a diagnostic tool in NF1 and also as a screening tool, by preselecting PNSTs that warrant further investigation with MRI or PET-CT to detect malignant transformation. This study aimed to gain additional insight on these applications.

Patients that had minor sonographic peripheral nerve involvement at inclusion remained in this category after 1 year of follow-up. These patients didn't show a large increase in nerve size or amount of PNSTs, and no new plexiform neurofibromas were found in their nerves. A large retrospective study on follow-up in NF1 with whole-body MRI also found that no plexiform neurofibromas developed over time in 100 patients without plexiform neurofibromas at inclusion in a total of 273 patient-years of observation.[4] Although the number of patients in our study was small and follow-up time was short, those findings could indicate that patients with minor sonographic peripheral nerve involvement on HRUS remain so, and that those patients may need less frequent follow-up for MPNST development. However, MPNSTs may arise at locations where plexiform neurofibromas were not detected previously,[7] and plexiform neurofibromas can be present at deeper-lying locations that cannot be assessed with HRUS (e.g. the lumbosacral plexus, or a thoracic, abdominal or pelvic localization).[11,12] Larger studies taking into account those factors should therefore be performed to determine if HRUS can adequately identify patients that can be excluded from follow-up.

Some patients with moderate peripheral nerve involvement showed diffusely enlarged nerve segments without PNSTs at inclusion. At follow-up, we expanded our HRUS protocol with longitudinal imaging over a longer tract. We reclassified several diffusely enlarged nerve segments at follow-up as segments with plexiform neurofibromas. We believe this reclassification reflects the improved detection of plexiform neurofibromas achieved by additional longitudinal imaging rather than the development of new plexiform neurofibromas in those segments during the follow-up period. Nonetheless, even with this extended protocol, we were unable to identify sonographic characteristics of a plexiform neurofibroma in some diffusely enlarged nerve segments (Figure 1C-D). We did, however, find a plexiform neurofibroma in at least one other nerve segment in patients with those diffusely enlarged nerve segments. Diffuse nerve enlargement in NF1 is therefore most likely a sign of the presence of a plexiform neurofibroma, and patients showing diffuse nerve enlargement may thus have a higher risk of developing a MPNST. Still, we could not perform biopsy of diffusely enlarged nerve segments without characteristics of a plexiform neurofibroma to confirm this origin. It remains therefore speculative if patients only showing this kind of abnormality should be suspected of having a plexiform neurofibroma and would therefore have to be monitored more closely for MPNST development.

Combined transverse and longitudinal sonographic imaging allowed good qualitative assessment of the peripheral nerves. All but one of the PNSTs identified at inclusion were also identified at follow-up. We found plexiform neurofibromas in peripheral nerves in arms and legs in 59% of patients, while another study on HRUS reported them in even 85% of patients.[5] In whole-body MRI-studies presence of plexiform neurofibromas was reported in 40-52% of patients,[4,7,11] but in those studies plexiform neurofibromas throughout the entire body were included. In one of these whole-body MRI studies, investigating the distribution of PNSTs in NF1, plexiform neurofibromas of the arms formed only a small proportion of the total amount of plexiform neurofibromas,[11] while in our study plexiform neurofibromas of arm nerves were detected in 53% of patients. A study by Zaidman et al., which included a few patients with neurofibromas, showed that HRUS was more sensitive than MRI when evaluating peripheral nerve pathology.[13] The higher frequency of plexiform neurofibromas in superficial peripheral nerves encountered in our study and that of Winter et al. also suggests that HRUS detects neurofibromas better in those peripheral nerves than MRI.[5] Therefore, HRUS seems a good choice as initial imaging technique to detect nerve pathology when patients with NF1 present with complaints suspect of peripheral neuropathy. As HRUS allows detailed assessment of nerve structure, the choice for this imaging modality could be especially advantageous when (plexiform) neurofibromas are only small.

Nerve size at inclusion and follow-up varied greatly in our cohort. Both substantial increase and decrease in nerve size over time was observed, especially in patients with severe peripheral nerve involvement and in nerve segments with large plexiform neurofibromas. In 60.6% of plexiform neurofibromas size remained stable or increased, while in 39.4% size decreased. A study on whole-body MRI tumor volumetry also observed a decrease in size over time in 35.5% of plexiform neurofibromas (median change -3.4%, range -35.9 to -0.07%),[4] but this represented change in PNST volume instead of the CSA investigated in our study. It seems unlikely that the large bidirectional changes we found in our study are all real anatomical changes in nerve or PNST size. HRUS is an operator-dependent imaging modality and intra- and inter-observer variability are issues that can hinder sonographic assessment of nerve CSA. Plexiform neurofibromas have a tortuous course and this most likely increases the intra- and inter-observer variability of HRUS substantially. Measurement of nerve size on 2D images proved to be unreliable to detect nerve growth in MRI imaging, and currently MRI tumor volumetry is used to detect this.[3,14] Based on our findings repeated measurement of nerve CSA with HRUS also seems to be unreliable to detect nerve growth, and unfortunately at present tumor volumetry with HRUS is not yet possible. In our study we performed an extensive standardized HRUS protocol in which nerve size was measured in a 2D plane at predetermined sites, PNSTs were measured at the site identified as maximally enlarged by the investigator, and in which the investigator was blinded for the results of previous HRUS investigations. Follow-up of a specific

plexiform neurofibroma might be more reliable with HRUS, because nerve CSA can be measured more often along the specific tract of that neurofibroma. Repeated sonographic assessment of specific solitary, or small plexiform neurofibromas during follow-up may therefore still be helpful, but a repeated sonographic protocol assessing nerve size at multiple standardized sites or PNST size of in multiple large plexiform neurofibromas seems not useful during follow-up.

In our study we did not perform MRI imaging of peripheral nerves. A study investigating both HRUS and MRI imaging (as gold standard) could give more insight on the amount of change in nerve size measured by HRUS attributable to intra-/inter-observer variability and the amount of actual change in nerve size. This would provide useful additional information on the applicability of HRUS as a screening tool in NF1. The detection of other sonographic parameters, e.g. vascularization or ill-defined margins, could be helpful to detect malignant transformation of plexiform neurofibromas as well,[5,15] and other (anatomical) landmarks, and supportive ultrasound modules enabling more easy identification of identical measurement sites at follow-up may improve the performance of HRUS as a screening tool. Furthermore, the development of 3D nerve ultrasound could be useful to detect PNSTs warranting further investigation for MPNST. However, larger studies will be necessary to determine the value of those features and techniques.

Conclusions

HRUS allows good qualitative assessment of peripheral nerves in NF1, which makes it advantageous as the imaging technique of first choice for NF1 patients with suspected peripheral neuropathy. Still, detection of a plexiform neurofibroma can be challenging, and transverse and longitudinal imaging should be combined to achieve optimal assessment. Patients with multiple plexiform neurofibromas have a higher risk to develop MPNSTs and should therefore be monitored more closely. Patients with minor sonographic peripheral nerve involvement may remain without plexiform neurofibromas, and may therefore need less frequent follow-up. Patients with severe sonographic peripheral nerve involvement remain in this state, but repeated assessment of nerve CSA in a 2D plane seems unreliable to detect growth of neurofibromas in those patients, especially when multiple large plexiform neurofibromas are present. Therefore, a repeated extensive HRUS protocol with multiple standardized sites of measurement does not seem useful during follow-up to preselect PNSTs warranting further investigation by MRI or PET-CT to detect malignant transformation. Sonographic follow-up of specific solitary or small plexiform neurofibromas might be of use, but this will have to be determined. Studies comparing HRUS and MRI could give additional insight on the usefulness of HRUS as a screening tool. Also, larger studies with a longer follow-up period will be necessary to confirm the findings in our pilot study and to determine the prognostic value of other sonographic features and techniques such as the detection of vascularization or 3D ultrasound.

