CLINICAL PHENOTYPING AND NEUROPATHOLOGICAL CORRELATES OF NEUROMUSCULAR DISEASE

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A thesis submitted in fulfilment of the requirements for the degree of Master of Philosophy (Medicine)

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2019
STATEMENT OF ORIGINALITY

This is to certify that to the best of my knowledge; the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes. I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Dr Dev Nathani
PUBLICATIONS

Chapter 1, 4.1 and 5


Chapter 3


Chapter 4

CONFERENCE PRESENTATIONS

18th Biennial Clinical Neurophysiology Workshop, Gold Coast, Australia, 2019

- Poster presentation – Paraproteinaemic neuropathy: The role for nerve biopsy

Campbelltown Hospital Neurology Department, University of Western Sydney, Sydney, Australia, 2019

- Paraproteinaemic neuropathy: The role for nerve biopsy
- Overview of the diagnostic utility of nerve biopsies in peripheral neuropathy

Australia and New Zealand Association of Neurologists Annual Scientific Meeting, Darwin, 2018

- Platform presentation: Role of nerve biopsies in vasculitic neuropathy

17th Biennial Clinical Neurophysiology Workshop, Gold Coast, Australia, 2017

- Poster presentation – Clinical and Neurophysiological Predictors of Vasculitic Neuropathy diagnosed on Nerve Biopsy

Brain and Mind Centre, University of Sydney, Sydney, Australia, 2017

- Overview of the diagnostic utility of nerve biopsies in peripheral neuropathy

AWARD

Motor Neurone Disease Research Scholarship (2017 – 2018)
ABSTRACT

Peripheral neuropathies have a significant population prevalence which is likely to increase in the future. They can impose a significant burden on the quality of life. The use of nerve biopsy is an important diagnostic tool in the assessment of patients with neuropathies. Newer techniques have emerged; reducing the reliance on nerve biopsies to assist with diagnosis. Nonetheless, the information obtained from histopathology may still help to distinguish clinical phenotypes which in turn can potentially modify the clinical management of patients. However, no clear guidelines currently exist to assist clinicians considering nerve biopsies for their patients. During this Master’s project, a comprehensive database was created based on the analysis of clinical and pathological information derived from the medical records of patients who were referred for nerve biopsy. This neuromuscular database was analysed to study the diagnostic utility of nerve biopsies in the clinical workup of selected types of neuropathies.

Patients with suspected vasculitic neuropathy were studied to determine the clinical parameters that influenced the presence of neuropathological findings supportive of a diagnosis of vasculitic neuropathy. Stepwise clinical progression, the presence of both sensory and motor features, and asymmetric or multifocal presentation best differentiated systemic and non-systemic vasculitic neuropathy from other conditions. Additionally, parameters that best differentiated systemic vasculitic neuropathy from other conditions included the presence of selected autoantibodies and elevated inflammatory markers. Nerve conduction studies (NCS) in patients with pathologically confirmed vasculitic neuropathy frequently demonstrated a pure axonal neuropathy
with sensorimotor abnormalities. In contrast, a diagnostic exclusion of vasculitis was highest in patients with normal NCS, patients with upper limb dominant symptoms, and in patients with cerebrospinal fluid (CSF) pleocytosis or CSF protein above 110mg/dL. Nerve biopsies in patients with symptoms that were purely sensory or chronic and symmetric as well as patients with dominant demyelinating features on NCS were unlikely to demonstrate evidence of vasculitis.

Patients with suspected paraproteinaemic neuropathy were studied to determine the clinical parameters that influenced the presence of neuropathological findings supportive of a diagnosis of paraproteinaemic neuropathy. The presence of an IgM paraprotein and absence of an IgG paraprotein best differentiated biopsy findings consistent with paraproteinaemic neuropathy from other diagnoses. Patients with an eventual clinical diagnosis of paraproteinaemic neuropathy were studied separately and it was determined that regardless of whether the nerve biopsy report suggested a diagnosis of paraproteinaemic neuropathy, there was no difference in the prevalence of changes to the management plan or to clinical outcomes.

Overall, this thesis has established that in a cohort of patients referred for nerve biopsy, several clinical parameters influenced the pathological outcomes of nerve biopsy. Consequently, decision aids were created for clinicians to assist in deciding whether a referral for nerve biopsy was appropriate.
PREFACE

All research involving the use of patient information has been done with the approval of the local research ethics committee. This study was undertaken at the Brain and Mind Centre, University of Sydney, and Royal Prince Alfred Hospital, part of the National Health and Medical Research Council Sydney Health Partners Advanced Health Research and Translation Centre.

Source of information: Patient medical records obtained from hospitals and/or the medical professionals involved in the care of the patient.

The research was undertaken under the guidance of my supervisors Professor Matthew Kiernan and Professor Michael Barnett. Other co-authors include Professor John Pollard, Dr Judith Spies and Dr Min-Xia Wang. The collaborators in my research played an advisory role and assisted in the editing of manuscripts that were submitted for publication. Additionally, the subject matter was their area of interest and I greatly benefited from their knowledge of neuropathology of the peripheral nerve.
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### List of abbreviations

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<tbody>
<tr>
<td>AL</td>
<td>Amyloid light chain</td>
</tr>
<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
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<tr>
<td>ANA</td>
<td>Anti-nuclear antibody</td>
</tr>
<tr>
<td>ANCA</td>
<td>Anti-neutrophil cytoplasmic antibody</td>
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<tr>
<td>BTU</td>
<td>Büllmann Titre Units</td>
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<tr>
<td>CANOMAD</td>
<td>Chronic ataxic neuropathy, ophthalmoplegia, monoclonal IgM protein, cold agglutinins and disialosyl antibodies</td>
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<tr>
<td>CMT</td>
<td>Charcot-Marie-Tooth</td>
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<tr>
<td>CIDP</td>
<td>Chronic inflammatory demyelinating polyneuropathy</td>
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<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DILS</td>
<td>Diffuse infiltrative lymphocytosis syndrome</td>
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<tr>
<td>EFNS</td>
<td>European Federation of Neurological Societies</td>
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<tr>
<td>EM</td>
<td>Electron microscopy</td>
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<tr>
<td>EMG</td>
<td>Electromyogram</td>
</tr>
<tr>
<td>ENA</td>
<td>Extractable nuclear antigens</td>
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<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
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<tr>
<td>GBE</td>
<td>Glycogen branching enzyme</td>
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<tr>
<td>GQ1b</td>
<td>Ganglioside Q1b</td>
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<tr>
<td>IVIG</td>
<td>Intravenous immunoglobulin</td>
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<tr>
<td>LSRPN</td>
<td>Lumbosacral radiculoplexus neuropathy</td>
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MADSAM  Multifocal acquired demyelinating sensory and motor
MAG  Myelin associated glycoprotein
MGUS  Monoclonal gammopathy of unknown significance
MMN  Multifocal motor neuropathy
MPO  Myeloperoxidase
MRI  Magnetic resonance imaging
MVA  Motor vehicle accident
NCG  Non-caseating granulomas
NCS  Nerve conduction studies
NGS  Next generation sequencing
NMC  Neuromuscular choristomas
NSVN  Non-systemic vasculitic neuropathy
PCR/qPCR  Polymerase chain reaction / qualitative polymerase chain reaction
PET  Positron emission tomography
PLEX  Plasmapheresis
PNS  Peripheral Nerve Society
POEMS  Polyneuropathy, Organomegaly, Endocrinopathy, M-protein, Skin changes
R-CP  Rituximab Cyclophosphamide Prednisolone
RF  Rheumatoid factor
TTR/ATTR  Transthyretin / Transthyretin amyloid
WM  Waldenström’s macroglobulinaemia
CHAPTER 1

LITERATURE REVIEW
### 1.1 Introduction

The understanding that nerves served as a signalling system developed around the first half of the third century. (1) In 1717, van Leeuwenhoek published the first histological description of myelinated nerve fibres. (2) With subsequent improvements in microscopy and histological techniques, the use of nerve biopsy as a diagnostic tool became established by the 1960s. (3)

In the modern era, the indications for nerve biopsy can be broadly categorised. Despite extensive investigation, no cause may be apparent in up to 10-25% of patients with peripheral neuropathy. (4) In these cases, nerve biopsy may become a consideration especially if there is clinical deterioration without an established diagnosis. (5) A further indication for nerve biopsy includes patients where management will involve therapies that have significant side effects. In such patients, clinicians may elect to undertake a nerve biopsy to achieve a formal diagnosis or to obtain greater diagnostic certainty prior to commencing treatment. A third, less common indication includes suspected hereditary neuropathy, where a nerve biopsy may guide targeted genetic testing when standard testing has been non-diagnostic. Separately, nerve biopsy in diseases such as vasculitis, (6) amyloidosis, (7) pure neuritic leprosy, (8) neurosarcoidosis, (9) and neurolymphomatosis (10) enables a definitive diagnosis. Notwithstanding the potential diagnostic benefits of referring patients for nerve biopsy, there are several issues that factor into the clinical decision-making process. The first part of this thesis will take the form of a literature review that critically dissects these issues and gives a detailed overview of the current indications for nerve biopsy. The following chapters represent the work done during my research in order to clarify the diagnostic utility of nerve
biopsies for selected indications for nerve biopsy. Consequent to a detailed review of the literature, each indication for nerve biopsy was assigned a category based on the relative importance of nerve biopsy for that indication: High, Moderate and Low. Highly important indications were those diagnoses for which the findings on nerve biopsy were highly specific and alternative diagnostic tools were less suitable. Moderately important indications were those diagnoses for which selected nerve biopsy findings had good specificity but where patient management was less reliant on a neuropathological diagnosis. Indications of low importance were those for which nerve biopsies were rarely required or helpful. Chapter 3 explores vasculitic neuropathy, which is a highly important indication for requesting a nerve biopsy. Chapter 4 explores paraproteinaemic neuropathy, which was considered a moderately important indication for requesting a nerve biopsy.

1.2 Literature review methodology

PubMed (1966, to June 2019) and Google Scholar (till June 2019) was searched using the following strategy. The term “nerve biopsy” was combined using the AND operator with one of the indications for nerve biopsy listed in this review or the terms “cost”, “yield” or “utility”. Additional articles were also identified by the co-authors. Only English language articles were considered. Indications for nerve biopsy exclusive to the paediatric population were excluded. For each identified article, the bibliography and list of citing articles was examined for potentially relevant articles. Emphasis was placed on articles published in the last 10 years.
1.3 Evolution of diagnostic modalities

With a rapidly expanding diagnostic armamentarium in recent decades, the most prominent reason for declining referrals for nerve biopsy relates to the availability of less invasive diagnostic modalities that can provide sufficient diagnostic certainty, particularly driven by advancements in molecular diagnostics.(4) Advances in related diagnostic modalities including peripheral nerve imaging, neurophysiological investigations and the advent of skin biopsy all combine to reduce biopsy rates.(11) While magnetic resonance imaging (MRI) and ultrasound are predominantly used for conditions that don’t require a nerve biopsy (entrapment neuropathy, traumatic neuropathy, space-occupying nerve lesion), these imaging modalities may provide information on nerve morphology, site and extent of damage. This is especially useful in areas that are difficult to evaluate with neurophysiological tests.(11) Several ultrasound-based scoring systems have been developed to better evaluate neuropathy and can be useful in patients with suspected inflammatory or hereditary neuropathies.(12)

1.4 Biopsy technique

In general, the skin and superficial fascia is dissected to allow for visualisation of the nerve. Difficulties with visualising the nerve can occur if the dissection is too deep or if the nerve appears paler or more wasted than expected due to pathology.(13) It is important to distinguish the nerve from adjacent tubular structures. Prior to biopsy, a single suture is placed proximally though the nerve, and the nerve is then cut proximally and distally. The biopsy specimen is then wrapped in damp saline swaps.
and transported to the laboratory. At all stages of the process, it is important that the specimen be minimally handled to prevent compression artefact.

1.5 Diagnostic yield of nerve biopsy

Previous studies that investigated the utility of nerve biopsy determined that a significant proportion of nerve biopsies do not provide clinically useful information. In one study of 38 patients with peripheral neuropathy of unknown cause, nerve biopsy led to a confirmed diagnosis in 37%, usually amongst those patients with asymmetric, non-chronic phenotype. In another study of 67 patients who underwent nerve biopsies, the results influenced the eventual diagnosis and management in 33% and 27% of patients respectively. In the subset of patients referred for polyneuropathy of uncertain origin, a diagnosis was established in 24% and led to new treatment in 20% of patients. A prospective series of 50 cases found that sural nerve biopsy altered diagnosis in 14% and affected management in 60%. Similarly, another study reviewing 234 patients underwent nerve biopsies determined that nerve biopsy was essential in 16%, and helpful in a further 22%. Clearly, much has changed in the diagnostic armamentarium since these studies were undertaken more than a decade ago.

1.6 Factors that affect diagnostic yield

Optimal outcome with nerve biopsy requires appropriate selection of patients, nerves and neuropathology techniques. No guidelines exist to inform patient selection for nerve biopsy. However, Dyck and colleagues counselled that nerve biopsy...
should be reserved for cases that have been carefully characterised by other clinical approaches first which have failed to provide a definite answer, and informed judgement is made that biopsy may be useful.

Nerve biopsy is more likely to be diagnostic in the acute and subacute phase rather than chronic phase. Diagnostic yield is also higher in patients presenting with asymmetric or multifocal symptoms. In one study, diagnostic yield of biopsy was 32.7% in patients with asymmetric or multiple mononeuropathy compared to 17.7% in patients with symmetrical neuropathy. (18) In another study, diagnostic yield was highest when there was clinical/ neurophysiological evidence of asymmetry (60%) and multifocal distribution (75%). (19) Diagnostic yield is also influenced by the pre-biopsy provisional diagnosis with studies showing higher yields with provisional diagnoses of vasculitic neuropathy, inflammatory demyelinating polyneuropathy, and hereditary motor and sensory neuropathy. (18, 19) The yield of nerve biopsies in cryptogenic neuropathies, while low, is not insignificant (0-37%). (14, 19-21) Indeed, important diagnoses like vasculitis have been made in such patients. (4)

With regards to neuropathology techniques, there is debate regarding the value that teased fibre analysis adds in the evaluation of nerve biopsies (19, 22) but it is useful in evaluating certain pathologies. (23) Examining serial frozen and paraffin/ resin-embedded sections has been shown to be useful. (19) Immunohistochemistry can add diagnostic and prognostic value. (22) One study found that electron microscopy (EM) added value in about 14% of cases. (19) Diagnostic yield for pathologies known to have patchy involvement also improves with larger amount of nerve tissue for examination. (4)
Apart from vasculitic neuropathy and neuropathy associated with amyloidosis and sarcoidosis, concomitant nerve-muscle biopsy can occasionally help to diagnose cholesterol embolism, intra-vascular lymphoma, mitochondrial disorders and storage diseases.(24)

With regards to appropriate nerve selection, identification of a focal lesion should ideally lead to a targeted biopsy of the lesion. Preference for the most clinically affected nerve needs to be balanced against the chance that severely affected nerves may have no residual nerve fibres to analyse.(25) Sural nerves are most often biopsied, being easily accessible pure sensory nerves that are usually affected in length-dependent neuropathies. The superficial branch of the radial nerve is preferred for upper limb dominant neuropathy. Biopsy of the superficial peroneal nerve may be preferable when vasculitic neuropathy is suspected as it is more likely to be pathologically involved and also allows for concomitant peroneus brevis biopsy through the same incision to improve yield.(13) Motor nerves are occasionally considered for biopsy as discussed above with the procedures having reasonable safety profiles.(26-28)

Proximal nerve biopsies can be useful in certain clinical contexts. For instance, a study of 112 patients who were selected to undergo targeted fascicular biopsy of the sciatic nerve resulted in an overall diagnostic yield of 84.8% with a wide range of diagnoses.(29) In this cohort, 4.5% developed permanent complications including persistent numbness in a peroneal division distribution on the biopsy side. Another study of 74 patients selected to undergo targeted brachial plexus biopsy reported an
overall diagnostic yield of 74.3% with a wide range of diagnoses. (30) In this study, worsening of numbness or weakness occurred in 4 and 3 patients respectively.

1.7 Complications of nerve biopsy

The frequency of complications is variable amongst the studies involving commonly biopsied nerves but in general, these include persistent numbness (72-100%), persistent pain (0-58%), wound infection (5-20%), delayed wound healing (1-12%), dysesthesia (11-60%), paraesthesia, haematoma and neuroma. (13, 15, 31) There is a suggestion that the presence of pre-existing hypoesthesia may correlate with reduced risk for post-operative pain, dysesthesia and paraesthesia. (32) Potential complications from reversing anticoagulation remain a further consideration. (13) Such complications and non-diagnostic biopsies account for some of the dissatisfaction expressed by patients in post-biopsy surveys. (16) In about 4% of biopsies performed, the tissue may contain blood vessel instead of nerve. For distal nerves, it is preferable to biopsy the whole nerve rather than fascicular biopsy (25) as the two approaches do not significantly differ in terms of post-operative pain or other complications. (16) It is currently recommended that 4-5 cm of nerve should be removed for sufficient diagnostic value, noting that the post-biopsy neurological deficit is independent of the length of the specimen. (5, 25)

1.8 Financial cost

Nerve biopsies are associated with significant costs. For instance, a German single centre study evaluated the cost-effectiveness of sural nerve biopsy in 80 patients and
determined that guideline-based laboratory workup, lumbar puncture and electrodiagnostic studies performed in all patients cost about 420 euros per patient. Sural nerve biopsy had diagnostic and therapeutic consequences in 36% and 23% of patients respectively and entailed a cost of 200 euros for surgery and 250 euros for neuropathology workup per patient. In Australia's government funded health system, basic examination of a nerve specimen with light microscopy costs AUD$274. Additional costs are associated with immunohistochemistry (AUD$90), electron microscopy (EM) (at least AUD$565) and specimen transport (~AUD$300). Further costs are associated with procedures performed in operation theatres and the management of potential complications. In contrast, nerve biopsy results that lead to change in management, especially where expensive therapy is consequently ceased or disability is reduced, will likely be cost saving.

1.9 Indications for nerve biopsy

A comprehensive literature review of the current indications for nerve biopsy is described in the following section. Vasculitic neuropathy and paraproteinaemic neuropathy will be separately covered in Chapters 3 and 4 respectively.

