TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

*SARJA - SER. D OSA - TOM. 849*

MEDICA - ODONTOLOGICA

# NEUROPHYSIOLOGIC DIAGNOSIS, CLINICAL SYMPTOMS AND NEUROPATHOLOGIC FINDINGS IN POLYNEUROPATHIES

by

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TURUN YLIOPISTO
Turku 2009

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ISBN 978-951-29-3924-4 (PRINT)
ISBN 978-951-29-3925-1 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2009

To my husband Jussi,
and our children Stella, Matias and Oliver

# ABSTRACT

Satu Laaksonen. Neurophysiologic diagnosis, clinical symptoms and neuropathologic findings in polyneuropathies. Department of Clinical Neurophysiology, Turku University Hospital. Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Finland, 2009.

**Backround**: Polyneuropathy (PNP) is a disorder of the peripheral nervous system that causes widespread, usually symmetric, abnormalities of peripheral nerves. Numerous underlying conditions can cause PNP.

**Aims**: To evaluate the subjective PNP symptoms and the most useful neurophysiologic tests for the diagnosis of uremic PNP, thalidomide induced PNP in myeloma patients, and PNP in Fabry disease. Another aspect of the study was to determine the correlation between subjective symptoms and neurophysiologic and neuropathologic findings in patients with PNP. In uremic patients, the aim was also to study the effect of one dialysis session on neurophysiologic parameters. Also the effect of dialysis on the function of autonomic nervous system was evaluated.

**Subjects and methods:** (I) 21 uremic patients, sensory and motor conduction studies, vibratory- and thermal detection thresholds before and after dialysis. The clinical findings and subjective symptoms were studied using a standardized questionnaire. (II) 12 myeloma patients with thalidomide therapy, the methods were the same as in study I. (III) 12 patients with Fabry disease, same methods as in studies I and II, also skin biopsy for the detection of intraepidermal nerve fibre density. (IV) 32 uremic patients, autonomic nervous system was studied with time-domain measures, thick myelinated fibers were studied with sensory neurography.

**Results**: The F- wave parameters, vibratory perception threshold from lower limbs, and the sural amplitude were the best parameters in the diagnosis of uremic PNP. Positive PNP symptoms in uremic patients correlated with vibratory perception threshold and sensory neurography. The neurophysiologic test can be done either before or after dialysis. Thalidomide PNP is predominantly sensory, but motor fibers are also slightly affected. The subjective sensory PNP symptoms did not correlate with neurophysiologic findings. In Fabry disease, women had more PNP symptoms than expected. Thick myelinated fibers are not affected in Fabry disease. In the diagnosis of small-fibre PNP, the skin biopsy and quantitative sensory tests complement each other. Effective dialysis therapy had a positive effect to cardiac autonomic function.

**Conclusions:** When diagnosing PNP, it is important to use standardized neurophysiologic tests that reflect the function of different types of nerve fibres. These tests should have proper reference values and they should be sensitive in detecting the particular type of PNP that is suspected. The patient's subjective symptoms, family history and clinical findings give valuable information, and should always be evaluated together with the neurophysiologic tests.

**Keywords:** Polyneuropathy, symptoms, IENFD, F-wave, Thalidomide, Fabry disease, uremia

# TIIVISTELMÄ

Satu Laaksonen. Polyneuropatian neurofysiologinen diagnostiikka, kliiniset oireet sekä neuropatologiset löydökset. Kliinisen Neurofysiologian yksikkö, Turun Yliopistollinen Keskussairaala. Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Finland, 2009.

**Tausta:** Polyneuropatia (PNP) on ääreishermoston sairaus, joka aiheuttaa laaja-alaisia, yleensä symmetrisiä vaurioita ääreishermostossa. PNP:aan johtavia syitä on satoja.

**Tavoitteet:** Löytää parhaat neurofysiologiset menetelmät uremian, myelooman hoidossa käytettävän talidomidin sekä Fabryn taudin aiheuttaman PNP:n diagnosoimiseksi. Fabryn taudissa tutkin lisäksi ohutsäieneuropatian aiheuttamia neuropatologisia löydöksiä iholta otetusta koepalasta. Tutkimuksissa kartoitettiin lisäksi PNP:n aiheuttamien subjektiivisten oireiden korrelaatio neurofysiologisten ja neuropatologisten löydösten kanssa. Munuaisten vajaatoimintaa sairastavilla potilailla tavoitteena oli tutkia dialyysihoidon tehon vaikutusta autonomisen hermoston toimintaan sekä yhden dialyysikerran vaikutusta neurofysiologisiin löydöksiin.

**Aineisto ja menetelmät:** I: Tutkittiin 21 uremiapotilaan sensoristen ja motoristen hermojen vasteet, värinä- sekä lämpötuntokynnykset ennen ja jälkeen hemodialyysin. Subjektiiviset PNP oireet kartoitettiin PNP oireita kysyvillä kaavakkeella. II:12 talidomidihoitoa saavaa myeloomapotilasta, tutkimuksen menetelmät olivat samat kuin tutkimuksessa I. III: 12 Fabryn tautia sairastavaa potilasta, edellä mainittujen neurofysiologisten tutkimusten lisäksi potilailta otettiin ihobiopsia säären alueelta. Ihobiopsiasta laskettiin ohuiden hermosyiden määrä koepalan värjäyksen jälkeen. Subjektiiviset PNP oireet kartoitettiin kyselykaavakkeella. Sydämen sykevaihtelu tutkittiin levossa taajuustason analyysillä. IV: 32 uremiapotilaan autonomisen hermoston toimintaa tutkittiin sydämen sykevaihtelun aikatason analysillä, paksujen myelinoituneiden säikeiden toimintaa tutkittiin perifeeristen sensoristen hermojen mittauksilla toistetusti noin 2.9 vuoden aikana.

**Tulokset:** Ureemisen PNP:n diagnostiikassa herkimmät tutkimukset ovat F-aaltojen parametrit alaraajojen motorisista hermoista, värinätuntokynnys alaraajoista sekä suralishermon amplitudi. Positiiviset PNP oireet uremiassa korreloivat värinätunto-kynnyksen sekä sensoristen hermojen neurografialöydösten kanssa. Neurofysiologisten tutkimusten ajankohdalla dialyysiajankohtaan nähden ei ole merkitystä. Talidomidi-PNP on pääasiassa sensorinen, mutta motoriset syyt ovat lievästi vaurioituneet. Talidomidi PNP:ssa subjektiiviset oireet korreloivat huonosti neurofysiologisten löydösten kanssa. Fabryn taudissa naisilla on oletettua enemmän ohutsäieneuropatian aiheuttamia oireita ja löydöksiä. Paksujen säikeiden löydöksiä ei tullut esiin. Ohutsäieneuropatian diagnostiikassa ihobiopsia ja kvantitatiiviset tuntokynnysmittaustestit täydentävät toisiaan. Tehokas dialyysi parantaa autonomisen hermoston toimintaa uremiapotilailla.

**Päätelmät:** Erityyppisten polyneuropatioiden diagnostiikassa pitää etukäteen valita PNP tyypille oikeat tutkimusmenetelmät raskaiden tutkimuspatterien vähentämiseksi sekä diagnostiikan parantamiseksi. PNP:n aiheuttamat oireet ja kliiniset löydökset pitää aina tutkia, mutta yksin ne eivät ole herkkiä PNP:n diagnostiikassa.

**Avainsanat:** Polyneuropatia, oireet, IENFD, F-aallot, Talidomidi, Fabryn tauti, uremia

# ABBREVIATIONS

| | |
|---|---|
| AAN | American Association of Neurology |
| AANEM | American Academy of Neuromuscular and Electrodiagnostic Medicine |
| AAPM&R | American Academy of Physical Medicine and Rehabilitation |
| AIDP | Acute inflammatory demyelinating polyradiculoneuropathy |
| AMPL | Amplitude |
| AMPL% | Decay |
| ANS | Autonomic nervous system |
| CAN | Central autonomic network |
| CAPD | Continuous ambulatory peritoneal dialysis |
| CIDP | Chronic inflammatory demyelinating polyneuropathy |
| CMAP | Compound muscle action potential |
| CMT | Charcot-Marie-Tooth |
| CNS | Central nervous system |
| CDT | Cold detection threshold |
| CT | Conduction time |
| CTS | Carpal tunnel syndrome |
| CV | Conduction velocity |
| DISP | Dispersion |
| DLAT | Distal latency |
| ECG | Electrocardiogram |
| EMG | Electromyography |
| ESRD | End-stage renal disease |
| FAMPL | F-wave amplitude |
| Fdisp | F-wave dispersion |
| Fmin | F-wave minimum latency |
| GBS | Guillain-Barré Syndrome |
| HD | Hemodialysis |
| HF | High frequency |
| HR | Heart rate |
| HRV | Heart rate variability |
| IENFD | Intraepidermal nerve fibre density |
| LF | Low frequency |
| LEP | Laser evoked potential |
| MAMPL | Motor action potential amplitude |
| MUP | Motor unit potential |
| NC | Nerve conduction |

| | |
|---|---|
| NIS | Neuropathy Impairment Score |
| NF200 | Neurofilament 200 |
| NSS | Neuropathy Symptom Score |
| PNP | Polyneuropathy |
| PNS | Peripheral nervous system |
| PTH | Parathyroid hormone |
| RMSM | Standard deviation of all RR intervals (also named SDNN) |
| rMSSD | The square root of the mean of the sum of the squares of differences between adjacent normal RR intervals. |
| RLS | Restless legs syndrome |
| SAMPL | Sensory amplitude |
| SFEMG | Single fibre electromyography |
| SCLC | Small cell lung carcinoma |
| SNAP | Sensory nerve action potential |
| SSR | Sympathetic skin response |
| TNF-α | Tumor necrosis factor α |
| TP | Total power |
| ULF | Ultra low frequency |
| VLF | Very low frequency |
| VPT | Vibratory perception threshold |
| WDT | Warm detection threshold |
| QST | Quantitative sensory threshold |

## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by the Roman numerals I-IV.

I Laaksonen S, Metsärinne K, Voipio-Pulkki LM, Falck B. Neurophysiologic parameters and symptoms in chronic renal failure. Muscle Nerve. 2002 Jun;25(6):884-890.

II Laaksonen S, Remes K, Koskela K, Voipio-Pulkki LM, Falck B. Thalidomide therapy and polyneuropathy in myeloma patients. Electromyogr Clin Neurophysiol. 2005 Mar;45(2):75-86.

III Laaksonen SM, Röyttä M, Jääskeläinen SK, Kantola I, Penttinen M, Falck B. Neuropathic symptoms and findings in women with Fabry disease. Clin Neurophysiol. 2008 Jun;119(6):1365-72.

IV Laaksonen S, Voipio-Pulkki L, Erkinjuntti M, Asola M, Falck B. Does dialysis therapy improve autonomic and peripheral nervous system abnormalities in chronic uremia? J Intern Med. 2000 Jul;248(1):21-26.



The studies were approved by the ethical committee of Turku University Hospital district and all patients gave their written informed consent for the studies.

# TABLE OF CONTENTS

# 1. INTRODUCTION

Polyneuropathy (PNP) is a disorder of the peripheral nervous system (PNS) that causes widespread, usually symmetric abnormalities. It is a large group of disorders leading to muscle weakness, sensory abnormalities and altered function of the autonomic nervous system (ANS). These symptoms affect patients' quality of life in different ways. Many PNPs affect all the different types of axons (motor, sensory and autonomic nerves), while others may selectively affect only one type of axon. PNP can lead to reduced sensation, pain, ulcerations, weakness, and many other symptoms and signs.

A search of the term "PNP" in the U.S. National Library of Medicine and the National Institutes of Health (PubMed database) in April 2008 resulted in 21,256 articles. Dorland's Illustrated Medical Dictionary defines PNP as follows: "*Neuropathy of several peripheral nerves simultaneously; called also multiple or peripheral neuropathy. Some conditions that are actually PNPs are called neuropathies*" (Dorland 2003), while Stedman's medical dictionary gives a very concise definition: "*A disease process involving several peripheral nerves (literal sense) 2. A nontraumatic generalized disorder of peripheral nerves, affecting the distal fibres most severely, with proximal shading (e.g. the feet are affected more severely than the hands) and typically symmetrically, most often affects motor and sensory fibres almost equally, but can involve either one selectively or very disproportionately; classified as axon degenerating (axonal) or demyelinating; many causes, particularly metabolic and toxic, familial or sporadic. SYN polyneuritis SYN multiple neuritis, symmetric distal neuropathy (poly-+ G.neuron, nerve + pathos, disease*) (Stedman 2000).

According to Thompson and Thomas, both of whom have done numerous landmark studies on PNPs (Thomas 1997; Thompson *et al.* 2005), the term "PNP" includes a variety of topographic, functional and pathologic disturbances, defining what part of the PNS has been affected. The pathologic process involves the PNS in a generalized manner, and the patient has a bilateral and symmetrical disturbance of motor, sensory and/or autonomic function (Thompson *et al*. 2005).

There are different ways to classify PNPs; by modality (motor, sensory or autonomic), by fibre-size-affected (myelinated axons, unmyelinated, thin fibres) or by anatomic distribution (distal symmetric, proximal), time course (acute monophasic, chronic monophasic, acute or chronic relapsing) and pathology (axonal and demyelinating) (Berger *et al.* 1995). Experienced neurologists are usually able to diagnose PNP in patients presenting with a characteristic history and neurologic findings. In spite of this, exact criteria for the diagnosis are not defined by internationally accepted criteria. In particular, the criteria for the diagnosis of distal symmetric PNP are debated (England *et al.* 2005).

PNPs may be caused by toxins, nutritional deficiencies, metabolic disorders or other systemic disorders, genetic diseases, or immune-mediated illnesses (Thompson *et al.* 2005). The underlying conditions that cause PNPs are numerous, and in this project only a few of them are included. In the western world, the most common cause of PNP is diabetes. However, in developing countries, PNP due to leprosy (causative agent Mycobacterium leprae), also known as Hansen's disease, is probably still the most common cause of PNP (Sabin *et al.* 2005), although intensive treatment and World Health Organization surveillance programmes are reducing the number of patients. PNP due to end–stage renal disease (uremia), is also common and often under-diagnosed. Uremic PNP affects the quality of life in many patients on maintenance dialysis (Krishnan *et al.* 2007).

A few PNPs, such as $B_{12}$ vitamin deficiency and many of the immune-mediated PNPs, are treatable. In some cases, the progression of PNPs can be slowed. This can be done especially in cases of PNP due to metabolic diseases like diabetes or uremia, if PNP is detected and the underlying disease is treated. In many toxic neuropathies, the progression of PNP can be slowed or stopped if the exposure to the toxin can be eliminated. Inflammatory neuropathies are often treatable and reversible, especially if they are detected early in the course of the disease. Unfortunately, for many PNPs there are no known treatments. The etiology of PNP can in some patients remain unclear, and there are no recent studies about the prevalence of cryptogenic PNPs. In studies done during 1975-1987, cryptogenic PNPs have been reported by Corvisier et al. in 11% of 432 patients, and in 20% of patients age 65 or over. Other authors have reported an undiagnosed cause of PNP in 13-24% of cases (Corvisier *et al.* 1987; Dyck *et al.* 1975; McLeod *et al.* 1984). Most PNPs affect both motor and sensory nerve fibres, usually more in one than in the other modality. Some PNPs specifically affect only sensory, motor or autonomic axons. Sensory neuropathies can be caused by the involvement of the dorsal root ganglion cells (neuronopathy or ganglionopathy), or by a peripheral distal axonopathy (Thompson *et al.* 2005). Motor neuropathy may be produced by primary degeneration of the anterior horn cells (neuronopathy) and proximal axons of the lower motor neurons within the spinal cord (Thompson *et al.* 2005).

The symptoms caused by PNPs are diverse, depending on the type of PNP. Often the symptoms are severe enough to significantly disturb the quality of life. The early detection of PNP and diagnosis of the etiology in treatable PNPs is crucial. The most common pattern of a generalized PNP is a symmetrical distal motor and sensory deficit. Weakness and sensory loss usually begin distally in the feet, and by the time the symptoms spread up to the level of the knee, symptoms appear in the hands (Thompson *et al.* 2005). The sensory loss in PNP patients presents as a distal "glove-and stocking" sensory loss, but with progression of the neuropathy, the sensory loss spreads proximally to the trunk. In the advanced stages of PNP, sensory loss progresses from the anterior abdominal

wall to the lateral aspect of the trunk, while also the vertex of the head and faces can be involved. In the most severe cases of PNP, sensory loss can be extensive, sparing only a midline strip over the posterior trunk and neck and the periphery of the face. Reduced tendon reflexes follow the same pattern, affecting first the ankle jerks, and then the lower limbs before the upper (Dyck 2005a). Neuropathic pain in the extremities is common, especially patients with small-fibre neuropathy report pain as the most debilitating symptom. In sensory neuropathies, ataxia is also common. With severe loss of sensation, the distal parts of the extremities are prone to wounds and wound infections; since the patients are unable to feel noxious stimuli. Deformities of the extremities may occur. Neuropathic osteoarthropathy, often called Charcot joint, is a rare complication of both peripheral and central neuropathies. It is characterized by progressive degeneration of weight-bearing joints, bony destruction, resorption and deformity. It has no single cause but represents the final common pathway in people who are predisposed to its development due to a varying overlap of several different factors (Jeffcoate 2008). Probably there is microtrauma to the joint, which is unnoticed by the patient due to the neuropathy. In addition, the injury may be enhanced by ANS dysfunction.

Peripheral neuropathies are difficult to study epidemiologically because PNPs are a heterogeneous group of disorders. Most epidemiologic studies of PNP have been done in diabetic patients, where the incidence and prevalence estimates may vary from 5-100% depending on how long a history of diabetes the patient has, and which diagnostic criteria have been used (Martyn *et al.* 1997; Burke *et al.* 2005; Gominak *et al.* 2001).

Early diagnosis of PNP and the detection of the etiology in treatable PNPs are crucial. Unfortunately, there are no universally accepted criteria for diagnosing PNP. Most studies utilize criteria set by the investigators themselves. The symptoms alone have relatively poor diagnostic accuracy in predicting the presence of PNP. In these studies, I have evaluated which symptoms, signs and electrodiagnostic findings are sensitive for the diagnosis of PNP in uremia, thalidomide-treated myeloma and Fabry disease.

# 2. REVIEW OF THE LITERATURE

## 2.1. Structure and function of the peripheral nerves

The nervous system consists of the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS comprises the brain and the spinal cord, while the PNS is composed of ganglia and peripheral nerves that lie outside the brain and spinal cord. The CNS and PNS are not two distinct functional entities; although they are anatomically separated, functionally they are interconnected and interactive. The PNS has two divisions, somatic and autonomic. The somatic division includes sensory neurons of dorsal root and cranial ganglia that innervate the skin, muscles and joints, and provide sensory information to the CNS about muscle and limb position, and about the environment outside the body. The axons of somatic motor neurons that innervate skeletal muscle and which project to the periphery are parts of the somatic division, even though their cell bodies are part of the CNS. The autonomic division of the PNS mediates visceral sensation as well as motor control of the viscera, smooth muscles, and exocrine glands. It is divided into three different anatomic subdivisions: the sympathetic, parasympathetic and enteric system.

*Structure of peripheral nerves.* A nerve fibre is an axon and its associated Schwann cells. The axon leaves the cell body at the axon hillock and gives rise to a varying number of collateral branches. It consists of a relatively firm gelatinous cord of neuronal cytoplasm, the axoplasm, which is enclosed by the axonal part of the neuronal cell membrane, the axolemma. The nerve fibres comprising the peripheral nerves are collected together in fascicles. Each fascicle is delineated by a perineural sheath, which is composed of flattened squamous cells connected together by tight junctions. The anatomy of the nerve fibre is divided into perifascicular connective tissues, lamellate sheath and intrafascicular tissue. The intrafascicular tissue is divided into the epineurium, perineurium and endoneurium. The individual fascicles are embedded in a collagenous matrix called the epineurium, the constituents of which are blood vessels, fibroblasts, adipose tissue, collagen and elastin fibrils (Kleitman *et al.* 1995). The amount of epineurium varies from nerve to nerve (from 35-75% of the total cross-sectional area of the nerve) (Berthold *et al.* 2005). The perineurium is the collagenous connective tissue, which is part of the membranous covering that ensheaths the nervous system. The perineurium covers each nerve fascicle and is made of 1 to 15 layers of distinct flattened perineural cells. Focal disruption of the perineurium (a “perineural window”) causes local demyelination. The endoneurium refers to the contents of a fascicle within the perineurium, including Schwann cells and axons (Berthold *et al.* 2005). The endoneurium contains supporting cells and the extracellular matrix that surround bundles of nerve fibres originating from

spinal and autonomic ganglia, as well as from motor neurons of the ventral spinal cord. The supporting tissues of the nerve include Schwann cells that ensheath or myelinate the axons in series along their length, fibroblasts, vascular elements and occasional mast and fat cells, as well as the extracellular matrix that surrounds these cells, providing strength and protection for the nerve (Kleitman *et al.* 1995).

The peripheral nerve comprises somatic motor, somatic sensory and unmyelinated C (somatic and sympathetic) fibres. The composition and type of unmyelinated fibres may vary by nerve (Low *et al.* 1997).

*Myelinated nerve fibres.* Peripheral axons are surrounded by Schwann cells and demarcated from the neighbouring connective tissue by the Schwann cell basement membrane. The Schwann cells are the myelinated glial cells found in the PNS. Compact myelin is formed by multilamellar spiral wrapping of Schwann cell plasma membranes. According to the organisation of Schwann cells, mature peripheral axons are classified as unmyelinated or myelinated. Myelination is a prerequisite for the rapid, saltatory conduction of action potentials, and also for the maintenance of the axonal structure. The peripheral myelinated nerve fibre consists of a single continuous neuronal process, the axon, and a set of Schwann cells arranged serially along the outside of the axon. A large myelinated fibre may have 250 to 300 turns of myelin wrapping around it, the actual axonal diameter thus being about 70% of the total diameter (Berthold *et al.* 2005). In myelinated nerves, the sheath is interrupted regularly along the axonal length by the nodes that have been named for their discoverer, Ranvier. The nodes of Ranvier are short (1μm) myelin-free segments, and are the only sites along a myelinated nerve fibre that support the fast de- and repolarisation process necessary for generation of action potentials. The intervening fibre segments correspond to the extension of one Schwann cell and are called internodes. Internodes are from 200 to 2000μm long; their length is positively correlated to axon size (Berthold *et al.* 2005). The nodes of Ranvier are the sites that enable rapid electrical signalling with relatively high nerve CVs in myelinated nerves. This ability is mainly due to the high concentration of voltage–sensitive $Na^+$ channels in the nodal axolemma, where it is free of myelin insulation. The internodal region of the axolemma has abundant $K^+$ ion channels, this is important for repolarisation of the axon membrane following the action potential.

The diameter of myelinated nerve fibres is 4-20 μm (Berthold *et al.* 2005). The CV of myelinated axons is proportional to the axon diameter (Berthold *et al.* 2005). The density of myelinated axons in the sural nerve between ages 10 and 60 years is 7,500-10,000/ $mm^2$ (Jacobs *et al.* 1985).

*Unmyelinated nerve fibres.* Unmyelinated axons vary in diameter from 0.2 to 3 μm; they do not have a multilamellar myelin sheath covering the nerve fibres. The unmyelinated axons are not completely devoid of myelin: the Schwann cells make just a single wrap

around the axon, often one Schwann cell encases several axons. In unmyelinated axon, the action currents flowing in local circuits progressively depolarize neighbouring regions of the membrane and the impulse travels continuously along the axon (Berthold *et al.* 2005).

The unmyelinated axons outnumber the myelinated axons; in most nerves, the ratio is 4:1 (Berthold *et al.* 2005). In mammals, unmyelinated axons comprise approximately 75% of axons in cutaneus nerves and dorsal spinal roots, 50 % in muscle nerves, and approximately 30 % in ventral roots. Autonomic nerve trunk contains only few myelinated nerves, and white rami communicantes contain two thirds unmyelinated axons (Berthold *et al.* 1995).

The nerves to the skin arise from sensory and motor neurons residing in dorsal root ganglia and sympathetic ganglia. When the nerve bundles enter the dermis, they form a horizontal subepidermal neural plexus. Epidermal nerve fibres branch from this plexus, and when they enter the epidermis from the dermis they lose their Schwann cell ensheathment and collagen collar (Kennedy *et al.* 2005).

## 2.2. Sensory system

*Sensory fibres, receptors and peripheral nerve endings.* The axons in cutaneous nerves are mostly afferent and the cell bodies of their primary somatosensory neurons are segmental, arranged in the dorsal root ganglion and in cranial root ganglia. Afferent sensory cutaneous fibres are divided according to their CV into C, Aδ, Aβ and Aα fibres, and by their anatomy into large myelinated fibres, small myelinated fibres and unmyelinated fibres (Lawson 2005).

Somatosensory neurons in dorsal root ganglia can be classified as nociceptive or non-nociceptive including unmyelinated (C) fibres or myelinated (A) fibres. These fibres respond to mechanical, thermal and/or chemical stimuli and they project to skin, muscle and blood vessels of the trunk and limbs or to visceral organs in the thorax or abdomen (Lawson 2005).