Table E1 Detailed sonographic findings in asymptomatic NF1 patients at follow-up

		Cut-off	Minor involvement at inclusion				Moderate involvement at inclusion						Severe involvement at inclusion						
Patient number		-	*3*	*9[a]*	*14*	*16*	*1[a]*	*2*	*6*	*10[b]*	*11*	*12[b]*	*4*	*5*	*7*	*8*	*13*	*15*	*17*
Median R	***PNST***	-	**L(13)**	NA	-	-	-	-	-	-	**P(14)[c]**	-	**L(14)**	**P(25)[c]**	**P(33)**	**P(56)**	**P(17)**	**P(17)**	**P(94)**
	WR	11	13	NA	12	11	12	10	13	10	16	9	13	14	16	20	13	14	10
	FA	9	5	NA	7	4	6	7	6	6	7	6	8	16	28	24	8	8	34
	UA	9	7	NA	9	9	9	10	11	9	13	13	13	24	26	56	16	17	55
Median L	***PNST***	-	-	NA	**L(11)**	-	-	-	-	**L(23)**	-	-	-	**P(24)[c]**	**P(31)**	**P(61)**	**P(24)**	**P(15)[c]**	**P(74)**
	WR	11	8	NA	10	9	10	8	12	12	14	8	14	16	14	15	12	12	10
	FA	9	6	NA	7	6	8	6	7	7	7	7	11	13	28	12	16	9	58
	UA	9	7	NA	10	9	11	8	11	12	12	12	12	18	27	31	12	15	49
Ulnar R	***PNST***	-	-	NA	-	-	-	-	-	**P(11)[c]**	-	-	**P(20)**	**P(15)**	**P(23)**	**P(85)**	**P(28)**	**P(13)[c]**	**P(81)**
	WR	7	5	NA	5	5	6	5	5	6	5	6	11	8	8	15	7	9	7
	FA	6	6	NA	5	5	6	4	5	6	6	6	11	10	12	19	12	7	17
	DS	9	5	NA	7	5	5	7	7	6	8	7	16	14	15	15	8	10	9
	SU	9	8	NA	8	12	9	5	9	8	10	7	17	14	10	13	13	9	10
	PS	9	5	NA	9	6	7	7	7	6	10	7	18	13	11	16	10	11	31
	UA	9	6	NA	7	5	8	6	7	6	10	7	15	15	23	18	12	13	81
Ulnar L	***PNST***	-	-	NA	-	-	-	-	-	**P(9)[c]**	-	-	**P(15)[c]**	**P(16)**	**P(24)**	**P(46)**	**P(25)**	**P(12)[c]**	**P(58)**
	WR	7	5	NA	5	6	6	6	6	4	5	6	8	7	9	7	7	5	8
	FA	6	4	NA	6	5	6	5	5	7	7	6	10	10	17	34	10	6	22
	DS	9	4	NA	7	5	6	6	7	6	8	6	9	13	14	15	9	10	17
	SU	9	5	NA	7	7	10	5	9	7	9	8	15	13	13	12	12	10	11
	PS	9	6	NA	8	6	8	6	8	6	7	9	11	16	20	16	11	10	28
	UA	9	5	NA	6	6	7	6	7	6	8	9	12	16	22	18	16	12	58
Plexus R	***PNST***	-	-	NA	-	-	-	-	-	-	-	-	-	-	-	**P(29)**	**P(79)**	-	-
	ST	8	4	NA	5	4	6	2	7	14	11	5	7	8	10	28	21	20	9
	MT	8	3	NA	3	3	6	4	4	5	11	5	6	10	9	29	24	21	5
	IT	8	4	NA	3	3	4	2	3	6	9	4	7	10	5	18	35	7	6
Plexus L	***PNST***	-	-	NA	-	-	-	-	-	-	-	-	-	-	-	**P(40)[c]**	**P(44)**	-	-
	ST	8	5	NA	4	4	7	2	5	8	10	5	8	6	12	40	44	10	7
	MT	8	6	NA	3	3	5	3	4	3	4	7	4	7	9	16	41	18	6
	IT	8	4	NA	2	3	5	3	4	3	4	6	8	6	6	13	31	10	6
Fibular R	***PNST***	-	**L(11)**	NA	-	-	-	-	-	**P(15)[c]**	**P(8)[c]**	**P(13)[c]**	**P(22)**	**P(30)[c]**	**P(15)**	**P(20)**	**P(131)**	**P(20)[c]**	**P(99)**
	FH	11	11	NA	8	8	8	9	14	13	11	7	17	30	12	17	14	10	14
	PF	9	6	NA	8	7	5	8	11	15	8	13	12	20	15	20	46	20	99
Fibular L	***PNST***	-	-	NA	-	-	-	-	-	**P(15)[c]**	-	**P(13)[c]**	**L(13)**	**P(20)[c]**	**P(25)**	**P(28)**	-	**P(16)**	**P(138)**
	FH	11	7	NA	10	8	10	11	14	15	10	7	11	20	14	17	-[d]	11	19
	PF	9	5	NA	8	6	8	8	12	15	7	13	13	20	25	28	-[d]	16	138
Tibial R	***PNST***	-	-	NA	-	-	-	-	-	-	-	-	-	-	**P(19)[c]**	**P(20)**	**P(39)**	-	**P(46)**
	MM	14	8	NA	11	10	13	11	15	12	12	12	10	23	19	20	39	13	46
Tibial L	***PNST***	-	-	NA	-	-	-	-	-	-	-	-	-	**P(27)[c]**	**P(21)[c]**	**P(44)**	-	-	**P(45)**
	MM	14	9	NA	12	10	13	11	18	13	12	12	11	27	21	44	-[d]	14	45
Sural R	***PNST***	-	-	NA	-	-	-	-	-	-	-	**L(6)**	-	-	**P(9)[c]**	**P(14)**	**P(12)**	-	**P(21)**
	PM	3	1	NA	2	2	1	2	2	2	2	6	2	6	9	14	4	4	9
Sural L	***PNST***	-	-	NA	-	-	-	-	-	**P(5)[c]**	-	-	**L(4)**	-	**P(5)[c]**	**P(13)**	-	-	**P(10)**
	PM	3	2	NA	2	2	2	2	3	5	2	2	4	7	5	13	-[d]	5	7
Enlarged segments			1	NA	2	1	3	1	6	6	5	5	7	11	12	12	9	10	11
Severely enlarged segments			0	NA	0	0	0	0	0	5	1	1	4	10	10	11	9	7	10
Segments with PNSTs			2	NA	1	0	0	0	0	6	2	3	6	7	10	12	9	6	10
Classification of nerve involvement at follow-up			Minor	NA	Minor	Minor	Minor	Minor	Moderate	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe

The cut-off values and cross-sectional area (CSA) measurements of the 17 individual NF1 patients at follow-up are shown in mm^2 for the standardized measurement points along the tract of the 12 investigated nerve segments (the bilateral median, ulnar, fibular, tibial and sural nerves and the brachial plexus). CSA measurements 100-150% above the reference value (mild enlargement) are marked light grey, CSA measurements >150% above the reference value (severe enlargement) are marked dark grey. The presence of PNSTs is also shown for each nerve segment (bold). Localized neurofibromas are marked with 'L'(CSA of localized neurofibroma), and plexiform neurofibromas are marked with 'P'(maximum CSA of plexiform neurofibroma along the investigated nerve segment). Data of the patients are grouped according to the degree of peripheral nerve involvement that patients showed at inclusion (1. Minor involvement, 2. Moderate involvement, 3. severe involvement).
WR=wrist, FA=Forearm, UA=Upper arm, DS=Distal of sulcus, SU=In sulcus, PS=Proximal of sulcus, ST=Superior trunk, MT=Medial trunk, IT=Inferior trunk, FH=Fibular head, PF=Popliteal fossa, MM=Medial malleolus, PM=Proximal of malleolus. PNST: peripheral nerve sheath tumor. a. First degree relatives. b. First degree relatives. c. Nerve segment reclassified as containing plexiform neurofibroma. d. Not measurable due to lower leg amputation.

Table E2 Detailed results on changes in nerve size after 1 year of follow-up

		Minor involvement at inclusion				Moderate involvement at inclusion						Severe involvement at inclusion						
Patient number		***3***	***9[a]***	***14***	***16***	***1[a]***	***2***	***6***	***10[b]***	***11***	***12[b]***	***4***	***5***	***7***	***8***	***13***	***15***	***17***
Median R	***PNST***	4	NA	-	-	-	-	-	-	1	-[c]	1	1	3	4	1	0	**28**
	WR	4	NA	2	1	1	2	3	1	**5**	0	1	-2	2	2	2	2	-2
	FA	0	NA	2	0	-1	1	-2	1	-1	0	3	-2	**10**	**17**	0	0	2
	UA	-2	NA	1	4	1	-2	3	0	0	0	-1	**7**	-1	**6**	0	0	**20**
Median L	***PNST***	-	NA	-1	-	-	-	-	0	-	-	-	**6**	**-5**	**23**	1	1	**10**
	WR	-1	NA	1	1	-1	-1	0	1	3	-4	1	0	-1	-2	2	2	-1
	FA	0	NA	1	0	0	1	-2	-3	-1	2	2	**5**	**10**	**7**	0	3	**6**
	UA	-1	NA	1	2	2	**-6**	0	2	2	-2	-4	0	-2	**8**	-1	1	**11**
Ulnar R	***PNST***	-	NA	-	-	-	-	-	2	-	-	**6**	-1	2	**18**	4	3	**-19**
	WR	1	NA	0	1	1	-1	-2	2	-2	2	4	-1	-1	0	0	1	2
	FA	1	NA	0	1	-1	1	1	1	-2	1	-1	2	-1	-4	2	-1	0
	DS	-3	NA	0	2	-2	-1	-2	-1	**-5**	-3	1	3	-1	-4	2	2	2
	SU	0	NA	2	-1	**-7**	-3	0	1	1	-1	1	-2	-4	-1	2	-4	2
	PS	-1	NA	1	1	-1	0	-3	0	1	0	3	-2	-3	-4	-1	0	0
	UA	1	NA	0	0	1	0	1	-1	0	1	-3	0	2	4	-1	3	**-19**
Ulnar L	***PNST***	-	NA	-	-	-	-	-	-1	-	-	-2	**-11**	1	3	**7**	-1	3
	WR	1	NA	0	2	0	1	-2	-1	-1	1	-1	-1	**-5**	-3	2	0	2
	FA	-3	NA	0	1	1	1	-1	3	0	0	-2	-3	**5**	-4	2	0	**6**
	DS	-2	NA	0	1	-1	1	0	-1	-1	-1	-2	-2	-2	0	1	-1	**-8**
	SU	-3	NA	-1	0	1	-2	1	0	4	1	-2	1	-3	-2	-1	-3	-4
	PS	-2	NA	0	1	-2	-1	-3	-2	-2	-2	-4	**-11**	2	0	3	0	2
	UA	-2	NA	1	2	1	2	-1	1	1	2	2	1	-1	**-5**	2	-1	3
Plexus R	***PNST***	-	NA	-	-	-	-	-	-	-	-	-	-	-	**8**	**20**	-	-
	ST	-1	NA	2	-1	0	0	3	2	-1	-1	4	-2	3	**9**	**5**	-1	-1
	MT	-1	NA	-2	0	2	1	-2	1	-1	0	3	-1	1	**8**	**5**	**6**	-4
	IT	1	NA	-1	0	1	0	-3	2	1	1	3	2	-1	**6**	**13**	-2	-2
Plexus L																		
	PNST	-	NA	-	-	-	-	-	-	-	-	-	-	-	**11**	**7**		
	ST	-1	NA	0	0	1	-1	0	0	3	-3	1	-2	3	**31**	**7**	2	0
	MT	1	NA	1	-4	0	-1	1	0	0	-1	0	-1	-1	**10**	**8**	**9**	1
	IT	-1	NA	-2	-1	1	0	1	-1	2	-1	0	0	0	**8**	2	3	-1
Fibular R	***PNST***	0	NA	-	-	-	-	-	**-7**	0	0	1	**-5**	-3	**-12**	2	-1	**-26**
	FH	0	NA	-3	1	-3	-2	4	**-5**	3	1	0	**-5**	-3	**-15**	4	-3	0
	PF	-1	NA	-1	3	-2	-4	0	**-7**	0	0	-3	0	-3	**7**	1	-1	**-26**
Fibular L	***PNST***	-	NA	-	-	-	-	-	4	-	0	-1	**-17**	-1	-4	-	-3	**-35**
	FH	-3	NA	-1	-1	-3	-2	2	4	1	-1	-1	**-17**	-1	4	-[d]	1	3
	PF	-4	NA	-1	1	-1	-3	0	4	-2	0	-1	-1	-1	-4	-[d]	-3	**-35**
Tibial R	***PNST***	-	NA	-	-	-	-	-	-	-	-	-	-	-2	**-6**	**13**	-	-4
	MM	-1	NA	0	-1	-2	-1	**5**	0	3	-2	-1	3	-2	**-6**	**13**	1	-4
Tibial L	***PNST***	-	NA	-	-	-	-	-	-	-	-	-	0	-1	**-14**	-	-	**-11**
	MM	0	NA	0	-2	1	**-5**	1	-2	2	3	-1	0	-1	**-14**	-[d]	-2	**-11**
Sural R	***PNST***	-	NA	-	-	-	-	-	-	-	1	-	-	0	4	-2	-	**-12**
	PM	-1	NA	0	0	-2	-2	-1	0	-1	1	-1	-2	0	4	1	-1	3
Sural L	***PNST***	-	NA	-	-	-	-	-	2	-	-	2	-	0	3	-	-	-12
	PM	0	NA	0	0	-1	-2	1	2	-1	-1	2	0	0	3	-[d]	1	2
Enlarged segments		0	NA	1	0	-1	-6	0	0	0	-2	1	0	1	0	0	-1	0
Severely enlarged segments		0	NA	0	0	-1	-1	0	2	-2	-1	-2	1	0	-1	0	1	0
Segments with PNSTs		2	NA	0	0	0	0	0	3	2	-1	2	5	3	0	0	2	0