1.9.1 Chronic inflammatory demyelinating polyneuropathy

CIDP can be a diagnostic challenge despite the availability of published criteria given its phenotypic heterogeneity. In patients meeting the European Federation of Neurological Societies (EFNS)/ PNS 2010 clinical diagnostic criteria for CIDP, definitive diagnosis is reliant on demonstrating prominent features suggestive of
demyelination on neurophysiological testing of motor nerves. (36) Where less prominent demyelinating features only suggest probable or possible CIDP, supportive criteria based on CSF analysis, MRI of spine/plexus, sensory electrophysiology, response to immunomodulatory treatment and nerve biopsy can be used to allow definitive diagnosis. (36) In a retrospective analysis of 146 patients with definite CIDP based on the EFNS/PNS 2006 criteria, 25% of patients required supporting criteria for definite diagnosis. This included 12% of patients where nerve biopsy was the supportive criteria. (37) Many of these patients however, would likely satisfy the conditions for a diagnosis of definite CIDP based on the additional supportive features in the newer EFNS/PNS 2010 criteria even without a nerve biopsy. This suggests that using the present criteria, where information from other supportive tests can be easily accessed, nerve biopsy would be rarely required to obtain definite diagnosis for CIDP.

Nerve biopsy light microscopy findings typically seen in CIDP are neither sensitive nor specific. Demyelination and mononuclear cell infiltration are the main findings. Onion bulbs can be seen in chronic cases. Secondary axonal loss may also be seen, usually accompanied by clusters of regenerating fibres. (38, 39) There is some evidence that the pattern of endoneurial perivascular macrophage clusters and the extent of matrix metalloproteinase-9 immunoreactivity can differentiate inflammatory from non-inflammatory neuropathy. (40) Immunoglobulin and complement deposits may be seen in some patients. (41) According to histopathologic criteria for CIDP proposed by the American Academy of Neurology, unequivocal evidence of demyelination and remyelination needs to be seen with greater than five demyelinated fibres on EM or evidence of demyelination/ remyelination in at least 12% of 50 teased fibres containing a minimum of 4 internodes each. (42) Evidence
of macrophage-mediated demyelination is characteristic of inflammatory neuropathy (acute and chronic) but is best seen on EM. It has been suggested that very severe CIDP may be misdiagnosed as chronic idiopathic axonal neuropathy in some patients and that EM is useful in detecting the demyelinating lesions in these cases. (43)

The utility of nerve biopsy is mainly for atypical CIDP, to rule out other differentials and when there is non-response to treatment. (44) Research has been conducted to identify non-biopsy methods to achieve greater certainty in diagnosing CIDP. Neurophysiological testing of additional limbs/motor nerves significantly improves the diagnostic yield of definite CIDP. (45) Since the pathological changes in polyradiculoneuropathies (CIDP and Guillain Barre Syndrome) particularly involve nerve roots and plexuses, it is important to test these regions by employing proximal stimulation, F-waves and evoked potentials. Novel neurophysiological methods have been suggested to increase the detection of demyelinating findings, an example being the triple-stimulation test to evaluate for very proximal conduction blocks that cannot be detected with standard neurophysiological testing. (46)

Plexus MRI can support a diagnosis of CIDP and help to differentiate it from multifocal motor neuropathy (MMN) and multifocal acquired demyelinating sensory and motor (MADSAM) neuropathy. In a study of patients with atypical CIDP, plexus MRI was shown to suggest a diagnosis of CIDP in 44% of patients. (47) However, changes suggestive of CIDP on plexus MRI did not correlate with finding demyelinating features on distal nerve biopsy and 75% of patients with plexus MRI that did not suggest CIDP had demyelination-remyelination abnormalities on distal nerve biopsy. (47) When
comparing CIDP with MMN and MADSAM, symmetrical abnormalities on MRI brachial plexus strongly suggest CIDP whereas asymmetrical abnormalities suggest MMN/ MADSAM.(48) Nerve ultrasound can help to distinguish CIDP from MMN and MADSAM (the latter 2 being sonographically similar) and may help in diagnosing early onset CIDP.(49) It can also help to differentiate CIDP from Charcot-Marie-Tooth (CMT).(50) Immune-mediated neuropathies tend to have patchy nerve enlargement compared to diffuse enlargement in hereditary neuropathies(51) although diffuse enlargement can occur in patients with longstanding, untreated CIDP.(52)

A study of patients with presumed CIDP who were refractory to 3-4 weekly IVIG infusions, plasmapheresis and/or steroids found that 54% eventually had alternate diagnoses. Certain clinical and neurophysiological features favoured true CIDP over alternate diagnoses. Most true CIDP patients demonstrated response to some of the following strategies: Increase IVIG infusion frequency to bi-weekly; Addition of plasmapheresis / corticosteroids; Addition of cyclophosphamide with or without fludarabine.(53)

1.9.2 Cryoglobulinaemic neuropathy

Cryoglobulinaemic neuropathy is usually clinically diagnosed in patients with peripheral neuropathy and mixed cryoglobulinaemia. Nerve biopsy can help to confirm the diagnosis. There are no standardised or validated diagnostic criteria for cryoglobulinaemic vasculitis.(54) Nerve biopsy usually demonstrates predominantly large fibre axonal degeneration without regeneration and is commonly accompanied by vasculitis or features suggesting a vasculopathy.(55) Intravascular deposition of
cryoglobulins without vasculitis can be seen in the vasa nervorum. Features of
demyelination can be seen and EM may show myelin sheaths with vacuoles
containing amorphous material.(56) Where immunoglobulin deposits are seen in the
context of monoclonal cryoglobulinaemia, EM can identify if they are of similar
composition.(43)

1.9.3 Neuropathy related to haematological malignancy

Peripheral neuropathy may be the initial manifestation of haematological malignancy
and in that setting, requires nerve biopsy for definite diagnosis.(10)
Neurolymphomatosis refers to the neoplastic infiltration of the peripheral nervous
system by a haematological malignancy, usually non Hodgkin lymphoma and rarely
leukaemia.(10) It can be phenotypically heterogeneous.(57) Neurolymphomatosis can
be the initial presentation of a haematological malignancy (primary) or occur in the
context of a known haematological malignancy (secondary), both associated with a
poor prognosis.(10) Biopsy is not required for secondary neurolymphomatosis unless
there is diagnostic uncertainty. MRI and PET scans have estimated diagnostic yields
of 80% and 88% respectively for neurolymphomatosis but findings can be non-
specific.(10) Cerebrospinal fluid (CSF) cytology can confirm or support the diagnosis.
In one study where diagnostic uncertainty persisted, nerve biopsy (often guided by
imaging findings) was performed with an 88% sensitivity for neurolymphomatosis.(10)
The imperfect sensitivity of nerve biopsy owes to the usually patchy distribution of
malignant cells.(39) In some patients with haematological malignancies that are latent
or in remission, nerve biopsy may be the only way to link the malignancy with the
neuropathy.(34) Polymerase chain reaction (PCR) of the lymphoid infiltrate in nerve
biopsies can prove monoclonality and help to distinguish between malignant and inflammatory infiltrates. (58)

1.9.4 Nerve and nerve sheath tumours and pseudotumours

Nerve biopsy is typically required to diagnose tumours of peripheral nerve; which may be benign or malignant. The most common benign tumours are schwannoma and neurofibroma, with perineurioma and ganglioneuroma less frequently encountered. Diagnosis can usually be made by characteristic histological appearances together with judicial use of immunohistochemistry with stains such as S100, EMA (epithelial membrane antigen), GFAP (glial fibrillary acidic protein) and CD34 (cluster of differentiation 34). Malignant tumours may arise from these or arise de novo and about 25-70% of malignant peripheral nerve sheath tumours occur in patients with Neurofibromatosis type 1. (59)

MRI and ultrasound are helpful in the assessment of peripheral nerve tumours and pseudotumours and allow confident diagnoses in patients with typical presentations of conditions such as traumatic neuroma, Morton’s neuroma, nerve sheath ganglion and lipomatosis of nerves. (59)

Pseudoneoplastic lesions of the peripheral nerve (60) such as neuromuscular choristomas (NMC) and inflammatory pseudotumours are extremely rare entities which require nerve biopsy for definitive diagnosis and importantly, for the exclusion of malignancy. Inflammatory pseudotumours usually present as painful, progressive mononeuropathies with weakness and sensory loss. MRI findings tend to demonstrate heterogeneity in signal characteristics and contrast enhancement. Nerve biopsy
findings tend to show chronic inflammatory infiltrates, interstitial fibrosis, excess vascularity and increased lipocytes. The lesions have been demonstrated to be steroid-responsive in some patients. NMCs are characterised by the presence of well-differentiated muscle (usually skeletal) fibres admixed amongst mature nerve fascicles. While diagnostic, nerve biopsies are frequently complicated by subsequent aggressive fibromatosis. Given that characteristic findings on MRI have been shown to be very sensitive and specific in diagnosing NMCs, patients fitting the clinical picture with such findings can probably be diagnosed with NMC and followed up clinically and radiologically to ensure check for stability.

1.9.5 Sarcoid neuropathy

Amongst patients with sarcoidosis, 5-16% have neurological involvement, including 1% with neuropathy. Sarcoid neuropathy is phenotypically heterogenous and rarely can be the sole (or initial) presentation of sarcoidosis. Various diagnostic criteria have been proposed for neurosarcoidosis, with just one including peripheral nervous system involvement. In patients suspected to have neurosarcoidosis on the basis of clinical manifestations and findings on MRI, CSF and/or NCS, a definitive diagnosis of sarcoid neuropathy requires demonstration of non-caseating granulomas (NCG) on nerve biopsy which cannot be explained by other causes of granuloma formation, primarily tuberculosis and leprosy. Sarcoid granulomas are usually found in the epineurium and perineurium. Small fibre involvement can be demonstrated on skin biopsy with reduced intraepidermal nerve fibre density. A subset demonstrates evidence of concomitant vasculitis. If nerve biopsy is not appropriate or negative, diagnosis of probable neurosarcoidosis
can be made using biopsy evidence of extraneural sarcoidosis accompanied by typical findings on MRI, CSF and/or neurophysiological testing.(63, 65) However, studies have found concomitant central nervous system involvement in sarcoid neuropathy to be uncommon (0-21%)(64, 68). One study(64) diagnosed probable sarcoid neuropathy in the presence of extraneural NCG accompanied by symptoms/ signs of limb neuropathy “judged to be related in time to histologic or radiologic evidence of active sarcoidosis”. Concomitant muscle biopsy is likely to improve the yield for sarcoid neuropathy, an additional benefit being that the presence of granulomas in muscle rules out tuberculoid leprosy which is muscle-sparing.(24) Nerve ultrasound and MRI neurography in sarcoid neuropathy patients may demonstrate higher cross sectional areas in certain nerves. Ultrasound findings have not been shown to correlate with electrophysiological findings or degree of disability.(69) MRI of the brachial or lumbosacral plexus can occasionally demonstrate nerve enlargement and enhancement of roots, plexus and nerves.(64)

1.9.6 Amyloid neuropathy

In amyloid neuropathy, the deposited amyloid fibrils are mainly composed of light chains (AL) or mutant transthyretin (ATTR) with other causes being rare.(70) In patients with pathogenic TTR gene mutation, definitive diagnosis requires at least two amyloidosis related symptoms and presence of amyloid deposits (See Figure 1) in the biopsied tissue.(7) While biopsy evidence may be sought from any involved organ, abdominal fat biopsy is less invasive. Non-cardiac biopsies are less sensitive in ATTR amyloidosis compared to AL amyloidosis.(71) Genetic testing can detect the specific TTR mutation. Sural nerve biopsy is about 80% sensitive for detecting TTR amyloid.(7)
and can establish amyloidosis as the cause for neuropathy. Concomitant muscle biopsy may increase the yield of amyloid deposits.(24, 72) Liver transplantation and therapies like Tafamidis, Patisaran and Inotersen have been shown to reduce disease progression hence the importance of early (even pre-symptomatic) diagnosis to preserve functional status.(7, 73, 74) However, abnormalities on sural nerve biopsy may be a late manifestation. Phenotypic heterogeneity frequently leads to misdiagnosis (up to 32%) and delayed diagnosis. CIDP is the most common misdiagnosis (about 20%) with up to 37% of patients having clinical findings meeting diagnostic criteria for CIDP.(75)

When AL amyloidosis is suspected, serum and urine immunofixation as well as serum free light chain assay is first undertaken with negative findings making the diagnosis unlikely. Definitive diagnosis requires histopathological demonstration of amyloid with further tests to confirm that the amyloid is light-chain related.(76) Abdominal fat pad biopsy has a sensitivity of about 80% and exceeds 90% if combined with bone marrow testing. Nerve biopsy can be considered if both biopsies are negative, but the index of suspicion remains high. The sensitivity of nerve biopsy is 30-100%.(77) Rectal and renal biopsy can also be used to find amyloid deposits.(70) Serial sections should be examined as the deposits tend to be focal. The typical pattern is one of severe nerve fibre loss, especially the small myelinated or unmyelinated fibres. Deposits of amyloid stained with Congo Red or Haematoxylin and Eosin stains may be found within epineurial and endoneurial connective tissue. The epineurial and endoneurial blood vessels may be thickened. EM may detect deposits that cannot be seen with light microscopy and confirm the characteristic periodicity of amyloid fibrils.(25)
Testing amyloid protein composition helps to guide management. For example, patients with presumed AL amyloidosis may instead have ATTR amyloidosis with coincidental monoclonal gammopathy on testing. Diagnosing AL amyloidosis is important as several treatment options are available including stem cell transplant and systemic chemotherapy.(78)

Novel diagnostic modalities are being evaluated to facilitate earlier diagnosis of amyloid neuropathy. MRI neurography may be able to identify peripheral nerve lesions in asymptomatic TTR mutation carriers. In the absence of monoclonal gammopathy, technetium-labelled cardiac scintigraphy can reliably diagnose cardiac ATTR amyloidosis with a positive predictive value of 100%. Other modalities include skin biopsy to evaluate for amyloid deposits(4) and evidence of small fibre neuropathy, feet electrochemical skin conductance to measure early dysautonomia and periumbilical ultrasound for amyloid deposits.(75)
Figure 1.1. Light microscopy of a sural nerve biopsy from a 60-year-old man with amyloid neuropathy that presented as a progressive painful neuropathy with prominent autonomic features. Congo red staining demonstrates deposits of amyloid in the endoneurium.
1.9.7 Pure neuritic leprosy

The cardinal symptom of leprosy is sensory loss, typically reflecting intracutaneous nerve damage.(79) Skin biopsy or skin scrapings therefore remain the cornerstone of diagnosis. Pure neuritic leprosy accounts for 4-8% of leprosy cases with nerve biopsy being the gold standard for diagnosis. Definite diagnosis requires the presence of acid fast lepra bacilli (See Figure 2) usually within foam cells and Schwann cells.(8) The presence of these bacilli is primarily a feature of lepromatous leprosy; they are rarely present in patients with tuberculoid leprosy which is characterised instead by the presence of epithelioid granulomas (sometimes with caseation).(8, 80) The sensitivity of nerve biopsy for leprosy can be increased using quantitative PCR (qPCR) testing for Mycobacterium leprae deoxyribonucleic acid (DNA). In patients with pure neuritic leprosy (no skin lesions and negative slit skin smear bacilloscopy), it is important to perform qPCR for Mycobacterium leprae DNA on slit skin smears and/ or skin biopsies as this increases the sensitivity for diagnosing leprosy and avoids nerve biopsy.(79) Fine needle aspiration cytology of the nerve can also demonstrate the lepra bacilli and epithelioid granulomas and has been shown to have comparable yields to nerve biopsy, while being less invasive.
Figure 1.2. Light microscopy of a sural nerve from a 49-year-old man leprosy neuropathy presenting clinically with painless leg ulcers, several enlarged nerves and a clinical picture of mononeuritis multiplex. Ziehl-Neelsen staining was positive for acid fast bacilli.
1.9.8 IgG4 related peripheral neural involvement

IgG4 related disease of a tissue is diagnosed when it is infiltrated by IgG4-positive plasma cells with accompanying elevated serum IgG4 levels. In the rare cases of peripheral nervous system involvement, IgG4 related perineural disease usually occurs in the ocular and paravertebral regions and is mostly asymptomatic. It appears as well-circumscribed soft-tissue perineural masses on MRI that responds to steroids. (81)

This condition is distinguished from paranodopathies which are mediated by autoantibodies (predominantly IgG4) against paranodal antigens such as neurofascin 155 and contactin-1 (41) and are not characterised by IgG4 plasma cell infiltration. (82)

The context of raised serum IgG4 levels and the presence of IgG4 related disease in other organs suggests the diagnosis but definitive diagnosis requires the presence of IgG4-positive plasma cell infiltrates on nerve biopsy. (81) Four cases (83-86) of peripheral neuropathy attributed to IgG4 related disease have been reported. The presentations were variable with mononeuritis multiplex seen in two cases. In one case, (83) sural nerve biopsy showed IgG4 positive plasma cells infiltrating the epineurium and moderate loss of myelinated nerve fibres. In the second case, (84) features of axonal degeneration were seen with epineurial infiltration of lymphocytes and eosinophils. Although not demonstrated on sural nerve biopsy, IgG4 plasma cell infiltration was seen on inguinal lymph node biopsy. In the third case, (85) the neuropathy was preceded by pleural effusion. Sural nerve biopsy was normal with the IgG4 plasma cell infiltration demonstrated on pleural biopsy instead. Symptoms rapidly
improved with oral Prednisolone in these three cases. The fourth case was steroid resistant. Sural nerve biopsy found “obstructive thromboangiitis with severe loss of myelin and axons” with IgG4 plasma cell infiltration diagnosed on bone marrow biopsy.

1.9.9 Storage disorders

Lysosomal and peroxisomal disorders are uncommonly of adult onset and can be associated with neuropathy. In most of these conditions, enzyme activity assays, genetic tests or biochemical tests are sufficient to make the diagnosis. Nerve biopsies for ultrastructural examination may be indicated with atypical presentations or if suspicion for the disease remains high despite the usual tests being non-diagnostic. Accumulation of intralysosomal material may be found in neurons or Schwann cells forming characteristic structures such as Zebra or Tuff stone bodies or prismatic inclusions in Metachromatic Leukodystrophies and related Sphingolipidoses. In Refsum’s disease, marked onion bulb formation may occur.

1.9.10 Hereditary neuropathies

Nerve biopsy is not required for most patients with hereditary neuropathies. However, in some patients, the initial genetic testing for the most prevalent mutations is negative or next generation sequencing (NGS) finds one or more variants of uncertain significance. In these patients, certain characteristic findings (Se Figure 3) on nerve biopsy features can suggest particular hereditary neuropathies which can lead to targeted genetic testing or indicate the pathogenic nature of variants found on NGS. Imaging findings may assist in differential diagnosis. In a study of CMT
patients, MRI showed bilateral brachial and lumbosacral plexus hypertrophy and diffuse symmetrical enlargement of peripheral nerves. The mid-thigh cross-sectional area of sciatic nerves was demonstrably larger compared to CIDP patients. Nerve ultrasound may assist in differentiating between axonal and demyelinating CMT.