The nociceptors (Latin nocere, to damage) are receptors that respond selectively to painful, tissue-damaging stimuli, and indirectly to others by means of one or more chemicals released in the damaged tissue. There are three subclasses of nociceptors, based on the stimulus that activates them: mechanical, thermal and polymodal nociceptors. Polymodal nociceptors are the largest subclass of these receptors and they are sensitive to destructive effects of a stimulus rather than to its physical properties (Basbaum *et al.* 2008).

Thermoreceptors are of two types; warm and cold receptors. Warm and cold or slow, burning pain is mediated by small unmyelinated C fibres from polymodal and thermal-mechanical receptors. Unmyelinated C nociceptors comprise the most numerous classes of somatic afferent nerve fibres. The majority of unmyelinated polymodal C-nociceptors are activated by mechanical, chemical and thermal stimuli (Torebjörk *et al.* 2005). Also unmyelinated C-mechano-insensitive receptors exist; they have higher activation thresholds for heat, and are not activated even by intense mechanical stimuli. About 10 % of the mechano-insensitive nociceptors show lasting activation by histamine, that parallels the itch sensation in humans; they are called the "itch fibres" (Torebjörk *et al.* 2005). Low thresholds C-fibres have a role in emotional touch (Torebjörk *et al.* 2005). The fibres that convey skin cooling (< 25 C) and hot temperatures (> 45 C) are small Aδ fibres. Aδ nociceptors are thinly myelinated; activation of them leads to the sharp, prickling pain and heat stimulation (burning pain). Aδ fibres can have long unmyelinated distal branches in the skin up to 5 cm (Torebjörk *et al.* 2005), which makes the differentiation between Aδ and C fibres in the periphery complicated.

Large myelinated fibres, Aα and Aβ, carry sensations of touch-pressure, skin stretch and vibration. The stimulus for vibration is experienced in Pacinian corpuscles on the skin. Touch–pressure is also mediated by Aδ, Aα and β fibres. The receptors for touch-pressure and position are Meissner's corpuscles, which are low-threshold, rapidly adapting receptors. Also the Merkel cell complex at the dermal epidermal junction and the slow adapting touch-pressure Ruffini receptors in the epidermis are activated by touch-pressure stimulus (Lawson 2005).

**Table 1.** Sensory axons; type, modality and receptor type

| Axon type | Modality | Receptor type |
|---|---|---|
| Unmyelinated C | Warm, cool pain, burning sensation | Cool receptors, polymodal nociceptors |
| Myelinated Aδ | Heat pain, cold, pin-prick sensation, limb proprioception (excess stretch or force) | Sensitive to hair movement, thermal: cool receptors and heat nociceptors, mechanical, thermal-mechanical, stretch sensitive free endings |
| Myelinated Aβ | Touch-pressure, skin stretch, vibration, limb proprioception | Muscle spindle secondary, joint capsule mechanoreceptors, Meissner corpuscle, Merkel disk, Pacinian corpuscle, Ruffini ending |
| Myelinated Aα | Touch-pressure, skin stretch, vibration, limb proprioception | Muscle spindle primary, Golgi tendon organ, Meissner corpuscle, Merkel disk, Pacinian corpuscle, Ruffini ending |

*The central pathways of the somatosensory system.* Irrespective of modality, all somatosensory information from limbs and trunk is conveyed by dorsal root ganglion

neurons, while the somatosensory information from cranial structures is transmitted by the trigeminal sensory neurons. The trigeminal sensory neurons are functionally and morphologically homologous to dorsal root ganglion neurons.

The cell bodies of sensation lie in a ganglion on the dorsal root of a spinal nerve. The axon has two branches, one projecting to the periphery, and the other to the CNS.

Sensory information from limbs and trunk is conveyed to the thalamus and cerebral cortex by two ascending pathways (Gardner *et al.* 2000). Tactile sensation and limb proprioception from large-diameter fibres are transmitted to the thalamus by the dorsal column-medial lemniscal system. Painful and thermal sensations from small fibres are transmitted to the thalamus by the anterolateral system.

The large-diameter dorsal root ganglion axons mediating touch and proprioception ascend ipsilaterally in the spinal cord to the brain stem. In the brain stem the axons above the level of Th6 terminate in the nucleus cuneatus, while the axons from the lower segments terminate in the nucleus gracilis. The nucleus cuneatus lays laterally to the nucleus gracilis in the brain stem. The second-order neurons in the dorsal column nuclei send axons across the midline in the medulla, where they form the medial lemniscus. When these axons ascend through the brain stem they shift laterally, joining fibres of the spinothalamic tract in the midbrain, before terminating in the ventral posterior lateral nucleus of the thalamus. Thalamic neurons mediating touch and proprioception send axons to the primary somatic sensory cortex in the postcentral gyrus.

The anterolateral tract consists of small fibres that convey pain and temperature changes. These fibres terminate on second-order neurons in the dorsal horn of the spinal cord, and the axons of these neurons cross the midline of the spinal cord. Thus, pain and temperature change ascend contralaterally in the spinal cord. Painful or thermal stimuli project to the primary sensory cortex, to the dorsal anterior insular cortex, and to the anterior cingulated gyrus (Gardner *et al.* 2000).

## 2.3. Motor system

*The motor unit.* Sir Charles Sherrington introduced the term “motor unit” for the combination of motoneuron and muscle fibres innervated by it (Liddell *et al.* 1925). Later, it became convenient to introduce the term “muscle unit” to denote the group of muscle fibres innervated by a given motoneuron (Burke 1967). The soma of the alpha motoneuron bodies lies in the anterior horn of the spinal cord or brain stem. The axon of each motor neuron exits the spinal cord through the ventral root or through the cranial nerve in the brain stem, and passes along peripheral nerves until it enters the muscle it controls.

The number of muscle fibres constituting a single motor unit varies greatly in muscles in different parts of the body. The size of a motor unit is explained in terms of the number of fibres in its muscle unit (the innervation ratio). These innervation ratios vary with muscle size. Mean innervation ratios can be calculated by dividing estimates of the number of total muscle fibres by the number of large efferent motor axons (Eccles *et al.* 1930). These average innervation ratios in humans vary with the size of the muscle, from less than 12 in intrinsic extraocular muscles to over a thousand in large limb and trunk muscles (McComas 1998). Except during development, each muscle fibre is normally innervated by only one motor neuron in only one place, usually near the muscle's midpoint (Loeb *et al.* 2000). The functional connection between a motor neuron and a target muscle is a chemical synapse, called the end-plate. The neurotransmitter in this neuromuscular unit is acetylcholine. End-plates are clustered in bands that extend across some or all of the muscle. In motor neuron diseases, the motor nerve cell bodies are affected, while in motor peripheral neuropathies, primarily the motor axons of the cell are affected (Loeb *et al.* 2000).

*The central motor pathways.* The motor cortex directly controls motor neurons in the spinal cord through two descending pathways, the ventral and lateral corticospinal tract. Specific regions of the motor cortex influence the activity of specific muscle groups. Fibers that originate in the primary motor cortex and terminate in the ventral horn of the spinal cord constitute a significant part of the corticospinal tract. The motor system has three levels of control, the spinal cord, brain stem and forebrain, organized both serially and in parallel.

## 2.4. Anatomy of autonomic nervous system

The autonomic nervous system (ANS) is a system of motor nerve fibres that supplies the cardiovascular system, smooth muscle and glands. It is also called the "visceral" or "vegetative nervous system". The central autonomic network (CAN) involves multiple areas distributed throughout the CNS. It includes the insular, anterior cinculate and ventromedial prefrontal cortices, central nucleus of the amygdala, paraventricular and other nuclei of the hypothalamus, periaqueductal gray matter, parabrachial nucleus, and nucleus of the solitary tract, ventrolateral medulla, ventromedial medulla, and the medullary lateral tegmental field (Benarroch 1997). The CAN is involved in tonic, reflex, and adaptive control of autonomic function, and its activity is state-dependent, it varies during the sleep-wave cycle or during adaptive behaviours. The CAN also coordinates autonomic function with endocrine, behavioural and antinociceptive responses to a variety of environmental stimuli. There is no anatomically identifiable descending autonomic pathway or tract in the spinal cord. The descending autonomic

fibres are diffusely disbursed in the spinal cord, all projecting to interomediolateral and interomediomedial pathways (Gardner *et al.* 2005).

The preganglionic nerve fibres are those that leave from the brainstem and spinal cord over certain cranial nerves and ventral roots, and synapse in peripheral autonomic ganglia. The postganglionic fibres are axons of the ganglion cells and are distributed to cardiac muscle, smooth muscle and certain glands. The locations, arrangements, connections and patterns of distribution of the preganglionic and postganglionic autonomic fibres are grouped, on the basis of an anatomic classification of subdivisions of the pathways, into sympathetic, parasympathetic and enteric divisions. Both sympathetic and parasympathetic divisions supply most viscera. Norepinephrine and acethylcoline are the predominant transmitters in the ANS (Gardner *et al.* 2005).

*Sympathetic nervous system.* The sympathetic nervous system comprises the preganglionic fibres that issue from the thoracic and upper lumbar levels of the spinal cord. The fibres travel in ventral roots and spinal nerves to reach peripheral sympathetic ganglia, where they synapse with ganglion cells. The sympathetic nerve trunk connects the ganglia vertically along the spine. The ganglia of the "sympathetic chains" lie along each side of the spine. Most of the preganglionic fibres that enter the sympathetic trunk synapse in the ganglia of the trunks and in accessory ganglia. Those fibres that do not synapse continue through and reach the ganglia of the prevertebral plexuses. Of the postganglionic fibres that arise from the trunk ganglia, some go directly to adjacent viscera and blood vessels; while the others return to spinal nerves and dorsal and ventral rami by way of rami communicantes. The fibres of both the ventral and dorsal rami eventually transport secretor fibres to sweat glands, to hair follicles and motor fibres to the smooth muscle of the blood vessels of limbs and walls of the trunk. Most postganglionic sympathetic neurons release norepinephrine (Gardner *et al.* 2005).

*Parasympathetic nervous system.* The parasympathetic system comprises the preganglionic fibres that issue from the brainstem by way of the 3$^{rd}$, 5$^{th}$, 7$^{th}$, 9$^{th}$, and 10$^{th}$ cranial nerves and from the sacral cord by way of its second and third or third and fourth ventral roots. The ganglion cells are usually in or near the organ to be innervated, hence, the postganglionic fibres are short. The ratio of preganglionic to postganglionic neurons is much smaller in the parasympathetic system than in the sympathetic system. This explains the specific, controlled and localized function of the parasympathetic nervous system (Gardner *et al.* 2005). Acetylcholine is released from parasympathetic nerve terminals (Gardner *et al.* 2005).

*Enteric nervous system.* The enteric system also consists of sympathetic and parasympathetic inputs. It is limited to the wall of the bowel and is relatively autonomous in its control of gut function. The enteric nervous system extends the length of the gastrointestinal tract from esophagus to rectum. It is composed of two major plexuses

of ganglion cells, as well as interconnecting fibres. The more internally located neurons (Meissner's plexus) are located in the submucosa of the gut wall, and the more externally located neurons (myenteric plexus, also called Auerbach's plexus) lie between the external longitudinal and internal circular smooth muscle layers of the muscular coat of the gut (Gardner *et al.* 2005).

## 2.5. Physiology of the autonomic nervous system

Parasympathetic-sympathetic balance is important in the maintenance of normal cardiac rhythm and in many other functions of the human body. Here the physiology of cardiovascular control is discussed in more detail.

*Cardiovascular control.* The ANS has a central role in regulating the arterial blood pressure, it is the most important variable in the cardiovascular system (Joyner *et al.* 1997). Heart rate (HR) depends on the net of excitatory and inhibitory action of sympathetic and parasympathetic systems. This is controlled by the CNS, which in turn depends on the inputs from cardiopulmonary receptors, arterial baroreflexes and muscle ergoreceptors. Rapid changes in HR are predominantly due to the vagus, and slower responses to sympathetic nervous system (Iversen *et al.* 2008). Resting HR is determined primarily by the parasympathetic tone, acting directly on the heart or interacting with the sympathetic nerves through several mechanisms in the brain and at the level of the heart. When alertness and activity are required, the sympathetic nervous system prevails. The tonic activity of both systems arises in the CNS. Parasympathetic-sympathetic balance is important in the maintenance of normal cardiac rhythm. Parasympathetic influence tends to lessen, and adrenergic stimulation to increase, the tendency for ventricular arrhythmias. Both parts of the cardiac ANS act in a synergistic manner, resulting in the phenomenon of accentuated agonism, whereby the negative chronotopic and inotropic action of the vagus is more pronounced when sympathetic tone is high. The high sympathetic activity can facilitate ventricular arrhythmias, whereas vagal activation increases electrical stability and minimizes the incidences of ventricular tachyarrhythmias and fibrillation.

## 2.6. The action potential

*Resting membrane potential.* The cell membranes of neurons are polarised: the inside of the neuron is negative relative to the outside (about -70 mV). This potential is established so that there is an unequal distribution of ions on the two sides of the nerve cell membrane when the membrane is not stimulated or being conducted. The resting transmembrane potential reflects 1) the relative concentrations of specific cations and anions between the extra- and intracellular compartments 2) the equilibrium potentials, 3) the permeability of the membrane to the ion species, and 4) the presence of an electrogenic, energy-dependent Na+/K+ pump (Brown *et al.* 2002).

Two ions are responsible for the membrane potential: sodium ($Na^+$) and potassium ($K^+$). An unequal distribution of these two ions occurs on the two sides of the nerve cell membrane. This is due to the active transport mechanism of the membrane, the $Na^+$-$K^+$ pump. Due to this active pump, there is a higher concentration of $Na^+$ on the outside than the inside, and a higher concentration of $K^+$ inside than outside.

The nerve cell membrane also contains special passageways for both $Na^+$ and $K^+$ ions that are commonly referred to as ion channels. These channels ($Na^+$ and $K^+$) represent the only way that these ions can pass through the nerve cell membrane. In a resting nerve cell membrane, all $Na^+$ gates are closed and some of $K^+$ gates are open. As a result, $Na^+$ cannot diffuse through the membrane and largely remains outside the membrane while some $K^+$ ions are able to diffuse out. $K^+$ ions begin to diffuse across the membrane from the compartment of higher concentration of $K^+$ ions to the lower concentration, driven by the concentration gradient of the $K^+$ ions.

The ever-increasing accumulation of positive ions in the extracellular space and the corresponding increasing negativity to the intracellular compartment produce the *diffusion potential.* However, the force exerted by the $K^+$ gradient is met by the mounting electrical resistance to further diffusion produced by the build-up of the positively charged $K^+$ ions on the extracellular side. Finally, when two forces, the concentration gradient for $K^+$ and the opposing electrical force, balance each other, any further net flux of $K^+$ ions ceases and the membrane reaches the equilibrium potential for $K^+$ ions (Brown *et al.* 2002).

This resting potential will be maintained until the membrane is disturbed or stimulated. Then, if it is a sufficiently strong stimulus, an action potential will occur.

*Generation of the action potential.* An action potential is a very rapid change in membrane potential that occurs when a nerve cell membrane is depolarized to the threshold of activating the action potential. The membrane potential goes from the resting potential (-70 mV) to some positive value (about +30 mV) in a time period of about 1 ms. The stimulus causes the $Na^+$ channels to open briefly. Because the $Na^+$ concentration on the outside is higher than on the inside of the membrane, $Na^+$ diffuses rapidly into the nerve cell. The positively-charged $Na^+$ rushing in causes the membrane potential to become positive and the inside of the membrane is now positive relative to the outside.

After that, the $K^+$ channels open, and because there is more $K^+$ inside the membrane than outside, positively-charged $K^+$ ions diffuse out. As these positive ions go out, the inside of the membrane once again becomes negative with respect to the outside (repolarisation).

*Propagation of the action potential.* The action potential occurs only when the cell membrane is stimulated (depolarized) enough so that the $Na^+$ channels open completely. This minimum stimulus needed to stimulate an action potential is called the threshold stimulus. The threshold stimulus causes the membrane to become less negative by

opening the $Na^+$ channels and allowing positive $Na^+$ ions to diffuse in. If the membrane potential reaches the threshold potential (5 - 15 mV less negative than the resting potential), all the voltage-regulated $Na^+$ channels will open. When the $Na^+$ ions diffuse inward, depolarization occurs. The action potentials occur maximally or not at all ("all-or-none law"), there is no such thing as a partial or weak action potential.

During and immediately after an action potential, a second stimulus will not produce a second action potential (no matter how strong that stimulus is). This is called the absolute refractory period. It corresponds to the period when the sodium channels are open (time period of < 1ms)

The relative refractory period corresponds to the period when the $K^+$ channels are open (time period of several milliseconds). Another action potential can be produced, but only if the stimulus is greater than the threshold stimulus. The cell membrane becomes more sensitive to stimulus, as the relative refractory period progresses. At the beginning of the relative refractory period, it takes a very strong stimulus to cause an action potential, whereas at the end of the refractory period, it takes only a slightly above threshold stimulus to cause an action potential.

The CV is dependent on the diameter of the nerve fibre, the temperature and the presence or absence of the myelin sheath. Myelinated axons conduct impulses faster than axons without myelin. The Nodes of Ranvier are places where the density of voltage–gated channels is high to allow the action potential to propagate by jumping from node to node, called "saltatory" conduction (Latin saltore = to jump) (Barnett *et al.* 2007). Because the impulse "jumps" over areas of myelin, the impulse travels faster along a myelinated neuron than along a non-myelinated neuron.

## 2.7. Pathophysiology and types of polyneuropathies

Different PNPs vary in their presentation. All types of fibres can be involved; sometimes the PNPs may be quite selective, involving, for example, only sensory fibres or only thin fibres. The distribution is usually distal and symmetric, but not always. The primary lesion may affect primarily the axon or the myelin, or selectivity may occur. There are different ways to categorize PNP; by (1) types of axons affected (motor versus sensory or autonomic), by (2) fibre size affected, (3) by anatomic distribution, (4) by pathophysiology or (5) by etiology.

*Types of axons affected.* Most common PNPs, especially diabetic PNP affect all different types of axons. There are, however exceptions to this and some PNPs affect only motor, sensory or autonomic nerves. Some neuropathies may affect one type of axons to a considerably greater degree than others.

*Motor.* Predominantly motor neuropathies include chronic inflammatory demyelinating PNP (CIDP), multifocal neuropathy with conduction block, and hereditary motor and sensory neuropathy (HMSN) I and II. Toxic neuropathies that lead to predominantly motor neuropathy include therapeutic gold compounds, amiodarone, dapsone, perhexilline, lead, mercury and Vacor (N-3-pyridyl methyl-N'-p-nitrophenyl urea), a rat poison (Sabin 2001). In chronic demyelinating motor neuropathies the muscle bulk can be preserved, although the patient has substantial weakness. In chronic axonal motor neuropathy, both weakness and atrophy occur. In pure motor syndromes it is crucial, however, to separate diseases which primarily affect the motor neurons themselves from the diseases that primarily affect the peripheral motor axons and / or myelin of the peripheral axons (Sabin 2001).

*Sensory.* In demyelination alone, without conduction blocks or axonal involvement, the sensory symptoms are mild or absent. In sensory demyelinating neuropathies without conduction blocks, many of the sensory fibres have to be affected before there are measurable elevations in sensory thresholds (Sabin 2001). Prominent positive sensory symptoms may be found without actual detectable sensory loss. Because the longest fibres are first affected in most PNPs, the sensory symptoms are distal. Paresthesias on the soles of the feet and later the palms can be prominent; a feeling of pins and needles. In sensory neuropathies, where there is large fibre sensory loss, loss of position and vibration sense is usually seen. Tingling and tightness in the limbs are also common by reported symptoms. Examples of sensory neuropathies with large fibre sensory loss include pyridoxine toxicity, ataxic subtype of AIDP (acute inflammatory demyelinating PNP) with large fibre sensory loss, chronic idiopathic ataxic neuropathies associated with paraproteins, carcinomatous neuropathy, cisplatin toxicity, and neuropathy associated with primary biliary cirrhosis (Vinken *et al.* 1987). When loss of deep sensibility reaches a critical level, sensory ataxia appears. Pseudochorea, involuntary palpatory movements of the outstretched upper extremities can occur (Sabin 2001).

*Small fibres.* Small fibres of the peripheral nerve subserve the sensory modalities of pain sensation and temperature, as well as the ANS (Sabin 2001). Small fibre neuropathy may occur isolated or, more commonly, in mixed PNPs affecting all categories of sensory and often also motor fibres. Since most small fibres are unmyelinated, the neuropathy affects primarily the axons. In neuropathies, where small-fibre neuropathy predominates or is the only pathologic finding, pain and autonomic disturbances are the most debilitating symptoms. The spontaneous pain in small-fibre neuropathy is often described as a burning, prickly, hot, stinging or freezing sensation (Boivie *et al.* 1993).

Clinically small-fibre neuropathy can be detected with reduced sensation for pinprick, cold and warm (Boivie *et al.* 1993). In isolated small-fiber PNP, the tendon reflexes are normal. Quantitative thermal thresholds are helpful in the diagnosis. From skin biopsy,

the density of small unmyelinated nerve endings can be calculated (Lauria *et al.* 2005). The most common cause of small-fibre involvement in PNP is diabetes. Isolated small-fibre neuropathies are rare; Fabry disease is a rare disorder affecting only small fibres (Karetova *et al.* 2006).

*Autonomic fibres.* Usually, autonomic neuropathy is seen together with the neuropathy of somatic fibres. The distributions of autonomic deficits in PNP are not so readily analyzed according to axonal length. Pure cholinergic and pure adrenergic neuropathies may occur in rare conditions (Sabin 2001). Adrenergic neuropathy is seen in rare hereditary conditions. Cholinergic neuropathy may be found in botulism and sometimes in Lambert-Eaton syndrome. In acute neuropathies, like AIDP, excessive or decreased activity in the ANS is common and may be life-threatening (Sabin 2001). Tachycardia and hypertension alternating with significant drops in blood pressure may occur. Also acute autonomic failure may occur, when there is an acute or subacute loss of both parasympathetic and sympathetic activity, but the non-autonomic fibres are spared. Toxic neuropathies, like thallium-, arsenic- and mercury-induced neuropathies may also have autonomic features, particularly affecting cardiac autonomic nerves by increasing the pulse and blood pressure (Windebank 1987). Dry mouth, alternations in sweating and in body temperatures, impotence and GI dysfunction manifested by vomiting, and constipation alternating with diarrhea may also occur (Windebank 1987; Sabin 2001).

*Distribution.* The distribution of peripheral nerve damage can vary depending on the underlying cause of the nerve damage. Most PNPs are relatively symmetric and affect the nerves in a length-dependent fashion. There are, however, other rarely occurring types of distributions. According to the report of the AAN (American Academy of Neurology), AANEM (American Academy of Neuromuscular and Electrodiagnostic Medicine) and AAPM&R (American Academy of Physical Medicine and Rehabilitation), the concept of distal symmetric PNP requires a clear definition of distal and symmetric in the context of PNP. Distal refers to those parts most distant from the center of the body. The PNP must begin in the feet. Symmetric indicates that the symptoms and signs are the same on both sides of the body (England *et al.* 2005).

*Symmetric, length-dependent.* Proximal-distal axonopathies, usually called distal axonopathies and dying-back neuropathies, are the most common presentation of generalized axonal neuropathies (Berger *et al.* 1995). The hallmark of the distal axonopathies is the sequential distal-to-proximal loss of sensory and motor function. When the longest and largest diameter fibres are affected, the symptoms and signs first appear in the toes and soles. At the early stages of the disease, complaints of pain, numbness and tingling are usually more prominent than weakness. Large- and small-fibre sensory modalities are usually affected, but diminished vibration sensation is usually the earliest abnormality found (Berger *et al.* 1995). Sensory disturbances extending from the

toes and soles up the feet to the distal legs, produce the "stocking" like numbness. When the mid-shins are affected, the fingertips become numb, presenting a "glove" distribution. In severe cases, even the distal portions of the intercostal nerves are affected, resulting in hyperesthesia over the mid-abdomen (Mendell *et al.* 2001). Vibration loss extends from the toes to the knees, eventually affecting the fingers and the iliac crest. Weakness spreads from distal muscles to proximal muscles. The sensorimotor abnormalities are usually seen symmetrically, but at the early stages of the disease there may be slight asymmetries in onset and severity. Tendon reflexes are diminished at the ankles, then the knees and only at the later stages at the arms. A substantial deviation from this pattern should suggest that another type of neuropathy is present (Berger *et al.* 1995).

In distal small-fibre neuropathy, there is distal paresthesia and allodynia, often accompanied by involvement of the postganglionic sympathetic nervous system, manifested by alternations in sweating and distal vasomotor instability (Devigili *et al.* 2008). The distributions of autonomic deficits in peripheral neuropathy are not so readily analyzed according to axonal length.