Changes in cross-sectional area (CSA) measurements of the 17 individual NF1 patients are shown in mm^2 for the identified peripheral nerve sheath tumors (PNSTs) and standardized measurement points along the tract of the 12 investigated nerve segments (the bilateral median , ulnar, fibular, tibial and sural nerves and the brachial plexus). An increase of nerve CSA of 3-4mm^2 is marked dark grey, and an increase of nerve CSA of ≥5 mm^2 is marked dark grey and bold. A decrease of nerve CSA of 3-4mm^2 is marked light grey, and an decrease of nerve CSA of ≥5 mm^2 is marked light grey and bold. Data of the patients are grouped according to the degree of peripheral nerve involvement that patients showed at inclusion (1. Minor involvement, 2. Moderate involvement, 3. Severe involvement).
WR=wrist, FA=Forearm, UA=Upper arm, DS=Distal of sulcus, SU=In sulcus, PS=Proximal of sulcus, ST=Superior trunk, MT=Medial trunk, IT=Inferior trunk, FH=Fibular head, PF=Popliteal fossa, MM=Medial malleolus, PM=Proximal of malleolus. PNST: peripheral nerve sheath tumor. a. First degree relatives. b. First degree relatives. c. PNST not identified at follow-up. d. Not measurable due to lower leg amputation.

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Chapter 9

Neurofibromatosis Type 2

Nerve Ultrasound shows Subclinical Peripheral Nerve Involvement in NF2

JA Telleman, MD Stellingwerff, GJ Brekelmans, LH Visser

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Abstract

Introduction: Neurofibromatosis type 2 (NF2) is mainly associated with central nervous system (CNS) tumors. Peripheral nerve involvement is described in symptomatic patients, but evidence of subclinical peripheral nerve involvement is scarce.
Methods: We conducted a cross-sectional pilot study in 2 asymptomatic and 3 minimally symptomatic patients with NF2 to detect subclinical peripheral nerve involvement. Patients underwent clinical examination, nerve conduction studies (NCS) and high-resolution ultrasonography (HRUS).
Results: A total of 30 schwannomas was found, divided over 20 nerve segments (33.9% of all investigated nerve segments). All patients had at least one schwannoma. Schwannomas were identified with HRUS in 37% of clinically unaffected nerve segments and 50% of nerve segments with normal NCS findings.
Discussion: HRUS shows frequent subclinical peripheral nerve involvement in NF2. Clinicians should consider peripheral nerve involvement as a cause of weakness and sensory loss in the extremities in patients with this disease.

Introduction

Neurofibromatosis type 2 (NF2) is a hereditary condition with a prevalence of 1 in 25,000.[1] The occurrence of bilateral vestibular schwannomas is the hallmark of the disease, but numerous other intracranial tumors can develop. Although NF2 is mainly associated with those intracranial tumors, schwannomas can develop in peripheral nerves. Peripheral neuropathy, with or without local nerve compression by a tumor, is reported in up to 66% of patients, though evidence is scarce.[1-3] Subclinical peripheral nerve involvement has also been reported based on nerve conduction studies (NCS), but this information is even more scarce.[3,4] However, this feature may be of importance, as neuropathic complaints could develop in the course of the disease.

High-resolution ultrasound (HRUS) is used increasingly in the analysis of polyneuropathies.[5] Sonographic characteristics of schwannomas have been described.[6-10] We found a large variation in subclinical sonographic peripheral nerve involvement in neurofibromatosis type 1 (NF1).[11] We performed the current study to determine if subclinical peripheral nerve involvement can be observed in NF2 as well.

Methods

We conducted a cross-sectional pilot study between January and July 2016 at the Elisabeth-Tweesteden Hospital, Tilburg, The Netherlands, a large general teaching hospital. The Brabant Regional Ethics Committee approved this study (NL54951.028.15) and all patients gave written informed consent. Known NF2 patients without neuropathic complaints were considered for inclusion at our outpatient department. Inclusion criteria were: 1) diagnosis of NF2 based on the Manchester diagnostic criteria,[12] and 2) age >18/<80. Exclusion criteria were: 1) Comorbidity associated with (poly)neuropathy (e.g. diabetes, alcoholism), and 2) inability to undergo HRUS.

Patients underwent a standardized clinical examination, nerve conduction studies (NCS) and HRUS following a previously published protocol.[11] In summary, one of the investigators (MS) obtained details on clinical history and investigated sensation and muscle strength. NCS of the median, ulnar, fibular, tibial and sural nerves were analyzed by a second investigator (GB). A limited, unilateral protocol was used to limit the burden for participants, but the investigator could choose to measure specific nerves bilaterally. A third investigator (JT) performed bilateral evaluation of the brachial plexus, median, ulnar, fibular, tibial and sural nerves (12 nerve segments total) with HRUS. A Toshiba ultrasound machine (Xario XG; Toshiba, Tokyo, Japan) with a 7-18 MHz linear-array transducer (PLT-1204BT) was used. Nerve cross-sectional area (CSA) was measured at predetermined sites and at sites at

which schwannomas were identified. CSA values measured at predetermined sites were compared to previously published reference values.[13] All investigators were blinded to results of the other testing modalities.

Results

Patient Characteristics

Five patients with NF2 were eligible for inclusion: 1 female and 4 males (age 30-66). Though all patients claimed to be asymptomatic upon entering the study, three of them reported mild complaints of sensory loss or weakness in an arm or leg (Patients 1, 2 and 5) during the patient history. We decided not to exclude those patients, as those complaints were vague and not clearly attributable to a specific peripheral nerve, and because NCS and HRUS could reveal a much wider scope of peripheral nerve involvement than the reported complaints would lead the investigator to suspect.

Clinical examination, NCS and HRUS findings

A total of 59 nerve segments was investigated with clinical examination and HRUS in the 5 patients; 1 nerve segment was not investigated because one patient had a sural nerve graft. Of all nerve segments, 28 were also investigated with NCS.

Clinical examination revealed hypesthesia of the right arm and lateral side of the right lower leg in patient 1, of the lateral side of the left foot in patient 2, and of the lateral side of the lower legs in patient 5. No loss of strength was found. Although symptoms were not clearly attributable to impairment of a specific peripheral nerve, a nerve segment was regarded as clinically affected if an identified area of hypesthesia involved part of the cutaneous region by that particular nerve segment. Therefore 8 nerve segments (13.6%) were regarded as clinically affected in further analysis (1 median, 1 ulnar, 3 fibular, and 3 sural nerves).

NCS showed abnormalities in 4 patients. Detailed results are shown in table e-1. In 2 patients, signs of subclinical carpal tunnel syndrome were found, and 1 patient had absent SNAPs of the sural nerves without other signs of polyneuropathy. Non-specific abnormalities not fitting a mononeuropathy or polyneuropathy were found in 9 nerve segments.

HRUS showed abnormalities in all 5 patients, with 30 abnormal nerve segments (50.8%) total. Detailed findings are shown in table 1. We found 30 schwannomas divided over 20 nerve segments (33.9%). Schwannomas were most often encountered in the median nerve (6/10), followed by the ulnar (5/10), fibular (4/10), and tibial nerves (4/10), brachial plexus (1/10), and sural nerve (0/9). All patients had at least one schwannoma (1-9

Table 1 High-resolution ultrasound findings of NF2 patients

Segments Investigated		Reference Values	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Median R	***No. of schwannomas (max CSA)***	-	2 (34)	-	3 (46)	1 (166)	2 (15)
	CSA at standard sites (mm ^{2}): Wrist/forearm/arm	11/9/9	**13**/7/9	10/8/**10**	**18**/9/**16**	9/5/**10**	8/4/9
Median L	***No. of schwannomas (max CSA)***	-	-	-	-	4 (428)	1 (9)
	CSA at standard sites (mm ^{2}): Wrist/forearm/arm	11/9/9	8/5/9	10/6/**10**	**17**/9/**13**	10/9/**15**	6/5/7
Ulnar R	***No. of schwannomas (max CSA)***	-	1 (89)	-	1 (11)	-	-
	CSA at standard sites (mm ^{2}): Wrist/forearm/distal sulcus/ sulcus/proximal sulcus/arm	7/6/9/ 9/9/9	**9**/**8**/**10**/ **10**/**11**/**15**	7/6/9/ 8/9/5	6/5/7/ **11**/**10**/7	7/5/7/ 6/7/6	3/4/5/ 6/7/5
Ulnar L	***No. of schwannomas (max CSA)***	-	-	1 (19)	-	2 (10)	2 (19)
	CSA at standard sites (mm ^{2}): Wrist/forearm/distal sulcus/ sulcus/proximal sulcus/arm	7/6/9/ 9/9/9	6/5/5/ **10**/8/8	6/6/6/ 7/**19**/4	5/6/6/ 8/8/9	6/**8**/7/ **10**/6/5	4/5/6/ 7/5/**19**
Plexus R	***No. of schwannomas (max CSA)***	-	-	-	1 (53)	-	-
	CSA at standard sites (mm ^{2}): Superior/median/inferior trunk	8/8/8	**29**/**24**/**10**	8/5/4	**10**/**10**/-†	5/4/4	4/2/4
Plexus L	***No. of schwannomas (max CSA)***	-	-	-	-	-	-
	CSA at standard sites (mm ^{2}): Superior/median/inferior trunk	8/8/8	2/5/3	6/6/3	7/6/7	5/5/5	5/5/6
Fibular R	***No. of schwannomas (max CSA)***	-	2 (185)	-	-	1 (11)	1 (27)
	CSA at standard sites (mm ^{2}): Fibular head/popliteal fossa	11/9	**15**/6	11/8	10/7	**16**/9	**14**/**10**
Fibular L	***No. of schwannomas (max CSA)***	-	-	-	1 (26)	-	-
	CSA at standard sites (mm ^{2}): Fibular head/popliteal fossa	11/9	9/5	10/**11**	**12**/**13**	**20**/8	11/6