Figure 1.3. Light microscopy of a sural nerve biopsy stained with Toluidine blue from a 13-year-old female with PMP-22 mutation who had experienced weakness and sensory loss from early life. At age 12, patient developed progressive severe weakness and bulbar symptoms. Well-formed onion bulbs and severe homogenous demyelination are demonstrated.
1.9.11 Adult polyglucosan body disease

Adult polyglucosan body disease is an extremely rare late onset illness that is often misdiagnosed initially. (90) Common clinical manifestations include neurogenic bladder, gait disturbance, distal lower limb sensory loss and mild cognitive impairment. Diagnosis is usually obtained with genetic testing and if equivocal, assay of glycogen branching enzyme (GBE) activity in skin fibroblasts or muscle is recommended. Sural nerve biopsy to demonstrate the polyglucosan bodies is recommended if the GBE activity assay is also equivocal. Polyglucosan bodies have also been discovered in other tissues. Diagnosis allows for prognostication, genetic counselling and potential enrolment into clinical trials. (91)

1.9.12 Pure motor neuropathies

In some patients with sporadic, recent onset lower motor neuron syndrome, differentiating motor neuropathy from progressive muscular atrophy can be difficult. Motor nerve biopsy can assist with making the distinction (28) and has therapeutic implications. Presence of upper motor neuron involvement can also be demonstrated with threshold tracking transcranial magnetic stimulation. (92) Peripheral nerve imaging using high resolution ultrasound and MRI can also be useful in discriminating between MMN and amyotrophic lateral sclerosis (ALS). (93) One study found that MMN patients tended to have larger peripheral nerve cross sectional area compared to ALS patients and used this finding to distinguish between MMN and ALS with a sensitivity of 87.5% and specificity of 94.1%. (49)
1.9.13 Other neuropathies

Nerve biopsy findings have been characterised for various other peripheral neuropathies, however none that rely on nerve biopsy for diagnosis.(94-96) Where neuropathy is suspected to be linked to diabetes and other metabolic disorders, the clinical utility of nerve biopsies is limited to excluding differentials that would usually require nerve biopsy.(97) Characteristic findings can be seen in toxic neuropathy secondary to medications like amiodarone and chloroquine as well as some industrial agents.(39) In the setting of occupational toxic exposure, the role of nerve biopsy is limited to clarifying ongoing diagnostic uncertainty despite extensive testing and assisting with prognostication.(98) While rarely indicated for this purpose, nerve biopsy can be used to diagnose cytomegalovirus induced neuropathy in immunosuppressed patients where other investigations have been inconclusive.(4) Patients with human immunodeficiency virus infection can rarely develop diffuse infiltrative lymphocytosis syndrome (DILS) which is characterised by CD8 T lymphocytosis and CD8 T cell infiltration into multiple organs, including nerves. Nerve biopsy is not required for diagnosing DILS but can suggest the diagnosis in the rare situation where peripheral nerve involvement is the sole or initial manifestation. DILS usually improves with anti-retroviral therapy.(99)

Finally, in patients with an idiopathic distal symmetric polyneuropathy, a previous review was unable to identify any articles that could be used to inform recommendations on the role of nerve biopsy in these patients.(100) In most patients with a chronic idiopathic sensory axonal neuropathy, sural nerve biopsies offer no benefit.(101) In the absence of clinical features suggestive of a treatable cause, nerve
biopsies may be considered in severe or progressive neuropathies to evaluate for potentially treatable aetiologies.\(^{(102)}\)

Nerve biopsy has also been useful in the research setting to investigate the pathological effect of novel autoantibodies in inflammatory neuropathies\(^{(103)}\) and to identify markers of pathogenicity or disease activity.\(^{(38)}\)
CHAPTER 2

METHODOLOGY
2.1 Patient selection

The total patient cohort comprised of all patients who had been referred for nerve biopsy between 1 January 2014 and 31 December 2016. Referrals originated from public and private facilities throughout New South Wales and the Australian Capital Territory. Biopsy specimens from autopsies or patients under the age of 15 were excluded from analysis. Specimens that could not be interpreted due to extensive degradation by artefact, insufficient specimen size or the lack of nerve in the specimen were excluded. The final patient cohort comprised of 350 patients.

2.2 Data collection and classification

For all patients forming the patient cohort, relevant clinical data was extracted from available clinical records. An exhaustive search was undertaken of all potential sources of information including the referral letter, consultation letters from all relevant clinicians, hospital medical records, pathology and imaging services.

Pre-biopsy assessment included patient demographics, clinical assessment and the results of all serological tests, imaging and electrophysiological investigations requested by the referring clinician as well as other treating clinicians for the diagnostic workup of the neuropathy. The results of biopsies from other tissues were also noted.

Biopsy-related parameters included referral, indications and subsequent pathological findings. Referral included the location from which the referral was made (postal code) and the specialty of the referring clinician. The provisional diagnosis and indications
for biopsy for each patient were determined from their medical records. Multiple provisional diagnoses or indications for biopsy could be documented for an individual patient.

Patient clinical records from the post-biopsy period were obtained till the end of June 2019 or until the patient was last reviewed, whichever was earlier. Post-biopsy information collected included the eventual diagnosis as well as the nature and outcome of any changes in management. The results of any investigations performed for the ongoing diagnostic workup or monitoring of the neuropathy were also noted. Any new potentially disease-modifying therapy trialled subsequent to biopsy constituted a change in management. Clinical status subsequent to biopsy was categorised as either improved, stable or progressive based on the documentation by the patient’s clinicians.

Presenting symptoms were categorised into pure sensory, pure motor and sensorimotor. Presence of pain, stepwise progression, asymmetric or multifocal symptoms, upper-limb dominance and length-dependence of symptoms was noted. Progression was deemed stepwise in the presence of acute, multifocal attacks separated by time. Comorbidities and family history were recorded. Duration of symptoms prior to initial presentation was categorised as acute (<1 month), subacute (1-3 months) and chronic (>3 months). Exposure to neurotoxins was documented. Determining whether a drug was neurotoxic was based on its inclusion in the well-maintained database of toxic neuropathies by the Neuromuscular Disease Centre at Washington University.(104)
All relevant abnormalities on blood and cerebrospinal fluid (CSF) analysis were incorporated into the database. For the purpose of analysis, an anti-nuclear antibody (ANA) titre of 1:160 or above was considered clinically relevant. Raised inflammatory markers referred to an elevation of C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) which could not be explained by other factors such as intercurrent infection. Elevated CRP was defined as clinically relevant when exceeding 10mg/L. Analysis for CRP level exceeding 50mg/L was performed separately. Where a pre-morbid ESR was available for comparison, an increase in ESR of at least 25% was considered clinically relevant. Otherwise, ESR above an age and sex-adjusted cut-off calculated as Age/2 for males or (Age plus 10)/2 for females was also considered significant. For assessment of degree of paraproteinaemia, the highest recorded concentration of paraprotein pre-biopsy was used.

Data from nerve conduction studies (NCS) was re-analysed and classified using published normative data, laboratory values and classification criteria.(36, 105-107) If abnormal, neuropathy was determined to be primarily axonal, primarily demyelinating or mixed pattern in addition to phenotypic classification as pure sensory, pure motor or mixed sensorimotor. The category of mixed axonal and demyelinating neuropathy on NCS included patients with both axonal and demyelinating features on NCS but where the demyelinating features only met the ‘probable’ or ‘possible’ electrodiagnostic criteria for CIDP.(36) In patients with NCS performed in both upper and lower limbs, findings were also determined as generalised or dominant in either upper or lower limbs. In patients with bilateral NCS performed, the presence of asymmetry was documented using published criteria.(108) Electromyography (EMG) findings were categorised as normal, isolated spontaneous activity, chronic
neurogenic, acute on chronic neurogenic or myopathic based on the EMG data. When performed, findings from neuroimaging studies and biopsies from other tissues were incorporated into the diagnostic workup.

2.3 Processing and analysis of biopsies

All nerve biopsies were processed at the state reference laboratory for peripheral nerve and muscle histopathology at the Brain and Mind Centre which is affiliated with the University of Sydney and the Royal Prince Alfred Hospital and houses an archive of 2000 nerve biopsies over 40 years. The nerve was divided into three to five sections, each about 5mm in length. One piece was fixed in picric acid, embedded in paraffin and sectioned transversely and longitudinally in 5 micrometre sections which were then stained with haematoxylin and eosin. Another piece was fixed in formalin and stained for 24 hours in 1% osmium tetroxide, macerated in glycerol and then teased apart under a dissecting microscope to separate individual nerve fibres. One piece was fixed in cold 2.5% glutaraldehyde in 0.1M cacodylate buffer for 3 hours or overnight followed by 2% osmium tetroxide for 2-5 hours. This tissue was then dehydrated in graded concentrations of ethanol, embedded in Spurr’s resin and cut transversely in 0.25-1.0 micrometre sections that were stained with toluidine blue. Another portion of the nerve was snap-frozen in iso-Pentane cooled liquid nitrogen and from this portion of the nerve 8 micrometre cryostat sections were cut for haematoxylin and eosin, Congo Red and direct immunofluorescence staining for immunoglobulins, complement and fibrinogen. Using paraffin, frozen and epoxy preparations as well as teased fibre analysis, nerve pathology was broadly determined as normal, axonal, demyelinating or mixed pattern by the reporting neuropathologist.
Teased fibre preparation was performed in all patients. Active axonal degeneration was deemed prominent if present in >15% of teased fibres.

2.4 Statistics

Statistical analyses were performed in SPSS Statistics for Windows Version 24.0 (IBM, Armonk, NY) using appropriate methods (T Test and either the χ2 or Fisher’s exact test). As the pre-biopsy workup was not standardized, there was variation in the nature and extent of investigations. For each parameter, the proportion of patients in each category was calculated using the number of patients for whom information about the parameter was available as the denominator.
CHAPTER 3

VASCULITIC NEUROPATHY
SUMMARY

Nerve biopsies have suboptimal sensitivity for the diagnosis of vasculitic neuropathy and are associated with a risk for complications. A study was undertaken to identify positive and negative pre-biopsy clinical predictors of a histopathological diagnosis of peripheral nerve vasculitis, aiming to identify a patient population in which the likelihood of vasculitis was low enough to obviate the need for nerve biopsy.

Clinical, laboratory, and neurophysiological parameters were analysed for consecutive patients referred for nerve biopsy with suspected vasculitis. Patients were assigned pathological categories of definite, probable, possible, or absent vasculitis using validated guidelines. Patients with definite or probable vasculitis were considered to have pathologically confirmed vasculitis. Vasculitis was considered pathologically unlikely in the remaining patients.

From a cohort of 138 patients, biopsy confirmed vasculitis in 27.5%. Strong discriminators between pathologically proven and pathologically unlikely vasculitis include the presence of a stepwise progression (34.2% vs 5.1%; p<0.001), the presence of both sensory and motor features (76.3% vs 49.0%; p<0.005), an asymmetric or multifocal presentation (73.7% vs 41.2%; p=0.001), and in the case of systemic vasculitis, the presence of myeloperoxidase antibodies (47.1% vs 5.1%; p<0.001), rheumatoid factor (57.9% vs 13.0%; p<0.001), cryoglobulinaemia (53.8% vs 8.3%; p<0.005) and elevated inflammatory markers (76.5% vs 27.1%; p<0.001). Patients with chronic, symmetric symptoms were less likely to have pathologically confirmed vasculitis (15.8% vs 42.2%; p<0.005). Pathologically unlikely vasculitis was
most frequently noted in patients with pure motor (85.0%) or pure sensory (84.6%) symptoms and in patients with findings on nerve conduction studies that were normal (90.0%) or predominantly demyelinating (85.7%). The findings from this study were combined with those in the existing literature to develop a decision aid for clinicians considering a referral for nerve biopsy for their patients with suspected peripheral nerve vasculitis.

3.1 Introduction

Vasculitic neuropathy is an inflammatory process that damages the vasa nervorum, affecting the associated axon with ischaemic sequelae.(109) The presentation ranges from acute to chronic.(5) Vasculitic neuropathy occurring as part of a primary systemic vasculitis or considered to be secondary to systemic autoimmune conditions, infections, malignancy and some drugs is termed systemic vasculitic neuropathy. Less common is non-systemic vasculitic neuropathy (NSVN) where vasculitis is isolated to the peripheral nerves.(108) Vasculitic neuropathy will hereon be referred to as vasculitis.

Definitive diagnosis requires histopathological confirmation and suspected vasculitis is the most frequent indication for nerve biopsy.(110) From a histological perspective, diagnosis of definite vasculitis requires inflammation within the vessel wall and associated vascular damage.(See Figure 3.1) In the absence of such findings, the presence of supportive histological features may contribute to a diagnosis of probable or possible vasculitis(6) and are detailed in the consensus diagnostic criteria developed by the Peripheral Nerve Society (PNS). Nerve biopsy is optional for patients
with clinicopathologically proven systemic vasculitis who develop a neuropathy phenotype typical for vasculitis. In contrast, nerve biopsy remains essential to the diagnosis of non-systemic vasculitic neuropathy (NSVN).

As no independent reference standard for vasculitic neuropathy exists, the sensitivity of nerve biopsy for vasculitic neuropathy can only be estimated; around 50%. Even for NSVN (where biopsy is essential to diagnosis), estimated sensitivity remains at 50% with sural nerve biopsy or combined superficial peroneal nerve and peroneus brevis muscle biopsy. A meta-analysis of patients with clinically suspected vasculitic neuropathy found that combined nerve and muscle biopsy increased the diagnostic yield by 5.1%, although this may be useful mainly when combined superficial peroneal nerve/peroneus brevis biopsy is undertaken rather than sural nerve/vastus lateralis. The PNS guidelines for vasculitic neuropathy recommend that concomitant muscle biopsy be undertaken if it can be obtained with the involved nerve through a single incision.

Given the suboptimal sensitivity of nerve biopsy and considering the issues surrounding cost and complications of biopsy as described above, a study was undertaken to identify positive and negative predictors of pathologically definite and probable vasculitis, aiming to identify a patient population in which the likelihood of vasculitis was low enough to obviate the need for nerve biopsy.
Figure 3.1. Light microscopy of a sural nerve biopsy stained with Haematoxylin and eosin specimen from a 15-year-old male with a history of right foot drop. Intense vascular and perivascular inflammation of an epineurial blood vessel is seen accompanied by fibrinoid necrosis. The patient was diagnosed with vasculitic neuropathy.
3.2 Methods

The cohort for this study comprised of all patients who were suspected to have vasculitic neuropathy prior to nerve biopsy. Patients with vasculitis listed as a provisional diagnosis or as the indication for biopsy were classified as clinically suspected vasculitis.

Pathological features from the PNS guidelines for definite, probable or possible vasculitis were used to categorise patients. Patients with either pathologically definite or pathologically probable vasculitis were combined to form a group referred to henceforth as pathologically confirmed vasculitis. Patients with either pathologically possible or pathologically absent vasculitis were combined to form a group representing patients in whom vasculitis was unlikely. Parameters were compared between the groups to identify predictors of pathologically confirmed vasculitis.

3.3 Results

From a total cohort of 350 patients, 138 (39.4%) were clinically suspected to have vasculitis, making this the most common indication for referral for nerve biopsy. The study was restricted to these 138 patients. Sural nerve was biopsied in 94.2% of patients; the remaining patients had biopsy of the superficial radial (2.9%), superficial peroneal (2.2%) or femoral cutaneous nerves (0.7%). Involvement of biopsied nerves was suspected based on clinical presentation. Concomitant muscle biopsy was performed in 26.8% of patients, with vastus lateralis the most frequently sampled (40.5%). Other frequently sampled muscles included peroneus longus (16.2%), deltoid
(16.2%), unspecified quadriceps muscle (13.5%) and tibialis anterior (10.8%). Evidence of vasculitis was seen in 5.4% (2 patients) of all muscle biopsies, with both patients also having evidence of pathologically confirmed vasculitis on nerve biopsy.

Based on histopathology, patients were categorised into definite (8.7%; 12 patients), probable (18.8%; 26 patients), possible (10.9%; 15 patients) or absent (61.6%; 85 patients) vasculitis. Therefore, vasculitis was pathologically confirmed in 27.5% of patients in whom vasculitis was clinically suspected prior to biopsy.

3.3.1 Demographics

Patients with pathologically confirmed vasculitis had a mean age of 62.0 years and 55.3% were female with neither parameter being significantly different in patients without vasculitis. Patients with pathologically confirmed vasculitis had higher odds of being referred by clinicians linked to immunological presentations (See Table 3.1), particularly rheumatologists, immunologists and haematologists compared to patients without pathologically confirmed vasculitis. In terms of referral bias, 92.0% of referrals were from large metropolitan specialist centres compared to 8.0% rural referrals.

3.3.2 Clinical Presentation

The initial pattern of symptoms was broad, including those patients with subsequent pathologically confirmed vasculitis. Patients who were subsequently diagnosed with pathologically confirmed vasculitis more frequently reported asymmetric and
sensorimotor symptoms that were of acute onset with features of a stepwise progression (See Table 3.1).

Pure sensory symptoms and signs were reported in 28.3% of the cohort. Vasculitis was pathologically confirmed in 15.4% of these patients, 50.0% of whom had evidence of motor involvement on NCS. Pure motor symptoms and signs were reported in 14.7% of the cohort. Vasculitis was pathologically confirmed in 15.0% of these patients, with evidence of sensory involvement on NCS in all patients in whom NCS was performed.

Upper limb dominant symptoms were present in 4.5% of the cohort with subsequent pathological confirmation of vasculitis noted in only 1 patient, from a superficial radial nerve biopsy. This patient initially presented with upper limb involvement but subsequently developed lower limb involvement after several months with NCS demonstrating mononeuritis multiplex affecting both upper and lower limbs. Of the remaining patients with upper limb dominant symptoms, 60.0% had a sural nerve biopsy due to evidence of lower limb involvement on either NCS or physical examination. The remaining 40.0% had a superficial radial nerve biopsy. The length-dependence of symptoms (25.0% vs 29.9%) and the presence of pain (73.7% vs 56.1%) did not distinguish pathologically confirmed vasculitis from those with other histopathological features although in the case of pain, a trend towards significance was noted (p=0.06). Chronic, symmetric phenotypes were less frequent across patients with pathologically confirmed vasculitis (See Table 3.1) than the rest of the cohort. Amongst patients presenting with a chronic, symmetric neuropathy, 75.0%
were subsequently found to have pathologically absent vasculitis while just 13.6% had subsequent pathologically confirmed vasculitis.