*Proximal.* In dysimmune neuropathies, like in Guillain-Barré syndrome (GBS) and sometimes in chronic inflammatory demyelinating PNP (CIDP) a proximal weakness may be observed and sensory symptoms may begin in the upper limbs (Schaumburg *et al.* 1991b). Also in acute intermittent porphyria, a proximal muscle weakness with proximal sensory loss may occur (Sabin 2001). In spinal muscular atrophy (inherited motor neuropathy) proximal weakness with upper limb involvement is characteristic (Meadows *et al.* 1969). B12-vitamin deficiency-induced neuropathy can begin in the upper limbs with sensory symptoms and clumsiness. Also in the type II form of hereditary amyloid neuropathy, sensory loss often begins in the upper limbs with bilateral median nerve lesions resulting from the compression of amyloid deposits under the flexor retinaculum of the wrists (Thompson *et al.* 2005). In Tangier disease, proximal sensory loss with relative sparing of the distal extremities is also seen (Kocen *et al.* 1967). The reason for selective proximal involvement is unknown. The answer may lie in the fact that the proximal portions of the limbs are supplied by larger diameter nerve fibres than those innervating the most distal regions and the trunk, and these fibres may have higher susceptibility to metabolic change and immunologic insults. In sensory gangliopathy, symmetrical or asymmetrical proximal sensory loss may begin in the upper limbs. Widespread sensory loss involving proximal and distal limbs is more commonly a clinical feature of a gangliopathy than a PNP (Thompson *et al.* 2005). Especially diabetics are prone to proximal neuropathies. The diabetic radiculoplexus neuropathies, also known as diabetic amyotrophy, present with sudden or subacute onset of pain and weakness in an asymmetrical pattern, and proximal segments are frequently involved. The same clinical phenotypes of radiculoplexus neuropathies also occur in nondiabetic patients, although they may be less frequent (Dyck 2005b).

*Mononeuritis multiplex.* Mononeuritis multiplex refers to a multifocal neuropathy that affects various sites in the PNS (Sabin 2001). Focal lesions attributed to specific nerves appear in a stepwise asymmetric way. A sudden onset of accumulating asymmetric deficits is typical. Sometimes, in the later stages of mononeuritis multiplex, the lesions can be confluent and appear similar to a distal symmetric PNP (Sabin 2001). A detailed history may be necessary to establish the asymmetry and patchy distribution, which point to mononeuritis multiplex. Mononeuritis multiplex is usually caused by vasculitis of the peripheral nerves (Kissel 2001; Thompson *et al.* 2005). Symptoms of numbness, tingling, contact hypersensitivity, pain and weakness begin in the region of a single peripheral nerve, but within days or weeks, other nerves become affected (Collins *et al.* 2005).

### 2.7.1. Pathophysiology

Neuropathies can be divided by their pathophysiology depending on which structure is predominantly affected. Most often the axons are affected, but sometimes the disease also or even predominantly affects the myelin sheath.

*Axonal PNP.* In most PNPs seen in neurologic practice, the primary degeneration affects the axon. A common accompaniment in axonal neuropathy, however, is secondary demyelination. Cell body chromatolysis can occur in severe cases (Berger *et al.* 1995). The underlying pathologic mechanisms in axonal neuropathy are debated, but the neuropathic insult is presumably at the cell body or some other point on the peripheral axon. Probably the failure of axonal transport results in degeneration of vulnerable distal nerve segments (Berger *et al.* 1995), and as the process continues, degeneration proceeds proximally toward the cell body. Initial morphologic changes occur in the distal parts of the axons, while in sensory nerves, both peripheral and central parts of the axons are affected. In the spinal cord the axons in the anterolateral and posterior columns, as well as the distal corticospinal tract, are also affected. In some cases, the proximally directed axons of the primary sensory neurons may be the most affected, while in others, the most affected axon is distally directed (Dyck *et al.* 2005a). Whether the primary abnormality is in soma, in proximal axons or in both axon and soma is not known. In arsenic and thallium intoxication, diabetes, uremia, alcohol, malnutrion and vitamin deficiency neuropathies, distal fibres undergo the changes typical of Wallerian degeneration. This type of degeneration is induced by destruction of nerve fibres leading to distal degeneration (Waller 1850). The mechanisms are not fully understood and these processes should be referred to as acute axonal degeneration (Dyck *et al.* 2005a).

According to another theory of the axonal PNP, the changes begin in a multifocal manner along the axons, not at the terminal parts of the axons. With continued damage the proximal spread of the degeneration is again multifocal, with short lengths of the

terminal axon appearing to undergo simultaneous degenerative changes distal to the nodal points at which giant axonal swellings have occurred (Sumner 1980).

It has been postulated that the axonal injury occurs on the basis of anoxia or ischemia, especially in diabetic PNP (Llewelyn *et al.* 2005). It appears likely that ischemia plays an important role in the pathogenesis of vasculitic and diabetic PNP (Stys *et al.* 1995; Low *et al.* 1989). Studies on human nerves have shown that ischemia initially causes a decrease in threshold, followed by an increase, then conduction block (Kugelberg 2008). Changes in threshold and accommodation during ischemia are thought to occur as a result of membrane depolarization, followed by inactivation of Na+ permeability, which leads to block of conduction (Baker *et al.* 1989a).

Acryl amide neuropathy has been used as a laboratory model of axonal neuropathy, because acrylamide (Ch2ChCoNh2) produces a pure axonal neuropathy in rats. Sensory axons are much more vulnerable to distal axonopathy than motor axons. One explanation may be the concept of toxin vulnerability and total cell size. In the case of lumbosacral root ganglion cells, the central extensions are lengthy. The total cytoplasmic volume of the cell can somehow determine the toxic susceptibility of the cell, with the consequent failure of the most distal peripheral and central axonal extensions (Sumner 1980).

A variety of disorders, metabolic, toxic and inherited causes, can lead to generalised axonal injury. In many neuropathies, distal fibres undergo the changes of Wallerian degeneration; examples of this are arsenic and thallium intoxication, diabetes, uremia, alcohol, malnutrition and vitamin deficiency, vasculitis, multiple myeloma, many toxic chemicals, drugs and heavy metals (Schaumburg *et al.* 1991a; Dyck *et al.* 2005a). As axonal degeneration begins in the distal portions of axons and spreads more proximally, the ”dying back” reaction or distal axonopathy has been used to determinate the pathology of axonal neuropathy. Atrophy in distal muscles, first in intrinsic foot muscles, occurs due to denervation (Schaumburg *et al.* 1991a).

In axonal neuropathies Wallerian degeneration results in denervation of muscle fibres. The denervated muscle fibre membrane potential decreases from -80 mV to -60 mV and acetylcholine receptors spread from end-plate area to other membrane areas. At the same time, the number and the conductance of sodium (Na) channels increase (Falck 2006). The resting potential of the muscle fibre membrane fluctuate and become unstable, producing fibrillation potentials and positive sharp waves. This abnormal spontaneous action can easily be detected with needle-EMG. Since one motor neuron innervates large number of muscle fibers, the axonal damage of one single axon results in the denervation of several hundred muscle fibres. The denervated muscle fibres attract collateral sprouts from surviving motor units and are reinnervated by so called collateral reinnervation. As a result of collateral reinnervation the number of muscle fibres in the motor units

increase. This increase of motor unit size can easily be detected with motor unit potential analysis on EMG (Falck 2006).

The term axonal dystrophy is used synonymously with axonal swellings or axonal spheroids, which are multifocal accumulations of cytoskeletal elements (Dyck *et al.* 2005a). The axonal spheroids are seen in giant axonal neuropathy (a disorder of children, probably recessively inherited, with large axonal spheroids in both peripherally and centrally directed primary afferent axon neurons) and neuropathies caused by many industrial toxins (Dyck *et al.* 2005a). In toxic neuropathies the distribution of axonal spheroids may be related to the dosage and potency of the toxins. The accumulation of a variety of organelles (glycogen, myelin figures, endoplasmic reticulum, degenerated mitochondria and lysosomes) may be encountered in both experimental and human neuropathies. Although these may not cause focal swellings, they may represent dystrophic changes (Dyck *et al.* 2005a). In the distal axonopathies, conduction velocities remain normal, or are mildly decreased. A fall in response amplitude (AMPL) is the main finding in neurography but secondary demyelination may occur, as discussed earlier. This is the case, for example, in hexacarbon neuropathy, in which CVs may be decreased (Berger 2001). The nerve fibres after hexacarbon poisoning may contain axonal swellings, which are associated with thinning of the underlying myelin, and retraction of the myelin sheath in the region of the nodes of Ranvier. In these kind of cases, secondary changes in the myelin caused by hexacarbon explain the slowing of conduction (Sumner 1980).

*Demyelinating PNP.* Demyelination is a common pathologic finding in peripheral neuropathy. In demyelinating PNPs the myelin sheaths are primarily affected, and the axons maintain their continuity to varying degrees. Often there is secondary involvement of the axons in the later stages of demyelinating PNPs. The demyelinating PNPs may be acquired, often caused by immune-mediated mechanisms, or hereditary due to genetic abnormalities (Ludwin 1995). In acute demyelination, damage to the myelin sheaths accompanies a variety of pathologic conditions, including physical trauma, chemical insult, immune reaction and infection (Rosenbluth 1995).

The neurophysiological hallmark of demyelination is a reduced nerve CV. In principle, two mechanisms might contribute to the decrease in maximum CV observed in the demyelinated nerve: 1) loss of the fastest conducting fibres, and 2) slowing of conduction in individual fibres (McDonald 1980). In fibres demyelinated by diphtheria toxin, Rasminsky and Sears (Rasminsky *et al.* 1972) showed that the slowing of conduction is due to increases in intermodal conduction time, and that the magnitude of the changes varies from segment to segment. Functional changes in conduction, including partial or complete conduction block, slowing, continuous conduction and spontaneous

activity, associated with demyelination or dysmyelination, depend on both the myelin abnormalities themselves and the secondary axonal abnormalities.

Physiologic studies have shown that the diabetic mouse develops a mild slowing of conduction with age, consistent with diabetic neuropathy (Rosenbluth 1995). The internodal myelin may become damaged, resulting in decreased internodal resistance and/or capacitance. The edges of myelin segments may retract from nodes, resulting in nodal widening with a corresponding increase in nodal capacitance. The edges of the myelin sheath may detach from the axolemma, either subtly or with obvious lifting and separation, breaking the paranodal junction and causing shunting of nodal currents under the myelin sheath. Also nodal sodium channels may be diminished in number, resulting in diminution of action currents (Rosenbluth 1995).

*Conduction block.* Persisting conduction block is caused by selective demyelination with preservation of axonal continuity. Conduction block has been demonstrated in demyelinating lesions produced in a wide variety of ways, including local compression, experimental allergic neuritis, and the local injection of lysophosphatidyl choline (McDonald 1980). PNP patients who have conduction block are suspected of having acquired neuropathy; the findings of conduction blocks in neurography distinguish acquired PNPs from familial neuropathies (Lewis *et al.* 1982; Lewis 2007).

The first complete conduction block was described by Erb (1876), who observed that in certain patients with complete paralysis of muscle due to traumatic peripheral nerve lesion, muscular contraction could be obtained by electrical stimulation of the nerve below the lesion but not above (McDonald 1980). Conduction block can be detected in motor neurography by comparing the M-wave obtained at proximal and distal stimulus sites. If there is a conduction block in the nerve segment, the MAMPL is significantly larger at the distal stimulus site. The commonly accepted criterion for a significant conduction block is >20% change in the MAMPL of the response and less than 15% dispersion of the response duration or >50% change in the MAMPL without consideration of dispersion (Uncini *et al.* 1993; Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force 1991; Lewis 2007). However, based on the recommendation of Oh et al., different criteria should be used for conduction block for different nerves, a 36% drop in tibial nerve, a 15% change in forearm segment of median and ulnar nerves, and a 20-33% change in other nerves and in upper arm segment should be used (Oh *et al.* 1994).

*Acute PNP versus chronic PNP.* In acute PNP the onset of PNP symptoms and findings occur in 50% of patients within two weeks prior to disease onset. The average time of progression is 5 to 10 days, with a range of 2 to 28 days. Inflammatory PNPs make up a major part of acute PNPs. The classic ascending paralysis in GBS results from the immune process being directed predominantly against myelin (Kissel *et al.* 2001a).

However 3-5% of GBS is mainly axonal, termed acute motor sensory axonal neuropathy (AMSAN) (Yokota *et al.* 1994b). The GBS includes acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor axonal neuropathy (AMAN), and AMSAN; weakness is predominant in all these syndromes. Distinguishing the axonal form from the demyelinating one requires neurography. Fisher syndrome (ataxia, areflexia, and ophthalmoplegia), acute panautonomic neuropathy and pure sensory acute neuropathy are also variants of GBS, but weakness is not predominant in these syndromes (Kissel *et al.* 2001a).

In acute PNP, the F-waves are of particular interest. F-waves are the first to react in immune-mediated demyelinating diseases, like GBS, and can be affected before the CV diminishes (Lachman *et al.* 1980). In GBS, the CVs in the legs and arms become clearly abnormal mostly in the third or fourth weeks or even later (Brown *et al.* 1984; Hughes *et al.* 2005), but the first conduction blocks in GBS can already be seen within two weeks of the paralysis (Brown *et al.* 1984; Lewis 2007; Hughes *et al.* 2005).

In the search for conduction blocks, the nerve conduction studies should be done bilaterally, and the tests should include n. peroneus studies over the fibula and n. ulnaris studies over the elbow. In addition, cervical and lumbar root stimulations can be done to search for proximal conduction blocks.

Sensory nerve conduction studies should be performed in both acute and chronic cases, even if the patient lacks sensory symptoms. Because of the lack of collateral innervation, the SNAPs react relatively early in some acute axonal PNPs, and are of great assistance in detecting acute axonal type of PNP. In subacute and chronic cases, MAPs are also diminished.

In the rare cases of acute panautonomic PNP, which is characterized by parasympathetic and sympathetic failure, the somatosensory or motor involvement is minimal or limited (Yokota *et al.* 1994a). SSR test and HRV should be included in the neurophysiologic studies if predominantly autonomic failure is suspected. The HRV can also be affected in other forms of GBS (Annane *et al.* 1999). This could result from demyelination of the reflex loop controlling respiratory oscillations in HR, and from desensitization of the arterial tree to an elevated plasma noradrenaline (Annane *et al.* 1999).

## 2.8. Etiologies of polyneuropathies

PNPs may be caused by toxins, nutritional deficiencies, systemic metabolic or other systemic disorders, genetic diseases, or immune-mediated diseases. The underlying conditions that cause PNPs are diverse, and in this project only a few of them have been studied. The most common cause of PNP in the western world is diabetes, which is discussed shortly here. PNP in end-stage renal disease (uremia) is common, and will

be discussed below in more detail. Fabry disease is an inherited metabolic disorder that leads to predominantly small-fibre PNP. Multiple myeloma causes PNP. Also thalidomide therapy, which is now widely used as an antiangiocenic theraphy in myeloma, causes PNP.

### 2.8.1. Metabolic neuropathies

Metabolic PNP includes a wide spectrum of peripheral nerve disorders. Diabetes, uremia, hypothyroidism, hepatic failure, polycythemia, amyloidosis, acromegaly, and porphyria, disorders of lipid/glycolipid metabolism, nutritional/vitamin deficiencies, and mitochondrial disorders, among others may cause PNP. The common hallmark of these diseases is predominantly axonal involvement of peripheral nerves by alteration of the structure or function of myelin and axons due to metabolic derangement.

#### *2.8.1.1. Uremia*

Despite adequate hemodialysis (HD), PNP is a common problem in ESRD patients (Bazzi *et al* 1991). Uremic PNP is one of the most frequent neurologic manifestations of chronic renal failure, typically presenting as a distal symmetric process (Bazzi *et al.* 1991; Krishnan *et al.* 2007; Mansouri *et al.* 2001). Neuropathy generally only develops at glomerular filtration rates of less than 12 ml/min (Brouns *et al.* 2004; Thompson *et al.* 2005). The condition is of insidious onset, progressing over months, and has been estimated to be present in 60-100% of patients on dialysis (Ackil *et al.* 1981b; Krishnan *et al.* 2007; Van den Neucker *et al.* 1998). It is regarded as the most reliable indicator of insufficient dialysis treatment. Although the exact pathogenesis of uremic PNP is unclear, the most consistent abnormality in peripheral nerves is axonal degeneration with secondary segmental demyelination (Ahonen 1981; Bolton *et al.* 1997; Pirzada *et al.* 1997; Rosales *et al.* 1988; Said *et al.* 1983). Patients may also develop autonomic dysfunction with impaired sweating, diarrhea, constipation or impotence (Krishnan *et al.* 2007), postural hypotension, arrhythmia, unstable blood pressure and intolerance to dialysis (Harnett *et al.* 1994). Plasma concentrations of noradrenaline are increased and reduced end-organ responses to noradrenaline have been reported (Kirvela *et al.* 1995; Thompson *et al.* 2005). Mixed axonal sensory and motor PNP is also common in the untreated or inadequately controlled uremic patients (Zochodne 2005). However, the symptoms of ANS and peripheral nerve dysfunction are often overshadowed by other uremic symptoms and may be unnoticed.

The development of uremic neuropathy has previously been related to the retention of neurotoxic molecules in the middle molecular (substances with a molecular weight of 300–12,000 Da) range, although this hypothesis lacked formal proof. These “middle molecules” include parathyroid hormone (PTH) and β-2-microglobulin (β-2M), and the concentrations of both these substances are elevated in patients with end-stage renal

failure (Furst *et al.* 1975). However, the only middle molecule with evidence of having a neurotoxin effect is PTH (Massry 1987). PTH has been shown to prolong motor nerve CVs in animal studies (Goldstein *et al.* 1978). However, in human studies, the effect of PTH on peripheral nerves has not been clear (Avram *et al.* 1978; Schaefer *et al.* 1980). Despite the shortcomings of the middle molecule hypothesis, the hypothesis that a dialyzable toxin may be involved in the pathophysiology of uremic PNP remains prevalent.

Studies utilizing axonal excitability techniques have recently shed further light on the pathophysiology of PNP (Bostock *et al.* 1998; Burke *et al.* 2001). Nerves of uremic patients have been shown to exist in a chronically depolarized state prior to dialysis, with subsequent improvement and normalization of resting membrane potential after dialysis. The degree of depolarization correlates with serum K+, suggesting that chronic hyperkalemic depolarization may play an important role in the development of nerve dysfunction in uremic patients. These recent findings suggest that maintenance of serum K+ within normal limits between periods of dialysis, rather than simple avoidance of hyperkalemia, is likely to reduce the incidence and severity of uremic neuropathy (Krishnan *et al.* 2007).

Successful treatment should correct the symptoms and signs of chronic uremia. During dialysis, the presence of ANS dysfunction may suddenly become symptomatic, because the normal defence against hypotension is mediated by the sympathetic nervous system. The defence against hypotension is mediated by increasing systemic vascular resistance and HR. This compensatory response may be defective if autonomic dysfunction is present.

*Neurophysiologic abnormalities in uremic PNP.* An earlier study (Nielsen 1973) has indicated that reduction of peroneal nerve motor CV is the most sensitive test for uremic neuropathy. Van den Neucker et al. (Van den Neucker *et al.* 1998) found that the H-reflex latency of the tibial nerve, sural nerve sensory CV, and deep peroneal nerve motor CV differed most significantly between chronic HD patients and control subjects. However, the sensitivity of neurophysiologic tests (proportion of abnormal results in patients undergoing HD) was not determined and F-waves were not studied.

Other studies have shown that the measurement of F-waves is useful in the detection of mild PNP (Ackil *et al.* 1981b; Andersen *et al.* 1997; Conrad *et al.* 1975). Tegner and Lindholm (Tegner *et al.* 1985) found the VPT of the foot to be the single most useful test of nerve dysfunction during HD, as it correlated with the clinical grading of the neuropathy. The clinical grading in this study included PNP signs (muscle power, tendon reflexes, sensation testing) but it did not include PNP symptoms. They compared the VPT to nerve conduction findings, but not to late responses. Also Nielsen (Nielsen 1972) found that the VPT were frequently abnormal in patients with uremic PNP, but also in

his study subjective symptoms were not assessed. Lindblom and Tegner (Lindblom *et al.* 1985) found that thermal sensation was abnormal in 30% of 64 uremic patients. They concluded that small-fibre uremic neuropathy exists as a separate entity.

The positive sensory symptoms are probably generated by increased or altered spontaneous firing of sensory axons. This altered firing can be documented using microneurography, which is a useful research tool but not suitable for clinical use (Torebjörk *et al.* 2005). Lowitzch et al. (Lowitzsch *et al.* 1981) demonstrated a marked shortening of the refractory period of the sural nerve after a single HD treatment without any change in sural nerve CV (Lowitzsch *et al.* 1981; Lang *et al.* 1977). In acute renal failure, Lang and Forsström found that CV, SAMPL, and rise-time of the compound action potential improved after one dialysis. They concluded that the metabolic disturbance due to dialysis causes transient inhibition of sensory nerve functions without morphological changes in the nerve (Lang *et al.* 1977). Nielsen (Nielsen 1973) suggested that the slowing of nerve conduction in chronic uremia is due to reversible inhibition of the sodium pump caused by humoral toxic factors in serum. This results in a decrease in the Na- K-ATPase activity, which maintains the polarization of the axon membrane. It has also been hypothesized that high concentrations of potassium, as a known neurotoxin, contributes to the process of uremic neurotoxity (Bostock *et al.* 2004).

Bischoff et al. (Bischoff *et al.* 1996) noted that A-waves are more common in patients with PNP than in others. A-waves are also seen in healthy subjects predominantly in the tibial nerve, with a frequency of about 25% (Puksa *et al* 2003a).

#### *2.8.1.2. Fabry disease*

Fabry disease is an x-linked lysosomal storage disease, caused by mutations in the gene coding for the lysosomal enzyme alfa-galactosidase in chromosome Xq22.1 (Eng *et al.* 1994; Ashley *et al.* 2001). As a result of the enzyme deficiency, neutral glycosphingolipids accumulate in most visceral tissues (Brady *et al.* 2000; Kaye *et al.* 1988). Typically, the first neuropathic symptoms, recurrent episodes of severe pain in the extremities during fever, occur during childhood or adolescence (MacDermot *et al.* 2001). Chronic pain in the extremities has also been reported. Later on, the most debilitating symptoms are pain and tingling paresthesias in their hands and feet, lasting from minutes to a day (Dutsch *et al.* 2002; Schiffmann *et al.* 2003).

Because the disease is X-linked, it has previously been postulated that only men are affected. In the majority of X-linked disorders, heterozygous women are asymptomatic. In Fabry disease, however, several recent studies have shown that the majority of heterozygous women have characteristic signs and symptoms of the disease, and many of them are severely affected (Maier *et al.* 2006; Mehta *et al.* 2004; Moller *et al.* 2006). The penetrance is close to 100%, an uncommon feature for women who are heterozygous

for an X-linked trait (Maier *et al.* 2006). Many women present with the full spectrum of disease manifestations, but with a later onset of symptoms, a slower rate of progression, and a higher phenotypic variability than men (Mehta *et al.* 2004). Life expectancy is reduced in both men and women, by about 20 and 30 years, respectively, due to renal, cerebral and cardiac complications (MacDermot *et al.* 2001; Maier *et al.* 2006; Mehta *et al.* 2004; Fellgiebel *et al.* 2006).

Earlier studies of the PNS in Fabry disease have shown that large myelinated axons are spared at the early stages of the disease, but small-fibre function mediated by unmyelinated C-fibres and small myelinated Aδ-fibres is affected in most patients (Dutsch *et al.* 2002; Luciano *et al.* 2002).

The PNP in patients with Fabry disease is length-dependent and is characterized by small-fibre neuropathy (Dutsch *et al.* 2002; Luciano *et al.* 2002; Scott *et al.* 1999). Cold perception is more frequently and severely impaired than warm perception (Luciano *et al.* 2002). Also sweating is decreased or sometimes absent (Cable *et al.* 1982; Schiffmann *et al.* 2003). Patients are intolerant to heat; pain attacks triggered by sudden changes in temperature are often the first symptoms of the disease (MacDermot *et al.* 2001). The exact pathophysiology of pain in Fabry disease is not fully understood, although deposition of glycosphingolipid in the dorsal root ganglia, with subsequent cell death, has been proposed as a possible mechanism (Gadoth *et al.* 1983). Small-fibre damage or anterolateral tract lesions are considered essential in the development of neuropathic pain after peripheral nerve damage or CNS lesions (Cruccu *et al.* 2004).

*Neurophysiologic abnormalities in Fabry PNP.* In previous studies (Luciano *et al.* 2002) it has been shown that thermal hypoesthesia to cold stimuli in quantitative sensory thresholds (QST) indicates damage to the thinly myelinated Aδ-fibres in Fabry disease. In line with QST findings, Aδ-fibre-mediated heat pain responses are also decreased in Fabry patients when evaluated with laser-evoked-potential (LEP) recordings (Valeriani *et al.* 2004). This occurs in combination with relative enhancement of C-fibre-mediated cortical LEP responses, and results in imbalance between the Aδ- and C-fibre system inputs (Valeriani *et al.* 2004). Because the disease affects only small fibres, neurography and vibration detection thresholds are within normal limits.