Table 1 Continued

Segments Investigated		Reference Values	Patient 1*	Patient 2*	Patient 3	Patient 4	Patient 5*
Tibial R	*No. of schwannomas (max CSA)*	-	-	-	-	1 (11)	1 (19)
	CSA at standard sites (mm²): Ankle	14	13	13	9	10	13
Tibial L	*No. of schwannomas (max CSA)*	-	-	-	1 (74)	-	1 (92)
	CSA at standard sites (mm²): Ankle	14	9	**17**	12	10	12
Sural R	*CSA at standard sites (mm²): Proximal to lateral malleolus*	3	3	-‡	**4**	2	**4**
Sural L	*CSA at standard sites (mm²): Proximal to lateral malleolus*	3	3	2	3	1	2
Total segments with abnormalities			5	5	7	6	7
Total segments with schwannomas			3	1	5	5	6

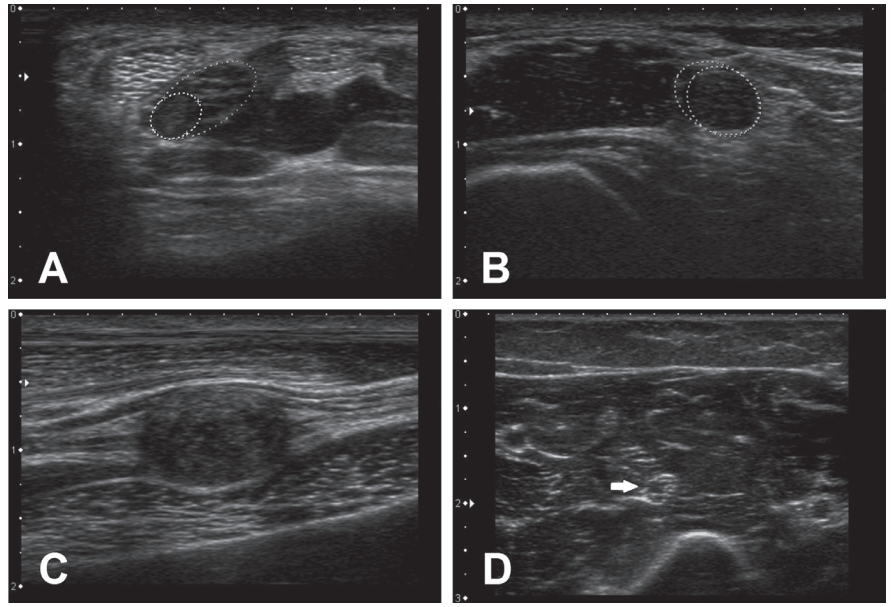
Sites with an increased CSA are shown in bold. The number of schwannomas (CSA of the largest schwannoma) is also shown for each nerve segment.
† Not identifiable. ‡ Missing due to sural nerve graft.

schwannomas/patient, size 3-428mm^2). Most schwannomas were hypoechoic, had clearly defined borders, and showed no vascularization. One patient had 2 'ancient schwannomas', which showed both hypoechoic and hyperechoic regions but no vascularization.

Apart from schwannomas, we found nerve enlargement along the tract of nerve segments. Enlargement was focal; no characteristics of a plexiform neurofibroma or diffuse enlargement were observed. At the focally enlarged sites we frequently observed abnormal, hypoechoic fascicles (Figure 1). In 10 nerve segments (16.9%) we only found focal enlargement without these abnormal fascicles. However, in 3 segments enlargement was only present at entrapment sites, and in 6 enlargement was only mild. One segment (right brachial plexus of patient 1) showed more severe enlargement. Though this enlargement was most likely due to a schwannoma, we were unable to classify it as such with certainty, as the brachial plexus is always hypoechoic on HRUS and we were unable to visualize a clear solitary hypoechoic lesion.

HRUS findings (brachial plexus excluded) were compared to the findings of clinical examination and NCS (Table 2). HRUS showed schwannomas in 37% of the clinically unaffected nerve segments and 50% of the nerve segments with normal NCS findings.

Figure 1 Examples of sonographic abnormalities in NF2



A. Schwannoma of the right median nerve in the forearm (Patient 3, CSA 9mm^2), B. Schwannoma of the left fibular nerve in the popliteal fossa (Patient 3, CSA 21mm^2), C. Longitudinal view of a schwannoma of the left median nerve in the forearm (Patient 4), D. Hypoechoic fascicle in the left median nerve in the forearm (white arrow, Patient 5).

Table 2 Correlation of clinically affected nerves, NCS, and HRUS

		HRUS (N=49): Number (%)			NCS (N=28): Number (%)	
		Normal	*Abnormal*	*Schwannoma*	*Normal*	*Abnormal*
Clinically affected	***No (N=41)***	18 (39%)	23 (56%)	15 (37%)	21 (88%)	3 (12%)
	Yes (N=8)	3 (38%)	5 (62%)	4 (50%)	3 (75%)	1 (25%)
NCS	***Normal (N=24)***	8 (33%)	16 (66%)	12 (50%)		
	Abnormal (N=4)	2 (50%)	2 (50%)	1 (25%)		

HRUS: high-resolution ultrasonography of the nerves, NCS: nerve conduction studies

Discussion

Subclinical peripheral nerve involvement in NF2 patients has been reported in NCS and whole-body MRI studies.[2,3] In our study we found multiple sonographic abnormalities in asymptomatic or minimally symptomatic patients. HRUS identified abnormalities more often than NCS, a discrepancy that is also observed frequently in other peripheral nerve diseases.[14]

Sonographic characteristics of schwannomas in our study were comparable to those in previous studies, presenting as solitary round or oval hypoechoic masses with clearly defined borders and no vascularization.[6,7,9,10] In our study all patients had one or multiple schwannomas. A recent study also found a high incidence of schwannomas on HRUS: in 8 of 10 NF2 patients presenting for routine visits at the outpatient clinic, at least one schwannoma was observed, but the correlation with clinical symptoms was not described.[10] Another recent study found that abnormal, hypoechoic fascicular structure was frequently observed in NF2 patients with neuropathy.[15] The authors did not find schwannomas in their patients, but only median nerves were investigated. In our study, we also frequently observed focal nerve enlargement and hypoechoic fascicles. Although we did not perform a biopsy to obtain a histopathological confirmation, those fascicular lesions are most likely schwannomatous. We did find nerve enlargement without an abnormal fascicular pattern at some sites, but this was only very mild or at entrapment sites only, which both are most likely incident findings. Several histopathological studies on peripheral nerves in NF2 showed endoneurial edema, Schwann cell complexes, and proliferations of endoneurial cells,[3,4,16] and an MRI study reported on non-compressive microlesions in nerves that correlated with the severity of the polyneuropathy.[17] Though NF2 is considered to be mostly associated with central nervous system tumors, these findings indicate subclinical peripheral nerve involvement in this disease. Although sensory loss and weakness were previously thought to derive mainly from central nervous system tumors, these findings in peripheral nerves should be taken into account when evaluating NF2 patients with such symptoms.

The current study had several limitations. Only 5 of our patients were eligible for inclusion and several of those reported some non-specific complaints during the history. Nonetheless, all patients had schwannomas of one or multiple clinically unaffected nerves, which confirms that peripheral nerves can be involved in NF2. Our findings and those of several previous studies indicate that this involvement may even be very frequent, but larger studies will be needed to determine the exact scope of subclinical peripheral nerve involvement in NF2. Also, a limited NCS protocol was used, meaning that subclinical peripheral nerve involvement may be more extensive than that found in this study.

In conclusion, HRUS shows frequent abnormalities of the peripheral nerves, confirming that NF2 is not only a disease with involvement of the central nervous system. Clinicians should consider peripheral nerve involvement as a cause of weakness or sensory loss in the arms and legs in these patients. HRUS appears to be a useful tool to evaluate this, as it is a quick and inexpensive method to investigate multiple nerves. Clinicians should seek anatomically meaningful relationships between abnormalities identified with HRUS and patients' symptoms, as schwannomas may remain asymptomatic, even if they are very large.

Table E1 Findings on nerve conduction studies

Motor conduction studies								
			Abnormal	**Patient 1 (R/L)**	**Patient 2 (R/L)**	**Patient 3 (R/L)**	**Patient 4 (R/L)**	**Patient 5 (R/L)**
Age (years)			-	66	55	52	45	30
Median	***Distal CMAP***	***mV***	<3.5	**2.2** / 4.0	- / 9.6	- / **2.4**	- / 17.7	- / 9.1
	DML	***ms***	>4.2	**4.5** / 4.7	- / 3.8	- / **5.1**	- / 3.7	- / 3.8
	CV forearm	***m/s***	<48.0	**35.3** / 48.0	- / 55.2	- / **38.7**	- / **45.5**	- / 54.0
Ulnar	***Distal CMAP***	***mV***	<2.8	7.3 / -	- / 12.8	- / 6.3	- / 16.9	- / 11.8
	DML	***ms***	>3.4	2.9 / -	- / **3.5**	- / 2.9	- / 3.1	- / 3.0
	CV forearm	***m/s***	<49.0	57.5 / -	- / 55.1	- / **47.7**	- / 56.5	- / 52.6
	CV across elbow	***m/s***	<50.0	55.5 / -	- / **46.6**	- / 84.6	- / 50.0	- / 60.5
Fibular	***Distal CMAP***	***mV***	<2.5	- / 7.0	- / 7.1	- / 5.5	- / 3.6	4.8 / -
	DML	***ms***	>5.5	- / 4.8	- / 5.0	- / 3.9	- / 5.4	4.0 / -
	CV lower leg	***m/s***	<40.0	- / 40.2	- / 40.1	- / 46.2	- / 41.2	45.8 / -
	CV across fibular head	***m/s***	<40.0	- / 52.0	- / 43.6	- / 63.6	- / 51.9	42.5 / -
Tibial	***Distal CMAP***	***mV***	<2.9	15.6 / 21.9	3.2 / -	10.5 / -	16.0 / -	8.7 / -
	DML	***ms***	>6.0	**6.7** / **8.6**	4.6 / -	4.7 / -	5.2 / -	4.3 / -
	CV lower leg	***m/s***	<41.0	**37.2** / **37.2**	44.9 / -	**40.7** / -	45.4 / -	42.1 / -
Sensory conduction studies								
			Abnormal	**Patient 1 (R/L)**	**Patient 2 (R/L)**	**Patient 3 (R/L)**	**Patient 4 (R/L)**	**Patient 5 (R/L)**
Median	***SNAP***	***µV***	<11/11/5*	**NR** / -	- / 31.5	- / **4.7**	- / **9.2**	41.1 / -
Ulnar	***SNAP***	***µV***	<11/7/5*	5.7 / -	- / 35.8	- / 7.3	- / 26.1	37.3 / -
Sural	***SNAP***	***µV***	<8/5/2*	- / 6.7	SG / -	**3.0** / -	**NR** / **NR**	- / -
	CV	***m/s***	<38.0	- / **37.1**	SG / -	38.7 / -	**NR** / **NR**	- / -

Abnormal values are shown in bold. A '-' symbol is shown if no measurement was performed. *per age category (18-39 years / 40-59 years / >60 years).
CMAP: Compound muscle action potential, CV: conduction velocity, DML: Distal motor latency, NR: no responds, SG: sural nerve graft, SNAP: sensory nerve action potential.