<table>
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<th>Parameter (%)</th>
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<th>Pathologically Unlikely (n=100)</th>
<th>p-value</th>
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<td>Sensory AND motor symptoms</td>
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<td>&lt;0.005</td>
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**Table 3.1.** Comparison of clinical features between the pathologically confirmed and pathologically unlikely vasculitis cohorts. Patients with pathologically confirmed vasculitis had a higher prevalence of symptoms that involved both sensory and motor modalities, were asymmetric or multifocal, and had features of a stepwise progression. Patients with pathologically confirmed vasculitis had a higher prevalence of positive p-ANCA (anti-neutrophil cytoplasmic antibodies), MPO (myeloperoxidase) antibodies, rheumatoid factor, cryoglobulinaemia and elevated ESR (erythrocyte sedimentation rate) or CRP (C-reactive protein). Pure sensory symptoms and a combination of symptoms that were both chronic (> 3 months) and symmetric phenotype were less prevalent in patients with pathologically confirmed vasculitis.
There was no significant association between the presence of systemic disease and pathologically confirmed vasculitis. History of a non-haematological malignancy (excluding basal and squamous cell skin cancer) was documented in 8.0% of the cohort with vasculitis subsequently found to be pathologically unlikely in 90.9% of patients with this history. However, the prevalence of a history of non-haematological malignancy was not increased amongst patients in whom vasculitis was pathologically unlikely (10.0% vs 2.6%; p=0.24). Hepatitis C was found in 5.8% of the cohort, 37.5% of whom had cryoglobulinaemia. Although not statistically correlated with pathologically confirmed vasculitis, vasculitis was eventually clinically diagnosed in 25.0% of Hepatitis C positive patients, 100.0% of whom had cryoglobulinaemia. There was no significant association with prescription medications or any neurotoxin exposure.

3.3.3 Pre-biopsy serological investigations

In patients with pathologically confirmed vasculitis, compared to the remaining cohort, there was a higher frequency of p-anti neutrophil cytoplasmic antibody (p-ANCA) positivity, myeloperoxidase (MPO) antibodies, rheumatoid factor (RF), cryoglobulinaemia and elevated ESR or CRP (See Table 3.1). Vasculitis was subsequently pathologically confirmed in 66.7% of MPO positive patients, 66.7% of RF positive patients, 70.0% of cryoglobulin positive patients and 41.0% of patients with elevated ESR or CRP. MPO antibodies, RF, cryoglobulins and ESR or CRP were tested in 80.4%, 55.1%, 41.3% and 85.5% respectively of patients. MPO positivity and p-ANCA positivity were analysed separately as 50.0% of the p-ANCA positive patients were MPO negative, with 2 (18.2%) of these patients subsequently diagnosed with
pathologically confirmed vasculitis. ANCA was tested in 80.4% of the cohort with 8 patients (21.6%) positive for c-ANCA, 4 of whom were proteinase 3 positive and 2 were MPO positive. Vasculitis was subsequently pathologically confirmed in 3 of the c-ANCA positive (or 2 of the proteinase-3 positive) patients. ANA and paraprotein were tested in 89.9% and 86.2% of the cohort respectively. The presence of ANA titre at or above 1:160 (27.8% vs 27.3%) and the presence of paraprotein (12.1% vs 16.3%) did not correlate with pathologically confirmed vasculitis.

3.3.4 Electrodiagnostic assessment

Routine NCS were performed in 94.2% of patients. They were reported as normal in 7.7% of patients, all except one of whom were subsequently confirmed not to have vasculitis. However, this patient presented with proximal lower limb symptoms and EMG demonstrated evidence of acute on chronic denervation with an eventual diagnosis of radiculoplexopathy. Across the cohort, pure sensory involvement was uncommon (10.3%) and pure motor involvement was rarely present (4.8%). Patients with pathologically confirmed vasculitis frequently had sensorimotor abnormalities (77.8%), pure axonal neuropathy (73.5%) and asymmetric or multifocal features (60.0%) on NCS although they were not significantly more frequent compared to patients with pathologically unlikely vasculitis. NCS exhibited primary demyelination in 11.3% of the cohort, 85.7% of whom had pathologically unlikely vasculitis. Of patients with pathologically confirmed vasculitis, just 5.9% had predominantly demyelinating findings on NCS, 50.0% of whom met the criteria due to the presence of conduction blocks. EMG assessment was performed in 52.2% of patients, 69.4% of whom had
fibrillation potentials and positive sharp waves with no correlation found with pathologically confirmed vasculitis.

3.3.5 Cerebrospinal fluid analysis and imaging investigations

Lumbar puncture was performed in 47.8% of patients. Of these patients, 54.5% had an elevated CSF protein (>45mg/dL) while 13.6% had CSF pleocytosis (>5 leukocytes/µL) ranging from 6 to 180 leukocytes/µL (median 17). There was no statistical correlation of either the prevalence of elevated CSF protein (41.2% vs 63.3%; p=0.11) or mean CSF protein (48 vs 57mg/dL; p=0.33) with pathologically confirmed vasculitis. A CSF protein exceeding 110mg/dL was only found in 3.0% of patients, none of whom had pathologically confirmed vasculitis. Only 22.2% of the patients with CSF pleocytosis had vasculitis on biopsy, with the degree of pleocytosis being mild (range 10 - 17 leukocytes/µL).

Brain MRI was performed in 36.2% of all patients. From this group, 4.0% had MRI changes involving the brain which, though non-specific, were documented to be consistent with vasculitis. Of these, 1 patient with a known diagnosis of microscopic polyangiitis had MRI findings of widespread periventricular and deep white matter changes without any focal diffusion restriction. Another patient had multifocal lesions, some of which exhibited restricted diffusion. Both patients subsequently had pathological confirmation of vasculitis. Plexus MRI was infrequently performed (5.8% of the cohort), however evidence of thickened nerve roots was demonstrated in 25.0% of these patients had with none subsequently diagnosed with pathologically confirmed vasculitis. Peripheral nerve MRI imaging was performed in 8.7% of the cohort. From
this group, 41.7% had demonstration of nerve inflammation or muscle denervation, 80.0% of whom subsequently had pathological confirmation of vasculitis. Of the patients with demonstrable nerve inflammation or muscle denervation on MRI, 80.0% had evidence of nerve enlargement or inflammation in the symptomatic limbs, and the remaining 20.0% had evidence of early denervation of an affected muscle.

3.3.6 Subgroup analysis

Amongst patients who had both pathological confirmation of vasculitis and eventual clinical diagnosis of vasculitis, 36.7% met the PNS criteria for NSVN. Comparisons involving the subgroups of patients with pathologically confirmed systemic vasculitis or NSVN was limited by the small number of patients within each subgroup. Patients with NSVN were on average 14.7 years younger than patients with pathologically confirmed systemic vasculitis (See Table 3.2). Selected autoantibodies and raised inflammatory markers were more prevalent in patients with pathologically confirmed systemic vasculitis compared to NSVN (See Table 3.2). Compared to patients with pathologically unlikely vasculitis, patients with pathologically confirmed systemic vasculitis more frequently had symptoms that were of acute onset, asymmetric, progressed in a stepwise manner with both sensory and involvement, and they had a higher prevalence of selected autoantibodies and raised inflammatory markers (See Table 3.3). Compared to patients with pathologically unlikely vasculitis, patients with pathologically confirmed NSVN were on average 9.3 years younger. They were more likely to have asymmetrical, sensorimotor symptoms with stepwise progression and less likely to have chronic, symmetric symptoms (See Table 3.4).
Table 3.2. Comparison of clinical features between patients with pathologically confirmed systemic vasculitis and pathologically confirmed non-systemic vasculitic neuropathy (NSVN). Amongst patients with pathologically confirmed vasculitis and an eventual clinical diagnosis of vasculitic neuropathy, those with systemic vasculitis had a higher mean age, an increased prevalence of autoimmune or inflammatory comorbidities and an increased prevalence of p-ANCA (anti-neutrophil cytoplasmic antibody) positivity, MPO (myeloperoxidase) positivity, rheumatoid factor positivity and elevated ESR (erythrocyte sedimentation rate) or CRP (C-reactive protein).

<table>
<thead>
<tr>
<th>Parameter (%)</th>
<th>Systemic Vasculitis (n=19)</th>
<th>NSVN (n=11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>68.4</td>
<td>53.7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Comorbidity: Autoimmune or inflammatory</td>
<td>36.8</td>
<td>0.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>p-ANCA positivity</td>
<td>58.8</td>
<td>9.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Anti-MPO positivity</td>
<td>47.1</td>
<td>0.0</td>
<td>=0.01</td>
</tr>
<tr>
<td>Rheumatoid factor positivity</td>
<td>57.9</td>
<td>12.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Elevated ESR or CRP</td>
<td>76.5</td>
<td>20.0</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The clinical presentation was suggestive of plexopathy in 9.4% of patients with suspected vasculitis with insufficient numbers to conduct subgroup analysis. In this subgroup, 23.1% had pathologically confirmed vasculitis and an eventual clinical diagnosis of vasculitis was made in 84.6%, CIDP in 7.7% and paraneoplastic plexopathy in 7.7%. Amongst those clinically diagnosed with vasculitis, 27.3% had systemic vasculitis, 18.2% had NSVN, 27.3% had diabetic lumbosacral radiculoplexus neuropathy (LSRPN) and 27.3% had non-diabetic LSRPN. Amongst all patients presenting clinically as a plexopathy, the onset was either acute or subacute in 84.6% while 76.9% had a painful, asymmetric neuropathy. Type 2 diabetes was seen in 30.8%, while 7.7% had Type 1 diabetes and 7.7% had post-pancreatectomy diabetes.
CSF was tested in 61.5% of with a clinical presentation suggestive of plexopathy with an elevated CSF protein found in 87.5%.

<table>
<thead>
<tr>
<th>Parameter (%)</th>
<th>Systemic Vasculitis (n=19)</th>
<th>Pathologically Unlikely (n=100)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms onset &lt;1 month</td>
<td>52.6</td>
<td>25.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Symptoms onset &gt;3 months</td>
<td>36.8</td>
<td>65.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sensory AND motor symptoms</td>
<td>73.7</td>
<td>49.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Asymmetric or multifocal symptoms</td>
<td>73.7</td>
<td>41.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Symmetric AND &gt;3 months</td>
<td>10.5</td>
<td>42.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Stepwise progression</td>
<td>42.1</td>
<td>5.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-Neurology referral</td>
<td>36.8</td>
<td>7.0</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>p-ANCA positivity</td>
<td>58.8</td>
<td>14.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-MPO positivity</td>
<td>47.1</td>
<td>5.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rheumatoid factor positivity</td>
<td>57.9</td>
<td>13.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Elevated ESR or CRP</td>
<td>76.5</td>
<td>27.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cryoglobulinaemia</td>
<td>53.8</td>
<td>8.3</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

**Table 3.3.** Comparison of clinical features between patients with pathologically confirmed systemic vasculitis and those in whom vasculitis was pathologically unlikely. Patients with pathologically confirmed vasculitis had a higher prevalence of symptoms that were acute, involved both sensory and motor modalities, were asymmetric or multifocal, and had features of a stepwise progression. Patients with pathologically confirmed vasculitis had a higher prevalence of positive p-ANCA (anti-neutrophil cytoplasmic antibodies), MPO (myeloperoxidase) antibodies, rheumatoid factor, cryoglobulinaemia and elevated ESR (erythrocyte sedimentation rate) or CRP (C-reactive protein).
### Table 3.4

<table>
<thead>
<tr>
<th>Parameter (%)</th>
<th>NSVN (n=11)</th>
<th>Pathologically Unlikely (n=100)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>53.7</td>
<td>63.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sensory AND Motor symptoms</td>
<td>90.9</td>
<td>49.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Stepwise progression</td>
<td>54.5</td>
<td>5.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Asymmetric or multifocal symptoms</td>
<td>90.9</td>
<td>41.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Symmetric AND &gt;3 months</td>
<td>9.1</td>
<td>42.2</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 3.4. Comparison of clinical features between patients with pathologically confirmed NSVN (non-systemic vasculitic neuropathy) and those in whom vasculitis was pathologically unlikely. Patients with pathologically confirmed NSVN had a higher prevalence of symptoms that involved both sensory and motor modalities, were asymmetric or multifocal, and had features of a stepwise progression. Patients with pathologically confirmed NSVN had a lower mean age and had a lower prevalence of phenotype characterised by the presence of both chronic (> 3 months) and symmetric symptoms.

### 3.4 Discussion

The present study has identified several clinical parameters that influence the pathological findings on nerve biopsy in patients who are suspected to have vasculitis. The parameters that best differentiated pathologically confirmed vasculitis from other conditions were a stepwise progression, the presence of both sensory and motor features, an asymmetric or multifocal presentation, and the absence of a chronic, symmetric presentation. In the case of systemic vasculitic neuropathy, additional parameters that best differentiated this diagnosis from other conditions were the presence of anti-MPO, RF, cryoglobulins and elevated inflammatory markers. In terms of functional assessment, sensorimotor abnormalities on NCS and the presence of a pure axonal neuropathy were most consistent with pathologically confirmed vasculitis.
In contrast, a diagnostic exclusion of vasculitis was highest in patients with pure motor or pure sensory presentations as well as patients with NCS that were normal or predominantly demyelinating.

Characteristic features of nerve vasculitis include pain, combined with asymmetric and multifocal nature of symptoms. When combined with an acute, subacute or relapsing presentation, vasculitis yield on biopsy becomes more likely.(6) Conversely, the presence of a chronic, symmetric phenotype would argue against the presence of vasculitis as demonstrated in our study.(108) Upper limb predominance is also uncommon in nerve vasculitis; it is was seen in 2.7% of patients with pathologically confirmed vasculitis which compares with 5% described in the literature.(108)

Vasculitis is not considered in the differential for pure lower motor neurone syndromes.(92) Although uncommon, vasculitis can present with pure sensory involvement. In the present study, 15.8% of patients with pathologically confirmed vasculitis had a pure sensory clinical phenotype though additional motor involvement on NCS was present in 50.0% of this group. Sjogren’s syndrome related neuropathy can present with pure sensory symptoms.(112) In the present cohort, 2.9% of the cohort had Sjogren’s syndrome related neuropathy with none having a pure sensory phenotype. A clinical diagnosis of vasculitis was eventually made in 75.0% of these patients, 66.7% of whom had pathologically confirmed vasculitis. Although pure sensory involvement is reportedly uncommon in patients with Sjogren’s related vasculitis,(112) the study of patient cohorts who undergo nerve and/ or muscle biopsy confers a selection bias that potentially skews inclusion towards patients with motor involvement.
In terms of other parameters, the mean age of 62.0 years in patients with pathologically confirmed vasculitis in the present series is similar to the mean age reported for patients with NSVN.\(^{(108)}\) Separately, it is unclear why patients with NSVN were overall younger than the rest of the cohort, and especially when compared to patients with systemic vasculitis. This difference in age between systemic vasculitis and NSVN has not been previously observed, apart from one study comparing NSVN to microscopic polyangiitis-associated neuropathy.\(^{(6)}\)

The association between Hepatitis C and vasculitis has been increasingly recognised. This is primarily mediated by cryoglobulins which are highly prevalent (up to 50%) in Hepatitis C positive patients.\(^{(113)}\) However, Hepatitis C positive patients have been shown to develop vasculitis even in the absence of cryoglobulinaemia with a neuropathy phenotype that is more acute and severe compared to Hepatitis C patients with cryoglobulinaemia.\(^{(113)}\)

Non-haematological malignancies were primarily present amongst patients in whom vasculitis was pathologically unlikely may be explained in part by paraneoplastic neuropathy. While none of the patients received an eventual clinical diagnosis of paraneoplastic neuropathy, it is known that paraneoplastic antibodies can be absent in this condition.\(^{(114)}\) One patient with a history of endometrial cancer was suspected to have dermatomyositis on a concomitant muscle biopsy; this potential association has been previously reported.\(^{(115)}\)

Amongst pathologically confirmed vasculitis patients, apart from neurologists, most referrals came from rheumatologists and immunologists. Such patients are clearly
more likely to have autoimmune and systemic inflammatory conditions to explain the correlation of vasculitis with referral networks. A strong correlation of autoantibodies including anti-MPO and RF with vasculitis has been previously identified.\(^{(6)}\)

In this study, 33.3\% (4 patients) of the anti-MPO antibody positive patients did not have pathologically confirmed vasculitis. In one patient with known sarcoidosis and suspected microscopic polyangiitis, this may be attributed to the imperfect sensitivity of nerve biopsy for vasculitis with biopsy showing evidence of perivascular inflammation and some active axonal degeneration but PNS criteria for definite or probable criteria were not met. The second patient had no symptoms or signs of neuropathy and had a normal NCS with myopathic features on EMG. Concomitant muscle biopsy in this patient was suggestive of myositis. In the third patient, nerve biopsy showed evidence of mild active axonal neuropathy however the biopsy contained a very short segment of nerve with significant fixation artefact. Nevertheless, this patient was diagnosed with eosinophilic granulomatosis with polyangiitis supported by findings on a concomitant muscle biopsy. The final patient had a biopsy consistent with diabetic neuropathy but had known diagnoses of Type 1 diabetes, pernicious anaemia and autoimmune thyroiditis. It is possible that the positive MPO-ANCA in this patient may reflect an underlying tendency towards autoimmunity.\(^{(116)}\)

The presence of CSF pleocytosis or CSF protein above 110mg/dL is rare in patients with peripheral nerve vasculitis, their estimated prevalence according to the literature is 4\% and 2\% respectively.\(^{(108)}\) The results of the present study have demonstrated similar findings. Patients with CSF protein above 110mg/dL would be expected to have
alternative pathologies such as chronic inflammatory demyelinating polyneuropathy.(41)

In terms of electrodiagnostic approaches, NCS are rarely normal in patients with vasculitis(117) as further confirmed by the present series. Furthermore, primary demyelination is rare in vasculitis, although such cases have been reported in the literature.(118, 119) Separately, when the observed demyelinating feature is a conduction block, it is important to perform serial studies. Disappearance of the conduction block would support the presence of a pseudo-conduction which is purported to be due to failure of focal axonal conduction.(120)

In patients with a non-diagnostic nerve biopsy, clinically probable vasculitis can be diagnosed in those with clinical or electrodiagnostic evidence of neuropathy, with a clinical profile typical for patients with vasculitis. Such a clinical profile is illustrated by the Brighton Collaboration case definition for peripheral nerve vasculitis.(121) For comparison, the Brighton criteria (See Table 3.5) was applied to patients in our series who had pathologically diagnosed definite, probable or possible vasculitis and sufficient information to apply the criteria. In the absence of nerve biopsy, only 33.3%, 32.0% and 20.0% of patients with definite, probable or possible vasculitis respectively, met the Brighton criteria for clinically probable vasculitis.
I. Evidence of peripheral neuropathy
   a. Electrodiagnostic evidence of an axonal neuropathy (symmetric or asymmetric)
      OR
   b. Clinical examination signs of peripheral neuropathy
      AND

II. Clinical presentation typical for neuropathy due to vasculitis
   a. Sensory-motor or sensory (not pure motor)
      AND
   b. Multifocal or asymmetric pattern at any time, AND this is not attributable to compression or entrapment of peripheral nerves or roots
      AND
   c. Lower limb predominant
      AND
   d. Painful
      AND
   e. One or more acute attacks (onset to maximum severity within 1 month followed by spontaneous stabilisation), or variable speed of progression, or improvement of motor or sensory deficit

Table 3.5. Criteria for a clinical diagnosis of vasculitis in the absence of nerve biopsy. Adapted from Hadden et al, 2017.(121)

The results of this study have been used to create a flowchart to assist clinicians when considering referring their patients for nerve biopsy on suspicion of vasculitis (See Figure 3.5). Patients with likely diabetic radiculoplexus neuropathy (diabetic amyotrophy) were assigned a separate category as they characteristically have a self-
limited course. The category of patients in whom there was a high suspicion of vasculitis consisted of patients who met the Level 3 criteria for vasculitis as outlined in Hadden et al, 2017.(121) All other patients in whom vasculitis was suspected were subdivided into categories based on the presence of positive or negative predictors of vasculitis. Positive and negative predictors of vasculitis were determined based on the literature(108) and influenced by the results of the present study.