#### *2.8.1.3. Diabetic polyneuropathy*

Diabetic neuropathy is the most common neuropathy in industrialized countries, and it is associated with a wide range of clinical manifestations (Dyck 1988). In diabetic neuropathy, the changes in PNS are the same in type I and type II diabetes. Most diabetic patients will develop neuropathy, if the duration of the disease is long enough and the most sensitive neurophysiologic tests are used. The most common type of PNP is the distal symmetrical form of the disorder that progresses following a fibre-length-

dependent pattern, with sensory and autonomic manifestations predominating (Gominak *et al.* 2001). It is characterized by a predominantly sensory involvement starting in the feet and subsequently involving the hands and the most proximal parts of the legs and arms, with or without pain, autonomic dysfunction, and cutaneous changes, while motor impairment is less prominent. This pattern of neuropathy is associated with a progressive distal axonopathy. Small-fibre involvement is common in diabetes, and the small unmyelinated and myelinated pain fibres are thought to be responsible for the neuropathic pain. From the clinical point of view, also a number of different types of PNS involvement other than PNP have been described, including cranial and spinal nerve mononeuropathy, autonomic neuropathy and plexopathy (Gominak *et al.* 2001; Llewelyn *et al.* 2005).

In diabetes, poor glycemic control and duration of diabetes have consistently been shown to be associated with neuropathy (Orchard *et al.* 1990). Other risk factors are age, height, male sex, and alcohol consumption, although for these the evidence is less consistent. Systemic hypertension, cigarette smoking, and raised concentrations of plasma lipids are associated with increased risk of neuropathy in insulin-dependent diabetes but not in non-insulin-dependent diabetes. Intensive treatment of diabetes lowered the risk of developing clinical neuropathy by more than 60% (Martyn *et al.* 1997).

A long-lasting hyperglycemia has been suspected to be one of the pathophysiologic factors in diabetic PNP. A high level of glucose alters the normal cellular metabolism in many types of cells and especially in neurons (Feldman *et al.* 1999). Several studies have shown that also genetic predisposition and insulin/C-peptide deficiencies may contribute to the onset of diabetic neuropathy (Baker *et al.* 1989b; Ido *et al.* 1997; Johansson *et al.* 1996; Sima *et al.* 1999), while reduced trophic support to neurons and nerve components (NTs, IGF-1) has been suggested as a possible cause of diabetic PNP (Sima *et al.* 1999).

Only one large population-based study has investigated the prevalence of autonomic neuropathy in diabetes. Using three tests of autonomic function based on cardiovascular reflexes, the Oxford Community Diabetes Study found that nearly 17% of diabetic patients had at least one abnormal test (Neil *et al.* 1989). Apart from erectile impotence, however, only 2.4% of the patients studied reported symptoms that could be attributed to autonomic dysfunction. Many other studies have also found that abnormal tests of autonomic function are common in diabetic patients, but symptoms are relatively rare. Some studies indicate that autonomic dysfunction in diabetes carries a poor prognosis; mortality was high in two follow-up studies of diabetic patients with abnormal tests of cardiovascular reflexes (Ewing *et al.* 1980; Flynn *et al.* 1995).

### 2.8.2. Toxic polyneuropathies

The most common toxic neuropathies are drug-related; most new pharmaceutical agents produce distal axonopathy, usually after prolonged use. Neuropathies from industrial agents (either from occupational or environmental sources), presenting after either limited or long-term exposure, are uncommon nowadays in Western countries. Many pharmaceutical agents can lead to distal axonal neuropathy after prolonged use. The PNP is caused by impaired axonal transport or other abnormalities in cell functions. There is more individual variation in nervous system vulnerability to some classes of drugs (antibiotics, anticancer agents) than to most occupational or environmental toxins. One reason for this variation is that persons exposed to occupational toxins are usually in good health with normal alimentary function, whereas individuals with acute systemic illness or chronic conditions (diabetes, asymptomatic hereditary neuropathy) receiving drugs may display increased vulnerability (Herskovitz *et al.* 2005). In the past, heavy metals, especially lead, arsenic, and thallium, accounted for many cases of neuropathy. Occupational exposure to solvents such as n-hexane, carbon disulphide, and methyl-n-butyl ketone was previously a cause of peripheral sensorimotor neuropathy but now, in the western world at least, occupational health legislation has resulted in strict control of permitted concentrations of these solvents in the workplace (Martyn *et al.* 1997).

#### *2.8.2.1. Thalidomide*

Thalidomide, N-phthaloylglutamimide, a glutamic acid derivative, was introduced as a sedative and hypnotic in 1956 in more than 40 countries. Three years following its clinical introduction it became evident that thalidomide caused sensory PNP; many patients experienced numbness in hands and feet, and signs of axonal degeneration were still evident four to six years later (McCredie *et al.* 1984; McCredie *et al.* 1984). The teratogenic effects of thalidomide in humans were recognized in 1961 (Fullerton *et al.* 1968). Other common adverse effects included constipation, sedation, skin rash and fatigue (Richardson *et al.* 2002). Due to its teratogenicity and the adverse effects, thalidomide was withdrawn from the market, but it never really disappeared. Thalidomide was reintroduced when it was found to be effective in the treatment of leprosy (Pelle *et al.* 2003; Nightingale 1998). Recently, thalidomide has been shown to have efficacy also in patients with advanced and refractory myeloma, including patients with relapses (Kumar *et al.* 2003b; Kumar *et al.* 2003b; Barlogie *et al.* 1999; Kumar *et al.* 2003a; Rajkumar 2001; Rajkumar *et al.* 2003). Preliminary data show that thalidomide is also an effective initial treatment for myeloma: a response rate of 66 % has been reported (Rajkumar *et al.* 2003). Angiogenesis is increased in multiple myeloma, and one of the suggested mechanisms of action of thalidomide has been its antiangiogenic effect (Singhal *et al.* 1999). It seems that thalidomide is also an immune modulator that induces apoptosis in myeloma cell lines (Hideshima *et al.* 2000).

Peripheral nerve toxicity is a known side effect of thalidomide, and the occurrence of peripheral neuropathy frequently leads to treatment being discontinued. Although thalidomide neuropathy has been known for a long time, thalidomide PNP remains incompletely characterized (Apfel *et al.* 2004). An important question is whether the PNP is related to the cumulative dosage or the daily dosage; there are different opinions in the literature (Cavaletti *et al.* 2004; Offidani *et al.* 2004; Briani *et al.* 2004). Some studies show a significant correlation between neurotoxicity and cumulative dose when the cumulative dosage exceeds 20g; for doses lower than that, the relation with onset of neuropathy seems to lose statistical significance (Cavaletti *et al.* 2004). The incidence of peripheral neuropathy during thalidomide therapy varies greatly in the literature (from 1% to 70%) (Harland *et al.* 1995; Sheskin 1980; Wulff *et al.* 1985). The most common clinical findings are paresthesias with tingling and loss of tactile and pinprick sensation of the extremities. It is not clear what mechanisms underlie thalidomide neurotoxicity (Plasmati *et al.* 2007). The drug causes a down-regulation of tumour necrosis factor α (TNF-α) synthesis, which might theoretically affect Wallerian degeneration of nerve fibres but, paradoxically, might also be behind the drug's beneficial effect on neuropathic pain, as indicated by George et al. (George *et al.* 2000).

*Neurophysiologic abnormalities in thalidomide-PNP.* The reduction in sural nerve SNAP has been confirmed as the earliest and most significant marker of thalidomide PNP (Lagueny *et al.* 1986). Also reduction of MAMPL has been reported (Plasmati *et al.* 2007). F-wave abnormalities have been reported by others; in some patients the only pathological findings have been F-wave abnormalities (Rao *et al.* 2000; Lagueny *et al.* 1986). Sadoh et al. (Sadoh *et al.* 1999) found increased chronodispersion of peroneal nerve F-waves in four patients treated with thalidomide after 32-130 supramaximal stimuli. Lagueny et al. also studied somatosensory evoked potentials (SEPs) and nerve conduction studies in 13 patients with discoid lupus erythematosus treated with thalidomide. They found changes that suggested involvement of both peripheral and central sensory pathways, affecting the sensory nerves and their corresponding central projection in the dorsal column. Whether the neuropathy is a length-dependent sensory axonal neuropathy or a ganglionopathy is not clear (Giannini *et al.* 2003). In fetal rats exposed to thalidomide, dorsal root ganglion degeneration has been demonstrated (McCredie *et al.* 1984).

### 2.8.3. Paraneoplastic polyneuropathies

Paraneoplastic neuropathies are associated with systemic malignancies, and their presentation may precede the clinical appearance of the neoplasm. As a group, their pathogenesis is not fully established, and their clinical presentation may precede the clinical appearance of the neoplasm. Involvement of sensory and autonomic ganglia

(ganglioradiculoneuritis) in paraneoplastic disorders may be focal or diffuse, and in the former it can be asymmetrical and localized to the cervical or lumbar region (Groves *et al.* 2005). Subacute sensory neuropathy is the best characterized of the paraneoplastic neuropathies (Spies *et al.* 2005). With the detection of specific antineuronal antibodies in the serum of patients with paraneoplastic encephalitis or sensory neuropathy, it has been recognized that these conditions are different manifestations of the same underlying disease process (Anderson *et al.* 1988). Anti-Hu antibodies react with the family of protein antigens of 35-40 kDa present in neurons and some tumours, especially in small cell lung carcinoma (SCLC) (Spies *et al.* 2005). In SCLC, most patients have in their serum and in CSF, polyclonal anti-Hu antibodies; these antibodies can exist also in patients suffering from neuroblastoma, breast or prostate carcinoma (Groves *et al.* 2005).

#### *2.8.3.1. Myeloma*

Neuropathy occurs in 1.4 to 13 % of all patients with multiple myeloma, less frequently in osteolytic than osteosclerotic myeloma (Kelly, Jr. *et al.* 1981; Kelly, Jr. *et al.* 1983). The usual presentation of the neuropathy in osteolytic myeloma is a sensorimotor painful PNP with a rapidly disabling course. The PNP can be purely or predominantly sensory. Osteosclerotic myelomas constitute only 3 % of all myelomas, but PNP is associated in 20 to 50% of cases (Kelly, Jr. *et al.* 1983). The PNP in myeloma does not differ from other axonal neuropathies (Ropper *et al.* 1998; Chassande *et al.* 1998). According to Kelly et al., the neuropathy associated with multiple myeloma without amyloidosis is a heterogenous disorder and bears a close resemblance to carcinomatous neuropathy (Kelly, Jr. *et al.* 1981; Kelly, Jr. 1985). Both carcinomatous neuropathy and myeloma neuropathy can present as a mild axonal sensorimotor, a pure sensory, or a subacute or remitting and relapsing PNP. Systemic amyloidosis can complicate multiple myeloma (Kelly, Jr. *et al.* 1981; Kissel *et al.* 2001b); some even believe it to be a rare and debatable cause of myeloma neuropathy (Kelly, Jr. *et al.* 1981). The clinical and electrophysiologic features of the neuropathy of primary systemic amyloidosis with or without myeloma do not differ in any substantial way (Kissel *et al.* 2001b).

*Neurophysiologic abnormalities in myeloma PNP.* There is no way of accurately differentiating the PNP caused by myeloma from PNP caused by thalidomide. In osteosclerotic myeloma, electrophysiologic studies disclose a subacute or chronic demyelination, with a symmetric sensorimotor deficit and features of secondary axonal degeneration (Leger *et al.* 2001). In osteolytic myeloma, the PNP is usually a sensorimotor painful PNP with a rapidly disabling course. In the majority of ostelytic myeloma cases, the electrophysiology discloses axonal features (Leger *et al.* 2001).

### 2.8.4. Hereditary polyneuropathies

Inherited neuropathies without known metabolic derangements are another group of disorders. The most common of these syndromes are known as "Hereditary Motor and Sensory Neuropathy" (HMSN) or Peroneal Muscular Atrophy, nowadays usually called Charcot-Marie-Tooth Disease (CMT) (Shy *et al.* 2005). Originally, this syndrome was believed to be one syndrome, but today it is known that peroneal muscular atrophy occurs in several disorders. The term HMSN was introduced by Peter Dyck (Dyck *et al.* 1968b; Dyck *et al.* 1968a) to encompass a broader group of syndromes than encompassed by peroneal muscular atrophy or CMT syndrome. With the detection of the genetic defects behind the different hereditary neuropathies, the trend has been to prefer the term CMT over HMSN. In 1968, Dyck and Lambert classified the inherited motor and sensory neuropathies into seven subclasses on the basis of different features (Dyck *et al.* 1968b; Dyck *et al.* 1968a). Population surveys have been carried out which show large geographic variations in the frequency of CMT disease: in Libya 8 per 100 000 population; in Nigeria 10 per 100 000; in south Wales 17 per 100 000; in northern Sweden 20 per 100 000; northern Spain 28 per 100 000; and in western Norway 41 per 100 000 (Martyn *et al.* 1997).

### 2.8.5. Inflammatory polyneuropathies

Inflammatory neuropathies are acquired, usually immune mediated, PNPs. The inflammation results in demyelinisation, and inflammatory neuropathies are demyelinating. The most important inflammatory neuropathies are GBS and CIDP (Gorson *et al.* 2001; Reid *et al.* 2001).

GBS is characterized by acute or subacute progressive, symmetric limb weakness with distal paresthesias and reduced or absent deep tendon reflexes. Electrophysiologic evidence of an acute neuropathy is the central feature in confirming the diagnosis (Gorson *et al.* 2001).

CIDP usually presents as a mixed sensorimotor neuropathy, but predominantly sensory or motor forms are also seen. In electrophysiologic studies, acquired demyelination is seen with conduction blocks, slowed CV, prolonged distal latencies and abnormal F-responses (Reid *et al.* 2001).

### 2.8.6. Infectious polyneuropathies

Leprosy is worldwide one of the most common causes of PNP (Ooi *et al.* 2004; Hietaharju *et al.* 2000). Leprosy is an important cause of treatable painful peripheral neuropathy (Hietaharju *et al.* 2000). Fortunately, multidrug treatment and World Health Organization surveillance programmes are having a major impact. In Europe and North America, the disease is only seen in immigrants. In leprosy, nerve enlargement is seen

at sites where the nerves are superficial and tissue temperature is cool (Ooi *et al.* 2004; Haanpaa *et al.* 2004). In neurography, the CV:s can be diminished before any sensory deficit appears. The cardinal symptom of leprosy is sensory loss, most often discovered through painless injury (Sabin *et al.* 2005). In tuberculoid leprosy, there may be only one or a few patches of intracutaneous sensory loss, the skin lesion and sensory loss develop together. In lepromatous leprosy, the sensory loss is not confined to the area of the skin lesion in the precise manner noted in tuberculoid leprosy (Sabin *et al.* 2005). Cutaneous sensory loss appears first in the coolest areas of the body: the lobes and helices of the ears are most affected (Sabin *et al.* 2005). In borderline leprosy, the nerve damage is largely limited to the same sites as lepromatous leprosy. However, occasionally borderline cases have the greatest potential for devastation of the PNS (Sabin *et al.* 2005). These cases are characterized by a degree of host resistance sufficient to result in prompt nerve dysfunction, but tissue response inadequate to prelude hematogenous dissemination of the disease. These patients show widespread nerve damage even early in the course of the disease and are the cases often reported as pure neural leprosy (Jopling *et al.* 1965).

## 2.9. Epidemiology of polyneuropathies

Except in the areas of diabetic neuropathy and GBS, there have been disappointingly few epidemiologic investigations of peripheral neuropathies. PNPs are difficult diseases to study epidemiologically because they are a heterogeneous group of disorders. In large-scale surveys of PNP one must rely on patients´ subjective symptoms, like paresthesias or painful dysesthesias, but even the accuracy and reproducibility of these subjective symptoms are variable. Another problem is that incidence and prevalence estimates may vary depending on which diagnostic criteria are used (Dyck 1988). For example, in diabetic neuropathy, if symptoms are used, about 5 % of diabetics are defined as having PNP (Dyck *et al.* 1993; Dyck *et al.* 1985). If vibration thresholds are taken, some 25% of diabetics are defined as having neuropathy (Burke *et al.* 2005). In some studies, the frequency of neuropathy in diabetics is as high as 59% (Dyck *et al.* 1993), and in other studies from 50 -100% (Burke *et al.* 2005). The exact criteria for the diagnosis of distal symmetric PNP must be stated in epidemiologic studies.

Peripheral nerve disorders are relatively common, in some studies they are shown to affect 1.6- 2.4% of the population (Beghi *et al.* 1998; Hughes 2002). The studies by Beghi and Hughes were done relying on patients' symptoms; by screening the neuropathic symptoms in all patients visiting a general practitioner's surgery first by a questionnaire, and if the patient had symptoms the PNP findings were evaluated by a neurologist. Neurophysiologic tests were not done to confirm the PNP. The Medline search by Hughes in 2002 from 11 epidemiologic PNP surveys found that the prevalence of PNP in a population may increase from 2.4 to 8.0% with advancing age (Hughes

2002; Martyn *et al.* 1997). These 11 studies included useful guidelines for the diagnosis and management of diabetic PNP but no guidelines on the diagnosis and management of generic PNP (Hughes 2002).

## 2.10. Symptoms caused by neuropathies

Symptoms must be directly evaluated because they (1) are what patients experience, (2) bring patients to doctors, (3) cannot adequately be inferred from other measurements, and (4) also convey the patients' reactions to primary experiences (Dyck 1988). The symptoms associated with PNP are classified as positive or negative; individual patients may experience both. Also motor and sensory positive and negative symptoms can coexist.

### 2.10.1. Positive symptoms and signs

Positive symptoms are not benign phenomena although the term may imply it. They are symptoms that are due to excess firing of diseased peripheral nerves, and they can occur in sensory, motor or autonomic nerve fibres. Typical positive symptoms are complaints of abnormal sensations or movement.

*Positive motor symptoms.* Positive motor symptoms and signs of PNP include muscle cramps, fasciculations, tremor and myokymia (Dyck 1988; Thompson *et al.* 2005).

Muscle cramps are a common symptom in PNP. They consist of painful contractions of part of a muscle related to active involuntary firing of motor units. Cramps are thought to originate from spontaneous activity in the terminal motor axons. They are common in partially denervated muscle and may mark the onset of muscle weakness (Harding *et al.* 1982).

While tremor is most often central origin, it may accompany PNP. Acquired and inherited axonal PNP with chronic denervation and reinnervation are often accompanied by a small-amplitude, rapid postural upper limb tremor. The tremor is caused by a co-contracting burst of agonist and antagonist muscle activity, and is attributed to weakness and muscle fatigue, leading to synchronous motor unit discharge (Thompson *et al.* 2005). In CMT1a, tremor is sometimes seen; previously it was called Roussy-Levy syndrome (Zubair *et al.* 2008). Nowadays, it is considered to be a part of the CMT1a phenotype (Thomas 1999; Zubair *et al.* 2008; Carvalho *et al.* 2005).

Fasciculations are visible spontaneous twitches of muscle caused by sporadic discharges of motor units. They are typically generated in terminal motor axons, but can arise from a variety of locations on the motor axon (Roth 1982). Fasciculations indicate altered exitability of the motor neuron soma or axon, often fasciculations are associated with

denervation (Thompson *et al.* 2005). However, benign fasciculations are not rare phenomena, particularly in distal foot muscles. MUP changes or fibrillations should not be seen in EMG in subjects with benigns fasciculations.

Myokymia occurs in diseases of motor axons, and is defined clinically as undulating, wavelike, vermicular or wormlike rippling of muscle. In electromyocraphy, "myokymia" refers to regular or semirythmic groups of motor units discharging in doublets, triplets or multiples. Neuromyotonia describes a clinical syndrome of delayed muscle relaxation after voluntary contraction of peripheral nerve origin. The syndrome is caused by continuous motor unit and muscle fibre activity resulting from peripheral nerve hyperexcitability (Thompson et al. 2005).

Painful legs and moving toes consist of slow, rhythmic toe movements, typically abduction-adduction and flexion extension movements, and leg pain. The origin of these movements has been speculated, but there is evidence of the peripheral origin of the diseased peripheral nerve of these movements (Alvarez *et al.* 2008).

Restless legs syndrome (RLS) is sometimes a feature of PNP. It is characterized by unpleasant "creeping, crawling" sensations felt deep within the legs, particularly between the knee and ankle, accompanied by an intense desire to move the legs and feet (Ekbom 1987). RLS is common in uremic neuropathy (Thomas 1978; Telarovic *et al.* 2007) and in diabetic neuropathy (Gemignani *et al.* 2007). Recent study indicates, that RLS is more prevalent among patients with hereditary PNP, but not in those with acquired PNPs (Hattan *et al.* 2009). RLS and PNP are both associated with positive symptoms such as pain, paresthesiae, and cramps. These are symptoms that may become more noticeable during lack of distraction, which tends to occur at night. This suggests that evaluation of RLS in patients with neuropathy must be performed with caution (Hattan *et al.* 2009).

*Positive sensory symptoms.* Positive symptoms occur in the absence of receptor stimulation or may occur as an exaggerated response to a sensory stimulation. Positive sensory symptoms are often uncomfortable. The positive sensory symptoms include pain, paresthesia (spontaneous sensations), allodynia (pain evoked by normally non-painful stimulus), dysesthesia (unpleasant sensations), hyperalgesia (exaggerated responses to painful stimuli, pain that is abnormally intense), hyperesthesia (increased sensitivity to stimuli), and hyperpatia (prolonged painful responses to repetitive stimuli, especially pinprick) (Dyck 1988; Price 1994; Thompson *et al.* 2005). The abnormal spontaneous sensations are often described as tingling, the feeling that "Novocaine is wearing off", or the feeling that the limb is "asleep". Dysesthetic pain is usually described as burning, raw and searing. Most patients find that dysesthesias are most troublesome at rest, especially in the night (Berger *et al.* 1995).

### 2.10.2. Negative symptoms

Negative neuropathic symptoms are due to loss of function, usually manifesting as weakness and loss of sensation.

*Negative motor symptoms.* Muscle weakness in voluntary movements is a negative symptom. Paralysis in demyelinating PNPs may be due to axonal loss or to conduction block caused by demyelination or axonal hyperpolarization, as has been demonstrated in experimental demyelinating neuropathies (Sumner *et al.* 1982; Cragg *et al.* 1964). If weakness is caused by a conduction block, muscle bulk may be maintained, electromyocraphic signs of denervation do not appear unless there is axonal loss, and recovery may be rapid and complete.

Axonal loss weakness is accompanied by atrophy of muscle. Atrophy becomes clinically evident within weeks (Thompson *et al.* 2005).

*Negative sensory symptoms.* The loss of afferent input results from conduction block or axonal loss. Loss of sensation may involve all sensory modalities, or the impairment may be restricted to particular forms of sensation. Small-fibre neuropathy leads to pain and temperature sensory loss (Mendell *et al.* 2001; Devigili *et al.* 2008; Dutsch *et al.* 2002). In large-fibre sensory loss, joint position sense and vibration sense are lost, and touch pressure sensibility is impaired (Mendell *et al.* 2001).

When stimuli fail to evoke a subjective sensory response, accompanying negative sensory symptoms of "numbness", hypoestesia (reduced sensitivity to touch or sensation) or hypoalgesia (decreased sensitivity to painful stimuli) occurs (Price 1994). The numbness is often described as a "wooden" feeling, and a feeling that the limb is wrapped in cotton or encased in cement. The feeling that patient is walking on stilts or on sand is also common. Negative symptoms rarely trouble patients if pain is absent. Severe loss of feeling can lead to painless injuries (Thompson *et al.* 2005).

Loss of tendon reflexes is a common sign in PNP (Mendell *et al.* 2001). The muscle stretch pathway involves activation of muscle spindles by muscle stretch, conduction of the ensuing spindle discharge in large diameter Ia afferent fibres to the spinal cord, and an efferent limb from the anterior horn cell muscle. Reflex loss is an early phenomenon in large-fibre neuropathies, neuronopathies, sensory gangliopathies affecting spindle afferent fibres and late in the course of small-fibre PNPs. Accordingly, tendon areflexia commonly reflects wide involvement of large-fibre sensory fibres (Thompson *et al.* 2005).

*Negative autonomic symptoms.* The ANS is affected in many diseases of the PNS. The postganglionic autonomic sympathetic fibres, both adrenergic and sudomotor, are often affected in the axonal neuropathies. Autonomic neuropathy results in decreased HRV,

which can lead to heart arrhythmias (Joyner *et al.* 1997; Paterson *et al.* 2005). In addition the regulation of blood pressure is affected, and orthostatic hypotension may occur (Joyner *et al.* 1997).

Anhidrosis displays a symmetrical distribution, and initially affects the lower legs. Anhidrosis is particularly common in diabetic PNP, and may lead to heat intolerance and excessive sweating over the upper parts of the body. It is a result of postganglionic sympathetic lesion in sudomotor nerve fibres (Thompson *et al.* 2005).

Disturbances of genitourinary function include dilated atonic bladder, which leads to voiding difficulties and postmicturition dribbling, retention and overflow incontinence. Failure of ejaculation resulting from failure of parasympathetic innervations often precedes impotence. Disturbances of large bowel function include diarrhea and/or constipation. Also vomiting may occur due to autonomic neuropathy (Thompson *et al.* 2005).

## 2.11. Tests for studying polyneuropathies

### 2.11.1. Estimation of subjective symptoms

In the studies that are included in this thesis, subjective symptoms and clinical findings were evaluated using questionnaires covering motor, sensory and autonomic symptoms. The basis of the questionnaire is a modified version of the "Neuropathy Symptom Score" (NSS) developed by Dyck (Dyck 2005a).