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Chapter 10

General Discussion

General discussion

For clinicians, diagnosing peripheral nerve disease can be difficult and treating it can sometimes feel like being a fortune teller, staring indistinctly into a crystal ball (**cover**). Patients' symptoms can vary greatly, discriminating treatment-responsive neuropathies from non-responsive mimics may be very difficult, and if a potentially treatment-responsive neuropathy is diagnosed it can be very hard to predict treatment-response. Currently, with nerve ultrasound, a new technique is emerging, which may provide vital clues on diagnosis and disease course. The goal of this thesis was to determine when nerve ultrasound is of added diagnostic and prognostic value, and in which circumstances nerve ultrasound does not contribute to management of peripheral nerve disease, thereby forcing the clinician to keep staring into his crystal ball.

Reliability of nerve ultrasound in routine practice and research

Nerve ultrasound is a diagnostic tool that is increasingly used in routine clinical practice. It is applied in the evaluation of mononeuropathies, polyneuropathies (**chapter 2**), and peripheral nerve trauma, and has already been incorporated in some diagnostic guidelines, e.g. the Dutch guideline for carpal tunnel syndrome.[1-4] It has several advantages over other commonly employed diagnostic tools, including nerve conduction studies (NCS) and MRI, as it is patient-friendly, cheap, often readily available, and it allows quick investigation of multiple nerves. On the other hand, nerve ultrasound requires training and is operator dependent, which could limit the scope in which it can be applied within the field of neurology.

In this thesis (**chapter 3**) we investigated interobserver variability of nerve ultrasound, which is a key feature of reliability and applicability in routine clinical practice.[5] We aimed to determine interobserver variability in a setting that approximated standard clinical practice, as such a setting gives most information on actual performance of nerve ultrasound in a general neurological practice. Therefore, we set up a multicenter study in which multiple investigators performed real-life investigation of patients (rather than performing nerve size measurement on still images), and investigators were allowed to use their preferred positioning of patients. In addition, we performed investigations on different sonographic devices, as hospitals often make use of different makes, and it is of importance to know if this introduces additional variability. Ideally, this study would have been performed by investigating the same patients in all participating hospitals and by all investigators, but unfortunately this was not feasible. Instead, we chose to perform investigation of all patients by a reference investigator and one of the local investigators, and constructed a multilevel mixed model to estimate the effect of multicenter sonographic investigation. To get a better grasp of factors leading to interobserver variability we not only analyzed data for systematic differences, but analyzed various

aspects of interobserver variability, and also tested multiple factors commonly encountered in routine clinical practice that might contribute to interobserver variability. In our study, we found that, overall, no systematic differences between investigators were present. The use of different devices, as well as the performance of ultrasound in different centers, had no influence on interobserver variability, while the investigated nerve sites had. As we performed an extensive ultrasound protocol including entrapment as well as non-entrapment sites, results of our study may be applicable on ultrasound investigation in both mono- and polyneuropathies, and the above-mentioned findings are all important when considering the applicability and reliability of nerve ultrasound.

First, the fact that no systematic differences were found indicates that nerve ultrasound can be readily applied in routine practice. Despite investigation in a different center and on a different brand of ultrasound device, clinicians may translate results of published studies directly to their clinical practice, which is different from NCS, which have significant interobserver variability.[6-8] Still, there are requirements to perform reliable translation of study results to clinical practice. Clinicians will have to be trained properly,[9] and characteristics of the investigated patients have to be comparable to those of the ones investigated in the published study. For instance, nerve size tends to be smaller in patients of Indian descent compared to patients of Dutch descent, and in such case published reference values may not be representative.[10,11] However, our study still indicates in countries with comparable patient populations, it is not necessary for each center to collect its own reference values. The high reproducibility of nerve ultrasound, therefore, implies that it can be applied reliably in routine practice, also in centers that did not participate in scientific research on the technique.

Also, the findings of our interobserver study have important implications for future research. To advance the knowledge on nerve ultrasound multicenter studies will be necessary, especially in more rare peripheral neuropathies.[1] The absence of systematic differences between centers identified in our study indicates that multicenter data on nerve size can be acquired and pooled reliably, and that future multicenter studies on nerve ultrasound are indeed feasible. The difference in interobserver variability observed at different nerve sites, i.e. high variation in brachial plexus and leg nerve sites, and low variation in arm nerve sites, is also important considering future research. Measurements at some nerve sites seem to be more reliable than others, and authors should take this into account when conceptualizing new studies and devising new diagnostic protocols.

Diagnostic value of nerve ultrasound in chronic demyelinating polyneuropathies

Within the group of acquired chronic polyneuropathies, treatment-responsive demyelinating polyneuropathies, including chronic inflammatory demyelinating polyneuropathy (CIDP) and multifocal motor neuropathy (MMN), have to be discriminated from much more common axonal types of polyneuropathy, as well as from other non-treatable diseases, such as amyotrophic lateral sclerosis (ALS).[12-15] Currently, NCS criteria are the centerpiece in diagnosing CIDP and MMN, but supportive criteria, e.g. an abnormal MRI of the brachial plexus or lumbar puncture, have been added to diagnostic criteria, because identification of these treatment-responsive polyneuropathies remains challenging.[14-18]

In a large study in the UMC Utrecht nerve ultrasound was found to be a diagnostic tool with high sensitivity and specificity for discriminating patients with a confirmed diagnose of CIDP and MMN (according to the international consensus criteria) from patients with disease mimics.[4] In this thesis (**chapter 5**) we validated this protocol in a multicenter setting in patients with suspected chronic demyelinating polyneuropathy, and found that an adjusted protocol, excluding measurements of the C6 and C7 nerve roots, had high sensitivity and moderate specificity, with comparable results in all participating hospitals.[19] In addition, we found that nerve ultrasound was able to identify up to 25% additional patients with a treatment-responsive polyneuropathy compared to the conventional NCS (**chapter 4**).[20] This feature was also observed in all centers participating in the multicenter study.[19] Therefore, nerve ultrasound seems not only to be a reliable diagnostic tool to detect CIDP or MMN, but even to improve detection of patients with potential treatment-response.

The diagnostic value of nerve ultrasound has also been shown in several other studies, though the approach differed among studies.[1,4,21-24] Establishing a diagnosis of CIDP or MMN in some cases revolved on mere presence of nerve enlargement at a non-entrapment site and in others on extensive scoring systems.[1,21-23,25] The short nature of our protocol, with inclusion of only three nerve sites that all have low inter-observer variability, may make it more preferable to use in routine clinical practice than more all-encompassing protocols, and it is currently the only protocol validated in a multicenter cohort of treatment-naïve patients clinically suspected of the investigated diseases.[1,5,19,20,25] Still, our protocol had only moderate specificity and diagnosing CIDP and MMN revolved on identification of nerve enlargement at set nerve sites only. There may be other morphological features that could optimize diagnostic yield. Not only the presence of nerve enlargement, but also the pattern of distribution of this nerve enlargement may provide diagnostic information on underlying pathology. For instance, in Charcot-Marie-Tooth (CMT) 1A profound diffuse enlargement is observed, whereas this is not the case in other types of (axonal) CMT, and nerve enlargement in leprosy is mainly distributed just

proximal of nerve entrapment sites.[1,24,26-28] Such characteristic patterns may also be present in CIDP and MMN or in disease mimics that are also associated with nerve enlargement (**figure 1**; unpublished data). Discerning these patterns may thereby aid in establishing a correct diagnosis, and in addition features, such as nerve vascularization, fascicular size, and echogenicity may also have discriminative value.[29] Future studies will have to determine which features are contributive to detect CIDP and MMN and which is the most reliable and feasible diagnostic ultrasound protocol in routine clinical practice.

Though further study is required to determine the optimal diagnostic protocol, the results in chapter 3 and 4 underline that nerve ultrasound has clear diagnostic value in CIDP and MMN, and, thus, that it should be incorporated in future consensus criteria for chronic demyelinating polyneuropathy.[4,19-23] Our short and easily applicable protocol, including only the median nerve in forearm and arm and the C5 nerve root, improved detection of treatment-responsive patients significantly. Therefore, nerve ultrasound could be added to the already existing supportive criteria within diagnostic guidelines, but given its large additional value it could also be a tool complementary to NCS, performed simultaneously or even prior to NCS. As nerve ultrasound has high sensitivity and lower specificity, as opposed to NCS, it may be applied first to exclude demyelinating neuropathy, and afterwards NCS could be performed in patients with abnormal ultrasound to confirm the diagnosis. In such a strategy a thorough evaluation should be performed in patients showing only nerve ultrasound abnormalities to exclude other causes because of the lower specificity of the technique, but trial-treatment should be considered in patients with the clinical phenotype of CIDP or MMN and no apparent other cause of symptomatology. However, though the results of our studies are promising, the exact timing and place of nerve ultrasound within diagnostic strategies to identify CIDP or MMN will have to be determined in future studies, also taking into account cost-effectiveness.

