

Introduction

Peripheral nerve biopsy is utilised as a final step for the diagnosis of peripheral neuropathy in selected cases.^{1,2} The invasive procedure results in sensory deficit, and may result in chronic pain in a proportion of patients.^{1,3} Histopathological diagnosis is indicated for elusive diagnoses and particularly those in which it will have a major impact on clinical management such as neurolymphomatosis and nonsystemic vasculitic neuropathy (NSVN).^{2,4} However, histopathology results can be more non-specific than expected,⁵ with findings of non-specific axonal injury often noted.

There is a degree of inherent uncertainty in such results because of the patchy nature of the pathology in certain disorders, including NSVN.^{2,6} The sensitivity of nerve biopsy to establish a diagnosis of vasculitic neuropathy is 50-60%, highlighting the importance of multi-modal assessment for a final clinical diagnosis.⁷ Guidelines published by the Peripheral Nerve Society recognise the limited evidence to guide pre biopsy evaluation.¹ Clinical predictors of increased biopsy yield include stepwise clinical progression, sensory and motor symptoms, asymmetry with shorter disease duration, non-neurology referral, presence of serum anti-myeloperoxidase (MPO) antibody and rheumatoid factor seropositivity.^{8,9} The utility of MRI or ultrasound to increase diagnostic yield remains unclear.^{1,8}

A recent review provides an overview of high, medium and low importance indications for nerve biopsy.² In this context, we performed a quality improvement audit aimed at

assessing the pre-biopsy assessment of patients and characterise the group of patients with actionable diagnostic nerve biopsy results.

Materials and Methods

We interrogated our database of all nerve biopsies performed at Oxford University Hospitals NHS Foundation Trust, Oxford, UK between June 2010 and June 2021. The Trust provides a regional neurosciences centre for Oxfordshire, Berkshire (Thames Valley), Buckinghamshire and South Northamptonshire including several district general hospitals. Nerve biopsies were referred by peripheral nerve specialist neurologists, general neurologists and other specialists including rheumatologists, within the hospital network. Patients were excluded if they had been referred from an external hospital network, or if there was a lack of clinical referral information.

Clinical data

Clinical case notes were reviewed to obtain clinical, serological, CSF, imaging and electrophysiological data pre-biopsy. Pre-biopsy evaluation was undertaken as deemed appropriate by the referring clinician. The pattern of neuropathy was determined by the findings on clinical examination. Nerve conduction studies were performed using standard techniques, in more than one laboratory, and by a number of different neurophysiologists. Results were reviewed by two authors (PK and AT). Standardised MRI sequences including T1-weighted, T2-weighted, short-tau inversion recovery (STIR) and T1 post Gadolinium contrast were performed on either 1.5 or 3 Tesla MRI. Data regarding concurrent skin or muscle biopsy were recorded. Complications rates of nerve biopsy were recorded, however, data regarding post operative pain and sensory loss were not systematically recorded, and so are not included in this audit.

Pathological selection criteria for vasculitis

Diagnosis of definite or probable vasculitis was in accordance with the Peripheral Nerve Society classification.^{4,7} Clinical diagnosis of peripheral nerve vasculitis was categorised into groups of systemic vasculitic neuropathy (SVN) and non-systemic vasculitic neuropathy (NSVN) as defined by the review of NSVN and The Chapel Hill Consensus Conference 2012.⁴ Biopsies were analysed at a single centre by neuropathologists with specific expertise in nerve histopathology. Peripheral nerve tissue was processed using Standard Operating Procedures (SOPs) in the contact of an NHS UKAS-accredited diagnostic neuropathology laboratory. Formalin-fixed paraffin-embedded tissue (FFPE) for standard histochemical and immunohistochemical characterisation and epoxy resin-embedded tissue for semithin sections were available routinely for patients. Teased nerve fibre preparations and electron microscopy were performed on selected biopsies.