This study has a number of limitations by design. First, in order to identify patients suspected to have vasculitis from the overall cohort, the patient’s medical records had to include vasculitis as either a provisional diagnosis or an indication for biopsy. It is possible that some patients were referred for biopsy on suspicion for vasculitis where this possibility had not been documented. Separately, the final cohort of 138 patients was referred by 83 different physicians with variable experience with vasculitis, so that each physician’s threshold for suspecting vasculitis would likely be different. Patients did not have a uniform diagnostic workup prior to biopsy. The lack of a uniform protocol for NCS and EMG resulted in further variation in the extent of neurophysiological testing and accordingly there would be variation in how the neurophysiological data was interpreted. For example, the presence of asymmetry on NCS could not be ascertained in 28.2% of patients due to a lack of bilateral testing. Decisions relating to the extent of NCS or EMG testing were likely influenced by the clinical presentation and therefore, the prevalence of findings like asymmetry on NCS or fibrillation potentials and positive sharp waves on EMG were likely higher than the true value. Likewise, the 46.2% of patients who had CSF testing were likely to have characteristics that differed from patients without CSF testing. The true mean CSF protein would likely be lower than calculated. Nevertheless, for all parameters, there
was no significant difference in the proportion of patients affected by this lack of information between patients with pathologically confirmed and pathologically unlikely vasculitis.

Another limitation relates to a type of NSVN patients who have prominent proximal involvement, termed radiculoplexus neuropathy (diabetic and non-diabetic). Compared to typical NSVN patients, these patients more frequently exhibit proximal limb weakness, weight loss and raised CSF protein and less frequently have necrotizing vasculitis on biopsy.(108) Microvasculitis, when associated with vascular damage, is typically seen in the diabetic radiculoplexus neuropathies.(108) However, insufficient subgroup size precluded analysis of either the radiculoplexus neuropathy (6 patients) or microvasculitis (1 patient) subgroups.

Biopsy of non-nerve tissue can strengthen the evidence for vasculitis, particularly when there is evidence of systemic vasculitis.(121) In one small study of patients with biopsy-proven peripheral nerve vasculitis or clinically probable vasculitis, cutaneous vasculitis was identified in some patients without nerve or muscle biopsy evidence of vasculitis, suggesting that concomitant full thickness skin biopsy may increase diagnostic yield.(122) Another small study of patients with sural nerve biopsy-proven NSVN found that quantification of perivascular macrophages on skin punch biopsies had a sensitivity of 94% and specificity of 79% for NSVN using a cut-off of 2.9 macrophages per vessel.(123) In a study of patients with peripheral neuropathies of various aetiologies, quantifying scattered macrophages in skin biopsy using a cut-off of 13 per mm² could differentiate vasculitic neuropathy from controls and other axonal neuropathies with a sensitivity of 71% and specificity of 79%.(124) In patients with
vasculitic neuropathy, a significant correlation between cutaneous and sural nerve perivascular inflammation was observed. Together, these studies suggest that concomitant full thickness skin biopsy may increase diagnostic yield for vasculitic neuropathy and indeed, the presence of perivascular mononuclear inflammation in skin biopsies taken concurrently with nerve biopsies feature in the Brighton Collaboration’s diagnostic criteria for histopathologically probable vasculitic neuropathy.

In terms of further approaches that may increase diagnostic yield, nerve ultrasound may provide supportive evidence for vasculitis. Patients with vasculitis tend to demonstrate focal nerve enlargement on ultrasound, less pronounced than changes observed in demyelinating neuropathies. By contrast, nerve enlargement is unusual in non-immune mediated axonal neuropathies. There is incomplete overlap between findings on nerve ultrasound and clinical and neurophysiological findings. A small study comparing patients with vasculitis to patients with non-inflammatory axonal neuropathy found that sonographic enlargement of arm nerves proximal to sites of nerve compression accurately identified patients with vasculitis with a sensitivity of 94% and specificity of 88%. Separately, ultrasound may also assist with identifying the most appropriate nerve to biopsy and possibly improve diagnostic yield. This is especially useful when the suspected vasculitic process predominantly involves motor nerves and is sural-sparing. Greater diagnostic value is expected with biopsy of the involved motor nerve and the risk can be mitigated by using ultrasound to guide a fascicular biopsy.
Figure 3.5: Flowchart to assist clinical decision-making when referring patients for nerve biopsy on suspicion of vasculitis.

*Comprising patients meeting Level 3 criteria for a diagnosis of vasculitis in the absence of nerve biopsy (See Table 3.5 adapted from Hadden et al, 2017)[121]

Strong positive predictors: Known primary vasculitis; stepwise clinical progression; presence of anti-myeloperoxidase antibodies, rheumatoid factor or cryoglobulins.

Moderate positive predictors: Presence of systemic diseases known to be associated with vasculitides; neuropathic pain; asymmetric or multifocal symptoms (clinically or electro-diagnostically) that cannot be attributed to an obvious alternate cause such as nerve or root entrapment; acute onset or rapid progression of symptoms within a month; erythrocyte sedimentation rate exceeding 50 mm/hour; C-reactive protein exceeding 50 mg/L.

Negative predictors: Normal nerve conduction studies; persistently demyelinating nerve conduction studies (excluding pseudo-conduction block); pure motor involvement (both clinically and electro-diagnostically); upper limb dominant presentation; cerebrospinal fluid pleocytosis; cerebrospinal fluid protein exceeding 110mg/dL.

NCS, nerve conduction studies; EMG, electromyogram; CSF, cerebrospinal fluid
In conclusion, stepwise progression, the presence of both sensory and motor features, an asymmetric or multifocal presentation, and in the case of systemic vasculitis, the presence of selected autoantibodies and elevated inflammatory markers are strong discriminators between pathologically proven and pathologically unlikely vasculitis. Chronic, symmetric symptoms, pure motor or pure sensory presentations, and NCS findings that are normal or predominantly demyelinating are good predictors that a diagnosis of vasculitis is unlikely. Nevertheless, if vasculitis is strongly suspected, nerve biopsy should be considered even in the presence of these negative predictors.
CHAPTER 4

PARAPROTEINAEMIC NEUROPATHY
SUMMARY

Paraproteins may be detected in some patients with a peripheral neuropathy. In the absence of an alternative explanation for the neuropathy, a paraproteinaemic neuropathy may be suspected. Many paraproteins are coincidental and proving their pathogenicity is difficult. Nerve biopsies can support this diagnosis but are non-contributory in many patients and entail risk. Two studies were performed. The first study aimed to identify parameters that influence the yield of nerve biopsy in diagnosing paraproteinaemic neuropathy. The second study aimed to evaluate the impact of nerve biopsy on management and clinical outcomes.

Clinical, laboratory and neurophysiological parameters were analysed for consecutive patients referred for nerve biopsy over 36 months. Biopsies reported to be supportive for paraproteinaemic neuropathy based on histopathological findings were identified. The first study included patients referred for suspected paraproteinaemic neuropathy and compared pre-biopsy parameters between patients in whom the biopsy report suggested this diagnosis and other patients. From a cohort of 22 patients, 45.5% had biopsy reports suggestive of a paraproteinaemic neuropathy. Of these patients, 90.0% had an IgM paraprotein while 10.0% had an IgG paraprotein (p<0.05). Of the remaining patients with a paraproteinaemia, 30.0% had an IgM paraprotein while 70.0% had an IgG paraprotein (p<0.05). Positive anti-nuclear antibody (ANA) with titre of at least 1:160 was found in 0.0% of patients in whom biopsy was suggestive of paraproteinaemic neuropathy compared to 55.6% of other patients (p<0.05). No statistically significant differences were found when comparing other pre-biopsy parameters between the 2 groups.
The second study included patients with an eventual clinical diagnosis of paraproteinaemic neuropathy and compared post-biopsy management changes and clinical outcomes between patients in whom the biopsy report suggested this diagnosis and other patients. From a cohort of 20 patients, 70.0% had a nerve biopsy suggesting a diagnosis of paraproteinaemic neuropathy. Change in management occurred in 71.4% of patients with supportive biopsies and 83.3% of other patients. After a median follow-up of 31 months, clinical stability was noted in 85.7% of patients with supportive nerve biopsies and 100.0% of other patients. These differences were not statistically significant.

This chapter has established that the presence of an IgM paraprotein is the best predictor of the presence of pathological findings on nerve biopsy that confirm the suspicion of a paraproteinaemic neuropathy. Conversely, nerve biopsies are unlikely to confirm a suspicion of paraproteinaemic neuropathy in patients with an IgG paraprotein. In patients with an eventual clinical diagnosis of paraproteinaemic neuropathy, management decisions and clinical stabilisation appear to be independent of the nerve biopsy result.

4.1 Introduction

4.1.1 Is the paraprotein causing the neuropathy?

Paraproteins are present in up to 3-5% of patients with peripheral neuropathy although likely coincidental in many patients, especially the elderly. (127) Although no specific tests can distinguish between coincidental and disease-causing paraproteins, the use
of indirect immunofluorescence, applying patient sera to normal nerve, may show binding to nerve components such as myelin, particularly in the case of IgM paraproteinaemia with anti-myelin associated glycoprotein (MAG) antibodies.

The issue of determining whether a paraprotein is causally linked to a patient’s peripheral neuropathy was explored within the EFNS/PNS guidelines in 2010. The guidelines describe that a causal relationship is highly probable in patients with a typical phenotype (IgM monoclonal gammopathy of unknown significance (MGUS) or Waldenström’s) who have either a high titre of IgM antibodies to MAG or ganglioside Q1b (GQ1b) or have nerve biopsy findings of IgM (See Figure 4.1) or complement deposits on myelin, or widely spaced myelin (See Figure 4.2) is found on electron microscopy. The guidelines also state that a causal relationship is probable in patients with high titres of IgM antibodies (IgM MGUS or Waldenström’s) directed towards other neural antigens (e.g. GM1, GD1a, GD1b, GM2, sulfatide etc.) and a slowly progressive predominantly distal symmetrical sensory neuropathy, or if the patient has IgG/ IgA paraproteins with nerve biopsy showing widely spaced myelin or IgG or IgA and/ or complement bound to myelin.

Neuropathy in the context of other paraprotein-related disorders including Waldenström’s macroglobulinaemia, POEMS (polyneuropathy, organomegaly, endocrinopathy, M-protein and skin changes) and CANOMAD (chronic ataxic neuropathy, ophthalmoplegia, monoclonal IgM protein, cold agglutinins and disialosyl antibodies) syndrome do not require nerve biopsy for diagnosis. Recently published diagnostic criteria for POEMS do not include nerve biopsy. It remains uncertain whether the M protein or cytokines such as vascular endothelial growth
factor are pathogenic, however treatment is indicated regardless. (127) When nerve biopsy is done, axonal degeneration and segmental demyelination can be seen with scant (if any) inflammation. (5) Characteristic but non-specific uncompacted myelin (43) can be seen on EM; this may help differentiate POEMS from other demyelinating peripheral neuropathies.

Figure 4.1. Light microscopy of sural nerve from 70-year-old man with a generalised sensory neuropathy accompanied by tremor in his hands. Nerve conduction studies were consistent with a DADS (Distal acquired demyelinating symmetric) phenotype and serum electrophoresis was positive for an IgM kappa paraprotein. Direct immunofluorescence demonstrating IgM bound to myelin which supports the pathogenic nature of the paraprotein.
Figure 4.2. Electron microscopy of a sural nerve from a patient with IgM paraproteinaemia. Widening of the myelin outer lamellae (arrows) is a characteristic finding of paraproteinaemic neuropathy.
4.1.2 Does nerve biopsy change management?

Establishing a link between the paraprotein and neuropathy can influence patient management. In atypical presentations that are treatment resistant or in progressive disabling neuropathy, clinicians may prefer greater diagnostic certainty prior to using higher-potency treatments that are associated with greater risks. In the presence of a paraprotein, nerve biopsy may be useful when a diagnosis of vasculitic neuropathy, amyloid neuropathy or malignant lymphoproliferative nerve infiltration is being considered;(77) specific treatments exist for these conditions. If the patient has multiple myeloma, they may require systemic therapy regardless of the severity of neuropathy, thus biopsy may not change management.(127)

Appropriate patient selection has the potential to improve diagnostic accuracy while reducing the frequency of diagnostically unhelpful biopsies. This chapter has two aims. The first is to understand the clinical parameters that influence the diagnostic accuracy of nerve biopsy when a paraproteinaemic neuropathy is suspected. The second aim is to evaluate the impact of nerve biopsy on management decisions and clinical outcome in patients with a clinical diagnosis of paraproteinaemic neuropathy.

4.2 Methods

A diagnosis of paraproteinaemic neuropathy was considered to be pathologically probable in patients suspected to have a paraproteinaemic neuropathy who had nerve biopsies that tested positive for deposition of IgM, IgG, IgA or complement on myelin or in whom electron microscopy confirmed widened myelin lamellae.(128) In the
absence of such findings, patients with nerve biopsies that demonstrated features considered by the neuropathologist to be suggestive of paraproteinaemic neuropathy were considered to have pathologically possible paraproteinaemic neuropathy. Patients with either pathologically probable or possible paraproteinaemic neuropathy were combined into a group which will henceforth be termed pathologically consistent paraproteinaemic neuropathy.

In Chapter 4.3, the cohort included all patients in whom paraproteinaemic neuropathy was suspected prior to nerve biopsy. The cohort was divided into 2 groups, those with pathologically consistent paraproteinaemic neuropathy and those other findings on nerve biopsy. Parameters were compared between the groups to identify predictors of pathologically consistent paraproteinaemic neuropathy.

In Chapter 4.4, the cohort included all patients in whom an eventual clinical diagnosis of paraproteinaemic neuropathy was made. A clinical profile was created for the subset of patients in whom the nerve biopsy result was pathologically consistent with paraproteinaemic neuropathy. Two parameters were compared between patients with pathologically consistent paraproteinaemic neuropathy and patients with other findings on nerve biopsy. These were the proportion of patients in whom there was a change in management and the proportion of patients who did not have clinical progression when last followed up.
4.3 **Results: Clinical predictors of paraproteinaemic neuropathy on nerve biopsy**

Paraproteinaemic neuropathy was documented to be either the provisional diagnosis or included in the differential diagnosis in 22 patients. The following results are an analysis of these 22 patients. Pathologically probable paraproteinaemic neuropathy was noted in 22.7% of these patients. All had known IgM MGUS with nerve biopsies demonstrating a demyelinating neuropathy with positive immunofluorescence for IgM. Of these, in 20.0%, the nerve biopsy demonstrated widened myelin lamellae. A further 22.7% of the cohort had nerve biopsies that represented pathologically possible paraproteinaemic neuropathy due to the presence of focal myelin swellings and irregular myelin sheath thickness. Therefore, pathologically consistent paraproteinaemic neuropathy was diagnosed in 45.5% of patients with suspected paraproteinaemic neuropathy.

4.3.1 **Clinical presentation**

The average age of patients suspected to have paraproteinaemic neuropathy was 71.0 years. When comparing between patients with pathologically consistent paraproteinaemic neuropathy and other patients, there was no difference in age (70.2 years vs 71.8 years) but there was a trend towards greater prevalence of a male sex (80.0% vs 41.7%; p=0.10).

There was no difference in the prevalence of symptoms that were pure sensory (70.0% vs 58.3%), sensorimotor (30.0% vs 41.7%), painful (50.0% vs 58.3%), asymmetric
(20.0% vs 33.3%), distal lower limb dominant (60.0% vs 25.0%), upper limb dominant (0.0% vs 16.7%), acute (0.0% vs 25.0%), subacute (10.0% vs 0.0%) or chronic (90.0% vs 75.0%) between patients with pathologically consistent paraproteinaemic neuropathy and other patients.

Most comorbidities were represented in only 1 or 2 of the 22 patients in this study. Analysis was performed on comorbidities found in at least 3 patients. There was no difference between patients with pathologically consistent paraproteinaemic neuropathy and other patients in terms of the prevalence of solid malignancies (30.0% vs 16.7%), prevalence of prior spinal surgeries (0.0% vs 25.0%), type 2 diabetes (10.0% vs 16.7%) or hypothyroidism (10.0% vs 16.7%). Prevalence of exposure to known neurotoxins did not differ between patients with pathologically consistent paraproteinaemic neuropathy and other patients (40.0% vs 41.7%).

4.2.2 Pre-biopsy serological investigations

There was variation in the type of blood tests performed on a patient prior to biopsy. Apart from routine full blood count and serum chemistry testing, patients were tested for autoimmune serology including ANA (81.8% of patients), ANCA (77.3% of patients) and RF (50.0% of patients). A significant number of patients were also tested for ESR and/or CRP (77.3% of patients) and Cryoglobulin (36.4% of patients). All patients were tested for the presence of a paraprotein. Statistical analysis of the results of a test was undertaken when the test was performed in at least 50% of patients.
Comparing patients with pathologically consistent paraproteinaemic neuropathy with other patients, there was no difference in the prevalence of ANCA positivity (12.5% vs 33.3%), elevated RF (33.3% vs 12.5%) or the prevalence of an elevated ESR or CRP (25.0% vs 11.1%). A positive ANA, however, was found in 0.0% of patients with pathologically consistent paraproteinaemic neuropathy compared to 55.6% of other patients (p=0.03).