### 2.11.2. Neurophysiologic methods for diagnosis of PNPs

In the objective diagnosis and characterization of PNPs, neurography, EMG and QST (vibration and thermal detection thresholds) are useful. If involvement of ANS is suspected, HRV tests and sympathetic skin response (SSR) can be used.

#### *2.11.2.1. Neurography*

Neurography gives information about the thick myelinated fibers. Sensory and motor electroneurography are among the most important methods used in the clinical neurophysiology laboratory. Neurography is essential in the diagnosis of both local and diffuse neuropathies; it can be used to characterize the pathophysiology of neuropathies and define the severity. Earlier, the method was called nerve CV measurements, because the main parameter studied was the CV. Nowadays, also other parameters, such as AMPL, shape and late responses (F waves) have become essential parts of the test. Therefore, the method has been called nerve conduction studies. A simple and well-defined term is electroneurography, or neurography for short, the abbreviation ENeG is useful. The term

neurography is clear and precise, but the abbreviation ENG should not be used, because it is used for electronystagmography (Falck 2003).

#### *2.11.2.2. Motor neurography*

A single supramaximal shock applied to the motor nerve activates all of motor axons in the nerve. The action potientials are conducted to the muscle and the individual motor unit potentials summate to form the compound muscle action potential, here called the M-wave. Because individual motor axons vary in CV and in terminal length the motor unit potentials summate slightly asynchronously (Kimura 2005). This M-wave, recorded from the surface of a muscle consists of the temporally dispersed sum of many motor unit action potentials located in the radius of the electrode (Kimura 2005).

The usual parameters that can be measured in motor neurography are discussed in detail below.

*Distal latency (DLAT)*. The distal latency is the time from the stimulus to the onset of the M-wave. The DLAT is composed of 1) the time it takes for the generation of the nerve action potentials at the stimulus site, 2) the conduction time (CT) from the stimulus site to the end plates of the fastest motor alpha axons, 3) the neuro-muscular transmission time, and 4) the time taken for the action potentials of the muscle fibres to travel from the end plate to the recording electrode. If the electrode is correctly placed in the end-plate region, there is no additional time from the end-plate region to the recording electrode. Most of the DLAT time consists of the time taken to propagate along the nerve to the stimulus site (Falck 2003).

*Conduction Time (CT)*. CT is the time it takes for the fastest axons to conduct over a nerve segment, i.e. the difference in proximal and distal latencies. The proximal latency is measured like the distal latency.

*Conduction velocity (CV)*. The motor conduction velocity (MCV) of a nerve segment used clinically is actually the CV of the fastest conducting alpha motor axons (Buchthal *et al.* 1966; Falck *et al.* 1995). In a healthy subject the CV in the upper extremities is around 55-60 m/s (Falck *et al.* 1991; Lee *et al.* 1975), and in the lower extremities 40-50 m/s (Hopf 1962). MCV depends on the diameter of the axon, the ratio between the diameter of the axon and the myelin sheath, and the distance between the nodes of Ranvier (Waxman *et al.* 1972). The variables that affect results from nerve conduction measurements are recording method, biologic factors (subject's age, height and gender) and physical factors (position of limb, length of muscle, temperature and nerve segment length).

In demyelinisation, the nerve CV is diminished, whereas in axonal neuropathies, CV tends to be within normal limits or only slightly reduced (Cornblath *et al.* 2001). In

normal myelinated axons there is an approximately linear relationship between CV and diameter, with a slope of 5.5 ms/µm. A decrease in the internode distance will decrease the CV (Waxman *et al.* 1972).

*The M-wave amplitude (MAMPL).* The M-wave is the sum of the action potentials generated by individual muscle fibres innervated by the stimulated motor axons (Lee *et al.* 1975; Falck 2003). The shape and size of the M-wave are determined by the MAMPL and the duration of the individual motor unit potentials, the number of motor units, and the temporal dispersion of the motor unit potentials (Lee *et al.* 1975). The individual motor unit potential recorded with surface electrodes has a biphasic negative-positive shape. The initial negative phase has duration of 5-6 ms, and the mean MAMPL is 96 µV (range 9-397uV) (Lee *et al.* 1975). This large variation is caused by the number of muscle fibres in the motor units and the proximity of the unit to the recording electrode. MAMPL is positively correlated with the muscle fibre diameter (Håkansson 1956).

*Decay and dispersion.* In motor neurography, the nerve is stimulated at two different sites. Even in healthy nerves there is dispersion of motor CVs. Usually the M-waves obtained at the proximal and distal stimulation sites in a healthy nerve are similar. Due to this dispersion of the nerve CVs the response at the more proximal site has a slightly lower MAMPL and longer duration than the M-wave obtained with distal stimulation. This propotional duration change is called dispersion (DISP). The fastest alpha motor axons in the upper extremities conduct around 55-60 ms (Dorfman 1984) (Falck *et al.* 1991; Lee *et al.* 1975), and the slower ones around 35 m/s (Hopf 1962). Because of this there will be an increasing temporal dispersion of the nerve action potentials and of the evoked motor unit potentials with increasing conduction distance (Falck 2003).

The proportional change in MAMPL of the proximal and distal stimulation sites is called decay (AMPL %) (Taylor 1993). The AMPL% should be assessed together with the DISP of M-waves when a conduction block is determined, because increased nerve conduction velocity dispersion gives rise to decay, as well.

In a healthy normal subject, the AMPL% in the upper extremities is >-25%, and in the lower extremities >-40% (Taylor 1993).

*F-Waves.* F-waves were first described by Magladery and McDougal over half a century ago (Magladery *et al.* 1950). Since the responses first recorded were from the foot muscles they called them F-waves. F-waves are recurrent discharges of antidromically activated motor neurons and they can be elicited in most muscles (Guiloff *et al.* 1991; Panayiotopoulos *et al.* 1977).

A supramaximal stimulus applied to a nerve elicits the F-wave following the M-wave. The recurrent discharges occur in each motor unit in 0-5% (Schiller *et al.* 1978) of the stimuli. For practical purposes, 20 stimuli are used in clinical studies (Chroni *et al.* 1994).

More stimuli would give more accurate results with better reproducibility (Guiloff *et al.* 1991). However, in clinical studies, more stimuli would increase the investigation time and adding more stimuli would be difficult for some patients to tolerate.

The recurrent activation of the alpha motor axon is probably generated at the axon hillock by the action potential of the soma dendritic membrane (SD spike). For a recurrent action potential to occur, the SD spike must occur after the 1 ms refractory period of the axon hillock and before the antidromically activated inhibition by the Renshaw cells. The time window for the occurrence of the F-wave is therefore very narrow, around 10-30 μs (Schiller *et al.* 1978). It is not clear whether the probability of F-wave generation depends on the size of the motor units, some studies indicate that there is no difference in the probability of large and small motor units in the generation of F waves (Kimura *et al.* 1984). On the other hand, another study indicates that the largest motor units with faster CVs are more likely to generate F-waves (Guiloff *et al.* 1991).

The advantage of F-waves is that they reflect the function of the entire motor unit (Fullerton *et al.* 1965; Kimura *et al.* 1984). F-waves are particularly useful in studies of PNPs, because the F-wave parameters may be abnormal even when CV and MAMPL are still within normal limits (Andersen *et al.* 1997).

*F-wave latency.* The most often used F-wave parameter is the F-wave minimum latency (Fmin), which is the shortest F-wave latency of 20 consecutive stimuli. The Fmin reflects the CV of the fastest conducting motor units and the length of the axon (Panayiotopoulos *et al.* 1978). The mean F-wave latency is the mean F-wave latency of the stimuli used, and the values are about 2 ms longer than Fmin (Puksa *et al.* 2003b). The maximum F-wave latency (Fmax) is the latency of the F-wave with the longest latency of the stimuli used.

In healthy subjects, the Fmin and the mean F-wave latency are highly correlated with each other (Puksa *et al.* 2003b)

*F-wave frequency.* The F-wave frequency, or persistence, is the number of traces with F-waves out of 20 stimuli, sometimes expressed in percentage (%) of traces containing F-waves. The number of F-waves recorded following a train of antidromic stimuli reflects, to some extent, the excitability of motor neurons (Rivner 2008; Fisher 1996). The problem with F-persistence is that it varies between subject and between nerves (Panayiotopoulos *et al.* 1996). The reference values published for F-wave number indicate that the number varies depending on the nerve and muscle studied; usually the tibial nerve shows many F-waves, while the peroneal nerve shows only few F-waves (Chroni *et al.* 1994; Guiloff *et al.* 1991; Puksa *et al. 2*003b). The persistence in the nerves of upper limbs varies from 60-100% (Chroni *et al.* 1994; Guiloff *et al.* 1991).

The F-wave frequency is reduced by conduction blocks, making it a useful way to verify conduction blocks in conventional neurography: If there is a significant conduction block the number of F-waves should be reduced (Guiloff *et al.* 1991). F-persistence may also provide the first and/or the only electrophysiologic abnormality in diseases such as Guillan-Barre syndrome (Panayiotopoulos *et al.* 1996). F-persistence is also low in diseases with depletion of motoneurones (anterior horn cell diseases) and motor axons (PNPs and radiculopathies) (Panayiotopoulos *et al.* 1996).

*F-wave dispersion.* The advantage of the compound F-wave population is the evaluation of the relative conduction properties of an individual's fast and slow conducting fibres of the nerve studied (Panayiotopoulos 1979). The variability of F-wave latencies has been proved to be important; it provides information about not only the fastest but also about many other motor fibres of the same nerve (Chroni *et al.* 1993; Panayiotopoulos 1979). F-chronodispersion has been defined as the scatter of dispersion of the relative latencies of statistically significant numbers of consecutively recorded F-waves (Panayiotopoulos 1979). It has been proposed as a sensitive method to detect the abnormal conduction of affected nerve fibres in mild neuropathies even before M-wave and Fmin are affected (Panayiotopoulos 1979)

*F-wave amplitude.* Single motor units generate the F-waves; usually one stimulus will generate one or a few F-waves. The F-wave amplitude (FAMPL) reflects the size of the motor units (Peioglou-Harmoussi *et al.* 1985). In chronic neuropathies with collateral reinnervation, the FAMPL is increased. Increased motor neuron excitability with activation of several motor units may also result in increased FAMPL.

*A-Waves.* A-waves are defined as late components following the M-wave with a constant shape and latency. There is no internationally accepted guideline for the definition of A-waves. One useful definition is that an A-wave has to be clearly discriminated from the baseline in at least 8 traces out of 20 stimuli with a jitter smaller than 0.5 ms (Bischoff *et al.* 1996). The waves should have an identical shape. To test whether the late component is an A-wave or a part of the M-wave, the stimulating electrode can be moved proximally. If the latency of the late response increases, it should be considered to be a part of the M-wave, and if the latency decreases it should be considered to be an A-wave. Around 75% of A-waves precede F-waves, but they may also be among the F-waves or follow F-waves (Falck 2003).

Fullerton and Gilliat (Fullerton *et al.* 1965) first described A-waves. They thought that these waves were axon reflexes, and called them A-waves. According to this hypothesis, the axons leading to the motor unit branch proximal to the stimulation site and the stimulus excite only a part of the axon braches. The stimulus travels proximally to the branching point and invades the unstimulated axon branch; the action potential in the muscle fibre generated by this branch will arrive later than the rest of the action potentials and is

seen as an A-wave. This type of A-wave should disappear when the stimulus intensity is increased. This is rarely seen; axon reflex is not a plausible explanation for the majority of A-waves seen in clinical studies. The generation of A-waves is not clear, and there are probably several different types of mechanisms. Roth and Egloff-Baer suggested in 1984 that A-waves are a motor axon loop (Roth *et al.* 1984). The authors suggest that the response is due to congenital looping of the axon within the nerve. Ephaptic response from a neighbouring axon is a third hypothesis (Tomasulo 1982). In a re-evaluation of the ephaptic hypothesis, Magistris and Roth came to the conclusion that ephaptic transmission could not account for the late responses and instead suggested proximal re-excitation of a motor axon as a generator of A-waves (Magistris *et al.* 1992). A local abnormality of the axon myelin would render the axon re-excitable. There are still several unanswered questions about the generation of A-waves (Puksa *et al.* 2003b).

H-reflex is a monosynaptic reflex that can easily be recorded from gastrocnemius muscle by weak stimulus intensity. Together with F-responses, it has been used in the diagnosis of uremic PNP (Ackil *et al.* 1981b; Ackil *et al.* 1981a). It is elicited by electrical stimulation of the muscle spindle afferents (Falck 2003). The latency of the stimulus is measured from the stimulus to the onset of the H-wave.

#### 2.11.2.2.1. Sensory neurography

The sensory nerve action potential (SNAP) is the sum of the action potentials generated by the myelinated axons of the sensory nerve. The main components of the SNAP are generated by fast conducting I (alpha) fibres with diameters exceeding 9 μm and CVs between 40 and 65 m/s in the upper extremities (Dorfman 1984; Falck 2003). The individual axons usually have a triphasic action potential with a small initial and terminal positive peak, and in between them, a large negative peak. Because of differences in the CVs of individual axons, there is an overlap of negative and positive components of different action potentials that result in cancellation of some components (Dorfman 1984). The first positive peak indicates the arrival of the fastest conducting action potentials, and the negative peak is the sum of action potentials generated mainly by thick myelinated axons with a diameter of more than 9 μm. The duration of the negative peak of the SNAP is proportional to the dispersion of CVs. In healthy nerves, the dispersion is relatively small, and the duration is around 1.5 ms (Falck 2003). In antidromic recordings from fingers, the SNAP shape is biphasic without a preceding positive peak. This is due to the volume conduction of the fingers (Falck 2003).

*Latency.* The latency refers to the time it takes for the stimulus travel from the stimulation site to the recording site. It is measured from the first positive peak of the SNAP, if there is no positive peak to the first deviation from the baseline in the negative direction.

*Conduction time (CT).* CT is the difference between the proximal and distal latencies if the nerve is stimulated at two different sites. The sensory CT depends on the same factors as the motor CT and has been explained before in the section on "motor neurography".

*Conduction velocity (CV).* CV is calculated as follows: CV (m/s) = segment length (mm)/ CT (ms). The sensory CV depends on the same factors as the motor CV, and has been described in the section on "motor neurography".

*Amplitude.* The SNAP amplitude (SAMPL) and the area over the duration of the negative spike are proportional to the stimulated axons. The exact relationship between the number of axons and the SAMPL and the area is complex and depends on many factors (Dorfman 1984).

Factors that influence the SAMPL include the density of sensory innervation and the subject's body mass index, the latter reflecting the depth of the nerve from the skin surface (Buschbacher 1998). Women tend to have greater SAMPLs than men, probably because the nerves lie more superficially and the smaller space for volume conduction in women than men (Horowitz *et al* 1992).

In diseased nerves, there can be increased dispersion of CVs, which results in an increased temporal dispersion of the sensory action potentials. Due to phase cancellation of the positive and negative components of the individual sensory action potentials, the SAMPL may be more reduced than expected from loss of axons. However, SAMPL and area can be regarded as a practical parameter that reflects the number of functioning axons of the nerve studied (Falck 2003).

*Orthodromic and antidromic sensory neurography.* Under physiologic conditions, sensory nerve action potentials are propagated from the receptors to the spinal cord. When a sensory nerve is stimulated along its course, the action potential spreads proximally in the orthodromic direction towards the spinal cord and distally in the antidromic direction towards the receptors. In the orthodromic technique, the recording electrode and reference electrodes are placed along the nerve to be studied, the recording being distal to the reference electrode. The nerve is stimulated distal to the recording electrode. In the antidromic technique, the recording and the reference electrodes are placed over a distal part of the nerve, with the recording electrode proximal to the reference electrode, the nerve is stimulated proximal to the recording electrode. The SAMPL of the antidromic recordings are higher than in orthodromic recordings, but the CVs are identical (Buchthal *et al.* 1966).

#### *2.11.2.3. EMG and SFEMG*

In axonal neuropathies, abnormal spontaneous activity (denervation and fibrillations), are seen in needle EMG first in the most distal muscles as a consequence of axonal damage

after Wallerian degeneration. Quantitative MUP analysis gives information about the motor unit changes due to reinnervation in PNPs. In single-fiber EMG (SFEMG), the variability of the interval between two single fibre potentials from the same motor unit, called the "jitter" is recorded (Stalberg *et al.* 1971). SFEMG is sensitive method in detection of axonal PNP (Shields, Jr. 1987) and in detection of axonal reinnervation (Bril *et al.* 1996; Stalberg *et al.* 1975). The fiber density is a measure of fiber type grouping in the muscle, and can be calculated in SFEMG. This grouping is increased in disorders in which fiber clusters of one histochemical type are found as a result of nerve sprouting and regeneration. Neurogenic muscle, therefore, shows an increase in the electrical fiber density as a result of nerve regeneration. Fiber density is thus a measure of reinnervation after denervation, and therefore a measure of an axonal property, specifically axonal sprouting (Bril *et al.* 1996).

#### *2.11.2.4. Tests of the autonomic nervous system*

The easiest and most common way to study ANS in the neurophysiologic laboratory are the sympathetic skin response (SSR) and heart rate variability tests (HRV). HRV tests give information about cardiac autonomic innervation, and short HRV tests without Holter registrations can also be done in neurophysiologic laboratories.

*Sympathetic Skin Response.* Measurement of spontaneously occurring or evoked electrodermal activity is an easily obtained index of sudomotor function. It refers to the electrical activity that originates from sweat glands and dermal tissues and is recorded with electrodes applied to the skin. Resting skin potential is consistently negative (-25 to -40 mV) relative to body interior. At normal ambient temperature, the palmar or plantar skin is usually 10-25 mV more negative than other skin regions, and spontaneous fluctuations in skin potential may be recorded from these skin sites (Schondorf 1997).

The electrical potential from electrodes on the skin (the sympathetic skin response; SSR) is dependent on an increase in sudomotor activity, and provides a measure of sympathetic cholinergic activity. Stimuli that induce an SSR include deep inhalation, auditory stimuli, emotional stimuli or electrical stimulation of peripheral nerve. The test can be used to detect the presence of sympathetic denervation in PNPs. (Low *et al.* 2005; Schondorf 1997).

*Heart rate variability test (HRV).* HRV is a measure of the variations in heart rate. It is calculated by analyzing a time series of beat–to-beat intervals from the ECG. There are two approaches to measurement of HRV: analysis in the time or in the frequency domain (Stein *et al.* 1994). Both measures are based on the analysis of interbeat intervals of normal beats determined from an ECG recording. Time domain analysis addresses the question of "How much variability is there? Time domain values result from simple, statistical calculations performed on the set of interbeat intervals. Frequency domain analysis addresses the question "What are the underlying rhythms?" (Stein *et al.* 1994).

Cardiovascular tests are sensitive and reproducible for detecting autonomic neuropathy. Reduced spontaneous, respiration-related variations in HR are common in patients with autonomic neuropathy; HRV reflects disease of the vagal function. There is also evidence of sympathetic denervation of the peripheral vasculature in patients with autonomic neuropathy, which is a contributing factor to the orthostatic hypotension in these patients (Tuck *et al.* 1997). Alternations (mostly reductions) in HRV have been reported in patients with heart failure, diabetic PNP or following heart transplantation (Paterson *et al.* 2005).

Heart rate is affeceted by external factors, such as excercice, meals and temperature changes. Drugs, like vasoactive drugs that either increase or decrease vascular resistance, affect the HRV (Paterson *et al.* 2005).

The tilt test can displace significant blood volume to activate both low- and high-pressure receptors to test neural control of HR. The valsalva manouver, cold pressure test and dynamic isometric exercise can be used to activate sympathetic efferent activity (Paterson *et al.* 2005).

Spectral analysis, using Fast Fourier transform, is an application in beat-to-beat time series. This provides an estimation of the amount of variation at given frequencies. Spectral analysis of cardiovascular waveforms from Holter monitoring studies have shown that patients with heart failure or diabetic PNP or following heart transplantation have low HRV with an impaired high-frequency (HF) component (vagal dysfunction) and an augmented low-frequency (LF) component (enhanced sympathetic activity). Quantification of HRV using fast Fourier transform analysis is useful in unmasking autonomic impairment in various pathophysiologic states. Two main peaks are identified with spectral analysis of cardiovascular waveforms: a HF and a LF fluctuation (Pomeranz *et al.* 1985). Changes in the LF/ HF ratio are thought to reflect an altered balance of the sympathetic and parasympathetic nervous systems (Bigger, Jr. *et al.* 1992).

The most often used frequency bands studied are:

1. Total power (TP), the total variance in the signal. It represents the sum of HF, LF, VLF and ULF.

2. High Frequency band (HF), between 0.2 and 0.4 Hz. HF is driven by respiration and appears to derive mainly from vagal activity of the parasympathetic nervous system.

3. Low Frequency band (LF) between 0.03 and 0.15 Hz. LF reflects both parasympathetic and sympathetic activity and has been hypothesized to depend on the delay in the baroreceptor loop.

4. Very Low Frequency band (VLF) band between 0.0033 and 0.04 Hz.

5. Ultra Low Frequency (ULF) is the band between 0 and 0.0033 Hz. ULF is the long-term day/night variation and therefore is only expressed in 24-hour recordings.

When using the time-domain HRV indices, standard deviation of all RR intervals, called RMSM or SDNN and the square root of the mean of the sum of the squares of differences between adjacent normal RR intervals (rMSSD), are the most often used measures. The time and frequency domain measures of HRV are related. For every frequency-domain measure, there is a time-domain measure that strongly correlates with it (Kleiger *et al.* 1991). HF correlates with rMSSD, and ULF with SDNN (Kleiger *et al.* 1991). The RMSM is comparable with the TP of the spectrum of RR variability, which measures the overall autonomic balance of the heart. The rMSSD is largely validated as a measure of the parasympathetic input to the heart and, therefore, correlates with the HF power of the spectrum (Stein *et al.* 1994).

#### *2.11.2.5. Quantitative sensory testing.*

Quantitative sensory tests (QST) are psychophysical tests used to evaluate sensation thresholds for sensory, thermal and nociceptive stimuli. Two different testing paradigms are used in QST (Bertelsmann *et al.* 1994). In the two-alternative forced-choice method, usually accompanied by the up-and-down transformation rule, the subject has to make a choice between two cutaneous stimuli. When the subject gives the correct answer, the difference between stimuli is reduced; if an error is made, the difference is increased (the up-and-down rule). After a series of stimuli, changes in direction (increase vs. decrease, or reversal points) are assessed. After six changes in direction, the procedure is terminated, with the mean of the six levels at which changes occurred being the estimate of the sensory threshold (Bertelsmann *et al.* 1994). Although theoretically accurate, this method is too time-consuming for clinical practice, which is why "the method of limits" is used clinically. In this method, the intensity of cutaneous stimuli is increased. The subject is asked to indicate the moment when she/he began to feel the stimulus. The procedure is repeated several times, the sensory threshold being the mean of consecutive measurements. The same method can also be used to assess the sensory disappearance threshold. There, the intensity of the cutaneous stimulus is decreased, and the subject is asked to indicate when the sensation disappears (Bertelsmann *et al.* 1994). The physiologies of thermal and vibratory thresholds are discussed in more detail below.

##### 2.11.2.5.1. Thermal thresholds

Thermal thresholds refer to sensation thresholds for cooling and warming. Usually the stimulus is presented to the skin using thermoids based on the Peltier elements. The Peltier elements are small metal plates on the thermode that cool or warm when

stimulated. Different sizes of thermoids have been designed for studies (small for the study of lingual area, bigger for the foot etc).

Cold receptors are innervated by Aδ fibres with a CV of approximately 8 to 15 m/s (Dyck *et al.* 2005b). A cooling pulse is experienced as a splash of coolness with a definite onset and offset. Warming and heat-pain sensations appear to be mediated by unmyelinated C fibres that have a CV of 0.5-2 m/s (Dyck *et al.* 2005b). A warming pulse has a less definite onset and offset, and there are regions of skin where (especially the foot and leg of old people) the first experience of a heat pulse is a small burning sensation. Pain is experienced when skin temperature exceeds 44 C (Dyck *et al.* 2005b). This temperature induces tissue injury, if maintained for a long time. To test only warm sensation, it is important not to give stimuli in the nociceptive temperature. Because the receptors for cool and warm are different, the sensation for warm and cool should be tested separately. The distribution and density of C and Aδ fibres are different; cooling receptors appears to be more evenly and densely distributed (Dyck *et al.* 2005b).

#### 2.11.2.5.2. Vibratory perception thresholds

Decreased ability to recognize rapid oscillations of a tuning fork (vibration) placed on a bony prominence (at the knee or the lateral malleolus of the ankle) was found to be an early sign of neurologic diseases like tabes dorsalis and diabetic PNP. Today, the sensation produced by low-frequency mechanical oscillations is referred to as flutter for frequencies of approximately 50 to 100 Hz, and as vibration or pallesthesia for frequencies greater than 100 Hz.

VPT is an indicator of Aαβ sensory axon function. The receptor-mediating vibration sensation is thought to be the Pacinian corpuscle, information on which is mediated by Aαβ sensory fibres, large spinal ganglion afferent somas, and their extensions into the posterior columns of the spinal cord (Gardner *et al.* 2000). Meissner's corpuscles provide information about the punctuate mechanosensation, whereas the role of vibration sensation in physiologic function is not as readily conceptualized (Gardner *et al.* 2000).