Prognostic value of nerve ultrasound

Predicting disease course and treatment effect in chronic inflammatory demyelinating polyneuropathies can be very hard. Response to intravenous immunoglobulin (IVIg) varies markedly, and only few prognostic factors have been identified in CIDP and MMN, including axonal loss on NCS and longer disease duration to start of treatment.[30-33] As a result, the search for new prognostic markers is ongoing, also because targeted treatment is preferable because of the high costs of IVIg treatment. In this thesis (**chapter 6**) we investigated the potential prognostic value of nerve ultrasound. Previous studies reported a correlation between decrease in nerve size and improved outcome measures, and normalization of nerve size in patients with positive treatment response.[22,34-37] In our study we could not replicate these findings. We saw a high variability in nerve size development, which was also the case in multiple investigated subgroups (e.g. pure motor CIDP, patients that reached remission). However, previous studies had methodological shortcomings,

Figure 1 Distribution of nerve enlargement in the median nerve in neuropathies

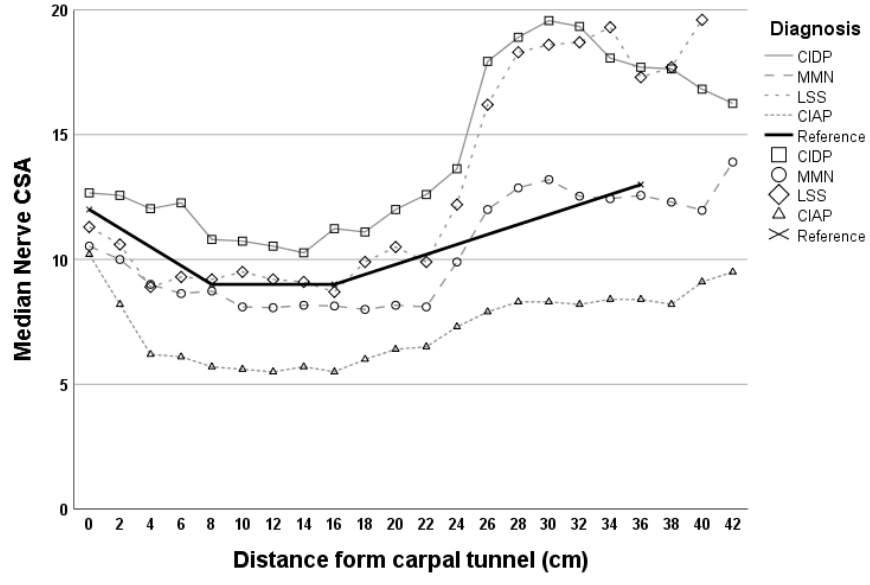

Figure 1 shows the mean nerve size in different disease types obtained by inching of the median nerve, and the distribution of sonographic nerve enlargement throughout the arm. Bilateral measurements of the median nerve were taken at every 2cm in the arm of patients with CIDP (n=15), MMN (n=15), LSS (n=5), and CIAP (n=5). Nerves in CIDP and LLS, and to a lesser degree in MMN are significantly larger than in CIAP, especially in the proximal segment of the median nerve.

e.g. small sample size, inclusion of already treated patients only, and a retrospective design. In addition, publication bias of only patients showing positive treatment response and nerve size decrease may play a role. Our study consisted of a large cohort of both newly diagnosed and already treated patients, which allowed us to gain a broad insight in development of nerve size and prognostic value of nerve ultrasound, and this most likely revealed the great heterogeneity in nerve enlargement and treatment response.

A variety of clinical phenotypes can be placed under the definition of CIDP. Patients' complaints may range from only confined sensory loss or tremor to extensive diffuse sensorimotor loss in arms and legs. It is, therefore, more likely that CIDP is a clinical syndrome with a large heterogeneity, rather than disease with a distinct pathophysiological process. Our ultrasonographic findings, with large heterogeneity in amount, distribution, and development of nerve enlargement support this hypothesis. Segmental de- and remyelination, inflammatory cell infiltrates and endoneurial edema, interstitial accumulation of amorphous substances, and fibrosis can all cause nerve enlargement, but measurement

of nerve size only cannot adequately discriminate between these processes.[38-40] We found that patients showing only nerve enlargement at the brachial plexus had better treatment response, while patients with diffuse of peripheral nerve involvement tended to have a worse response. This may be caused by different pathophysiological processes underlying sonographic nerve enlargement. In an MRI study enlargement of the brachial plexus was not associated with disease course, but in this study no MRI evaluation of peripheral nerves was performed.[41] Further studies will have to be performed to determine if patients with different distributions of nerve abnormalities have indeed different disease types, or if these different distributions are rather dependent on temporal evolution in a distinct disease process.

The fact that presence of nerve enlargement had prognostic value, but that the exact amount of enlargement is very heterogenic in patients may also suggests that nerve enlargement is more of an epiphenomenon of the disease, indicating the presence of a disease process, rather than being a disease modifying factor itself. However, it is also possible that nerve enlargement is an expression of active or previously experienced disease activity. In multiple sclerosis, a disease characterized by demyelination in the central nervous system, active lesions can be identified with MRI, but inactive lesions remain visible as well.[42] It could be possible that in MMN and CIDP, which are characterized by demyelination of the peripheral nervous system, imaging techniques reveal comparable results. If this would be the case, nerve ultrasound could potentially be used to detect new (active) lesions in case of clinical worsening in patients. It is unlikely that these new active lesions can be picked up reliably with only the CSA measurements performed in our study. However, previous studies in leprosy showed hypervascularization in active lesions and a reduction in vascularization in patients with favorable treatment response.[43-45] In addition, hyperechoic alterations in nerve morphology have been associated with chronic lesions showing fibrosis.[38] Further studies will have to performed to determine if extended sonographic evaluation and improved sonographic measurement tools can aid in the detection of active peripheral nerve lesions.

At this moment the value of nerve ultrasound in prognostics and follow-up of treatment-response in CIDP and MMN seems limited, and repeated performance of nerve ultrasound during follow-up should not be encouraged. The pattern of distribution of nerve enlargement may give an indication on treatment response, but as there is large heterogeneity in nerve size and nerve size development conventional CSA-measurement only seems insufficient to provide clinicians with meaningful prognostic information. The assessment of additional parameters, e.g. echogenicity, may improve the prognostic performance of nerve ultrasound,[34,35,46] but new, standardized, easily applicable measurement tools will have to be developed before nerve ultrasound may be applied as a useful prognostic tool in routine clinical practice in CIDP and MMN.

Apart from the prognostic value of nerve ultrasound in acquired chronic demyelinating neuropathies, we also investigated the potential value of nerve ultrasound in neurofibromatosis, a relatively common hereditary disease with frequent peripheral nerve involvement and severe complications of this involvement including the development of malignant peripheral nerve sheath tumors. In this disease the applications of peripheral nerve imaging had not been studied in detail previously.

In neurofibromatosis type 1, we found a large variability in sonographic abnormalities (**chapter 7**), ranging from no abnormalities at all to diffuse plexiform neurofibromas.[47,48] Plexiform neurofibromas can undergo malignant transformation, and the search for screening tools for such malignant transformation is ongoing. As nerve ultrasound can identify patients with these nerve tumors, it may have such applications. However, reliable measurement of change in tumor size is difficult with conventional cross-sectional area measurement (**chapter 8**).[48] In addition, malignant peripheral nerve sheath tumors may arise at locations where plexiform neurofibromas were not detected previously, and may develop in the thorax, abdomen or pelvis, which cannot be visualized with high-resolution ultrasound.[49-51] Currently, nerve ultrasound could help in identifying patients with an increased risk of malignant transformation and instructing these patients to seek medical care in case of symptoms suspect of a malignant peripheral nerve sheath tumor. Also, it may be able to select patients that would benefit from a frequent screening program. However, nerve ultrasound is currently not suitable to identify a malignant tumor, which still requires (whole-body) MRI or PET-CT investigation.[52,53]

To advance the knowledge on the applications of nerve ultrasound as a screening tool in neurofibromatosis type 1, further studies will have to be performed. We only conducted a small pilot study in asymptomatic patients in the Elisabeth-Tweesteden Hospital in Tilburg, a center with a relatively small population of neurofibromatosis patients. Future studies will have to be conducted in a multicenter setting, will have to include asymptomatic as well as symptomatic patients, and will require a longer follow-up duration. A study combining nerve ultrasound and (whole-body) MRI could be very useful, as it may give insight in the correlation of findings of both imaging modalities. Moreover, such a study could determine the extent of plexiform neurofibromas that can't be detected by nerve ultrasound, and it could be determined if nerve ultrasound is more sensitive in detecting plexiform neurofibromas of the peripheral nerves (as our study and others on nerve ultrasound reported a higher prevalence of plexiform neurofibromas than MRI studies).[48-50,54-56] Our study focused only on measurement of tumor size with cross-sectional area measurement. Whole-body MRI using tumor volumetry has improved this imaging technique significantly.[52] Development of volumetric measurement tools for nerve ultrasound could also improve the reliability of nerve ultrasound in detecting tumor growth. However, tumor growth only is not a reliable marker of malignant transformation,

and detection of changes in tumor metabolism seems just as important. FDG-PET/CT is a technique currently employed that detects these metabolic changes reliably,[53,57] but a significant downside to this technique as a screening tool is that the repeated exposure to radiation may increase the risk of malignant transformation itself. Development of new and improved sonographic tools to detect changes in tumor metabolism, e.g. standardized tools to assess vascularization and echogenicity, may therefore also be very helpful in developing a safe and reliable screening program for NF1 patients. As all these applications are currently unavailable, the exact value of nerve ultrasound in this disease is still unclear.

Future directions

Nerve ultrasound underwent a significant transformation in recent years. From an experimental technique it has developed into a tool with a multitude of applications.[46,58,59] In common entrapment neuropathies, e.g. carpal tunnel syndrome and ulnar neuropathy at the elbow, it has proven diagnostic value, and evidence on its value in diagnosing polyneuropathies is ever increasing.[1-3,19,20] With the transformation of nerve ultrasound from an experimental technique to an established diagnostic tool, a transition in the routine neurological practice will have to be made as well. A technique that was initially performed in few specialized clinics will have to be incorporated in general neurologic departments. Up to what degree general departments will have to be able to perform nerve ultrasound will have to be determined, but with evidence accumulating, the capacity of performing nerve ultrasound at common sites of entrapment seems at least a necessity in a general neurologic department. To make nerve ultrasound available in these practices, many changes will have to be facilitated. For instance, hospitals will have to create budget to finance suitable equipment, and adequate training will have to be provided to general neurologists and lab technicians. Initially, this could be achieved by in-service training, but eventually training in nerve ultrasound will have to be incorporated in a neurologists basic residency program. These challenges will have to be overcome the coming years, on an (inter)national as well as a local level, to allow optimal availability of this advantageous new tool in the field of neuromuscular disease.