Serum paraprotein was detected in 90.9% of patients. For the remaining 2 patients, it is unclear why the clinician considered a differential of paraproteinaemic neuropathy, but it was noted that 1 patient had positive GM1 IgG and the other patient had elevated free kappa light chains. Of those with a detectable paraprotein, 55.0% were IgM and the remaining were IgG. An IgM paraprotein prior to biopsy was present in 90.0% of patients with pathologically consistent paraproteinaemic neuropathy compared to 30.0% of other patients (p=0.02). Likewise, an IgG paraprotein was rarely seen in patients with pathologically consistent paraproteinaemic neuropathy compared to other patients (10.0% vs 70.0%; p=0.02). In terms of light chains, 70.0% were kappa and the remaining were lambda. Prevalence of kappa light chains was not significantly different between patients with pathologically consistent paraproteinaemic neuropathy compared to others (80.0% vs 60.0%). An IgG lambda paraprotein was noted in 15.0% of patients, none of whom had pathologically consistent paraproteinaemic neuropathy. Antibodies against MAG were tested in only 31.8% of the cohort. Of these patients, MAG was positive in 28.6% with all patients subsequently diagnosed with pathologically consistent paraproteinaemic neuropathy. Anti-ganglioside antibodies were tested in 50.0% of the patients. Of these, anti-ganglioside antibodies were
positive in 18.2%, none of whom had pathologically consistent paraproteinaemic neuropathy.

In some patients, the concentration of the paraprotein was too low to be accurately quantified and termed ‘trace’ in the pathology reports. The median paraprotein level just prior to nerve biopsy was 2.35g/L in patients with pathologically consistent paraproteinaemic neuropathy compared to a median of 4.70g/L amongst other patients. The proportion of patients with a paraprotein level <5.0g/L was 90% in patients with pathologically consistent paraproteinaemic neuropathy compared to 50% in other patients (p=0.14).

4.3.3 Electrodiagnostic assessment

A predominantly demyelinating nerve conduction study was seen in 60.0% of patients with pathologically consistent paraproteinaemic neuropathy compared to 25.0% of other patients, however this was not statistically significant (p=0.37). A predominantly axonal neuropathy was seen in 10.0% of patients with pathologically consistent paraproteinaemic neuropathy compared to 41.7% of other patients (p=0.16). Most patients had both sensory and motor involvement on nerve conduction studies and this feature did not distinguish pathologically consistent paraproteinaemic neuropathy (100.0%) from other patients (83.3%).

In terms of distribution of neuropathy on nerve conduction studies, there was no difference between pathologically consistent paraproteinaemic neuropathy and other patients when the neuropathy was generalised (50.0% vs 54.5%), upper limb
dominant neuropathy (0.0% vs 18.2%) or lower limb dominant neuropathy (50.0% vs 27.3%). Bilateral testing was performed in 81.8% of patients. In these patients, the presence of asymmetry did not distinguish between pathologically consistent paraproteinaemic neuropathy (50.0%) and other patients (40.0%).

EMG was only performed in 28.6% of the cohort which precluded statistical analysis. It was noted that active denervation was noted in 33.3% of these patients, none of whom had pathologically consistent paraproteinaemic neuropathy.

4.3.4 Cerebrospinal fluid analysis

Only 45.5% had CSF testing. Amongst the 40.0% of these patients subsequently found to have pathologically consistent paraproteinaemic neuropathy, CSF Protein ranged from 0.34g/L to 0.93g/L (median 0.35g/L). The remaining patients had CSF protein ranging from 0.27g/L to 1.23g/L (median 0.74g/L). Elevation of CSF protein above 0.45g/L was seen in 25% of patients with pathologically consistent paraproteinaemic neuropathy compared to 83.3% of other patients. There were no other relevant findings amongst the other CSF tests done.

4.3.5 Imaging investigations

In terms of imaging, 31.8% of the cohort had documentation of an MRI of the brain with the only abnormality being the presence of extensive small vessel ischaemia in 57.1% of these patients. An MRI of the spine (cervical, thoracolumbar, lumbar or whole spine) was documented in 63.6% of the cohort. Apart from degenerative changes of
varying severity seen in 78.6% of these patients, there were no other diagnostic findings. CT of the chest, abdomen and pelvis as a screen for malignancy was performed in 31.8% of the cohort, with negative findings for malignancy in all patients.

A bone marrow biopsy was undertaken prior to nerve biopsy in 36.4% of the cohort. Bone marrow biopsy was consistent with a diagnosis of Waldenström’s macroglobulinaemia in 25.0% of these patients, 50.0% of whom were subsequently diagnosed with pathologically consistent paraproteinaemic neuropathy.

4.3.6 Pre-biopsy management

There was no dramatic benefit amongst the patients who tried IVIG prior to biopsy, 40.9% of the cohort. Plasma exchange was trialled for 3 to 6 months in just 9.1% of the patients prior to biopsy with no effect seen in these patients.

4.3.7 Neuropathological features

Sural nerves were biopsied in 95.2% of patient with the remaining patients having a radial nerve biopsy. Amongst patients with pathologically consistent paraproteinaemic neuropathy, the nerve biopsy had predominantly demyelinating features in 66.7% and mixed axonal and demyelinating features in 33.3%. Amongst the other patients, the nerve biopsy features were predominantly axonal in 58.3%, predominantly demyelinating in 16.7% and mixed axonal and demyelinating in 25.0%. Additional electron microscopy was performed in 23.8%, 20.0% of which demonstrated widened myelin lamellae.
4.3.8 Post-biopsy diagnosis and management

In terms of implications of nerve biopsy, it was noted that all patients in whom biopsy showed pathologically consistent paraproteinaemic neuropathy (45.5% of the cohort) received a final clinical diagnosis of paraproteinaemic neuropathy. There was a change in management in 70.0% of these patients. (See Table 4.1 for details)

Amongst the patients in whom the nerve biopsy did not show pathologically consistent paraproteinaemic neuropathy, the nerve biopsy contributed to an important diagnosis and consequently changed management in 25.0%. These included diagnoses of vasculitic neuropathy and AL (light-chain) Amyloidosis (see Tables 4.2 and 4.3; Patients 1, 9 and 11).
<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre-biopsy management</th>
<th>Post-biopsy management</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Supportive care</td>
<td>IVIG started</td>
</tr>
<tr>
<td>2</td>
<td>Supportive care</td>
<td>IVIG started but ceased after 40 months as ineffective. PLEX effective but ceased due to line sepsis. PLEX re-commenced after 14 months using peripheral line.</td>
</tr>
<tr>
<td>3</td>
<td>IVIG initially effective but benefit waned so trialled PLEX for 3 months to no effect</td>
<td>Rituximab given</td>
</tr>
<tr>
<td>4</td>
<td>IVIG to good effect</td>
<td>IVIG continued</td>
</tr>
<tr>
<td>5</td>
<td>Supportive care</td>
<td>IVIG started</td>
</tr>
<tr>
<td>6</td>
<td>Trialled 3 months of R-CP to no effect, off treatment for 10 months leading up to biopsy.</td>
<td>Rituximab given 5 months post-biopsy. IVIG started 8 months post-biopsy and dose escalated.</td>
</tr>
<tr>
<td>7</td>
<td>Supportive care</td>
<td>Supportive care</td>
</tr>
<tr>
<td>8</td>
<td>Supportive care</td>
<td>IVIG started; dose escalated to 3-weekly after 6 months. Weaned to monthly after further 16 months.</td>
</tr>
<tr>
<td>9</td>
<td>Monthly IVIG for 4 months was ineffective. Commenced Azathioprine and Prednisone 3 months prior to biopsy.</td>
<td>Continued IVIG, Azathioprine and Prednisone 5mg daily.</td>
</tr>
<tr>
<td>10</td>
<td>Failed 9-month trial of IVIG, off treatment for 23 months prior to biopsy</td>
<td>Commenced 3 weekly PLEX 9 months post-biopsy. Trial of 4 weekly PLEX for 6 months resulted in disease progression hence resumed 3 weekly PLEX.</td>
</tr>
</tbody>
</table>

**Table 4.1.** Description of changes in management instituted post-biopsy in patients with pathologically consistent paraproteinaemic neuropathy. IVIG, intravenous immunoglobulin; PLEX, plasma exchange; R-CP, rituximab, cyclophosphamide, prednisolone
<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre-biopsy differentials</th>
<th>Biopsy result</th>
<th>Post-biopsy diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vasculitis; related to multiple myeloma</td>
<td>Suggestive of vasculitis</td>
<td>Vasculitic neuropathy</td>
</tr>
<tr>
<td>2</td>
<td>Paraproteinaemic neuropathy; CIDP (bone marrow biopsy consistent with WM)</td>
<td>Mild, chronic axonal neuropathy.</td>
<td>Paraproteinaemic neuropathy (based on bone marrow biopsy result and no response to IVIG)</td>
</tr>
<tr>
<td>3</td>
<td>Paraproteinaemic</td>
<td>Non-specific chronic, active axonal neuropathy</td>
<td>Suspected paraproteinaemic</td>
</tr>
<tr>
<td>4</td>
<td>Inflammatory; Paraproteinaemic</td>
<td>Chronic axonal neuropathy</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>Paraproteinaemic; Amyloid</td>
<td>Chronic active inflammatory neuropathy with demyelinating features</td>
<td>Paraproteinaemic neuropathy</td>
</tr>
<tr>
<td>6</td>
<td>CMT; Inflammatory; Paraproteinaemic</td>
<td>Chronic predominantly axonal neuropathy</td>
<td>Paraproteinaemic neuropathy</td>
</tr>
<tr>
<td>7</td>
<td>Vasculitis; Paraproteinaemic; Amyloid</td>
<td>Chronic active neuropathy with mixed axonal and demyelinating features and some ischaemic features</td>
<td>Vasculitic neuropathy</td>
</tr>
<tr>
<td>8</td>
<td>Vasculitis; Paraproteinaemic; Amyloid</td>
<td>Active neuropathy with axonal and demyelinating features</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>Paraproteinaemic; Immune-mediated; Genetic</td>
<td>Acquired inflammatory demyelinating neuropathy</td>
<td>Paraproteinaemic neuropathy</td>
</tr>
<tr>
<td>10</td>
<td>Diabetic; Paraproteinaemic</td>
<td>Both axonal and demyelinating features. Features of diabetic neuropathy and previous ischaemic injury</td>
<td>Unclear diagnosis – likely diabetic.</td>
</tr>
<tr>
<td>11</td>
<td>Related to multiple myeloma</td>
<td>Severe active axonal neuropathy with amyloid deposits</td>
<td>AL Amyloidosis</td>
</tr>
<tr>
<td>12</td>
<td>Paraproteinaemic</td>
<td>Mild axonal neuropathy</td>
<td>Chronic idiopathic axonal polyneuropathy</td>
</tr>
</tbody>
</table>

**Table 4.2.** Description of the impact of nerve biopsy on diagnosis in patients not found to have pathologically consistent paraproteinaemic neuropathy. AL, amyloid light chain; CIDP, chronic inflammatory demyelinating polyneuropathy; CMT, Charcot-Marie-Tooth; IVIG, intravenous immunoglobulin; POEMS, polyneuropathy, organomegaly, endocrinopathy, M-protein, skin changes.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre-biopsy management</th>
<th>Post-biopsy management</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Supportive care</td>
<td>Steroids and Azathioprine</td>
</tr>
<tr>
<td>2</td>
<td>IVIG initially effective then benefit plateaued</td>
<td>Commenced Rituximab, Cyclophosphamide and Prednisone (in view of bone marrow biopsy results)</td>
</tr>
<tr>
<td>3</td>
<td>Supportive care</td>
<td>Supportive care</td>
</tr>
<tr>
<td>4</td>
<td>Supportive care</td>
<td>Supportive care</td>
</tr>
<tr>
<td>5</td>
<td>Failed trial of PLEX (3 months) and Prednisolone (7 years). IVIG started 2 months pre-biopsy</td>
<td>Continued IVIG. Disease progression after 1 year of stability. Commenced Rituximab.</td>
</tr>
<tr>
<td>6</td>
<td>IVIG started 2 months pre-biopsy to good effect</td>
<td>IVIG continued</td>
</tr>
<tr>
<td>7</td>
<td>Supportive care</td>
<td>Pulsed methylprednisolone followed by weaning course of Prednisolone. Commenced Mycophenolate</td>
</tr>
<tr>
<td>8</td>
<td>Supportive care</td>
<td>Supportive care</td>
</tr>
<tr>
<td>9</td>
<td>IVIG with partial response</td>
<td>High-dose steroid induction 14 months post-biopsy was ineffective. PLEX commenced at 20 months post-biopsy with improvement.</td>
</tr>
<tr>
<td>10</td>
<td>IVIG trial (3 months) to no effect</td>
<td>Steroids trialled for 1 year but ongoing progression</td>
</tr>
<tr>
<td>11</td>
<td>Supportive care</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>12</td>
<td>Supportive care</td>
<td>Trialled IVIG for 5 months to no effect</td>
</tr>
</tbody>
</table>

**Table 4.3.** Description of the impact of nerve biopsy on management in patients not found to have pathologically consistent paraproteinaemic neuropathy. IVIG, intravenous immunoglobulin; PLEX, plasma exchange; WM, Waldenström’s macroglobulinaemia.
4.4 Results: Clinical profile of a patient with paraproteinaemic neuropathy; Impact of nerve biopsy on management and clinical outcomes in patients with paraproteinaemic neuropathy

From a database of 350 patients who had been referred for nerve biopsy, 20 patients were eventually clinically diagnosed with paraproteinaemic neuropathy, 70.0% of whom had a biopsy demonstrating pathologically consistent paraproteinaemic neuropathy. Paraproteinaemic neuropathy was documented amongst the differential diagnoses in 75.0% of the cohort. Of the rest, the differentials included chronic inflammatory demyelinating polyneuropathy (CIDP), vasculitis and amyloidosis. The following sections will first characterise the typical patient with clinically diagnosed paraproteinaemic neuropathy in whom biopsy was pathologically consistent with paraproteinaemic neuropathy. This will be followed by a comparison between patients with biopsies demonstrating pathologically consistent paraproteinaemic neuropathy and patients with biopsies demonstrating other findings.

4.4.1 Clinical presentation

The average patient was 70.8 years old and 78.6% of the cohort was male. Most of the patients presented with symptoms that were purely sensory (78.6%) and symmetrical (78.6%). Some patients with an initial pure sensory presentation developed weakness during post-biopsy follow-up, such that the eventual proportion of patients with both sensory and motor symptoms was 42.9%. The symptoms were rarely pure motor (0.0%) or predominantly affecting the upper limbs (0.0%). Pain was present in 50.0% and symptoms predominated in the lower limbs in 64.3%. The
median duration of symptoms between symptom onset to initial presentation to a Neurologist or relevant specialist (one patient was referred for biopsy by a Haematologist) was 17 months.

With regards to comorbidities, a solid organ malignancy was found in 21.4% of the cohort. Co-existing inflammatory conditions were present in 14.3% of the cohort and included rheumatoid arthritis and polymyalgia rheumatica. Information pertaining to family history was available for 64.3% of the cohort. Only 11.1% of these had a positive family history for neuropathy. One patient had a daughter who was diagnosed with familial amyloid polyneuropathy on sural nerve biopsy. Exposure to a potential neurotoxin was documented in 42.9% of the cohort and included Allopurinol, chronic heavy alcohol use, Levodopa, Phenytoin and a statin.

4.4.2 Serological investigations

Information was available for all patients however, there was variation in the workup for each patient. ESR or CRP was tested in 78.6% and was found to be elevated in 18.2%. In terms of autoimmune serology, ANA was tested in 92.9% of the cohort and found positive in 7.7% of these. ANCA was tested in 71.4% of the cohort and found to be positive in 10.0%; MPO and Pr3 was however negative). Extractable nuclear antigens (ENA) were tested in 85.7% of cohort and found to be negative in all. Other autoimmune serological tests were infrequently performed. Cryoglobulin was only tested in 35.7% and was found to be negative in all. Rheumatoid factor was tested in 28.6% of the cohort and found to be positive in 50.0%; the levels were mildly elevated (≤20). Anti GM1 antibodies were tested in only 21.4% and found to be negative in all.
A serum paraprotein was demonstrated in all patients, of which 92.9% were IgM and 7.1% was IgG. As for light chain subtypes, 85.7% were kappa and 14.3% were lambda. The median paraprotein level prior to nerve biopsy was 2.35g/l. Apart from 1 patient with a paraprotein level of 22g/l, the remaining patients had a level of ≤5g/l. Tests for MAG antibodies were undertaken in 64.3%, including some patients tested subsequent to nerve biopsy. MAG antibodies were positive in 77.8% (median 32252 Bühlmann Titre Units (BTU); range of 9388 to 134422 BTU).

4.4.3 Electrodiagnostic assessment

Nerve conduction studies were performed in all patients. The pattern was found to be predominantly demyelinating in 50.0%, mixed axonal and demyelinating in 42.9% and predominantly axonal in 7.1%. There was evidence of both sensory and motor involvement in all studies. Both upper and lower limb studies were performed in 92.9% of the cohort, with evidence of generalised involvement in 38.5% while the remaining had predominantly lower limb findings. Bilateral nerve conduction studies were performed in 78.6%, 54.5% of whom had evidence of asymmetry on testing. Only 28.6% of the cohort had an EMG performed, of which 50.0% were normal and 50.0% demonstrated chronic neurogenic changes.

4.4.4 Cerebrospinal fluid analysis and bone marrow biopsy

Lumbar puncture was performed in 35.7% of the cohort, 40.0% of whom had elevated CSF protein levels (range 0.77 - 0.93g/L). None of the patients had CSF pleocytosis.
Bone marrow biopsy was performed in 42.9% of the cohort, 83.3% of which were reported normal and the remaining demonstrated lymphoplasmacytic lymphoma.

### 4.4.5 Imaging investigations

MRI of the brain was performed in 35.7% of the cohort. Abnormal findings include extensive microvascular ischaemia in 20.0% and a glioma in another 20.0%; the latter was confirmed on a subsequent PET scan. MRI of either the whole spine or selectively of the cervical or lumbar spine was performed in 35.7% of the cohort, none of which demonstrated findings that explained the patient’s presentation. A screening CT of the chest, abdomen and pelvis was performed in 64.3% of the cohort with no significant findings noted. A PET scan was performed in 28.6% of the cohort, 75.0% of which were reported normal, the remaining 25.0% was suggestive of a low-grade glioma (see above).

### 4.4.6 Biopsy features

Evidence of IgM deposition on the myelin was noted in 64.3% of the biopsies. The remaining patients had features on nerve biopsy that were suggestive of paraproteinaemic neuropathy such as focal myelin swelling. Additional electron microscopy was performed in 42.9% of the cohort, 50.0% of which demonstrated widened myelin lamellae. The presence of widened myelin lamellae was seen only in patients with evidence of IgM deposition.
4.4.7 Post-biopsy follow-up

A summary of individual patient data pertaining to changes in management subsequent to nerve biopsy is provided in Table 4.4. Half the cohort only had supportive therapy prior to nerve biopsy, with 71.4% of them being commenced on an immunomodulatory therapy subsequent to biopsy, intravenous immunoglobulin (IVIG) in all cases.