Assessing VPT is useful in detection, characterisation and following the course of PNP (Dyck *et al.* 2005b). In disease, only raised thresholds (hyposensitivity) and not lowered threshold (hypersensitivity) have been shown to be abnormal (Dyck *et al.* 2005b). Increased VPT can represent abnormality of receptors, Aαβ fibres or the sensory pathways in the CNS.

The vibrometer is a device that has readout of the AMPL of the vibrating probe and provides quantified stimuli. The algorithm of testing and the expression of abnormality are left to the user.

The VPT is simple and is therefore often used in epidemiologic surveys and clinical trials (Hilz *et al.* 1995; Tegner *et al.* 1985; Olney 1998). The VPT can be used alone or preferably with other neurophysiologic test results to develop composite scores that can be used to set criteria for assessing the occurrence and severity of neuropathy (Dyck *et al.* 2005b).

### 2.11.3. Histologic methods

In clinical practice, histologic studies of the nerves, nerve endings in the epidermis and receptors have been used. For histologic tests, a biopsy of nerve or skin is taken. After staining, the biopsy is studied in the neuropathology laboratory.

*Intraepidermal nerve fibre density.* During the last decade quantitative analysis of intraepidermal nerve fibre density (IENFD) has become a widely used tool in the study of unmyelinated nerve fibres in the epidermis (Lauria *et al.* 2005). A 3 mm punch biopsy of the skin is minimally invasive and multiple sites can easily be used to confirm the presence and distribution of small-fibre involvement (Lauria *et al.* 2005). The method is much less invasive than sural nerve biopsy. IENFD is also more sensitive than nerve biopsy in detecting small-fibre neuropathy; it is also less complicated and takes less time to analyze than nerve biopsy (Lauria *et al.* 2005). The PGP 9.5 antibody is currently the most commonly used marker for IENFD, and the one we also used in our study. It is a polyclonal panaxonal marker that stains both Aδ- and C-fibre intraepidermal nerve-fibre endings (Koskinen *et al.* 2005). Reference values for IENFD of our own laboratory were used.

*Nerve biopsy.* Nerve biopsy may be considered when the patient appears to have an interstitial process of the nerve, such as vasculitis, amyloidosis, leprosy, granuloma, lysosomal storage disease, or when other diseases recognizable by their tissue alternations is suspected, and other less invasive tests have been negative (Dyck *et al.* 2005a). Also, if the patient has an undiagnosed symptomatic and progressive focal nerve lesion and a putative focal nerve enlargement or enhancement of an accessible nerve (for biopsy) has been identified. In vasculitic disorders, nerve biopsy may show inflammatory or characteristic patterns of ischemic injury to the nerve. Nerve biopsy may also be needed to define the pathologic basis of focal nerve enlargement on magnetic resonance imaging (MRI) (Dyck *et al.* 2005a).

Nerve biopsy is an invasive procedure and may cause disabling neuropathic symptoms. Patients may complain of allodynia and discomfort for months afterwards (Theriault *et al.* 1998). The purpose of the biopsy and the likely benefit to the patient must be determined. It can be a helpful diagnostic procedure in the determination of the etiology of a PNP, but it should be reserved for cases when other methods have failed to provide a definite answer (Said 2002).

The nerve biopsy is of limited or no value in explaining such symptoms as paresthesia, pain, cramps and other manifestations of neural hyperactivity. Study of nerve tissue must be limited to a small sample of accessible tissue, and it can only be done on a single or at most a few occasions. Often, a detailed study is only possible postmortem on poorly preserved tissue (Dyck *et al.* 2005a).

*Muscle biopsy.* Neurogenic changes are often seen in muscle biopsies taken from patients with PNP, also myopathic changes or mixture of both can be seen (Harding 2005; Zochodne 2005). The findings of myopathic changes are more prominent in chronic denervating diseases, presumably because of the greater denervation that takes place. With longstanding disease there is more fibre breakdown and cellular response in the muscle, which Drachman et al (Drachman *et al.* 1967) refer as the "myopathic" changes of chronic neuropathy.

# 3. AIMS OF THE STUDY

The present study using different neurophysiologic methods was undertaken to:

1. Evaluate to what degree different types of nerve fibres are affected in uremic PNP (I, IV), PNP induced by thalidomide (II), and PNP in Fabry disease (III).

2. To study the correlation between PNP symptoms and the various test parameters used (I, II, III).

3. To study the effect of single dialysis on nerve conduction studies (I) and the effect of long term dialysis to HRV and nerve sensory conduction studies (IV).

# 4. SUBJECTS AND METHODS

## 4.1. Subjects

The patients examined in different studies are described below.

*Neurophysiologic parameters and symptoms in chronic renal failure.* Neurophysiologic parameters and symptoms in chronic renal failure were studied in 21 patients. The patients were undergoing chronic maintenance HD treatment three times weekly, for 3.5 to 5 hours per session. The median length of dialysis treatment before the examination was 12.5 months (mean 23.9 months; SD 19.2 month) Patients with other possible causes of peripheral neuropathy such as diabetes, alcoholism, amyloidosis, or other systemic illness were excluded.

*Thalidomide therapy and PNP in myeloma patients.* Twelve patients who were treated with thalidomide and followed up for at least five months were studied. The mean age of the patients was 61 years (sd 10 years, range 42-79 years); eight patients were male and four were female. None of the patients had amyloidosis. The mean duration of thalidomide therapy was 10 months, (sd 5, range 5-20 months). Prior to thalidomide therapy all patients were treated with chemotherapy that included vincristine, and seven patients had also been treated with cisplatin. During the thalidomide treatment, one patient received concomitantly cisplatin (180 mg), and another vincristine (3.2 mg).

Treatment with thalidomide was initiated if patients did not respond to conventional chemotherapy or relapsed after autologous allogenic stem cell transplantation. Inclusion criteria also required a life expectancy of more than three months. All patients underwent clinical neurologic examination and neurophysiologic tests before thalidomide treatment was initiated. The drug manufacturer suggested monthly neurologic controls; therefore, we carried out monthly follow-ups with neurophysiologic tests.

The thalidomide dose was 200 mg per day for the first two weeks, and subsequently, the dose was slowly increased by 100-200 mg every month up to 800 mg, if no severe side effects appeared. The thalidomide dose was adjusted to such a level that side effects such as sedation, fatigue, constipation and rash, were acceptable to the patient. The mean cumulative thalidomide dose adjusted to body surface area was 35.1 g/m$^2$ (sd 12.6 g/m$^2$).

*Neuropathic symptoms and findings in women with Fabry disease.* The study group consisted of 12 women with Fabry disease aged 17-63 yrs (mean 45.5, SD 15.1 yrs). The diagnoses were confirmed by mutation analysis in nine patients, and by skin biopsy in one, while two were obligate carriers. Eleven of the patients agreed to have a skin biopsy;

one patient did not give her consent for a biopsy, but gave consent for the other tests. Serum creatinine was normal in all patients and the renal function was also considered normal. No-one had diabetes or any other systemic disease that could lead to PNP.

*Does dialysis therapy improve autonomic and peripheral nervous system abnormalities in chronic uremia?* In the study of ANS dysfunction and PNP in uremic patients, 32 patients with chronic uremia were studied. None of these patients were included in study I. Sixteen patients received hemodialysis (HD) and 16 continuous ambulatory peritoneal dialysis (CADP) therapy. Thirteen were women and 19 men; age 52 ± 12 years. The patients had undergone dialysis treatment for a median of two months before the first examination.

## 4.2. Methods

### 4.2.1. Symptoms

Positive symptoms (paresthesias, pain, or cramps), negative symptoms (loss of sensation), and autonomic symptoms (orthostatic hypotension or impotence) were evaluated. Symptoms related to systemic diseases like uremia (fatigue, weakness, or itching) were not included. A positive response was given a value of 1, and a negative response a value of 0. The questionnaire used for PNP scoring is seen at the end of the book as appendix 1, (Table 2).

The questionnaire is a modified version of the "neuropathy symptom score" NSS, developed by Dyck et al. (Dyck 2005a). The NSS is a checklist of symptoms, including muscle weakness and negative and positive symptoms. The NSS has been useful for screening and characterization purposes (Dyck 2005a).

The questionnaire on positive symptoms had 12 questions with a maximum score of 12 points. The negative sensory symptoms questionnaire maximum score was 3 points, the autonomic symptoms 9 points, and the motor function 5 points. A total neuropathy score was calculated by adding the scores of each subgroup (positive, negative, and autonomic PNP symptoms) together. The maximum number of points from the score was 29. A patient with a total score of one point or less was regarded as asymptomatic

In the study on Fabry disease, a modified questionnaire that resembled the "NSS positive and negative" score was used. The section on subjective sensory symptoms had 10 questions about positive and negative PNP-related symptoms. Four of the questions in the questionnaire concerned the type of pain. The autonomic symptoms questionnaire had eight questions. If a patient had a symptom, one point was given. Items are shown in detail in Table 3.

**Table 3.** Sensory and autonomic symptoms

| Sensory symptoms | Autonomic symptoms |
|---|---|
| Decreased feeling of surface features, size, shape or texture when touching | Feeling faint, only on sitting or standing |
| Decreased recognition of hot from cold | Repeated nausea |
| Decreased feeling of pain, cuts or other injuries | Persistent diarrhea, especially at night |
| Continuous "dead feeling" like novocaine without prickling | Loss of bladder control, not due to gynecologic/prostate problems |
| Continuous "prickling" or "tingling" feeling | Loss of rectal control |
| Unusual sensitivity or tenderness | Impotence |
| Sharp needle-like pain | Dryness of eyes |
| Burning discomfort | Dryness of mouth |
| Deep aching pain | |
| Other pain | |

Additionally, a separate pain questionnaire that evaluated the severity, type and distribution of pain was used in the Fabry study. This questionnaire had questions from the "Graded chronic pain status" (Von Korff *et al.* 1990; Dworkin *et al.* 1992; Smith *et al.* 1997) and the SCL-90R questionnaire (Derogatis *et al.* 1973). The questionnaire is used routinely in our pain clinic. In this questionnaire, a numerical rating from 0-10 points was used. The patient indicated whether the pain was intermittent or continuous. The questionnaire had questions about pain now, worst pain and average pain within the last six months. It also included a "social disability score", which reflected the effect of pain on social life, family life, past-time activities and ability to work and carry out domestic duties during the last 6 months on a scale from 0 to 10. "Social disability score" was the mean of the sum of the questions on the effect of pain on social life. The questionnaire also had separate questions to evaluate depressive symptoms (20 questions) like loneliness or difficulties falling asleep. It also had a section for non-specific somatic symptoms (11 questions), for example, "feeling unwell, nonspecific symptoms in the stomach". One to four points were given for each positive answer, regarding the severity of the symptom asked about. The points were added together and divided by the number of questions in the questionnaire. Depressive symptoms were graded as severe, if the patient scored >1.105 points, moderate 0.535-1.105 points, and normal < 0.535 points. The somatic symptom score was considered normal (<0.5 points) moderate (0.5-1) and severe (> 1 point).

#### 4.2.2. Clinical findings

The scoring of clinical findings was done using the "Neuropathy Impairment Score", "NIS" (former called the neurologic disability score, NDS) described by Dyck (Dyck 2005a). The NIS has been extensively validated and used in clinical trials (Dyck *et al.*

2001; Dyck *et al.* 1986a; Dyck *et al.* 1994; Dyck *et al.* 1991b). The score has been developed as a standard sum-score of neurologic findings based on standard grading of muscle weakness, reflex loss and decreased sensation so as to express overall severity of PNP. The clinical findings included in NIS were: (1) the strength of the limb and trunk muscles on a modified MRC scale, (2) biceps brachi, triceps, patellar and Achilles tendon reflexes, (3) clinical sensory examination; touch-pressure was tested with long fibre cotton wool, pin-prick with straight pins. The tests were done on the dorsal surface at the base of the nail of the terminal phalanx of the index finger and great toe, (4) joint position, measured by moving the terminal phalanx of the index finger and great toe.

*Muscle strength.* The muscle strength was estimated by manual testing by NIS (Dyck 2005a), which is modified from the MRC-scale. The MRC (Medical Research Council, 1943) approach is employed worldwide in neurologic practice, but the scaling is not linear. In NIS the muscle weakness has been scored linearly, (0 = normal, 1 = 25% weak, 2 = 50% weak, 3 = 75% weak, 3.25 = movement against gravity just possible, 3.5 = movement with gravity eliminated just possible, 3.75 = flicker, and 4 = paralyzed).

On the MRC scale the numerical rating is: 0 = no contraction, 1 = flicker or trace of contraction, 2 = active movement with gravity eliminated, 3 = active movement against gravity, 4 = active movement against gravity and resistance, and 5 = normal power.

*Tendon reflexes.* The tendon reflexes were studied from biceps brachi, triceps, patellar and Achilles bilaterally. Normal tendon reflexes were scored as 0, reduced as -1, and absent as -2.

*Sensation.* Sensation of the dorsum of the index fingers and great toes near the base of the nails was graded as 0 = normal, 1 = decreased, and 2 = absent. The sensation is tested with cotton wool (tactile), pinprick, vibration (a standard 100 Hz tuning fork), and joint position and motion.

### 4.2.3. The diagnosis and overall severity of polyneuropathies

The overall severity of PNP in diabetics has been classified by Dyck (Dyck 1988; Dyck *et al.* 1992; Dyck *et al.* 1993; Dyck *et al.* 1997). We have used the same classification in our study. This method combines symptoms and neurophysiologic tests to obtain the severity of PNP. The method is good for classifying axonal large-and also small- fibre neuropathies. The classification of PNP was done at 4 different stages, stages 0-3, (Table 4).

**Table 4.** The severity of PNP

| | |
|---|---|
| Stage 0 | No neuropathy. Fewer than two abnormalities on NC or QST. |
| Stage 1. | Asymptomatic neuropathy. NSS normal, Two or more abnormalities on NC or QST. |
| Stage 2. | Neuropathic symptoms not disabling. Two or more abnormalities on NC or QST. |
| Stage 3. | Neuropathic symptoms disabling. Two or more abnormalities on NC or QST. |

### 4.2.4. Neurography

The measurements were done using Medtronic Keypoint EMG equipment (Skovlunde, Denmark) and the appropriate analysis programmes. Stimulation was done using surface electrodes (Nihon Kohden NM 420 S, Japan). The length of a nerve segment was measured from the centre of the cathode at one stimulation site along the nerve to the centre of the cathode at the next stimulation site. The nerve conduction studies used in these studies were done antidromically. A constant–current stimulator was used. The temperature of the hands was to be at least 30°C and of the feet 28°C. If they were cooler than the stated temperature, the limbs were warmed.

#### *4.2.4.1. Motor neurography*

For the recording of the motor response, single-use silver/silver-chloride tape surface electrodes (Ambu NF-50-k/W Blue Sensor) were used. The recording electrode was placed over the end-plate region of the muscle, over the middle of the muscle belly in the end-plate area. The stimulation was 8 cm proximally from the recording site

*Distal latency (DLAT).* The DLAT was measured from the stimulus to the first deflection of the signal from the baseline. The electrode was properly placed when the first deflection from the baseline was in the negative direction at the distal stimulation site.

*Compound nerve action potential amplitude (MAMPL).* The MAMPL of the action potential was measured from the first positive peak to the highest negative peak.

*Conduction velocity (CV).* The CV of the fastest axons was calculated as follows: CV (m/s) = CT (ms) / segment length (mm).

*F-Waves.* F-waves were recorded with surface electrodes placed exactly like in motor neurography, and stimulation was carried out at the most distal stimulation site used in motor nerve conduction studies. The stimulus intensity was supramaximal to ensure that all alpha motor axons in the nerve are stimulated, and the cathode was placed distally to anode. The optimal stimulus frequency was 1 Hz, and 20 stimuli were given. Linear regression models for age- and height-matched reference values were used. For Fmin and Fdisp, Z-score > 2 was considered abnormal. The distribution of the F-waves could not be normalized and, therefore, if the number of F-waves was below the 2.28 percentile, the finding was considered abnormal.

#### *4.2.4.2. Sensory neurography*

The electrodes were bipolar fixed electrodes with an inter-electrode distance of 25 mm from cathode midpoint to anode midepoint that were mounted on a plastic bar. The recording electrodes used were identical to the stimulating electrodes. The distance between stimulation and recording site was 140 mm. In the recordings from the nerves

of the hand, the recording electrodes were in the finger in which innervation was studied (4th and 5th finger in ulnar nerve and 2nd and 3rd in median nerve), and the stimulation was done 140 mm proximally in the wrist area on the nerve studied. In the leg, the sural nerve was studied: the recording electrode was placed behind the lateral malleolus and the stimulating electrode 140 mm proximal in the mid-calf. The temperature of the hands had to be at least 29°C and of the feet 27°C. The reference values for DLAT, SAMPL and CV were obtained from a large database in our department standardized for age and height. Linear regression models for age- and height-matched reference values were used. Values are expressed as Z scores.

#### *4.2.4.3. Vibratory perception threshold*

Vibratory perception threshold (VPT) in the foot was measured over the middle of the 1st metatarsal bone on the dorsal side of the foot with a 100 Hz vibratory device (Vibrameter type II, Somedic AB, Solna, Sweden). The vibration AMPL of the probe was steadily increased until the patient first perceived vibration. The VPT was taken as the vibratory threshold. The VPT was measured as the probe's vibration AMPL in μm. A null trial was not done, but the test was repeated five times, the largest and smallest test results were discarded, and the average of the remaining three test results was used. If the patient's results differed by more than 10 μm from each other, the test was repeated until reproducible results could be obtained. For VPT, Z scores > +2 were considered abnormal.

#### *4.2.4.4. Cold and warm detection thresholds*

The CDT and WDT were measured from the foot with Thermotest Type 1 equipment (Somedic AB, Solna, Sweden). The probe was placed over the extensor digitorum brevis muscle in the foot and over the thenar eminence in the hand. The probe cooled or warmed, depending on the direction of the current applied. The subject reversed the current by turning a switch as soon as cooling or heating of the element was perceived; the CDT and WDT were studied separately. The measurement was repeated five times, with both cooling and warming, and the thresholds were taken as the average of these measurements. For thermal thresholds, the Z-scores > +2 were considered abnormal.

### 4.2.5. Tests of the autonomic nervous system

The studies of the ANS system were done using Medtronic Keypoint EMG equipment (Skovlunde, Denmark) and the appropriate analysis programmes.

*Sympathetic Skin Response (SSR)*. Disposable surface electrodes (Ambu NF-50-k/W blue sensor) were used. The active electrodes were placed on the volar surface of hands and feet and the reference electrodes were placed on the dorsal side of the hands and feet. The stimuli given to get the SSR response were: electric stimulus to the median

nerve abruptly and with notification to the patient, voice stimulus (technician clapped the vibratory fork to metal instrument table, about 80 dB) and a sudden deep inhalation (patient breaths deeply once, technician records the response during breathing). If no response were recorded from two or more tests, the test result was considered abnormal, if a notable response was seen, the test result was considered normal.

*Heart rate variability test (HRV) during rest and controlled breathing.* For HRV tests the patient was lying supine on the bed for 20 minutes before the recording. No recommendations for food, drinking, exercise etc. before the test were given. The placement of single-use surface electrodes (Ambu NF-50-k/W blue sensor) were 1) recording electrode at the midaxillary line left side at nipple level and 2) reference electrode on the scapula right side.

HRV during normal breathing was recorded in supine for position one minute (time-domain analysis) or 20 minutes (frequency-domain analysis), depending on the study. The HR range (maximum-minimum), RMSM and rMSSD were calculated. In the 20-minute study, frequency-domain measures (HF and LF bands) were calculated. The results were compared with the reference values of our laboratory for normality.

In the one minute study, the time-domain measures were used. HRV at rest and at breathing frequency 6 breaths / minute were studied. HR response to deep breathing was studied after normal breathing in the one minute test setting. The subject was asked to breathe maximally at a rate of 6 breaths / minute, inspiratory and expiratory cycles of 5 seconds each. The HR range (maximum-minimum) / mean HR ratio was calculated. The method was identical with the method from the Department of Clinical Neurophysiology at the University Hospital in Uppsala, Sweden, and their reference values were used (Stalberg *et al.* 1989).

#### 4.2.6. Intraepidermal nerve fibre density (IENFD)

Punch biopsies of the skin were taken with a 3 mm disposable circular punch after a local infiltration with 5 % lidocaine; no suture was needed. Biopsy specimens were obtained 10 cm above the lateral malleolus from the non-dominant side and fixed with Zamboni's fixative. Ten micrometer-thick sections (Koskinen *et al.* 2005) were stained with a polyclonal panaxonal marker PGP 9.5. The light microsope examination of the nerve fibres was done under the Olympus BX51 microscope and the number of intraepidermal nerve fibres was morphometrically analysed using the Olympus Soft Imaging system (Cell*) with Olympus Colourview IIIu microscope. Only fibres clearly penetrating into the epidermis were counted. Unmyelinated fibres were considered as separate if 1) the distance between two different perpendicular sections of the immunoreactive nerve exceeded five times the diameter of an axon, or 2) there were clearly two individual parallel fibres. Nerves in the underlying dermis and sweat glands served as internal

positive controls. The densities of nerve fibres / mm were calculated. The normal values for IENFD of our own laboratory were used. Our technique was the same as used in the study of Koskinen et al (Koskinen *et al.* 2005), which had almost same reference values. Most other laboratories use 20 μm thick sections for staining.

#### 4.2.7. Biochemical measurement and assessment of dialysis efficiency

In the studies on uremic PNP, the Kt/V, an index of fractional urea clearance, was used to measure the dialysis efficiency. The Kt/V is defined as dialyzer urea clearance (K) multiplied by the dialysis session length (t), divided by the urea distribution volume (V). (Basile *et al.* 1990). In our study, dialysis efficiency was regarded as good if Kt/V was > 1.25, and poor if Kt/V was <1.25.

#### 4.2.8. Reference values

To increase the sensitivity of the neurophysiologic methods, reference values were based on age, height and gender, if necessary, using multiple linear regression models. The measured values were compared to the reference models and the deviation was expressed as a Z-score. The Z score is the measured value minus the expected value divided by the standard deviation. The Z –score indicates how much the measured values differ from the expected reference values. In the studies, a Z score ± 2 was considered abnormal. Because the measured neurophysiologic abnormalities can only be in one direction (for instance CV, can only be reduced), a Z-score of -2 means that the measured value has a 2.23% probability of being normal. For nerve CV and response AMPLs, Z scores < –2 were designated as abnormal; for Fmin, Fdisp, thermal thresholds and VPT, Z scores > +2 were considered abnormal.

The simple approach to use reference values is to use reference limits. These are either 95 % confidence limits or limits calculated from the mean ± two standard deviations. In neurography simple reference limits are not optimal, because many parameters depend on one to three independent variables, which make it difficult to deal with reference values in simple tables of reference limits. A practical solution is to construct linear regression models from the reference value database (Falck *et al.* 1991).

# 5. RESULTS

In this section each study is discussed independently.

## 5.1. Neurophysiologic parameters and PNP symptoms in uremic patients

*The effect of a single dialysis.* To study the sensitivity of neurophysiologic parameters in uremic PNP, 21 patients who were undergoing chronic maintenance HD treatment three times weekly were examined. No significant change in any F-wave or other neurophysiologic parameters was seen after a single HD treatment (paired-samples t-test). The comparison was done on the whole group of patients and on PNP groups 1 and 2. The effect of dialysis efficacy (Kt/V) on neurophysiologic parameter change was also tested by grouping the patients according to the Kt/V value (<1.25 and >1.25), but no significant differences could be found (paired samples t-test). The serum Mg concentration did not change significantly after HD, nor did it correlate with conduction velocities in any nerve.

*Sensitivity of different neurophysiologic parameters.* In upper limbs, median nerve motor studies were often abnormal. Because the tests were done on the side of the arteriovenous fistula, data from the median, ulnar, and radial nerves were omitted from further analysis. The VPT on the foot was a sensitive test: 13 out of 17 patients with PNP had an abnormal VPT, yielding a sensitivity of 76%. Thermal thresholds were performed in only 10 patients, 8 of whom had a PNP. The CDT was more sensitive (50%) than the WDT, where the sensitivity was only 13%. A-waves were seen in all nerves and were most common in the tibial nerve. They were seen in the tibial nerve in six studies before HD (35% of all tibial nerve studies) and in five studies (29%) after HD. In only two patients (12%) were tibial nerve A-waves seen both before and after HD: four patients (24%) did not have A-waves after HD, whereas in three patients (18%) the tibial nerve A-waves were first seen after HD. The frequency of A-waves before or after HD did not change in a systematic way.

*Correlation between subjective symptoms and neurophysiologic parameters.* There was a statistically significant correlation between positive neurologic symptoms and neurophysiologic tests (Publication 1, Table 3, page 4). Muscle strength was not associated with any neurophysiologic parameter. Thermal thresholds (WDT and CDT) did not correlate with any subjective symptoms. However, these tests were performed in only 10 patients. Other parameters were not significantly associated with subjective symptoms.