Nowadays, a frequently encountered debate is whether nerve ultrasound could replace NCS. As nerve ultrasound is a patient-friendly alternative to often cumbersome NCS, this seems an attractive scenario. However, one has to keep in mind that the two techniques investigate very dissimilar facets of peripheral nerve disease. Ultrasound focusses on nerve anatomy, and NCS on nerve function. In brain disease the MRI and electroencephalography (EEG) are employed to investigate anatomy and function respectively. Both tools provide very different information on brain function and each tool has its own applications and indications, which vary depending on the suspected cerebral pathology. Likewise, in peripheral nerve disease ultrasound and NCS both can provide useful information. Nerve ultrasound is able to identify anatomic abnormalities underlying nerve disease which

require a therapeutic intervention that would not be detected by NCS, e.g. presence of an intraneural ganglion in fibular neuropathy.[60] On the other hand, NCS is able to test sensory and motor function of nerves independently to determine the extent of nerve pathology; something that is not possible with ultrasound.[59] Also, there are numerous examples in which both ultrasound and NCS can be helpful. For instance, both techniques are able to detect carpal tunnel syndrome, but they do not identify identical patients, and thereby increase each other's diagnostic yield.[2] In ALS, NCS can identify denervation in multiple regions, while ultrasound can aid in the identification of fasciculations, and debate is currently ongoing on whether to incorporate both testing modalities in new diagnostic criteria.[61-65] Similarly, in this thesis we found that both ultrasound and NCS could identify patients with CIDP and MMN, even though the other test was negative (**chapters 4 & 5**).[19,20] In our study, ultrasound seemed particularly helpful in excluding CIDP or MMN, and NCS to confirm it. Though our study shows that a chronic inflammatory neuropathy cannot be excluded without performance of nerve ultrasound, nerve ultrasound and NCS are, therefore most likely complementary techniques, each with their own specific indications. The use (and order of) nerve ultrasound and NCS in diagnostic strategies will most likely depend on the suspected nerve disease, and cost-effectiveness of such strategies will have to be determined, but replacement of one technique by the other entirely seems rather unlikely.

Similarly to ultrasound versus NCS, a question is often posed whether ultrasound or MRI is the most preferable technique. Nerve ultrasound is often readily available, allows investigation of multiple nerves in short time, and has higher spatial resolution than MRI.[59,66] In addition, nerve ultrasound allows dynamic imaging, which can be very helpful, for instance to detect fasciculations in ALS.[63-65] These features may make nerve ultrasound more preferable, especially when investigating superficial peripheral nerves.[56,67] On the other hand, nerve ultrasound is less suitable to investigate deeper-lying structures due to the use of high-frequency probes, and in our study we found higher inter-observer variability of deeper-lying nerve roots C6 and C7.[5] In traumatic brachial plexus injuries, there are studies that show high sensitivity of nerve ultrasound,[68,69] but imaging of these structures is most likely highly operator-dependent and requires extensive training. When specifically investigating (traumatic) plexopathies performance of MRI may thus be more favorable.[59,70] In addition, MRI allows volumetric measurement of nerves, which can be especially useful when investigating conditions associated with extensive nerve tumors, such as neurofibromatosis, and this is currently not possible with ultrasound.[47,48,52] Also, there are conditions in which both ultrasound and MRI independently have diagnostic use, such as ALS, CIDP and MMN.[71-73] Thus, as in ultrasound and NCS, it seems that MRI and ultrasound are complementary techniques, and that preference and choice of imaging modality depend on the diagnostic dilemma posed.

Research on nerve ultrasound in past years mainly focused on nerve cross-sectional area, as this is an easily obtainable and reliable parameter. Investigation of other parameters and development new sonographic techniques may improve diagnostic and prognostic value of nerve ultrasound in the future. Quantification of intra-neural blood flow may be one of the tools that improves therapeutic monitoring of patients. Studies already showed changes in intraneural blood flow in patients with end-stage kidney disease and leprosy, in which disappearance of this intraneural blood flow was associated with positive treatment-response.[45,74] Development of new measurement tools, such as Superb Micro-Vascular Ultrasound Imaging (SMI), could potentially enable standardized assessment of this intra-neural vascularization. Also, probes with frequencies of up to 70MHz are currently being developed, that allow even more detailed assessment of nerves microstructural architecture, which could further improve monitoring pathophysiological changes within the nerve.[75] Shear-wave nerve elastography, which investigates nerve stiffness, and has potential diagnostic applications is also currently under investigation.[76] Future studies will have to determine the usefulness of those newly introduced techniques, and whether application of these techniques in routine clinical practice is feasible.

Another relatively new ultrasonographic technique is 3D-ultrasound. It allows assessment of structures in 3 different planes simultaneously. The technique is already frequently employed in other fields of medicine such as gynecology or cardiology. Some studies have explored this technique at entrapment sites,[77-80] but the applications in peripheral nerve disease are currently still limited. Recently, we conducted a pilot study on 3D-ultrasound in several types of peripheral nerve disease, including CIDP, MMN, and neurofibromatosis, as well as healthy controls (unpublished data). We performed nerve ultrasound of the median and ulnar nerve in forearm and upper arm and made use of a regular probe with an attached 3D-sensor. Through this 3D-sensor an ultrasonographic device is able to reconstruct a sagittal and coronal plain in addition to the regularly obtained transversal plain (**Figure 2**). Obtaining such images took only a few additional seconds compared to regular ultrasound evaluation. With this technique we were able to evaluate peripheral nerves in the arm in multiple plains simultaneously, and were also able scroll through already obtained images to follow the peripheral nerves along its tract in the arm, comparable to CT and MRI. Currently, in cooperation with Canon Medical Systems Netherlands we are developing a new tool to perform volumetric measurements on these sampled images (rather than the now standard cross-sectional area measurements) and are exploring the possibility of 3D-reconstructions of peripheral nerves. These developments could pose numerous advantages in sonographic assessment of peripheral nerves in the future. Volumetric measurement of nerves could further decrease interobserver variability, and improve reliable follow-up of peripheral nerve abnormalities. For instance, in neurofibromatosis type 1 this could improve assessment of temporal and spatial evolution of large plexiform neurofibromas, which could make nerve ultrasound a more reliable screening

Figure 2 3D-Nerve ultrasound

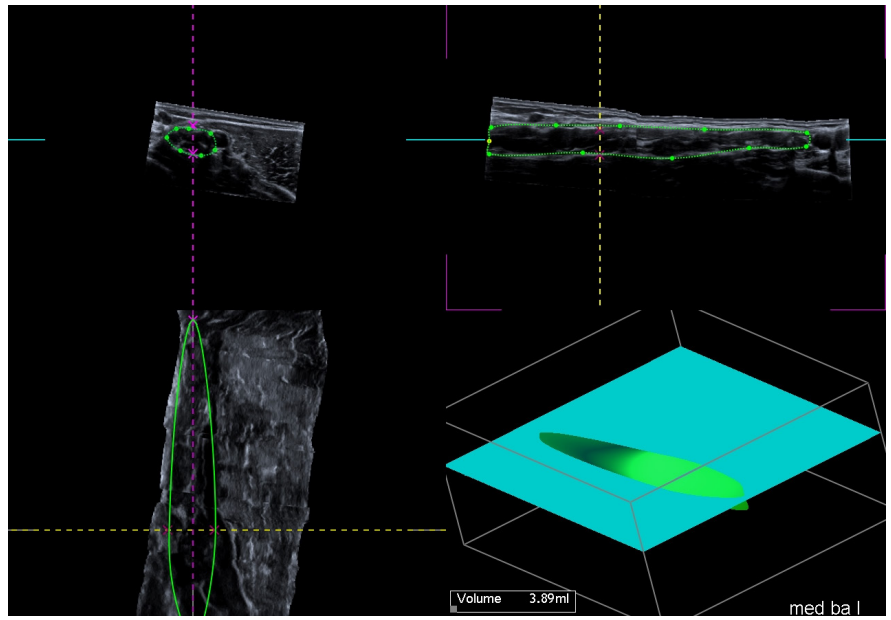

Image obtained with 3D-nerve ultrasound in a patient with neurofibromatosis type 1 and a plexiform neurofibroma in the left median nerve. The upper left panel shows the regular transverse sonographic image with the nerve measured within the hyperechoic rim. The right upper panel shows the simultaneously obtained longitudinal view of the median nerve. In the left lower corner a coronal view of the same nerve is shown, and in the right lower panel a 3D reconstruction of the region of interest with a calculated volume of the nerve (created by the measurements performed in transversal and longitudinal plane). At this moment optimization of the visualization of the coronal plain of view and the 3D-reconstuction is still necessary. Volumetric measurements are currently still based on an assumed spherical shape, but development of a tool to determine volume with an assumed cylindrical shape (which is regularly the shape of nerves) is ongoing to be able to perform volumetry of the nerve with ultrasound..

tool for malignant transformation in this disease in the future. The capability to follow the tract and distribution of abnormalities in peripheral nerves on still-stored images could also be advantageous in detecting specific patterns of nerve enlargement, evaluating temporal evolution of peripheral nerve tumors, planning nerve biopsy, and assessing traumatic nerve lesions and planning surgical treatment for such lesions. Though these applications are still under development, and further studies will have to be performed to determine the actual value of 3D-nerve ultrasound, this technique seems promising, and could lead to further improved diagnostics and prognostics in peripheral nerve disease.

Conclusions

In this thesis, we explored the diagnostic and prognostic value of nerve ultrasound in peripheral nerve disease. The indications for this technique are rapidly increasing, and we found that it is a technique that can be reliably incorporated in routine neurological practice. Nerve ultrasound can make substantial contributions in diagnostics, not only by identifying patients also detected with more cumbersome techniques, but also by identifying additional treatment-responsive patients. Prognostic value of nerve ultrasound currently seems more limited, though with the development of new techniques, including 3D-nerve ultrasound, it may have applications as a follow-up and screening tool. Nonetheless, nerve ultrasound is a very useful addition to the general neurological practice, and it should be incorporated in standard work-up of peripheral neuropathy.