Subsequent to nerve biopsy, IVIG was newly commenced 42.9% of the cohort but subsequently ceased in 33.3% of these patients due to inefficacy. Among the patients in whom IVIG was ceased for inefficacy, 1 patient benefitted from a switch to plasmapheresis and the other patient opted for supportive care.

Subsequent to nerve biopsy, plasmapheresis was newly commenced in 14.3% of the cohort with benefit demonstrated in all patients. Likewise, subsequent to nerve biopsy, Rituximab was trialled in 7.1% with subsequent stabilisation. A regimen of IVIG, Azathioprine and Prednisone that had been started just prior to nerve biopsy was continued in 1 patient with resultant clinical stabilisation.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre-biopsy management</th>
<th>Post-biopsy management</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IVIG</td>
<td>IVIG continued</td>
</tr>
<tr>
<td>2</td>
<td>Supportive care</td>
<td>IVIG started but ceased after 30 months as ineffective.</td>
</tr>
<tr>
<td>3</td>
<td>Supportive care</td>
<td>IVIG started but ceased after 40 months as ineffective. PLEX effective but ceased due to line sepsis. PLEX recommenced after 14 months using peripheral line.</td>
</tr>
<tr>
<td>4</td>
<td>Supportive care</td>
<td>Supportive care</td>
</tr>
<tr>
<td>5</td>
<td>IVIG ineffective after initial benefit. 3-month trial of PLEX was ineffective.</td>
<td>Rituximab started</td>
</tr>
<tr>
<td>6</td>
<td>IVIG effective</td>
<td>IVIG continued. 36 months post-biopsy, IVIG frequency reduced from 3 to 4 weekly but failed hence resumed 3 weekly IVIG after 9 months.</td>
</tr>
<tr>
<td>7</td>
<td>Supportive care</td>
<td>IVIG started, ceased after 26 months.</td>
</tr>
<tr>
<td>8</td>
<td>Long term Methotrexate for rheumatoid arthritis</td>
<td>IVIG started.</td>
</tr>
<tr>
<td>9</td>
<td>Supportive care</td>
<td>IVIG started.</td>
</tr>
<tr>
<td>10</td>
<td>3 months (6 cycles) of R-CP (for WM) with improvement. 10 months off treatment prior to biopsy.</td>
<td>Rituximab given 5 months post-biopsy. IVIG started 8 months post-biopsy and dose escalated.</td>
</tr>
<tr>
<td>11</td>
<td>Supportive care</td>
<td>Supportive care. Patient declined IVIG and Rituximab on multiple occasions.</td>
</tr>
<tr>
<td>12</td>
<td>Supportive care</td>
<td>IVIG started. After 6 months, frequency increased to 3-weekly. After further 16 months, resumed 4-weekly IVIG.</td>
</tr>
<tr>
<td>13</td>
<td>Monthly IVIG for 4 months was ineffective. Commenced Azathioprine and Prednisone 3 months prior to biopsy.</td>
<td>Continued IVIG, Azathioprine and Prednisone 5mg daily.</td>
</tr>
<tr>
<td>14</td>
<td>Failed 9-month trial of IVIG, off treatment for 23 months prior to biopsy.</td>
<td>Started 3 weekly PLEX 9 months post-biopsy. Unable to wean to 4 weekly PLEX as it resulted in disease progression.</td>
</tr>
</tbody>
</table>

**Table 4.4.** Description of the changes in management of patients diagnosed with pathologically consistent paraproteinaemic neuropathy. IVIG, intravenous immunoglobulin; PLEX, plasmapheresis; R-CP, Rituximab, Cyclophosphamide, Prednisolone; WM, Waldenström’s macroglobulinaemia
In summary, subsequent to reviewing the nerve biopsy report, a change in management was determined for 71.4% of the cohort. This change was implemented in all but one patient who elected to continue a trial of supportive care. IVIG was used as monotherapy at some stage (pre- or post-biopsy) in 78.6% of the cohort. Of these, IVIG monotherapy was ineffective in 45.5%. Plasmapheresis was trialled in 60.0% of the patients who failed IVIG, with efficacy demonstrated in 66.7%. The remaining 33.3% had disease stabilisation with Rituximab.

In terms of clinical outcome, prior to nerve biopsy, 85.7% had progressive symptoms regardless of whether a trial of therapy was undertaken. The remaining 14.3% had shown some improvement on therapy prior to biopsy. Subsequent to biopsy, there was clinical improvement followed by stabilisation in 57.1%, clinical stabilisation in 21.4% and disease progression in 21.4%. Amongst the patients with disease progression, 66.7% were only on supportive therapy and the remaining 33.3% did not trial any therapy after failing IVIG. The details are summarised in Table 4.5 as well as Figure 4.3 below.
Figure 4.3. Flow diagram of treatment regimens and their outcome in patients with clinically and pathologically consistent paraproteinaemic neuropathy. Ongoing disease progression was only noted amongst patients who did not receive any immunomodulatory therapy or did not trial a new immunomodulatory therapy after failing IVIG (intravenous immunoglobulin). PLEX, plasma exchange; R-CP, Rituximab, Cyclophosphamide, Prednisolone.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre-biopsy status</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Slowly progressive dysesthesia and weakness.</td>
<td>Improved dysesthesia. Clinically stable with persistent deficits.</td>
</tr>
<tr>
<td>2</td>
<td>Rapidly progressive neuropathy (predominantly sensory symptoms)</td>
<td>Slow progression.</td>
</tr>
<tr>
<td>3</td>
<td>Progressive pain and paraesthesia.</td>
<td>Improved initially, then stabilised. Gradual progression when not on PLEX.</td>
</tr>
<tr>
<td>4</td>
<td>Slowly progressive pain and sensory neuropathy.</td>
<td>Minimal progression.</td>
</tr>
<tr>
<td>5</td>
<td>Slowly progressive numbness and weakness.</td>
<td>Initially stable. Subsequent progression stabilised with further Rituximab.</td>
</tr>
<tr>
<td>6</td>
<td>Progressive paraesthesia initially; improved with IVIG</td>
<td>Improved and clinically stable.</td>
</tr>
<tr>
<td>8</td>
<td>Insidious onset of paraesthesia</td>
<td>Slow improvement then stabilised.</td>
</tr>
<tr>
<td>9</td>
<td>Numbness and paraesthesia with unclear rate of progression.</td>
<td>Clinically stable.</td>
</tr>
<tr>
<td>10</td>
<td>Slowly progressive sensory symptoms. Post 3 months of R-CP (for WM), had 3 months of improvement, then stable.</td>
<td>Disease progression 2 months post-biopsy. Initial improvement on therapy and then stabilised.</td>
</tr>
<tr>
<td>11</td>
<td>Very slowly progressive sensory and autonomic symptoms.</td>
<td>Slow progression until patient deceased (MVA) 24 months post-biopsy.</td>
</tr>
<tr>
<td>12</td>
<td>Progressive weakness and numbness</td>
<td>Initial improvement. Subsequent disease progression stabilised with increased IVIG frequency.</td>
</tr>
<tr>
<td>13</td>
<td>Progressive sensory neuropathy</td>
<td>Clinically stable with residual deficit.</td>
</tr>
<tr>
<td>14</td>
<td>Progressive pain and sensory symptoms.</td>
<td>Initial improvement. Clinically stable.</td>
</tr>
</tbody>
</table>

**Table 4.5.** Description of impact on clinical outcome in patients diagnosed with pathologically consistent paraproteinaemic neuropathy. IVIG, intravenous immunoglobulin; MVA, motor vehicle accident; PLEX, plasmapheresis; R-CP, Rituximab, Cyclophosphamide, Prednisolone; WM, Waldenström’s macroglobulinaemia
4.4.8 Subgroup analysis of patients with clinically diagnosed paraproteinaemic neuropathy comparing those with nerve biopsy demonstrating pathologically consistent paraproteinaemic neuropathy to those with other nerve biopsy findings.

From the source database of 350 patients, 6 patients with clinically diagnosed paraproteinaemic neuropathy were identified in whom the nerve biopsy did not suggest this diagnosis. Amongst patients with clinically diagnosed paraproteinaemic neuropathy, when comparing those in whom nerve biopsy demonstrated pathologically consistent paraproteinaemic neuropathy to those with other nerve biopsy findings, the primary difference between the groups was that the former had a higher prevalence of IgM paraprotein (92.9% v 16.7%; p<0.005) and MAG antibodies (77.8% vs 0.0%; p=0.02). Additionally, in the former, there was a trend towards significance for a higher prevalence of both sensory and motor involvement on nerve conduction studies (100.0% vs 66.7%; p=0.08). The median CSF protein was 0.56g/L among patients in whom nerve biopsy demonstrated pathologically consistent paraproteinaemic neuropathy and 1.04g/L in patients with other nerve biopsy findings. Table 4.6 summarises the results of all the comparisons made between the 2 groups.

There was no difference between patients with pathologically consistent paraproteinaemic neuropathy and patients with other diagnoses on nerve biopsy when comparing freedom from disease progression at time of last follow-up (78.6% vs 100.0%) or the proportion of patients who had a change in management (71.4% vs 83.3%).

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A new clinical recommendation for non-IVIG immunotherapies (plasmapheresis, Rituximab, Cyclophosphamide or Azathioprine) subsequent to nerve biopsy was noted in 28.6% of those with pathologically consistent paraproteinaemic neuropathy compared to 66.7% of those with other findings on nerve biopsy (p=0.16). Therefore, there was no clear difference in management choices or clinical outcomes regardless of whether the clinical diagnosis was supported by nerve biopsy.

A trial of IVIG monotherapy at any time (pre or post biopsy) was failed by 5 of 11 (45.5%) patients in whom the biopsy supported the diagnosis compared to 5 of 6 (83.3%) patients in whom biopsy did not support the diagnosis. Immunosuppressive therapy such as Rituximab or Azathioprine was trialled in 2 of 14 (14.3%) patients in whom the biopsy supported the diagnosis compared to 3 of 6 (50.0%) patients in whom biopsy did not support the diagnosis. A flow diagram of therapies trialled by patients with clinically diagnosed paraproteinaemic neuropathies that was not supported by nerve biopsy is shown in Figure 9.
Table 4.6: Clinical parameters that were compared between patients with pathologically consistent paraproteinaemic neuropathy and patient with other findings on nerve biopsy. All patients had an eventual clinical diagnosis of paraproteinaemic neuropathy. **Bold** is used to highlight significance.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Biopsy supportive</th>
<th>Biopsy not supportive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>70.8</td>
<td>73.0</td>
<td>0.69</td>
</tr>
<tr>
<td>Gender (%)</td>
<td>78.6</td>
<td>50.0</td>
<td>0.30</td>
</tr>
<tr>
<td>Pure sensory symptoms (%)</td>
<td>78.6</td>
<td>50.0</td>
<td>0.30</td>
</tr>
<tr>
<td>Sensorimotor symptoms (%)</td>
<td>21.4</td>
<td>50.0</td>
<td>0.30</td>
</tr>
<tr>
<td>Pain (%)</td>
<td>50.0</td>
<td>50.0</td>
<td>1.00</td>
</tr>
<tr>
<td>Symmetry of symptoms (%)</td>
<td>21.4</td>
<td>33.3</td>
<td>0.61</td>
</tr>
<tr>
<td>Lower-limb dominance (%)</td>
<td>64.3</td>
<td>33.3</td>
<td>0.34</td>
</tr>
<tr>
<td>Chronic symptoms (%)</td>
<td>85.7</td>
<td>83.3</td>
<td>1.00</td>
</tr>
<tr>
<td>ANA positivity (%)</td>
<td>7.1</td>
<td>40.0</td>
<td>0.16</td>
</tr>
<tr>
<td>ENA positivity (%)</td>
<td>0.0</td>
<td>0.0</td>
<td>1.00</td>
</tr>
<tr>
<td>ANCA positivity (%)</td>
<td>0.0</td>
<td>0.0</td>
<td>1.00</td>
</tr>
<tr>
<td>Raised ESR or CRP (%)</td>
<td>18.2</td>
<td>0.0</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>IgM paraprotein (%)</strong></td>
<td><strong>92.9</strong></td>
<td><strong>16.7</strong></td>
<td><strong>&lt;0.005</strong></td>
</tr>
<tr>
<td>Kappa light chain (%)</td>
<td>85.7</td>
<td>66.6</td>
<td>0.55</td>
</tr>
<tr>
<td>Median paraprotein level (g/l)</td>
<td>2.35</td>
<td>4.7</td>
<td>NA</td>
</tr>
<tr>
<td><strong>MAG positive (%)</strong></td>
<td><strong>77.8</strong></td>
<td><strong>0.0</strong></td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>NCS: Axonal neuropathy (%)</td>
<td>7.1</td>
<td>33.3</td>
<td>0.21</td>
</tr>
<tr>
<td>NCS: Mixed axonal and demyelinating neuropathy (%)</td>
<td>42.9</td>
<td>16.7</td>
<td>0.35</td>
</tr>
<tr>
<td>NCS: Demyelinating neuropathy (%)</td>
<td>50.0</td>
<td>50.0</td>
<td>1.00</td>
</tr>
<tr>
<td>NCS: Sensory involvement (%)</td>
<td>0.0</td>
<td>16.7</td>
<td>0.30</td>
</tr>
<tr>
<td>NCS: Motor involvement (%)</td>
<td>0.0</td>
<td>16.7</td>
<td>0.30</td>
</tr>
<tr>
<td>NCS: Sensorimotor involvement (%)</td>
<td>100.0</td>
<td>66.7</td>
<td>0.08</td>
</tr>
<tr>
<td>NCS: Generalised involvement (%)</td>
<td>38.5</td>
<td>66.7</td>
<td>0.35</td>
</tr>
<tr>
<td>NCS: Lower limb predominant (%)</td>
<td>61.5</td>
<td>16.7</td>
<td>0.14</td>
</tr>
<tr>
<td>NCS: Asymmetry (%)</td>
<td>54.5</td>
<td>50.0</td>
<td>1.00</td>
</tr>
<tr>
<td>Post-biopsy: Management change (%)</td>
<td>71.4</td>
<td>83.3</td>
<td>1.00</td>
</tr>
<tr>
<td>Post-biopsy: Disease progression (%)</td>
<td>14.3</td>
<td>0.0</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Figure 4.4. Flow diagram of treatment regimens and their outcome in patients with clinically diagnosed paraproteinaemic neuropathy that was not supported by nerve biopsy findings. Multiple treatment modalities were trialled, and clinical stability was achieved in all patients. IVIG, intravenous immunoglobulin; PLEX, plasmapheresis; R-CP, Rituximab, Cyclophosphamide, Prednisolone; WM, Waldenström's macroglobulinaemia
4.5 Discussion

This chapter has established that the typical patient with a clinical diagnosis of paraproteinaemic neuropathy that is supported by nerve biopsy is an older male who present with a gradual onset of progressive, symmetrical sensory symptoms. The investigations typically identified an IgM kappa paraprotein often associated with MAG antibodies. Most patients would test negative for autoantibodies like ANA, ENA and ANCA and not have elevated inflammatory markers. Electrodiagnostic testing established a generalised or predominantly lower limb sensorimotor neuropathy with demyelinating features. Most patients experienced clinical improvement or stabilisation with the following treatments when given alone or in combination: IVIG, plasmapheresis, steroids, azathioprine or rituximab.

By contrast, patients clinically diagnosed with paraproteinaemic neuropathy without a supportive nerve biopsy did not have positive MAG antibodies and were unlikely to have IgM antibodies. Instead, most of this group had an IgG paraproteinaemia. It is known that in patients with IgM paraproteinaemic neuropathies, that the most common target antigen is MAG.(130) However, patients can have an anti-MAG neuropathy without the presence of IgM paraproteinaemia.(131) Additionally, the presence of widened outer myelin lamellae is rarely found outside of IgM paraproteinaemic neuropathies.(130) The pathological features in patients with IgG paraproteinaemic neuropathy can be similar to those seen in CIDP and can include demyelination with IgG or complement deposition on myelin lamellae but biopsies in some patients can show predominantly axonal degeneration.(132) In comparison to IgM paraproteinaemic neuropathy, widened lamellae are more common in the inner and
middle layers of the myelin sheath in IgG/ A paraproteinaemic neuropathy.(80) Immuno-EM may be used to demonstrate binding of monoclonal IgM or IgG to target antigens, and such studies implicate the Ig as pathologic and also show the mechanism of injury.(43)

When considering the usefulness of nerve biopsy as a diagnostic tool for paraproteinaemic neuropathy, it is not possible to evaluate for parameters such as positive or negative predictive values or specificity and sensitivity in the absence of an established gold standard. Nerve biopsies are not 100% sensitive for paraproteinaemic neuropathy(130) which is also reflected this study where 6 patients with clinically diagnosed paraproteinaemic neuropathy were identified in whom the nerve biopsies did not suggest the diagnosis. However, 83.3% of these patients had IgG paraprotein and the literature has yet to prove a causal association between IgG or IgA paraproteins and peripheral neuropathy.(127)

In terms of specificity, however, the presence of widened myelin lamellae or IgM bound to myelin has been shown to be very specific for IgM paraproteinaemic neuropathies.(130) However, widened myelin lamellae have been demonstrated in patients who test negative for direct immunofluorescence for IgM.(130) As such, when patients are referred for biopsy with suspected paraproteinaemic neuropathy and direct immunofluorescence is negative for IgM bound to myelin, the specimen should be sent for electron microscopy to assess for widened myelin lamellae. This was infrequently done in our cohort. Amongst nerve biopsies for suspected paraproteinaemic neuropathy, 22.7% had a positive direct immunofluorescence for IgM but only 5.9% of the remaining biopsies were referred for electron microscopy.
Other findings on nerve biopsy such as demyelination, inflammatory changes, axonal degeneration and regeneration, tomaculous changes, irregular myelin sheaths and onion bulb formations are common\(^\text{(5, 133, 134)}\) but non-specific. However, the presence of some of these features may raise suspicion for a paraproteinaemic neuropathy as was the case amongst patients in the study who had biopsies that were classified as pathologically possible paraproteinaemic neuropathy. For example, in the overall cohort of 350 nerve biopsy reports that were analysed for this thesis, 15 reports were classified as pathologically possible paraproteinaemic neuropathy, 5 of whom were eventually clinically diagnosed with paraproteinaemic neuropathy and therefore included in our studies.