Pathological Grouping

Patients were grouped into two groups, either the diagnostic group or non-specific/normal group. The diagnostic group included patients with defined histopathology diagnosis including vasculitis (definite or probable), infiltrative pathology including lymphoma or amyloidosis, and granulomatous disease. The diagnostic group reflects conditions indicated to be of high or medium importance indication for nerve biopsy.² The non-specific/normal group included patients with acute and chronic axonal loss without a specific aetiology having been identified and patients with a normal biopsy result. The group level analysis focused on histopathology demonstrating axonal neuropathy, common to both diagnostic and non-specific/normal groups. The distinct clinical phenotype of demyelinating

neuropathy confounds comparison of clinical parameters if including in the group level analysis. Furthermore, demyelinating neuropathy is considered a low indication for nerve biopsy,² and histopathology did not reveal specific aetiology.

Data Analysis and Ethics

Statistical analysis was performed using GraphPad Prism 9.2.0 (GraphPad Software, San Diego, California) using Mann-Whitney rank sum test for age and duration of neuropathy as data were not normal distributed. Fisher's exact test were used for contingency table analysis given the small sample size, with Baptista-Pike method used for calculation of odds ratio (OR). A p-value less than 0.05 was considered statistically significant. Due to variation of pre-biopsy work up, the denominator for each parameter involved the number of patients for a given parameter. Reference range for elevated results included white cell count $> 11 \times 10^9/L$, hyper-eosinophilia $> 1.5 \times 10^9/L$, erythrocyte sedimentation rate (ESR) $> 20 \text{ mm/h}$ and antinuclear antibodies (ANA) $\geq 1:160$. Anaemia was defined by haemoglobin level in male $< 130 \text{ g/L}$ and females $< 120 \text{ g/L}$. The clinical quality improvement activity was registered with the Oxford University Hospitals NHS Foundation Trust Audit and Quality Improvement Team (Number 7257).

Results

Sixty-six patients underwent nerve biopsy during the study period. Two patients were excluded due to lack of clinical data availability. Nerve biopsy site comprised of sural nerve 75.4%, superficial peroneal nerve 13.8%, superficial sensory radial nerve 4.6%, sciatic nerve 1.5%, anterior interosseus nerve 1.5%, intermediate cutaneous nerve of thigh 1.5% and posterior interosseus nerve 1.5%. Concurrent muscle biopsy was performed in 21 cases (32.8%) and skin biopsy in 8 cases (12.5%). Muscle biopsy provided definitive diagnosis of vasculitis in one patient and AL amyloidosis in another (4.8%), both results were concordant with the nerve biopsy result. Skin biopsy provided a concordant diagnosis to the nerve histopathology in two cases (one each of lymphoma and vasculitis).

Electron microscopy was performed in 53.2% of total cases. Patients in the non-specific/normal group had electron microscopy performed in 43.3% of patients. Considering the pre-biopsy referral diagnoses in the non-specific/normal group, electron microscopy was performed 38.8% of vasculitis referrals, 50% neurolymphomatosis, 60% of amyloidosis and none of the sarcoidosis. All results on electron microscopy were concordant with initial the histopathology, except for a single case with AL amyloidosis diagnosed post electron microscopy.

The final histopathology diagnosis is presented in Figure 1. The diagnostic group comprised of 21 patients, including definite vasculitis (six patients), probable vasculitis (nine patients), neurolymphomatosis (four patients), neurosarcoidosis (one patient) and AL amyloidosis

(one patient). SVN comprised of 60% of histopathological diagnosed vasculitis, including four with definite vasculitis and five with probable vasculitis. The non-specific/normal group comprised of 30 patients, including normal biopsy result (five patients), acute axonal loss (15 patients) and chronic axonal loss (ten patients).

Patients excluded from group level analysis comprised of ten patients with demyelinating pathology, two with inadequate nerve tissue for definitive histopathological opinion and one patient with histopathology features supportive of the pre-biopsy clinical diagnosis of POEMS. The ten patients with demyelinating histopathological diagnoses all had features of demyelination on nerve condition studies prior to the biopsy. Seven patients had the provisional diagnosis of CIDP prior to biopsy, with the diagnosis remaining unchanged post biopsy. The biopsy was undertaken in this group due to clinical referral information of inadequate response to treatment for CIDP and assessment of alternative aetiology such as hereditary neuropathy or para-proteinemic related neuropathy. One other patient had a pre-biopsy clinical diagnosed immune-mediated inflammatory polyneuropathy overlapping with known CMT which was confirmed on biopsy. Two patients had speculative biopsy in context of concurrent CNS features and demyelinating neuropathy evaluating for hereditary neuropathy.