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Appendix

Nederlandse samenvatting

Zenuwen zijn de snelwegen die onze hersenen verbinden met de rest van ons lichaam. Door het voortgeleiden van prikkels zorgen ze ervoor dat we kunnen zien, voelen, spreken en bewegen. Omdat de zenuwen een sleutelrol hebben bij het voortgeleiden van deze prikkels kunnen zenuwziekten een groot scala aan klachten veroorzaken en voor grote beperkingen zorgen. Het is voor patiënten dus van groot belang dat er snel een goede diagnose kan worden gesteld en een goede behandeling kan worden gegeven.

Het vaststellen van een zenuwziekte kan voor artsen soms zeer lastig zijn en het voorspellen van het verloop van neuropathieën kan soms voelen als een waarzegger die in een glazen bol staart (kaft). Het electromyogram (EMG) is van oudsher het instrument om een neuropathie vast te stellen, maar dit onderzoek, dat met behulp van elektrische stroomschokken de functie van de zenuw onderzoekt, kan erg belastend zijn voor patiënten en is regelmatig niet conclusief. In de afgelopen jaren is er met zenuwechografie een nieuwe techniek bijgekomen in het arsenaal van de arts. De echo is een techniek waarmee goedkoop en in korte tijd de anatomie van de meerdere zenuwen kan worden bestudeerd, wat cruciale informatie zou kunnen opleveren over de oorzaak van een zenuwziekte of het te verwachten effect van een behandeling. Het is daardoor mogelijk dat er bij complexe neuropathische problematiek door de echo voor de arts eindelijk een antwoord opdoemt in zijn glazen bol.

In dit proefschrift worden verschillende aspecten van de diagnostische en prognostische waarde van zenuwechografie onderzocht. In hoofdstuk 2 wordt de literatuur over de waarde van zenuwechografie bij polyneuropathie geëvalueerd. Polyneuropathie is een veelvoorkomende aandoening van de zenuwen, waarbij onder andere krachts- en gevoelsverlies kunnen optreden. Er zijn vele verschillende soorten polyneuropathie, waaronder axonale varianten (welke zich kenmerken door schade aan de axonen) en demyeliniserende varianten (welke zich kenmerken door inflammatie van de myelineschede van de zenuw) en er zijn erfelijke en verworven oorzaken. Uit de literatuur blijkt dat deze typen polyneuropathie zich met verschillende echografische afwijkingen presenteren, waardoor de echo kan ondersteunen bij het vaststellen van de oorzaak van een polyneuropathie.

In hoofdstuk 3 wordt de inter-observer variabiliteit van zenuwecho onderzocht. Dit is een zeer belangrijk aspect van een onderzoekstechniek, aangezien een techniek alleen betrouwbaar ingezet kan worden in de dagelijkse praktijk als er geen groot verschil in metingen wordt gevonden tussen verschillende onderzoekers. In deze multicenter studie vinden we dat er geen grote systematische verschillen tussen onderzoekers zijn en dat er geen verschil in resultaten is als het onderzoek wordt uitgevoerd in verschillende

ziekenhuizen of op verschillende typen echoapparaten. Deze bevindingen ondersteunen dat zenuwecho een betrouwbare techniek is om de zenuwen te bestuderen en dat de echo breed toegepast kan worden in de algemene dagelijkse praktijk.

In hoofdstuk 4 en 5 onderzoeken we de diagnostische waarde van zenuwecho bij chronische demyeliniserende polyneuropathieën. Dit zijn ontstekingsziekten van de zenuwen die in korte tijd tot veel klachten kunnen leiden, maar die in essentie ook behandelbaar zijn. Het onderscheid met axonale polyneuropathieën en andere ernstige neurologische aandoeningen, zoals amyotrofische lateraal sclerose (ALS) kan zeer moeilijk zijn, maar is van het grootste belang aangezien deze ziekten tot op heden niet behandelbaar zijn. In de studies in hoofdstuk 4 en 5 onderzochten we 100 patiënten in het UMC Utrecht en 100 patiënten in een multicenter cohort die op basis van hun klachten verdacht werden van een demyeliniserende polyneuropathie. Uit deze studies blijkt dat met een kort echoprotocol bestaande uit 2 meetpunten in de nervus medianus en 1 in de brachiale plexus niet alleen zeer betrouwbaar kan worden vastgesteld of er sprake is van een demyeliniserende polyneuropathie, maar ook dat de echo zelfs +/- 25% meer patiënten identificeert met een behandelbare polyneuropathie in vergelijking met het nu standaard toegepaste EMG. De echo is daarom een zeer nuttig diagnostisch instrument bij deze typen polyneuropathie en op basis van onze bevindingen bevelen we daarom ook aan dat de echo wordt toegevoegd aan de diagnostische criteria voor deze ziekten.

In hoofdstuk 6 wordt ook de prognostische waarde van zenuwecho bij chronische demyeliniserende polyneuropathieën onderzocht. Deze polyneuropathieën vereisen vaak langdurige behandeling met immunoglobulines, wat zeer prijzig is, belastend kan zijn voor patiënten en meerdere bijwerkingen kan hebben. Tot op heden is het behandeleffect bij deze aandoeningen moeilijk te voorspellen. In een multicenter cohort studie, waarin we 237 patiënten met een demyeliniserende of axonale polyneuropathie gedurende een periode van 1 jaar vervolgden, vonden we dat de ontwikkeling van klachten en zenuwafwijkingen sterk verschillen per patiënt. Alleen bij patiënten met multifocale motor neuropathie (MMN), één van de subtypen van demyeliniserende polyneuropathie, was er enig verband tussen de mate van zenuwverdikking en de reactie op behandeling. In tegenstelling tot bij de diagnostiek naar chronische demyeliniserende polyneuropathieën lijkt de echo in zijn huidige vorm dus slechts een beperkte rol te hebben bij de prognostiek. Op basis van onze studie wordt het herhalen van de echo tijdens de behandeling van patiënten om het effect hiervan te bepalen derhalve ook niet aanbevolen.

In hoofdstuk 7 en 8 onderzoeken we de waarde van zenuwecho bij neurofibromatose type 1 (NF1). Dit is een erfelijke aandoening, waarbij er zenuwtumoren kunnen ontstaan zonder dat patiënten daarvan klachten hebben. Deze zenuwtumoren zijn goedaardig, maar kunnen zich in sommige gevallen kwaadaardig ontwikkelen en hebben dan een

hoge mortaliteit. Op dit moment is er nog geen betrouwbaar screeningsprogramma. In onze studies vonden we dat patiënten met NF1 zonder neuropathische klachten een zeer uiteenlopende betrokkenheid hebben van het perifere zenuwstelsel. Terwijl sommige patiënten geen zenuwtumoren hadden, werden bij andere patiënten zeer grote tumoren gevonden in meerdere zenuwen. De zenuwecho zou daarom kunnen helpen bij het identificeren van patiënten met een hogere kans op het kwaadaardig ontwikkelen van een zenuwtumor. Aan de andere kan blijkt uit onze studies dat de zenuwecho op dit moment nog niet in staat is om betrouwbaar groei van een tumor over de tijd aan te tonen. Op dit moment lijkt de echo daarom niet geschikt om patiënten met een hoger risico op een kwaadaardige tumor te vervolgen. Een betrouwbare screeningsstrategie voor kwaadaardige zenuwtumoren bij NF1, met een mogelijke rol voor zenuwecho hierin, zal daarom nog verder moeten worden ontwikkeld.

In hoofdstuk 9 worden de echografische bevindingen bij neurofibromatose type 2 (NF2) beschreven. Deze aandoening wordt met name geassocieerd met brughoektumoren, welke bij het centrale zenuwstelsel zijn gelokaliseerd. In onze studie vonden we daarnaast echter ook betrokkenheid van de perifere zenuwen bij alle patiënten. Dit is belangrijk omdat er bij patiënten met NF2 met klachten van krachts- of gevoelsverlies dus niet alleen gedacht moet worden aan een tumor bij de hersenen als oorzaak, maar ook aan een tumor van de zenuw.

Concluderend werden in dit proefschrift de diagnostische en prognostische waarde van zenuwecho onderzocht. Zoals bij onderzoek van de hersenen EEG en de MRI elkaar aanvullen met informatie over functie en anatomie, vullen het EMG en de echo elkaar aan bij het onderzoek van de zenuwen. De zenuwecho is een reproduceerbare techniek met zeer nuttige toepassingen als diagnostisch instrument, in het bijzonder bij de identificatie van chronische demyeliniserende polyneuropathieën. Het staat niet alleen detectie toe van patiënten die anders alleen door meer belastende onderzoekstechnieken kunnen worden geïdentificeerd, maar verbetert zelfs de detectie van patiënten die op behandeling reageren. De rol van zenuwecho als prognostisch instrument en screeningstechniek is op dit moment beperkter in de dagelijkse praktijk. Echter, met het doorontwikkelen van de techniek, bijvoorbeeld door het ontwikkelen van 3D-zenuwechografie (waaraan momenteel door ons wordt gewerkt), kan de echo in de toekomst mogelijk ook van toegevoegde waarde zijn op dit gebied. Desalniettemin tonen de studies in dit proefschrift aan dat de zenuwecho een zeer nuttige aanvulling in de dagelijkse neurologische praktijk is en dat deze goed reproduceerbare techniek zou moeten worden toegevoegd aan de standaard work-up van patiënten met perifere neuropathie.

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Curriculum vitae

Johan Telleman was born on November 23, 1986 in Geldermalsen, The Netherlands. He attended the gymnasium at the RSG Lingecollege and graduated in 2005. Afterwards he started medical school at Utrecht University. During his medical school he was involved in research projects at the Department of Vitreoretinal Surgery and Department of Otolaryngology, Head and Neck Surgery and the DHS Julius Center in the UMC Utrecht. After graduating in 2011 he worked as a resident in Neurology at the Groene Hart Hospital in Gouda and the Isala Clinics in Zwolle. In 2014 he started as a resident in Neurology at the Elisabeth-Tweesteden Hospital in Tilburg and was subsequently admitted to the Neurology specialisation programme. In 2015 he started his PhD-programme on diagnostic and prognostic value of nerve ultrasound at the Elisabeth-Tweesteden Hospital and UMC Utrecht, Brain Center Rudolf Magnus under supervision of prof. dr. L.H. Visser and prof. dr. L.H. van den Berg. During the past years he has combined research and clinical training and he is currently a member of the Workgroup Neuromuscular Ultrasound of the Dutch Society of Clinical Neurophysiology, a member of the Rotterdam 2020 9th International Society of Peripheral Nerve Imaging Conference Organising Committee, and a teacher in Nerve Ultrasound for the Workgroup Neuromuscular Ultrasound.