## 5.2. Thalidomide neuropathy

The thalidomide treatment had to be discontinued in five patients because of various side effects (42% of patients); no one interrupted the therapy because of severe PNP. One patient experienced diffuse generalized weakness, and one patient had to discontinue the treatment because of severe rash and bone marrow suppression, two patients because of disturbing fatigue and weakness, but without neurophysiologic signs of PNP, and one patient because of severe constipation and rash.

*Severity of neurophysiologic PNP.* The severity of neuropathy of the PNP before thalidomide treatment and at the end of the treatment using the different scales is shown in Table 1. (Publication 2). Prior to thalidomide treatment, four patients had no neurophysiologic signs of PNP, seven had minimal and one slight PNP. The neurophysiologic PNP findings deteriorated in 10 patients during the thalidomide treatment. At the end of the study, one patient did not have neurophysiologic PNP, four patients had minimal, and four slight PNP, three patients had moderate PNP, and none had severe PNP.

*PNP symptoms and neurologic findings.* Positive and negative PNP symptoms increased significantly during the thalidomide treatment (p=0. 015). Autonomic symptoms did not change significantly during the treatment.

No significant increase was detected in muscle weakness, measured as the total score of the manual testing. Subjectively, one patient experienced diffuse generalized weakness and the thalidomide treatment was discontinued (patient number 6). On neurophysiologic tests, no significant change in PNP was observed at this time. The generalized weakness the patient experienced was not due to peripheral neuropathy.

The Achilles and Patellar tendon reflexes significantly decreased during the treatment (p=0.019).

*Neurophysiologic findings.* In both motor nerves, A-waves were seen before the thalidomide therapy and at the end of the therapy. The incidence of A-waves did not change systematically during the study.

At the beginning of the study, no patient had pathologic sural nerve SAMPL, whereas at the end of the study, 58% had abnormal sural nerve SAMPL. The sural nerve SAMPL decreased by 61% on average during the study, while the radial nerve SAMPL decreased by 43%. The CV of the sural nerve also decreased (paired samples t test, p=0.05); no patient had abnormal CV at the beginning, but 50% had slightly abnormal values at the end, the relative mean decrease in CV being 7%. Peroneal nerve MAMPL decreased significantly (p=0.02); 83% of peroneal nerve MAMPL Z scores were slightly pathological already at the beginning of the study, and at the end of the study 83% were still pathologic (the

relative mean change being -31%). Also the peroneal nerve CV decreased (p=0.034): 8% had reduced CV at the beginning, and 58% at the end (relative change -13%). The CVs in the upper extremity nerves did not change significantly, nor were they abnormal.

The peroneal nerve Fmin increased (p = 0.03); 8% of patients had abnormal findings at the beginning and 33% at the end**.** The relative mean increase in latency was 14%. The peroneal nerve F-wave number did not change significantly. Peroneal nerve Fdisp decreased (p=0.05) as a result of the thalidomide treatment (relative mean change of -45%). No F-wave parameter in the median nerve changed significantly.

There were no changes in QST tests, in WDT, CDT or VPT. The change in neurophysiologic parameters in patients without neurophysiologic signs of PNP prior to treatment with thalidomide was not significantly different from that in patients with minimal or slight PNP (independent samples t-test).

*Thalidomide dose in relation to change in neurophysiologic parameters.* There was no significant correlation between the cumulative thalidomide dose and the change in the neurophysiologic parameters in the 12 patients (bivariate correlation, Pearson correlation coefficient).

*Neurophysiologic abnormalities and correlation with change in neuropathic symptoms.* When the neuropathic symptoms at the last follow-up study were compared with the neurophysiologic parameters, only muscle strength correlated with the symptoms (r=0.64, p=0.03).

The correlation between change in sural nerve CV and change in neuropathic symptoms was significant (r=0.616, p=0.03). Regarding the changes in other parameters, no correlation with changes in neuropathic symptoms was found.

The main reasons for discontinuing the treatment were primary disease or other than neurologic side effects.

The most sensitive parameter in detecting thalidomide-induced neuropathy was the sural nerve SAMPL, but also radial nerve SAMPL and sural and peroneal nerve CVs are sensitive, even though the neuropathy is predominantly axonal. The correlation between subjective symptoms and neurophysiologic findings was poor.

## 5.3. Neuropathic symptoms and findings in women with Fabry disease

The main results are summarized in Tables 2a and 2b (Publication 3, pages 5 and 6).

*Subjective symptoms.* Pain in the extremities, continuous or discontinuous, was reported by 10 patients; the youngest patient did not report any pain. Burning pain in the extremities during fever and / or hot conditions was present in 8 out of 12 patients. Deep pain in the extremities was the most often reported type of continuous pain; present in 4 out of 12 patients. Other symptoms were nonspecific (Table 2a, Publication 3, page 5). Symptoms related to CTS were excluded from the analysis of symptoms due to Fabry disease. One patient did not answer the questions about pain now, worst pain, average pain and pain affecting social life. The pain affected patients' social life in all five patients with continuous pain. In four of them the effect on social life was severe. These patients also had high sub-scores on the question that referred to current pain. Average pain experienced by the patients within six months was from 2-7 /10, two of the youngest patients did not report any pain during the last six months, the second youngest patient had had pain earlier only during fever (Table 2a, Publication 3, page 5).

*Symptoms of depression and nonspecific somatic symptoms.* Nine out of 12 patients filled in the questionnaire screening depressive and nonspecific somatisation symptoms. Two patients had scores indicating severe depressive symptoms. They also had elevated scores for nonspecific somatic symptoms. In addition, they had neuropathic pain according to the pain questionnaire, together with moderately decreased IENFD. Three patients had scores indicating moderate depressive symptoms. The same patients also had scores indicating nonspecific somatic symptoms. Of the patients who answered, 50 % had normal scores indicating no depression; one of them had moderately elevated scores on nonspecific somatic symptoms (Table 2a, Publication 3, page 5).

*Clinical findings.* Clinical neurologic examination, done in ten patients, was mostly normal. In the foot, only one patient did not feel the pin prick (patient 9). Ankle reflex was diminished but not absent in patient 10. The tendon reflexes and muscle strength were normal in all the other patients.

*Neurography.* There was slowing of the median nerve sensory CV at the wrist in three patients, indicating median nerve entrapment at the wrist. All had subjective symptoms compatible with CTS. One patient (patient 6) had severe CTS with no other neurologic symptoms, but slight abnormalities in sensory sural nerve neurography; Z-score of sural nerve SAMPL was -2.3. Her median nerve sensory responses from the fingers were absent, and the median nerve DLAT from the wrist to the thenar muscles was markedly increased (9.1 ms, Z-score 15. 5). Moderate CTS was seen in patient 5 with increased median nerve DLAT, and sensory CV of the median nerve at the wrist was slow. This patient also had mild PNP (> two abnormalities in neurophysiologic tests, and sensory symptoms; abnormal sensory radial (Z -2. 2), sural SAMPL (Z -3. 7) and tibial nerve MAMPL (Z -2. 3). The third patient had mild CTS with mild sensory median nerve CV (Z -2, 6), and normal median nerve motor DLAT. The IENFD was close to the lower limit

of normal (Z -1.6), but other tests were normal. One (patient 11) had slightly decreased sural nerve SAMPL (Z -2. 3), while neurography was otherwise normal. Her legs were markedly swollen, which probably caused the reduced sural nerve SAMPL. In the other patients, neurography was normal.

*Thermal and vibration perception thresholds.* Four patients (33%) had abnormal CDT in the foot. Two of them were also the oldest patients in the study group with the longest disease duration. One patient did not feel cold at all in the foot, but CDT was normal in her hand. Two of these patients also had abnormal WDT in the foot. VPTs were normal in all patients (Table 2b, Publication 3, page 6).

*Sympathetic skin response (SSR).* Eleven out of 12 patients had normal SSR responses from both upper and lower extremities. One patient (number 12) did not have recordable responses in the foot, but showed normal response in the hand. She also had autonomic symptoms; feeling faint, dryness of eyes and mouth.

*Heart rate variability test (HRV).* HRV frequency-domain measures were measured in eight patients. One patient had atrial fibrillation and the HRV tests were not performed, while in three patients, the programme was not available at the time the studies were done. The HRV variables in the frequency and time-domains were normal in all patients studied.

*Intraepidermal nerve fibre density (IENFD).* IENFD was decreased compared to normal values of our laboratory in three out of 11 patients. Three patients had borderline abnormality and only one patient had clearly normal IENFD (Table 2b, Publication 3, page 6). The IENFD was related to age ($p = 0.04$), older patients having fewer intraepidermal nerve fibres compared to young patients. Associations were found between subjective symptoms and findings in neurophysiologic, QST and neuropathologic tests.

Low Z-scores of IENFD were associated with high pain symptom sub-scores within the last six months. Subjective symptoms (pain, sensory or autonomic) were not associated with the CDT or WDT test in either hand or foot. The thermal thresholds did not correlate with IENFD. Age and the findings in QST did not correlate significantly. Nor did age reach significant association with subjective symptoms. However, two of the youngest patients did not score any points on the pain questionnaire.

## 5.4. The effect of dialysis therapy

Table 2 (Publication 4 page 4) shows the HRV change in the HD and CAPD groups during the follow-up. Six patients changed their HRV subgroups markedly during the study without experiencing any known major clinical event, such as myocardial infarct

or sepsis. The number of patients was not large enough to examine the association between the etiology of uremia and HRV response, because the number of patients in each etiologic subgroup was too small. Patient age was not significantly associated with the HRV response (Kruskal-Wallis test). Tables 4 and 5 (publication 4, page 4) shows the relationships between HRV change and dialysis adequacy.

During HD, improvement in the HRV time-domain measures occurred only in patients with a mean Kt/V > 1.20 (Fisher's exact test, $P < 0.002$) (Table 4 publication 4, page 4). Progressive deterioration of autonomic neuropathy was associated with Kt/V-values <0.87. During CAPD (Table 5, publication 1, page 4), a corresponding trend was observed ($P < 0.18$). The overall correlation of HRV response with the estimated adequacy of CAPD treatment did not reach statistical significance ($P = 0.18$).

Diabetic patients ($N = 4$) differed from other uremic patients as their HRV was severely abnormal already at the beginning and did not improve significantly during the study.

*Sensory neurography abnormalities and efficacy of dialysis.* Twenty patients had no sensory nerve conduction abnormalities at the beginning of the study. With the exception of two patients who developed moderate PNP, the neurography parameters did not deteriorate significantly during the study. Seven patients had mild PNP, of whom one improved. None of the patients with moderate ($n = 1$) or severe PNP at the beginning of the study showed any improvement. The observed changes in PNP status were not associated with dialysis efficiency in this study. The severity of the PNP did not correlate with the ANS dysfunction (Spearman correlation coefficient not significant). Two of the diabetic patients had moderate and one had severe PNP, and their nerve CVs and SAMPLs did not show any improvement or deteoriation during the study.

# 6. DISCUSSION

## 6.1. Different types of PNPs – how representative are the studied PNPs and the patients?

Our understanding of the causes and pathophysiology of PNPs have increased significantly during the last 50 years with the development of chemical, histologic, electrophysiologic and genetic methods (Dyck *et al.* 1985; Dyck *et al.* 1987; Dyck 1988; Gilliatt 1982; Lauria *et al.* 2005; Thomas 1997). The exact number of different causes of PNP is not known; my own estimate is that the number of causes is greater than 300. The pathophysiology, symptoms and neurophysiologic findings differ depending of the type and classification of PNP. Obviously it is not possible to have a representative sample of all different types of PNP in four studies. Both myelinated large-fibre and unmyelinated thin- fibre PNPs are studied here. Uremic, thalidomide-induced and myeloma PNPs are axonal. Also Fabry disease, a small-fibre PNP, can be considered as an axonal PNP. Both sensory and motor fibres are affected in uremia and myeloma PNP; thalidomide PNP is predominantly sensory, but as was evident in study II, motor fibres can also be affected. There are no primarily demyelinating acquired or hereditary PNPs in these studies. During the collection of material, patients with hereditary demyelinating PNPs were also collected, particularly patients with hereditary liability to pressure palsies.

In uremia, the most consistent abnormality in peripheral nerves is axonal degeneration with secondary segmental demyelination of myelinated axons (Ahonen 1981; Bolton *et al.* 1997; Pirzada *et al.* 1997; Said *et al.* 1983). This was also seen in studies I and IV included in this thesis. In uremic PNP, also autonomic fibres are affected (Vita *et al.* 1990; Said *et al.* 1983; Pirzada *et al.* 1997; Kirvela *et al.* 1995; Harnett *et al.* 1994; Bolton *et al.* 1997; Ahonen 1981). This mainly affects the cardiac autonomic fibres, and is seen as decreased HRV and variations in blood pressure in patients with ESRD (Vita *et al.* 1990). Cardiovascular disease is the largest single cause of death in patients on dialysis, accounting for 50 % of total deaths (Harnett *et al.* 1994). The study IV found a correlation between dialysis efficacy and improvement of HRV. Based on the results in the study IV, if the dialysis can be done effectively, the cardiac autonomic dysfunction should improve and the risk for cardiovascular death diminish. The efficacy of dialysis therapy to HRV has not been studied earlier. The earlier studies done to predialysis and dialysis patients have found that the disturbance in sympathetic and also parasympathetic activity is more severe in predialysis than dialysis patients (Campese *et al.* 1981; Heidbreder *et al.* 1985).

Signs of autonomic dysfunction have been reported also in Fabry disease, and they have been shown to correlate with lipid deposition in autonomic neurons (Cable *et al.* 1982).

However, the study III did not find significant autonomic involvement in patients with Fabry disease with either of the two methods used. Five of twelve patients reported autonomic symptoms, which did not affect their daily life. In the study III, HRV was studied with frequency domain-measures. The study group was small, which is probably the reason for normality. The study done by Kampmann et al. (Kampmann *et al.* 2008), however, found decreased HRV indexes in male pediatric Fabry patients, but not in females. This can be due to the earlier manifestation of the disease in males. ANS involvement in Fabry disease has also been reported by others, mainly in the form of reduced saliva and tear production (Cable *et al.* 1982). In study III, only women with Fabry disease were evaluated. In other neurophysiologic studies on Fabry disease, the patients have mostly been men. The disease is X-linked, so the manifestations can be milder and occur later in women than homotsygotic men. However, women affected with Fabry disease do have symptoms and neurophysiologic findings compatible with peripheral thin fibre PNP, as do men (Gibas *et al.* 2006; MacDermot *et al.* 2001) but study III shows that clinical examination alone is insensitive in detecting small fibre PNP in these patients.

Fabry disease has been considered a pure axon length-dependent small-fibre PNP also in many other studies (MacDermot *et al.* 2001; Luciano *et al.* 2002; Scott *et al.* 1999; Moller *et al.* 2006). Large myelinated axons are spared (Dutsch *et al.* 2002; Hilz *et al.* 2004; Luciano *et al.* 2002; MacDermot *et al.* 2001; Moller *et al.* 2006; Valeriani *et al.* 2004). This was evident also in our study (III). The small unmyelinated C-fibres are affected, and the other nerve-endings that are affected do not have myelin. Based on the study III, this leads to the conclusion that the axons have to be the ones that are affected, so the PNP in Fabry disease is considered as axonal.

## 6.2. How sensitive are the diagnostic tests and how well do they reflect abnormalities of different types of axons?

The neurophysiologic methods used in these studies are used routinely in the study of peripheral nerves worldwide. To minimize methodologic errors the measurements were done rigorously according to the quality manual of our laboratory (accredited to the ISO 17025 standard) by the highly qualified staff of our laboratory. This should have minimized technical errors.

*Age, height and temperature.* The reference values, which included age and height, were used. Effects of aging increase the difficulties in diagnosing PNP. With aging there are both anatomic and functional degenerative changes in the nervous system (Jacobs *et al.* 1985; Kawamura *et al.* 1977; Tohgi *et al.* 1977). Slight degeneration and demyelination of myelinated fibres become increasingly common after 60 years of age (Jacobs *et al.* 1985). Also, consistent reduction in the density of unmyelinated axons has been seen even

in the fifth decade (Jacobs *et al.* 1985). Loss of anterior horn cells has been documented with aging (Kawamura *et al.* 1977), while loss of whole motor units has been found in a study by Edström (Edstrom *et al.* 1987). Edström and Larsson also found that in old age the reinnervation of previously denervated muscle fibres is incomplete (Edstrom *et al.* 1987). The CV of both motor and sensory nerves decreases by around 1 m/s per decade in adults (Buchthal *et al.* 1966; Stetson *et al.* 1992; Trojaborg *et al.* 1992). The perception thresholds for vibration, cold and warm increase with age. All these changes are similar to the abnormalities caused by PNPs. In addition, people may be exposed to various neuropathic toxins, especially alcohol, alimentary toxins, industrial agents and drugs over the years.

The temperature of the limbs has an effect on the CV and AMPLs of the nerves (Dioszeghy *et al.* 1992), and the standardization of control of temperature should always be recognized. In a study by median sensory nerve, Buchthal an Rosenfalck (Buchthal *et al.* 1966) found a change of 2 m/s/C° over the entire length of nerve between the axilla and the finger over a temperature range of 20C° to 36C°. The local cooling causes an increase in SAMPLs (Lang *et al.* 1981). In the studies included in this thesis, the temperature of the limbs was measured and the limbs were warmed if the temperature was above the stated. One limitation of the studies was that the effect of temperature was not taken into consideration in statistical analysis. With better standardisation of temperatures, the diagnostic accuracy of these studies could have increased.

*Amplitudes.* We came to the same conclusion (I) as Tilki et al. (Tilki *et al* 2007), that the peroneal nerve Fmin and sural nerve SAMPL are useful parameters for the detection of mild-to-moderate PNPs in ESRD patients undergoing chronic dialysis. By Tilki et al., sural nerve SAMPLs, peroneal nerve CV and Fmin were also correlated with the severity of the clinical findings in these patients, suggesting that these parameters can be used in follow-up studies of uremic patients (Tilki *et al* 2007). The MAMPL depends mainly on the number of motor units and the motor unit size, and is less affected by moderate conduction slowing than the SAMPL. The MAMPL is not a true indicator of the number of axons in slowly developing axonal neuropathies, such as uremic PNP, due to fact that denervation of a number of muscle fibres could be compensated for by collateral reinnervation. Thus, the MAMPL can remain within normal limits in mild-to-moderate axonal PNPs provided the reinnervation capacity is normal, while the SAMPL shows a definite abnormality due to the absence of compensatory mechanisms to maintain the SNAP (Tilki *et al* 2007).

In axonal PNP of sensory nerves there is no collateral reinnervation to maintain the SAMPL in slowly progressing PNP, and the changes in SAMPL are seen at relatively early stages of the disease. In toxic thalidomide-induced PNP, the SAMPLs are the most sensitive indicators of PNP (Chaudhry *et al.* 2002; Apfel *et al.* 2004; Briani *et al.* 2004;

Cavaletti *et al.* 2004). Also in our study (II), the SAMPLs of the sural and radial nerves were the most sensitive parameters for detecting the thalidomide-induced neuropathy; also, the proportional change in nerve AMPL during the treatment was much greater than the other parameters.

The SAMPL and the area of the compound sensory nerve action potential best reflect the degree of axonal involvement in axonal neuropathies. Unfortunately, the coefficient of variation in repeated studies of the SAMPL is around 30% (Bleasel *et al.* 1991). With this high degree of variability, the sensitivity for detecting a small change with SAMPL is low. Another issue is that during axonal reinnervation, the reinnervating axons conduct slowly and are not detected at all by this technique. The regression models of the reference values for the SAMPLs have a coefficient of determination (R2) close to 60% which explains only 10-30% of the variation (Falck *et al.* 1991). Temperature of the limbs affects the AMPL; a decrease in temperature increases the AMPL. In future studies, the effect of temperature should be more carefully considered when analysing the results statistically. For Fmin, the coefficient of variation is 45-60% (Falck *et al.* 1991). In future studies, more sensitive methods based on large age, height and BMI related reference values are needed. Using more distal sensory nerves in the feet may be helpful. Some authors have suggested the plantar nerves (n. plantaris medialis and n. plantaris lateralis) (Sylantiev *et al.* 2008), but they are not any more distal than the superficial peroneal nerve. Superficial peroneal nerve was not included in our study protocol. If we had used the superficial peroneal nerve, it would probably have increased the sensitivity of detecting mild PNPs. With the use of well standardized methods and good quality reference values the sensitivity of the diagnosis can be increased without loss of specificity.

*Conduction velocity.* In our study on ESRD patients, only one third of patients had abnormal motor CVs, and less than 15% had abnormal sensory nerve CVs (I). In a previous study on ESRD patients (Nielsen 1973), reduction of peroneal nerve motor CV was the most sensitive test for uremic neuropathy. This difference is probably due to the bias in previous studies where less attention was paid to the response AMPL or F-waves than to the CV. Because the PNPs studied here were mainly axonal, the finding that CVs were not among the most sensitive tests detecting PNP was not surprising. In Fabry disease, the large fibres are not affected if uremia does not coexist and nerve conduction studies are within normal limits.

*F-waves.* The Fmin of the lower extremity nerves were by far the single most sensitive neurophysiologic parameter in the detection of uremic PNP (I). If the F dispersion and the number of F-waves were also taken into consideration, the overall sensitivity of F-wave studies of the tibial nerve increased, although the sensitivity of these other parameters alone was lower. The F-waves are valuable in detecting subclinical PNP

because they reflect the function of the entire motor axon and the excitability of the soma, whereas other tests provide information only about restricted segments of the nerve (Puksa 2003).

During thalidomide treatment (II), slight abnormalities in the peroneal nerve motor CV, in the MAMPL and in the Fmin were also seen. On the basis of these findings, it is clear that the PNP induced by thalidomide is not purely sensory. F-wave abnormalities have also been reported by others; in some patients the only pathologic findings were F-wave abnormalities (Lagueny *et al.* 1986; Rao *et al.* 2000). In our patients, peroneal nerve Fdisp did not increase significantly during the treatment, while Sadoh et al. (Sadoh *et al.* 1999) found increased chronodispersion of peroneal nerve F-waves in four patients treated with thalidomide after 32-130 supramaximal stimuli, whereas we used only 20 stimuli.

*A-waves*. In our study (I), A-waves were occasionally seen in all nerves, in dialysis patients either before or after dialysis, most commonly in the tibial nerve. Bischoff et al. (Bischoff *et al.* 1996) noted that A-waves are more common in patients with PNP than among others. A-waves are also seen in healthy subjects predominantly in the tibial nerve, with a frequency of about 25% (Puksa *et al.* 2003a). The number of A-waves did not appear or change in a consistent fashion in our study (I); in some patients they disappeared and in others they occurred first after dialysis.

*Autonomic tests*. HRV tests at rest and SSR were used to screen the function of ANS. These tests are easy to obtain in the laboratory of clinical neurophysiology, in our laboratory the tests are performed with the same computer as nerve conduction tests.

Althought the ANS study on uremia (IV) was retrospective and the HRV study was measured with time-domain measures, a positive correlation between dialysis adequacy and cardiac autonomic function was seen. In our study, we recorded the overall cardiac autonomic function; we did not differentiate between the sympathetic and parasympathetic dysfunction. Also a more complete set of ANS tests, especially HRV tests with 6/min controlled breathing in the Fabry study (III), tilt test and Valsalva manouver might have increased the yield of abnormalities and specified the possible parasympathetic dysfunction.

SSR was used in combination with HRV as a measurement of autonomic function in the study on Fabry patients (III). SSR is easy to perform and helpful in the assessment of sudomotor autonomic function in PNPs. However, the evaluation of SSR test results is not simple. A shortcoming of the SSR is the variability of the response even in healthy subjects. This is why I used only the absence of the response as the criteria for abnormality. The SSR also reflects not only the function of the PNS but also the sympathetic function of the CNS, which can lead to misdiagnosis in some cases. However, I had one patient

in this small study group that did not have responses in the SSR study, she also had autonomic symptoms.

*QST and IENFD.* IENFD as a quantitative pathologic test and thermal QST as a psychophysiologic test complement each other in the diagnosis of small-fibre dysfunction, as was evident in our results (III). QST, with the advantage of simplicity and noninvasiveness, provides no information regarding anatomic localization. Skin biopsy, on the other hand, can assess the pathologic substrate of elevated sensory thresholds (Pan *et al.* 2001). Based on the findings in the Fabry study (III), when small fibres are suspected to be affected, decreased number of small fibres at skin biopsy, together with findings in thermal detection threshold tests, is enough to make a diagnosis of small-fibre PNP.

Also Pan et al (Pan *et al.* 2001) came to the conclusion that thermal thresholds and IENFD should be an integrate package for detection of small–fibre PNP. In their study, IENFD was reduced in 80% of patients with sensory PNP, 54 % had abnormal WDTs, and 71% had abnormal CDTs.

In 2004, a Task Force was set up under the auspices of the European Federation of Neurological Societies (EFNS) with the aim of developing guidelines on the use of skin biopsy in the diagnosis of PNP (Lauria *et al.* 2005). In the EFNS guidelines, quantification of IENFD closely correlated with WDT and heat-pain thresholds, and appeared more sensitive than the sensory nerve conduction study and sural nerve biopsy in diagnosing small-fibre sensory neuropathy. The diagnostic efficiency and predictive values of this technique were very high (level A recommendation) (Lauria *et al.* 2005).