Referral Diagnoses

The referral clinical diagnosis was concordant with the histopathological diagnosis in 76.2% of patients in the diagnostic group. Patients with a histopathological diagnosis of vasculitis

had a referral diagnosis of vasculitis in 93.3% of patients. All six patients with definite vasculitis had a clinical referral for vasculitis. Eight of the nine patients with probable vasculitis had a clinical referral for vasculitis, and one for paraneoplastic neuropathy. The subgroup of patients within the diagnostic group with neurolymphomatosis (four patients), AL amyloidosis (one patient) and neurosarcoidosis (one patient) had lower concordance with prebiopsy clinical diagnosis of 33.3%. The non-specific/normal group clinical referral indication consisted of vasculitis (60%), neurolymphomatosis (20%), amyloidosis (16.7%) and sarcoidosis (3.7%).

Pre-Biopsy Assessment

Clinical Predictors of Diagnostic Group

Clinical predictors of the diagnostic group are summarised in table one, with all parameters assessed in the supplement (supplement table one).

History and Examination Features

Patients from the diagnostic group had a median age of 64 years (S.D. 17.1) with 61.9% female, which did not differ from the non-specific/normal group (median age 69 years, $p=0.180$; female 43.3%, $p=0.351$). The diagnostic group had a shorter duration of neuropathy (median 3 vs 12 months, $p=0.006$), more often had a history of stepwise clinical progression (81% vs 20%, OR 15.6 (95% CI 3.5-51.7)) and neuropathic pain (85.7% vs 56.7%, OR 4.6 (95% CI 1.2-16.7)). In regards to examination findings, the diagnostic group more frequently had features of asymmetric neuropathy (90.5% vs 60%, OR 5.3 (95% CI 1.1-25.9)), vasculitic rash (23.8% vs 0%, $p=0.009$) or mononeuritis multiplex (57.1% vs 10%, OR 13.5 (95% CI 3.2-

50.1)). Patients from the diagnostic group tended to have more motor weakness (71.4 vs 50%, $p=0.156$), and systemic features (57.1% vs 36.7%, $p=0.167$). There was no difference in the presence of cranial neuropathy (23.8% vs 16.7, $p=0.722$) or autonomic neuropathy (4.8% vs 6.7%). CNS presenting features including upper motor neuron weakness, seizures and stroke were more often present in non-specific/normal group (23.3% vs 4.8%, $p=0.119$). One patient in diagnostic group with SVN due to eosinophilic granulomatosis with polyangiitis also presented with stroke. The subgroup of normal biopsies had significantly more CNS presenting features compared to the diagnostic group (80% vs 4.76%, OR 114, CI 8.5-1382)). Non-neurology referral source occurred in 13.7% of patients, with no difference between groups (diagnostic group 19.1% vs 10%, $p=0.427$).

Laboratory Findings

The diagnostic group had significantly higher elevated white cell counts (47.6% vs 16.7%, OR 4.5 (95% CI 1.3-15.6)) and ANCA with positive MPO titre results (19.1 vs 0%, $p=0.0249$). Other investigations including presence of anaemia (diagnostic group 33.3% vs non-specific/normal group 30%, $p>0.999$), elevated ESR (50% vs 37.9%, $p=0.401$), positive ANA (23.8% vs 8%, $p=0.249$), positive rheumatoid factor (23.5% vs 13%, $p=0.432$), and hyper-eosinophilia (14.3% vs 3.3%, $p=0.293$) did not differ significantly between groups. CSF analysis was conducted in 52.4% of the diagnostic group and 86.7% of the non-specific/normal group. Raised CSF protein count (diagnostic group 63.6% vs 57.7%, $p>0.999$), presence of a raised CSF lymphocyte count (18.2% vs 11.5%, $p=0.623$), and matched oligoclonal bands (44.4% vs 40%, $p>0.999$) did not differ between groups. Other positive

test results included cryoglobulinemia in one patient with definite vasculitis, and a positive ENA in one patient with probable vasculitis.