This chapter determined that nerve biopsy influenced a change in management plan for the majority (71.4\%) of patients in whom paraproteinaemic neuropathy was diagnosed. It was noted however, that 50\% of all patients had not trialled an immunomodulatory therapy prior to nerve biopsy. Given that 54.5\% of the patients initiated on IVIG monotherapy in this study achieved improvement or stabilisation of disease progression, it is likely that subjecting all patients to a trial of IVIG prior to considering nerve biopsy would reduce the number of patients referred for nerve biopsy. In terms of the impact of nerve biopsy on clinical outcomes, nerve biopsy likely facilitated a halt in disease progression with the proportion of patients with ongoing disease progression dropping from 85.7\% pre-biopsy to 21.4\% post-biopsy. However, it was noted that of the 11 patients who achieved clinical improvement or stability, 54.5\% only required IVIG monotherapy while 45.5\% required riskier therapies like plasmapheresis, Azathioprine and Rituximab. This would again support a practice of trialling IVIG prior to considering referral for nerve biopsy.
The comparison of patients with clinically diagnosed paraproteinaemic neuropathy in whom the nerve biopsy did not suggest this diagnosis with patients with clinically diagnosed and pathologically consistent paraproteinaemic neuropathy found no clear differences in the rate of management changes, the rate of commencing therapies such as plasmapheresis or Rituximab or the rate of achieving freedom from disease progression. Despite the small numbers in each group, this was an interesting observation. The likely explanation for this finding is that in the face of ongoing disease progression resulting in accruing disability, the risk-benefit profile would increasingly favour aggressive management strategies such as Rituximab.

Indeed, recent consensus guidelines stipulate that in patients with IgM and Waldenström-associated peripheral neuropathies, nerve biopsies are primarily indicated in the setting of atypical, treatment-unresponsive, progressive, disabling presentations.(77) Given the safety profile of IVIG,(135) it is unclear why some patients in the present study did not have a trial of IVIG prior to nerve biopsy as adequate response to IVIG would normally preclude a nerve biopsy. Plasmapheresis, while a relatively safe procedure,(136) and can be used long-term,(137) requires specialised equipment and large-bore central venous access which entails serious risks including infection, thrombosis, pneumothorax and vessel perforation. Hence, nerve biopsy may be reasonably indicated when ongoing plasmapheresis is required and especially if immunotherapies such as Rituximab or Azathioprine are being considered. In the present study, subsequent to a nerve biopsy report supporting a diagnosis of paraproteinaemic neuropathy, patients were commenced on rituximab or plasmapheresis and 1 patient continued azathioprine with resultant clinical improvement or stabilisation.
This chapter has also determined that in these patients suspected to have paraproteinaemic neuropathy, nerve biopsy could confirm the diagnosis in 45.5%. The only pre-biopsy parameter that predicted a diagnosis of paraproteinaemic neuropathy was the presence of an IgM paraprotein. Conversely, the only pre-biopsy parameter that predicted an alternate result was the presence of an elevated ANA above a titre of 1:160. It is possible that the elevated ANA reflects an alternate underlying immune-mediated pathology that may be the cause of the neuropathy.

In terms of limitations, it is accepted that there may have been selection bias. For instance, patients who were referred for nerve biopsy were unlikely to represent all patients with paraproteinaemic neuropathy. For example, patients with high levels of MAG antibodies and a typical clinical phenotype may have been treated without nerve biopsy. Second, the reporting neuropathologist was not blinded to the clinical history and was often aware of the presence of a serum paraprotein. There remained a potential for this knowledge to influence the requisition of additional testing (e.g. electron microscopy) and to have influenced the interpretation of pathological findings. Separately, in order to identify patients suspected to have paraproteinaemic neuropathy from the overall cohort, the patient’s medical records had to include this as either a provisional diagnosis or an indication for biopsy. Finally, physicians had variable experience with diagnosing paraproteinaemic neuropathy, so that each physician’s threshold for suspecting this diagnosis would likely be different.

It is accepted that patients did not have a uniform diagnostic workup prior to biopsy. This lack of uniformity may have been influenced by the clinical presentations and suggests potential differences between patients who had particular investigations (like
CSF tests) versus patients who did not have those investigations. The lack of a uniform protocol for NCS and EMG resulted in further variation in the extent of neurophysiological testing and accordingly there would be variation in how the neurophysiological data was interpreted. For example, the presence of asymmetry on NCS could not be ascertained in some patients due to a lack of bilateral testing. Also, the terminal latency index is typically low in patients with IgM paraproteinaemic demyelinating neuropathies (127) but could not be calculated for this study as the true distal distance was uncommonly recorded on nerve conduction study reports.

Testing for anti-MAG, anti-ganglioside antibodies and bone marrow biopsy were infrequently undertaken, especially prior to nerve biopsy which can result in unnecessary referral for nerve biopsy. For example, amongst the patients with suspected paraproteinaemic neuropathy in whom nerve biopsy demonstrated pathologically consistent paraproteinaemic neuropathy, only 30.0% were tested for MAG antibodies pre-biopsy. A further 30.0% were tested for MAG antibodies after nerve biopsy, all of which were positive. Follow-up data was available for a mean of 30.8 months (range 5 to 46 months) so it is possible that patients deemed to be stable at present may subsequently have ongoing disease progression despite current therapy.

In conclusion, when patients with a peripheral neuropathy and a monoclonal gammopathy were suspected to have a paraproteinaemic neuropathy and subsequently referred for nerve biopsy, the presence of an IgM monoclonal gammopathy was predictive of a biopsy that suggested a diagnosis of paraproteinaemic neuropathy. Amongst patients clinically diagnosed with
paraproteinaemic neuropathy, IgM monoclonal gammopathy and MAG antibodies were significantly more prevalent in patients with a nerve biopsy that suggested a diagnosis of paraproteinaemic neuropathy while IgG monoclonal gammopathy was significantly more prevalent in patients with biopsies suggesting other diagnoses. Subsequent to nerve biopsy, most patients had changes to their management plans and subsequently had improved outcomes. However, the degree to which these changes can be attributed to the findings on nerve biopsy are uncertain as similar degrees of changes to patient management and clinical outcome were observed in patients receiving a clinical diagnosis of paraproteinaemic neuropathy in whom the nerve biopsy report did not suggest a diagnosis of paraproteinaemic neuropathy.
CHAPTER 5
SUMMARY AND CONCLUSIONS
While the utility of nerve biopsy in the clinical setting has reduced over recent years, it continues to play a role in the diagnostic workup of highly selected patients. Given the risks and costs associated with nerve biopsy, it is important to consider factors that would maximise the diagnostic yield of this investigation. This includes the clinical features of the neuropathy, appropriate nerve selection, consideration of combination tissue biopsies and the appropriate use of neuropathology techniques.

Chapter 3 established that nerve biopsy was more likely to demonstrate vasculitis in patients who had a stepwise clinical progression or in patients with presence of serum anti-MPO antibodies, rheumatoid factor or cryoglobulin and unlikely to demonstrate vasculitis in the context of NCS that were normal or primarily demyelinating.

Results from the studies in Chapter 4 demonstrate that the results of a nerve biopsy are unlikely to affect the management or clinical outcome of patients with paraproteinaemic neuropathy. However, a suspected paraproteinaemic neuropathy is most likely to be confirmed on nerve biopsy if the patient has an IgM paraprotein. Conversely, nerve biopsy is unlikely to suggest a diagnosis of paraproteinaemic neuropathy in patients with an IgG paraprotein. A decision to refer patients for a nerve biopsy should consider the results of this study in the context of existing literature. To guide such a process, a decision aid for nerve biopsies has been constructed in the form of a flowchart (Figure 5). This decision aid is to be used with the accompanying tables (Tables 5.1 – 5.3) that have been organised based on the relative importance of nerve biopsy for a given indication.
CHAPTER 6

FUTURE PERSPECTIVES
This thesis has focussed on the role played by nerve biopsy in two diseases. Further studies are therefore indicated to evaluate the utility of nerve biopsies for other common indications. One common reason for referral in the present cohort was the suspicion of a predominantly demyelinating pathology, as this may indicate the presence of a potentially treatable immune-mediated neuropathy. Another common indication for referral in the present cohort was the absence of a clear provisional diagnosis despite extensive diagnostic workup.

Additional studies should be considered to investigate the role of incorporating the use of artificial intelligence in the interpretation of biopsies. Such work has been reported in the literature, especially relating to the diagnosis and prognosis of malignancies and the detection of microorganisms. Studies have demonstrated that the use of artificial intelligence software has the potential to reduce workload and assist with triaging biopsies requiring urgent attention. Additionally, there is the potential for the discovery of as yet unknown diagnostic or prognostic information consequent to deep machine learning from a large dataset of biopsies. However, several challenges need to be addressed prior to widespread adoption.

Finally, the use of proteomics in nerve biopsies can be of value especially for the identification of specific proteins. In amyloid neuropathies for example, immunohistochemical staining may not be able to subtype the amyloid protein in some patients. Proteomic analysis has been shown to overcome such a challenge. The use of proteomics may also be helpful in the providing diagnostic and prognosis information for neoplastic and infective pathologies of the nerve.
Figure 5.1. Flowchart to assist clinical decision-making when referring patients for nerve biopsy.

Inflammatory demyelinating polyneuropathy

Patient with neuropathy, Diagnostic workup:
1. Phenotype
2. NCS/EMG
3. Blood investigations
4. CSF
5. Genetics
6. Imaging
7. Biopsy of extraneural tissue

Provisional diagnosis made

Cryptogenic neuropathy despite extensive investigations. Is the neuropathy severe or progressive enough to require treatment?

Are any of the following clinical features that may increase the diagnostic yield of biopsy present?
1. Acute/subacute (<6 months) onset
2. Asymmetric or multifocal

Yes

Conservative management or satisfactory response to appropriate management.

Suspected hereditary neuropathy

Nature of proposed management requires greater confidence in the diagnosis AND the provisional diagnosis is one of the following:
1. Vasculitic neuropathy
2. Paraproteinemia neuropathy
3. Amyloid neuropathy
4. Sarcoid neuropathy
5. Malignant infiltration of nerve
6. CIDP
7. Pure neuritic leprosy
8. IgG4 related disease involving nerve
9. Distinguish motor neuropathy from PMA
10. Primary neoplasm of nerve or nerve sheath

No

Nerve biopsy is less likely to be helpful

Nerve biopsy is not required. Patient should have ongoing follow up to monitor for change in phenotype.

Yes

Consider nerve biopsy

Nerve biopsy may be required. See Tables 1-3 for details.

Research purpose

Focal mass lesion of nerve detected

Poor response or deterioration despite appropriate management
<table>
<thead>
<tr>
<th>Suspected Diagnosis</th>
<th>When to biopsy?</th>
<th>When diagnosis can be made without biopsy</th>
<th>Performance of biopsy*</th>
<th>Other diagnostic tools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasculitic neuropathy</td>
<td>Most cases of vasculitic neuropathy</td>
<td>• Typical neuropathy phenotype in presence of clinicopathologically proven systemic vasculitis.</td>
<td>Sensitivity: ~50% (108)</td>
<td>• Muscle biopsy: Can increase yield by ~5% (111).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Diabetic radiculoplexus neuropathy phenotype</td>
<td></td>
<td>• Skin biopsy: Potential to increase diagnostic yield.</td>
</tr>
<tr>
<td>Neurolymphomatosis</td>
<td>• Primary neurolymphomatosis</td>
<td>Secondary neurolymphomatosis diagnosed using CSF, PET-CT or MRI.</td>
<td>Sensitivity: 88% in one study (10)</td>
<td>• Nerve US: Can show focal nerve enlargement and can inform nerve selection for biopsy.</td>
</tr>
<tr>
<td></td>
<td>• Secondary neurolymphomatosis:</td>
<td>• If diagnosing neurolymphomatosis will not change management (e.g. active haematological malignancy).</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Persistent diagnostic uncertainty despite CSF Cytology and imaging workup (MRI/ PET)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary Nerve/ Nerve sheath neoplasm/ pseudo-neoplasm</td>
<td>• To exclude malignant tumours</td>
<td>Typical phenotype accompanied by characteristic MRI/US findings can establish diagnosis in some subtypes (59)</td>
<td>Not applicable</td>
<td>MRI and US: Can help in differential diagnosis and guiding biopsy.</td>
</tr>
<tr>
<td></td>
<td>• Diagnosis of some atypical benign tumours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Symptomatic relief</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure neuritic leprosy</td>
<td>Almost always (for definite diagnosis)</td>
<td>If fine needle aspiration cytology of the nerve demonstrates M.leprae bacilli or epithelioid granulomas</td>
<td>Sensitivity: 33.3% - 75.9% (141)</td>
<td>• Skin biopsy (even if no sign of skin involvement): Can demonstrate presence of bacilli</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• qPCR: M.leprae DNA on slit skin smears and/or skin biopsies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Nerve US: Guide nerve selection for biopsy</td>
</tr>
</tbody>
</table>

Table 5.1. Conditions in which nerve biopsies are of high importance

*Data regarding performance of nerve biopsy for individual disorders needs to be taken in the context of there being no true reference gold standard for many of these disorders.

US, ultrasound; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; qPCR, quantitative polymerase chain reaction; DNA, deoxyribonucleic acid; PET-CT, positron emission tomography – computed tomography; M.leprae, Mycobacterium leprae
<table>
<thead>
<tr>
<th>Suspected Diagnosis</th>
<th>When to biopsy?</th>
<th>When diagnosis can be made without biopsy</th>
<th>Performance of biopsy*</th>
<th>Other diagnostic tools</th>
</tr>
</thead>
</table>
| Para-proteinenaemic neuropathy | To link paraprotein to neuropathy especially if severe/progressive or treatment resistance | • Multiple myeloma (may need treatment regardless)  
• POEMS or CANOMAD  
• High titre anti-MAG / GQ1b IgM in context of typical phenotype | Widened myelin lamellae:  
Sensitivity: 60-96%(130, 142, 143) for anti-MAG neuropathy. Very specific. | Bone marrow biopsy: To detect haematological malignancy |
| Amyloid neuropathy | Other tissues not amenable to biopsy or have negative biopsy. | Amyloidosis pathologically demonstrated in other tissue. | • Sensitivity: 80% for TTR Amyloid(7) and 30-100% for AL Amyloid(77) | • Extraneural biopsy: amyloidosis obviates need for nerve biopsy.  
• MR Neurography: Peripheral nerve lesions in asymptomatic TTR mutation carriers.  
• Tc-labelled cardiac scintigraphy: Diagnose cardiac ATTR amyloidosis  
• Skin biopsy: SFN  
• ESC: Early dysautonomia  
• Periumbilical US: Amyloid deposits |
| Sarcoïd peripheral neuropathy | Definitive diagnosis | Probable SPN: extraneural NCG in context of typical phenotype and evidence of neuroinflammation on CSF studies or MRI. | Sensitivity: 90.5% in one study(64). | Muscle biopsy: Can improve yield. Granulomas in muscle rules out tuberculosis leprosy.  
• Nerve US and MR Neurography: Higher CSA in specific nerves.  
• MR Plexus: enlargement/enhancement of roots, plexus and nerves |
| IgG4 related perineural disease/neuropathy | Biopsy required for definite diagnosis | Probable IgG4 related perineural disease: typical phenotype in the presence of extraneural IgG4 related disorder and raised serum IgG4 levels. | No data | Extraneural biopsy: Evidence of IgG4 disorder  
MRI in IgG4 related perineural disease: Well circumscribed soft tissue perineural mass that is steroid responsive |

**Table 5.2. Conditions in which nerve biopsies are of moderate importance**

*Data regarding performance of nerve biopsy for individual disorders needs to be taken in the context of there being no true reference gold standard for many of these disorders.*

POEMS, polyneuropathy, organomegaly, endocrinopathy, M-protein, skin changes; CANOMAD, chronic ataxic neuropathy, ophthalmoplegia, monoclonal IgM protein, cold agglutinins and disialosyl antibodies; MAG, myelin associated glycoprotein; AL, light chain amyloid; ATTR, amyloid transthyretin; SFN, small fibre neuropathy; ESC, electrochemical skin conductance; US, ultrasound; MRI, magnetic resonance imaging; SPN, sarcoïd peripheral neuropathy; NCG, non-caseating granuloma; CSF, cerebrospinal fluid; CSA, cross sectional area
<table>
<thead>
<tr>
<th>Suspected Diagnosis</th>
<th>When to biopsy?</th>
<th>When diagnosis can be made without biopsy</th>
<th>Performance of biopsy*</th>
<th>Other diagnostic tools</th>
</tr>
</thead>
</table>
| CIDP                | • Atypical phenotype  
   • Non-response to treatment | Most patients are diagnosed based on clinical presentation, neurophysiology and other diagnostic tools. | • Sensitivity: 71.4% in 1 study(144)  
   • EM: 79% sensitivity and 91% specificity(145)  
   • Teased fibre: 50% sensitivity and 83% specificity(145) | • Triple stimulation test: Very proximal conduction block  
   • Plexus MRI: hypertrophy/ enhancement  
   • Nerve US: Patchy nerve enlargement |
| Hereditary neuropathy | • NGS identifies variants of unknown significance  
   • Inconclusive initial genetic panel: To guide further genetic testing.  
   • Adult polyglucosan body disease: High index of suspicion despite negative genetic testing and GBE1 activity | Not required for diagnosis in most patients. | Certain findings are characteristic but not specific for particular neuropathies. These are reviewed elsewhere.(88) | • MRI in CMT: Can show diffuse, symmetrically enlarged nerves and plexus hypertrophy  
   • Nerve US: May help to discriminate axonal vs demyelinating CMT |
| Motor neuropathy vs MND | Unable to distinguish between MND and motor neuropathy despite thorough testing | If UMN dysfunction can be proven clinically or with ancillary tests like PET and TT-TMS | Overall sensitivity 95% in 1 study(28) | MR Neurography and US: Higher nerve CSA in MMN compared to MND |
| Cryptogenic neuropathy / Other aetiology | Following other aetiologies are suspected despite extensive inconclusive workup:  
   • Neuropathy due to certain toxins  
   • CMV induced neuropathy  
   • Diffuse infiltrative lymphocytosis syndrome isolated to nerves | Cryptogenic: Biopsy will not alter management for most patients. Other aetiologies: Usually diagnosed by other means. | Cryptogenic neuropathy: Yield of useful information is 0-37% (studies published 1990-2007)(14, 19-21) | - |

Table 5.3. Conditions in which nerve biopsies are of low importance

*Data regarding performance of nerve biopsy for individual disorders needs to be taken in the context of there being no true reference gold standard for many of these disorders.

CIDP, chronic inflammatory demyelinating polyneuropathy; US, ultrasound; MRI, magnetic resonance imaging; CMT, Charcot-Marie-Tooth; NCS, nerve conduction studies; EM, electron microscopy; AAN, American Academy of Neurology; PPV, positive predictive value; GBE1, glycogen branching enzyme 1; MND, motor neuron disease; UMN; upper motor neuron; EEG, electroencephalogram; TT-TMS, threshold tracking – transcranial magnetic stimulation; MMN, multifocal motor neuropathy; NGS, next generation sequencing; CSA, cross sectional area; CMV, cytomegalovirus
REFERENCES