The PGP 9.5 antibody is currently the most commonly used marker for IENFD, and also the one we used in our study (III). It is a polyclonal panaxonal marker that stains both Aδ- and C-fibre intraepidermal nerve-fibre endings (Koskinen *et al.* 2005). Other markers for staining are also used; one of them is calcitonin gene-related peptide (CGRP) that is expressed by all types of primary afferents in the rat (Ruscheweyh *et al.* 2007). Different types of neurochemical markers, such as neurofilament 200 (NF200), substance P (SP), and isolectin B4 (IB4) have been useful to distinguish between A- and C-fibre neurons (Ruscheweyh *et al.* 2007). NF200 seems to be limited to neurons with A-fibres in the rat (Lawson *et al.* 1991). However, the expression patterns of these markers change after peripheral nerve injury, so that it is not clear whether they still distinguish between fibre types in models of neuropathic pain (Ruscheweyh *et al.* 2007).

*EMG and SFEMG in the diagnosis of PNP.* In the studies included here, EMG was not used. In axonal neuropathies spontaneous denervation activity is seen in EMG in distal muscles, together with motor unit (MUP) and interference pattern changes. If EMG is used in the study of PNP, quantitative MUP analysis should be done. However, reference values for the size of MUPs are still missing in most laboratories. How much EMG

findings contribute to the diagnosis is not clear. Qualitative analysis of MUPs probably does not give any more information than neurography. Needle EMG is uncomfortable and requires a doctor. If the PNP symptoms are symmetrical, the tests used in this study are enough to make the diagnosis of PNP. The role of needle EMG in the diagnosis of CTS has been discussed, there seems to be different opinions of the utility of EMG in the diagnosis of CTS (Gnatz *et al.* 1999a; Gnatz *et al.* 1999b). Usually, fibrillations and signs of collateral reinnervation in EMG are seen several days to weeks later than small changes in neurography, in SAMPLs and Fmin. The generation of MUP changes due to reinnervation, especially high amplitude MUPs, usually takes months and may be detected in chronic PNPs. EMG is definitely useful in differential diagnosis of PNP and focal neuropathies, particularly if there is suspicion of multiple radiculopathies in patients with spinal stenosis. Also, in some follow-up studies, the increase in spontaneous activity and MUP changes in EMG can be used as one marker of progression of axonal PNP. EMG may also be useful in establishing coarsely the age of a PNP: in an acute PNP there are no signs of collateral reinnervation, in a chronic neuropathy there are signs of collateral reinnervation. To some extent EMG may also reflect the rate of progression in a chronic PNP: in a slowly progressing PNP the denervated muscle fibers are reinnervated readily and there is little abnormal spontaneous acitivity, while in a rapidly progressing PNP the rate of denervation is greater than reinnervation, resulting in more abundant spontaneous activity.

SFEMG was not used in the diagnosis of axonal PNP in these studies. Earlier study of diabetic PNP has proposed that SFEMG may provide a way to follow responses to therapy in patients with diabetic neuropathy, as a more precise electrophysiological tool to study nerve regeneration than conventional nerve conduction studies (Bril *et al.* 1996). SFEMG from distal leg muscle could have increased the sensitivity of diagnosing PNP, at least in the mildest cases. However, jitter is an unspecific finding for PNP, the study is time consuming and a bit uncomfortable for the patient. Jitter is also increased in myasthenia gravis (Stalberg *et al.* 1974) and in other neurological disorders like ALS and distal hereditary myopathy (Stalberg *et al.* 1969; Stalberg *et al.* 1994).

## 6.3. Diagnosis and characterization of PNP

Unfortunately, there are no universally accepted criteria for diagnosing PNP. During this project, I found that most studies utilize criteria set by the investigators themselves. In epidemiologic studies, the diagnosis is usually based on symptoms and clinical findings. In mild PNPs, these clinical criteria are neither sensitive nor specific. However, laboratory investigations must be combined with the subjective symptoms and clinical findings. Neurophysiologic tests, neurography in particular, have commonly been used in combination with QST (Dyck *et al.* 1987; Dyck 1988; Dyck *et al.* 1991a; Dyck *et*

*al.* 1992; Olney 1998; Tegner *et al.* 1985). Because QST consists of psychophysiologic tests, some authors suggest that QST should be used in combination with neurography or autonomic tests to ensure that the subjective psychophysiologic tests do not result in false positive findings (Dyck *et al.* 1999). During the last ten years also histologic methods, mainly IENFD but also nerve biopsy has been used. Nerve biopsy is a relatively invasive procedure and is reserved for special diagnostic problems, especially for the diagnosis of PNP caused by amyloidosis or vasculitis. IENFD, on the other hand, is a well tolerated method that has become a standard in the diagnosis of small-fibre neuropathy (Lauria *et al.* 2005).

The lack of unequivocal, simple diagnostic criteria for the diagnosis of PNP is probably due to the wide variability of findings and symptoms in different types of PNP. In diabetic PNP, Dyck et al. have done numerous studies both on the diagnosis and on the grading of severity of PNP (Dyck *et al.* 1985; Dyck *et al* 1986b; Dyck *et al*. 1987; Dyck 1988; Dyck *et al.* 1991a; Dyck *et al.* 1992; Dyck *et al.* 1993; Dyck *et al. 1*997). In a study of diabetic PNP by Dyck et al. (Dyck *et al.* 1987), nerve conduction studies (AMPL, CV, area, DLAT) employing the criterion of abnormality in two or more out of four nerves were the most sensitive tests in detecting PNP. The sensitivity of SAMPL is not surprising, because diabetic PNP is also predominantly axonal. In this diabetic study by Dyck et al., neither F-waves nor IENFD were studied. Nerve conduction was followed by symptoms, NIS, VPT, and rather surprisingly for diabetic neuropathy, where small fibres usually also are affected, last by CDT. The sensitivity of nerve conduction studies naturally depends on the criteria used. The percentage of diabetics with abnormality of nerve conduction declined progressively from 80% to 21% when the criterion changes from abnormality in one to four out of four nerves. The use of the criterion of abnormality in two separate nerves appears to be a reasonable one for PNP, as this degree of abnormality is the first increment greater than can be accounted for by a mononeuropathy (Dyck *et al.* 1987). The same study also indicated that at least 16% of patients had other abnormalities (in NSS, NIS, VPT or CDT) not recognized by abnormality of nerve conduction studies (Dyck *et al.* 1987). However, there are no international policies for the strategy of neurophysiologic studies while having a suspected PNP patient on consultation, and nor are there criteria for neurophysiologic findings and symptoms in PNP.

## 6.4. Utility of different methods in the diagnosis of PNP

The methods used in the diagnosis of PNP of unknown etiology should cover different types of fibres; both small myelinated and unmyelinated and large myelinated fibres. Neurography, including F-wave studies and VPT are included in the PNP study for the study of large fibres. The parameters of interest in a neurophysiologic study should cover axonal and demyelinating types of PNP. This is usually the case in normal neurography,

where both AMPLs and CVs are taken into consideration. F-wave studies are informative in detecting demyelination, and at least Fmin should be included in PNP studies. Sensory and motor nerves from upper and lower extremities should be used in the study to determine the distribution of PNP.

It is useful to do the clinical neurologic assessment for detecting PNP before neurophysiologic studies. Tendon reflexes and muscle strength from both upper and lower limbs should be examined. Also light touch with a ball of cotton wool and pin-prick sensation by using disposable pins should be tested. Cold and warm detection can be tested with water filled tubes or with metal rollers. Walking in the study room should be observed and possible ataxia recognized.

*Correlations between neurophysiologic findings and symptoms.* The evaluation of PNP symptoms is essential in the diagnosis of PNP; electrophysiologic studies provide only indirect and incomplete information about (1) neuropathic symptoms, (2) neuropathic deficits, (3) dysfunction of small fibres, and (4) specific neuropathologic abnormality (Dyck 1988). However, to my knowledge, there is only one other study (Devigili *et al.* 2008), where symptoms have been correlated with dysfunction and neuropathology of small fibres. This study was done after the publication of our Fabry study (III). Earlier, Dyck has correlated the symptoms and neurophysiologic tests with sural nerve biopsy (Dyck *et al.* 1985). According to that study, although the symptoms, neurologic deficits and abnormalities of nerve conduction are statistically associated, they should be evaluated separately to provide an adequate characterization. However, in follow-up studies and multi-centre studies, PNP symptoms and test results from NIS might give rather variable results because the method of testing and evaluating of results might vary considerably among physicians (Dyck 1988). The studies included here were done mainly by the author (SL) and the same qualified technicians, so the variability depending on the investigator should have been minimized.

The positive sensory symptoms are probably generated through increased spontaneous firing of sensory axons. This altered firing has been documented using microneurography, which is a useful research tool but not suitable for clinical use (Torebjörk *et al.* 2005). The neurophysiologic methods used in the clinical routine mainly reflect loss of function, not positive symptoms. However, it is interesting to note that in uremic patients (I), many of the parameters reflecting reduced peripheral nerve function, such as the VPT, the SAMPL of the sural nerve, as well as the tibial nerve motor CV and FLAT, were highly correlated with subjective positive neuropathy symptoms. These correlations of both motor and sensory parameters with positive sensory symptoms probably indicate that the positive sensory symptoms are related to the severity of the PNP. Unfortunately, IENFD was not available in our laboratory when the uremic PNP study was done, and the small fibre involvement was studied only with QST. In the study of Fabry disease,

the correlations between PNP symptoms and neurophysiologic tests were weaker. The PNP in Fabry patients was milder than in uremic patients, and large fibres were not affected. Pain, as a positive symptom, was associated with a decrease in IENFD in Fabry disease but not significantly with QST tests. The same kind of result was seen later in the study by Devigili et al (Devigili *et al.* 2008), where clinical examination with evaluation of negative (hypoesthesia) and positive (evoked pain) signs correlated with the diagnosis of small-fibre PNP in about half of 67 patients with small-fibre PNP (no-one with Fabry disease), showing that IENFD had a higher diagnostic efficiency than QST. The advantage of this study was that QST and IENFD were analyzed from the same area, which to my knowledge, has not been done in previous studies. In our study, the QST were done more distally than the IENFD. This was due to the lack of normal values for these different tests at the same site. Most researchers avoid taking the IENFD at distal sites to minimize the risk of complications. In our study, no complications were seen at the biopsy site used, 10 cm above the lateral malleolus. However, pathology of small fibres also was seen at this site. The correlation with QSTs and IENFD could have been better, if the biopsy site had been more distal, taken at the same site as QST.

In PNP, positive symptoms, pain, paresthesias etc. are usually the most disturbing symptoms, and are not easily measured with neurophysiologic studies. One of the reasons for the weak correlation with overall symptoms and neurophysiologic findings can be that mild negative symptoms, complaints of loss of function or sensibility, are probably not recognized when they first appear. Probably, we could also have obtained a better correlation with symptoms and neurophysiologic findings, if the spectrum of the severity of PNP in our patients had been broader, from very mild to very severe PNP.

However, in axonal neuropathies, at least some of the symptoms are usually recognized at the early stages of the disease. This is not the case in hereditary demyelinating PNPs, where sensory symptoms are usually minimal. In the studies of demyelinating PNP, the symptom profile we used in axonal PNPs is probably not informative enough.

*Confounding problems caused by focal neuropathies.* We found increased incidence of CTS among Fabry patients; 25% of patients had CTS (III). In women overall, the incidence is 2.7% -7.4% (de Krom *et al.* 1992; Atroshi *et al.* 1999). The study group was relatively small; of course there is a risk that the high prevalence of CTS could be explained by chance. However, increased prevalence (27%) of CTS in patients with Fabry disease has also been reported by Luciano et al. (Luciano *et al.* 2002). The precise cause of this predisposition is not known; perhaps glycosphingolipids also accumulate in the carpal tunnel and in tendons passing through it, thus thickening them and causing CTS.

In our study on ESRD patients (I), some patients may have had mild subclinical CTS, and access fistulas may have exerted mechanical pressure on superficial sensory nerves,

especially the branches of the radial nerve. Moreover, iatrogenic mononeuropathies resulting from the placement of forearm arteriovenous fistulas may have occurred. In our patients, the frequently abnormal radial nerve CV and SNAP, despite the relative preservation of conduction in the lower extremities, may have been partly due to the dialysis fistula. Mononeuropathies are a frequent clinical complication in ESRD patients, and most typically occur in the median, ulnar, and femoral nerves (Krishnan *et al.* 2007). CTS, due to the accumulation of amyloid deposit, uremic tumoural calcinosis, and the placement of arteriovenous fistulas (Bicknell *et al.* 1991), is the most common mononeuropathy in ESRD, with prevalence rates varying from 6% to 31% (Bicknell *et al.* 1991; Hirasawa *et al.* 2000; Jain *et al.* 1979; Schwarz *et al.* 1984).

Because of the increased risk of having CTS in uremia and Fabry disease, recordings from the median nerve in the diagnosis of PNP should be avoided. In patients with uremia, the radian nerve also can be affected due to the fistula. The nerve conduction test battery for PNP diagnosis from the upper limbs should be planned carefully, keeping in mind these complications which are not due to PNP.

## 6.5. Strategies for testing and screening PNPs

As concluded by the report of the AAN, AANEM and AAPM&R (England *et al.* 2005), no single reference standard defines distal symmetric PNP. There are many recommendations regarding NCS criteria for the diagnosis of PNP, but no formal consensus exists. By their recommendation, unilateral studies of sural, ulnar and median sensory nerves, and peroneal, tibial and ulnar motor nerves with F-waves should be done. This recommendation is close to what was done in studies included in this thesis, except that the median nerve was avoided due to the possibility of CTS.

PNP should be adequately described by a composite score encompassing impairment of motor, sensory and autonomic fibres: which other components are chosen and how they are weighted should be based on careful judgment (Dyck *et al.* 1999). Such a composite score might include measures of clinical neurologic abnormality (NIS), nerve conduction studies, QST, and autonomic tests. Nerve conduction and autonomic tests should always be included to ensure that objective measures, uninfluenced by the will of the patient, are used (Dyck *et al.* 1999). When studying small-fibre PNP, IENFD can not be influenced by the patient. However, the studies by Dyck leave the judgment of which tests should be used in different type of PNPs, (other than diabetes, that has been studied widely) to the investigator. Nor is advice given as to where the studies should be performed; should the neurography always be done bilaterally, and should the QST always be done from hands and feet.

In PNP the symptoms are symmetric. If there is no suspicion of other reasons behind the symptoms and the patient does not have local nerve entrapments, local lesions or lower back problems, unilateral neurophysiologic recordings can be done. However, if conduction blocks are looked for (if acquired demyelinating PNP is suspected), the recordings should be done symmetrically.

*Screening for PNP in a patient with known etiology.* In cases where the patient has a known disease causing PNP, the test battery can easily be chosen to follow the fibres that should be affected. In follow-up studies, when the patient already has a known PNP, the pattern of neurophysiologic tests can be narrower than in a first-time study.

In pure small-fibre PNP (Fabry disease) patients do not normally have large-fibre involvement, and studies can concentrate on small-fibre function. Even we did not found autonomic PNP in patients with Fabry disease, autonomic fibres can be affected in small-fibre PNPs. Simple autonomic tests (HRV during normal and controlled breathing and SSR) are useful additions to the neurophysiologic study.

The following tests should be done in Fabry disease: unilateral CDT and WDT from hand and foot, followed by skin biopsy (IENFD) from foot. SSR and HRV studies should be done to screen the autonomic involvement, if tests are available. If there is any suspicion that the patient already has renal involvement, also large-fibre studies should be performed. Peroneal and ulnar motor nerve studies, including F-wave studies, and sural and radial nerve sensory studies from the same side as CDT and WDT should also be evaluated. VPT should also be studied from hand and foot, if large fibres are suspected to be affected.

In axonal PNPs like uremic and diabetic PNP, both small and large fibres can be affected, and all the studies mentioned above should be done. Thalidomide PNP is axonal and predominantly sensory, even though we found a slight motor component. These tests are good at detecting the PNP also in thalidomide and myeloma-induced axonal PNP.

*Diagnosing PNP in a patient without a previously known cause.* In planning the screening strategy for PNP of unknown cause, the patient's anamnesis is crucial. The symptoms of different types of PNP should be thoroughly asked about. The symptom profile we used has a great advantage in detecting different types of symptoms. Positive and negative sensory and motor symptoms should be asked about separately. The anamnesis of the patient should cover pharmaceutical history including non-prescribed vitamin substitutions and natural medicines, occupational history, relatives suffering from PNP or any neurologic disease, alcohol consumption, and dietary restrictions because of for example celiac disease. In addition, the timing of the appearance of PNP symptoms and findings is critical. Did the symptoms begin within a day or week, or slowly over months or years?

If the patient has symptoms of PNP but the etiology is not known, the screening battery should be broad. The findings in neurophysiologic tests are helpful in searching for the possible cause of PNP. For example, conduction blocks are usually seen in inflammatory PNPs, whereas there is only sensory involvement in carcinomatous or some toxic PNPs.

Patient's symptoms can be easily screened with symptom chart. The patient draws on the human feature in what parts of the body he/she feels the symptoms. The area of numbness is coloured in yellow, pain in red, and loss of sensation in blue.

## 6.6. Future developments

The international policies for neurophysiologic studies in the different types of PNPs should be stated. The diagnostic criteria for PNP should be confirmed, and they should be the same in all laboratories.

The neurophysiologic studies on PNP should include focused questionnaires for PNP symptoms. These questionnaires should be filled in by the patient before the neurophysiologic studies are done. The questionnaires can concentrate on small-fibre PNP in pain patients, or large-fibre function (sensory or weakness) in other patients.

In small-fibre PNP studies, IENFD studies can be more specific in staining different types of small fibres. PGP 9,5 is currently the most often used antibody for staining the small fibres, but it is non-specific and stains all the fibres in the epidermis. The technique and staining properties of more specific staining methods still need to be improved to be a reliable option.

# 7. CONCLUSIONS AND SUMMARY

Early diagnosis of PNP and the detection of the etiology in treatable PNPs is crucial. Unfortunately, there are no universally accepted criteria for diagnosing PNP. Most studies utilize criteria set by the investigators themselves.

The diagnosis of PNP requires skilful combination of information from different sources. Based on our results, clinical examination and symptoms alone may be insesitive in detecting PNP. More objective diagnostic tools are therefore needed. When diagnosing PNP, it is important to use standardized neurophysiologic tests that reflect the function of different types of nerve fibres, these tests should have proper reference values and they should be sensitive in detecting the particular type of PNP that is suspected. The patient's subjective symptoms, family history and clinical findings give valuable information, and should always be evaluated. Histologic methods, genetics and clinical chemistry are valuable tools in making the diagnosis. The neurophysiologic methods, when chosen correctly, are the cornerstone of the diagnosis of PNP.

# 8. ACKNOWLEDGEMENTS

The present study was carried out at the Department of Clinical Neurophysiology, Turku University Hospital during the years 1998-2009. I want to thank all the patients who participated in these studies during these years. Without their contribution, this work would never have been possible.

I wish to express my warmest thanks to Professor Jaakko Hartiala, Head of Clinical Physiology and Nuclear Medicine, and Professor Jorma Viikari, Head of the Department of Medicine, for the possibility to carry out these studies in their departments.

I owe my deepest gratitude to my supervisors, both of who have guided me with endless patience, support and encouragement during these years. It has been a privilege to work with them. Docent Björn Falck always had faith in this study. I truly admire his enthusiastic attitude towards scientific work and his experience in clinical studies. He always had patience and willingness to solve the problems, educate me in the principles of statistical analysis, and demystify various computer programs all over and over again.

Docent Liisa-Maria Voipio-Pulkki always had faith in my possibilities and encouraged me continuously. She introduced me to the fields of scientific work, and her contribution to the writing processes of the articles and the thesis has been valuable.

Docent Tapani Salmi and Docent Aki Hietaharju, the official reviewers of this thesis, are gratefully acknowledged for their kind and valuable challenging, which helped to improve this thesis.

I wish to express my sincere gratitude to all the co-authors of these studies. Docent Satu Jääskeläinen, the current Head of Department of Clinical Neurophysiology, I express my deepest gratitude for friendship, support and for her wide scientific knowledge in the area of pain. I am grateful to Docent Matti Erkinjuntti, who enabled my entrance to Clinical Neurophysiology when I was preparing my fist study. I thank Docent Matias Röyttä, who kindly advised me in the analysis of skin biopsies and did enormous amount of work with creating the new analysis system of intraepidermal nerve fibres in our hospital. I also thank Professor Hannu Kalimo and the skilled staff in the laboratory of neuropathology, who studied and started the new staining method of intraepidermal nerve fibres in our hospital for the benefit of my study.

Docent Kaj Metsärinne and Markku Asola, MD, I thank for valuable comments in the area of kidney diseases and dialysis. Docent Kari Remes and Kari Koskela MD, I thank for their advice in the field of myeloma and thalidomide therapy. Professor Ilkka Kantola I thank for his valuable comments concerning Fabry disease and for leading the Fabry

project. Maila Penttinen, MD, I thank for her advice in the area of genetics in Fabry disease.

I warmly thank Docent Heli Forsell and Anna Valjakka M.Sc., for their advices in the use of pain questionnaires, and Docent Tero Taiminen for his valuable advice concerning the use of depressive and somatisation questionnaires.

I express my deepest gratitude to the skilled and energetic personnel of Department of Clinical Neurophysiology. Without good co-operation and friendly atmosphere with all the technicians and secretaries, this work would not have ever been possible to perform. The technicians have made enormous amount of work with the patients in this study. I am deeply grateful for the technicians who took part in the studies: Pirkko Koskinen, Eeva-Liisa Kärpijoki, Anne Enala, Tuula Jokela and Pia Aalto. I am thankful also to Leila Viita, Marja Alaspää and Ulla-Maija Aaltonen for their assistance during the first studies.

I would also like to express my deepest gratitude to Tarmo Kärki M.Sc. for his help with the HRV studies and for his endless patience in solving my computer problems. My colleagues Maria Peltola, Marja Koivu, Hannu Mikola, Antti Puhakka, Irina Virtanen, Markus Müller and Santeri Aura I thank for friendship and creating a warm and friendly atmosphere at the department.

Further, I thank Jacqueline Välimäki, MA, for revising the English language of this thesis.

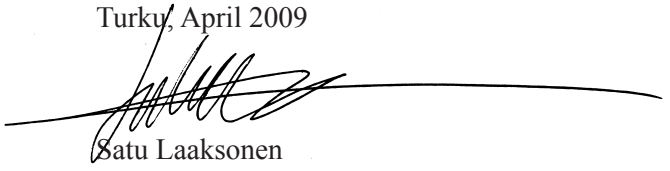
My dear friends, Katariina Korhonen, Kirsi Leino, Heidi Nurmi, Mia Mäkinen and Maria Peltola I thank for support and friendship. I also warmly thank all my other friends in Finland and abroad for keeping this study out of my mind when needed.

I am deeply grateful to my parents Tuula and Erkki Virtanen, to my sisters Outi, Laura and brother Timo, and to my mother-in-law Marja Laaksonen for their love, support and help with children during these years.

Finally, I owe my deepest love and gratitude to my husband Jussi for his love, patience and support, and to our children Stella, Matias and Oliver, for constantly reminding me what is truly important in life.

This study was financially supported by the Turku University Foundation, the EVO-funding of Turku University Hospital and the Finnish Kidney Foundation.

Turku, April 2009



Satu Laaksonen

# 9. APPENDIX

Table 2. Subjective PNP symptoms and clinical findings

PNP SCORING

One point / symptom

I: Muscle strength

1. Face _____
2. Upper limbs _____
3. Hand _____
4. Lower limbs _____
5. Foot _____

Total _____

II: Symptoms

***A*** Negative symptoms

6. Inability to recognise food in mouth _____
7. Inability to recognice objects with hands _____

(Numbness in hands, do you get scars on your hands without noticing it)

8. Walking unstable _____

(Numbness in feet, do you get scars on your feet without noticing it)

Total _____

***B*** Positive symptoms

Hands

9. Paresthesia (numbness, tingling) _____
10. Dysesthesia (altered sensation) _____
11. Hyperesthesia (increased sensibility to stimuli) _____
12. Allodynia (normal tactile stimulus painful) _____
13. Hyperalgesia (pain sensation increased) _____
14. Hyperpathia (pain continues after stimulus is removed) _____

Feet

15. Paresthesia (numbness, tingling) _____
16. Dysesthesia (altered sensation) _____
17. Hyperesthesia (increased sensibility to stimuli) _____
18. Allodynia (normal tactile stimulus painful) _____
19. Hyperalgesia (pain sensation increased) _____
20. Hyperpathia (pain continues after stimulus is removed) _____

Total _____

Autonomic symptoms

21. Have you fainted more than once during the last year? ____

If yes, in what kind of situation?

22. Have you vomited during the last 3 months?

(Exclude viral infections or vomiting due to chemotherapy) ____

23. Do you have diarrhoea at night? ____
24. Has your sweating decreased? ____
25. Do your hands sweat at all, has it decreased? ____

Increased? ____

26. Does your feet sweat at all, have it decreased? ____

Increased? ____

27. Do you sweat while eating? ____
28. Men: Do you have erectile difficulties? ____
29. Do you feel like fainting when standing up? ____

Sum of autonomic symptoms ____

Total _____

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