Neurophysiology

The majority of patients had axonal neuropathy features on nerve conduction studies (diagnostic group 85.0% vs non-specific/normal group 66.7%, $p=0.191$). The diagnostic group included three patients (15%) with axonal neuropathy with additional superadded demyelinating features (conduction slowing and/or prolonged F waves). Histopathological diagnoses from this subgroup of axonal neuropathy with superadded demyelinating features included neurolymphomatosis, neurosarcoidosis and amyloidosis.

Neurophysiological evidence of demyelinating neuropathy did not occur in the diagnostic group compared with 14.8% in the non-specific axonal/normal group ($p=0.131$).

Neurophysiology of the biopsied nerve showed absent or reduced sensory nerve action potentials (SNAP) in the majority of patients (diagnostic group 87.5% vs non-specific/normal group 100%, $p=0.146$). Normal sensory nerve action potential in biopsied nerve was present in the diagnostic group in 12.5% (one histopathological diagnosis of probable vasculitis and one neurolymphomatosis), and none in non-specific/normal group. The time between neurophysiology test and biopsy in these cases were one month and four months respectively.

Peripheral Nerve Imaging: MRI or Ultrasound

Imaging was performed in 41.2% of patients, with MRI of peripheral nerve or plexus in the vast majority (95.2%). Brachial plexus imaging was performed in 42.9%, lumbar plexus imaging 38.1%, lower limb peripheral nerve 14.3%, and upper limb peripheral nerve 9.5%. Abnormal imaging findings included focal nerve swelling, thickening and/or enhancement which was significantly higher in the diagnostic group (77.8% vs 25%, OR 10.5 (95% CI 1.2-62.7)). Abnormal imaging findings were proximal to the site of nerve biopsied in all cases.

Complications of nerve biopsy

Nerve biopsy resulted in wound infection in 3.1 % of the total cohort, including one requiring surgical intervention for wound dehiscence. There was inadequate nerve for complete histopathological analysis in 3.1% of patients.

Discussion

The diagnostic group had a tendency to be female and elderly, in keeping with previous studies in vasculitic neuropathy.^{8,12,13} Patients with normal biopsy results were associated with central nervous system features at presentation such as seizure, stroke, reduced conscious state and upper motor neuron weakness. Several clinical parameters that were associated with a specific histopathological aetiology are concordant with previous studies in peripheral nerve vasculitis.^{8,9,12,13} Features of history and examination were of significant value, including stepwise clinical progression, shorter duration of neuropathy, neuropathic pain, mononeuritis multiplex and asymmetry pattern of neuropathy. Our study did differ in finding vasculitic rash, an elevation of white cell count but not elevated ESR, and peripheral imaging abnormalities were associated with specific histopathological diagnosis. We did not find an association of non-neurology referral which has been demonstrated previously.⁸

Multi-modal assessment is essential in this patient cohort due to limitations in the sensitivity of nerve biopsy, previously reported to be 50-60% in vasculitic neuropathy.⁷ Patients with non-specific/normal results does not exclude important treatable diagnoses. Evaluation of the diagnostic group in our quality improvement project provided insights into pre-biopsy evaluation and patient characteristics that had actionable histopathology results.

The diagnostic group had concordant pre-biopsy referral diagnosis in 76.2% of patients. The concordance was much lower in neurolymphomatous, amyloidosis or granulomatous diagnoses compared to vasculitis. These are often elusive diagnosis, with significant value of a diagnostic nerve biopsy. The presence of serum markers may contribute to the higher rate of concordant provisional diagnosis in systemic vasculitis. Nathani and colleagues published

a clinical flow chart to guide the decision to biopsy has predominantly systemic vasculitis markers as the strong positive predictor indication to biopsy in vasculitic neuropathy.⁸

Future studies should consider larger cohorts with specific sub-populations of elusive diagnoses including NSVN, neurolymphomatosis, sarcoidosis and amyloidosis.

The role of imaging as a clinical parameter to predict certain nerve pathologies remains uncertain.^{1,8} Nathani and colleagues study examining clinical predictors of nerve vasculitis was unable to determine the value of peripheral nerve MRI due to a small minority of patients undergoing the investigation.⁸ MRI has been utilised in previous case series to guide selective fascicular plexus or proximal motor nerve biopsy.^{6,15} The current study did not show that imaging guided the site of biopsy. However, it did indicate that there may be a role in peripheral nerve imaging to improve biopsy yield, even when the biopsy site is distal to the imaging findings. The presence of imaging findings may represent a more active pathological process that associates with an increase the yield of specific histopathological diagnosis. Further prospective studies are required to utilise imaging to improve the decision to proceed with biopsy and to guide selection of the biopsy site. Sonographic enlargement of nerves in the upper limb proximal to compressible sites with sparing of the brachial plexus is indicative of vasculitic neuropathy.¹⁶ Standard MRI sequences were utilised in this study. Advanced MRI imaging techniques such as diffusion tensor imaging may be more informative.^{17,18}

The clinical audit provided valuable insights to our peripheral nerve biopsy patient population and instigated quality improvement measures to improve patient management (refer to Box 1).

- Consider referral to nerve specialist clinic prior to nerve biopsy;

- Nerve biopsy request form that includes essential clinical parameters pre-biopsy;
- Assessment of target nerve with multimodal assessment including clinical, nerve imaging (MRI or Ultrasound) and neurophysiology testing followed by multidisciplinary discussion with neurologist, neurophysiologist, imaging expert and surgeon to target selected nerve;
- Consider nerve biopsy in patients with clinical parameters associated with a diagnostic histopathological result;
- Speculative peripheral nerve biopsy in CNS disorders is not indicated;
- Consider electron microscopy when the initial histopathological evaluation does not confirm a diagnosis.

Normal biopsy results are relatively uncommon but may be avoidable as a significant number of these patients had presented with features of a CNS disorder. Reviewing the notes these had often been requested on a more speculative basis of multi-system involvement (for instance angiocentric lymphoma). Clinical review in a sub-speciality nerve specialist clinic may aide in reducing the frequency of such speculative biopsies.

The study had several limitations including limited patient numbers and the retrospective nature of the data. Pre-biopsy work up was conducted across many hospital sites by various clinicians, and data was gathered from clinical notes. Complication rates may be underestimated due to lack of collection of data regarding complications of sensory loss or pain over the long term. The results of this quality improvement project have not been externally validated to other patient cohorts.

In conclusion, careful consideration is required to focus nerve biopsy to those individuals in which it is most likely to impact clinical management. Our study highlights several important clinical parameters to improve diagnostic yield, including peripheral nerve imaging. Current practices suggest that in a significant proportion of patients the nerve biopsy findings will have significant impact on the final clinical diagnosis. However, several quality improvement measures are proposed to enhance multi-modal evaluation in our cohort. Further prospective studies are required to understand the utility of peripheral nerve imaging before a decision is made to proceed with nerve biopsy and target site.

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Figure Legends

Figure 1: cases presented as per histopathological diagnostic category of diagnostic group, non-specific/normal group and excluded.

Figure 2: visualisation of the binary classification tree with each node showing a fitted class and the associated probability (expressed as a percentage).

Tables

Table one: Clinical parameters with significant difference between diagnostic group and non-specific/normal group, expressed as total (%) unless stated.

	Diagnostic group n=21	Non-specific/ normal group n=30	Statistical Analysis
Duration Neuropathy Months (Median, SD)	3 (15.8)	12 (54.6)	p= 0.006 *
Stepwise History	17 (81.0)	6 (20.0)	OR 15.6 (95% CI 3.5-51.7) **
Neuropathic Pain	18 (85.7)	17 (56.7)	OR 4.6 (95% CI 1.2-16.7) **
Vasculitic Rash	5 (23.8)	0	p= 0.009 **
Asymmetrical Neuropathy	19 (90.5)	18 (60.0)	OR 5.3 (95% CI 1.1-25.9) **
Mononeuritis Multiplex	12 (57.1)	3 (10.0)	OR 13.5 (95% CI 3.2-50.1) **
Elevated White Cell Count	10 (47.6)	5 (16.7)	OR 4.5 (95% CI 1.3-15.6) **
ANCA positive with positive MPO titre	4 (19.1)	0	p= 0.0249 **
Abnormal Imaging Plexus or Peripheral Nerve	7 (77.8)	3 (25)	OR 10.5 (95% CI 1.2-62.7) **

* Mann-Whitney test ** Fisher's exact test