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RESEARCH ARTICLE

High Resolution Nerve Ultrasound in the Diagnosis of Pure Neuritic Leprosy

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ABSTRACT

Objectives: We aimed to compare the clinical, electrophysiologic, and sonographic characteristics of commonly affected peripheral nerves in suspected cases of pure neuritic leprosy (PNL) and to describe an ultrasound-guided fine needle aspiration cytology (FNAC) procedure to improve diagnostic capabilities in PNL.

Methods: This retrospective study was carried out at Roy Neuro Care Centre, Ranchi, Jharkhand, India through revisiting charts of suspected cases of PNL from October 2022 to June 2024. The charts of individuals with a history, examination, and nerve ultrasound consistent with PNL were reviewed. We retrospectively analyzed the diagnostic steps, including the skin biopsy (which had been guided by electrophysiology and/or nerve ultrasound) and the ultrasound-guided FNAC of nerves that had been sent for histopathological examination, acid-fast bacilli (AFB) staining, and polymerase chain reaction (PCR).

Results: Charts of 52 patients derived from the records of those with mononeuropathy or mononeuritis multiplex suspected to have PNL during October 2022–June 2024 were evaluated. The skin biopsy was diagnostic of PNL in 23 patients, while 29 patients had an inconclusive result. Those with an inconclusive result had an ultrasound-guided FNAC followed by cytology and AFB staining. The review of the records of these cases revealed that in 24 (82.8%) cases there were features suggestive of PNL, while 5 patients had negative cytology and AFB staining. Three of these individuals had PCR, with a positive result in 1 (33.3%). A positive correlation was noted between the duration of illness and markers of progressive involvement of nerves, as determined by electrodiagnosis and ultrasound.

Conclusion: Nerve ultrasound and ultrasound-guided nerve FNAC can assist in the early diagnosis of PNL.

1 | Introduction

Leprosy is a disease of peripheral nerves and the skin having the longest incubation period among all communicable diseases and is difficult to detect and eliminate. Of all the variants, the least common is pure neuritic leprosy (PNL), in which the absence of skin lesions has been most commonly reported from the Indian subcontinent, and there has

been a rising proportion of cases of this subtype (Khadilkar et al. 2021). Nerve damage in PNL ultimately leads to deformities, so early diagnosis is important.

Leprosy continues to be an important burden on the health care system in India (Shah et al. 2021), and there are districts where leprosy is still endemic (Sharma and Singh 2022). An important aspect contributing to this high prevalence could be the underdetection of PNL, where diagnosis continues to be a challenge due to the absence of skin lesions, low sensitivity of conventional methods of diagnosis, paucity of effective bedside tools for suspicion, and lack of diagnostic paradigms (Khadilkar et al. 2021). Furthermore, nerve biopsy, which is the gold standard investigation of PNL, is not widely available at all centers and is invasive and painful.

In this context, high resolution ultrasound (HRUS), an investigative modality to assess peripheral nerves, has gained popularity. HRUS features of nerve involvement in leprosy include increased cross-sectional area (CSA) indicating nerve enlargement; increased blood flow (vascularity), a sign of inflammation; and changes in echo texture and fascicular architecture of the nerves (Razdan et al. 2024). Additionally, HRUS can also help guide the site of biopsy of the skin according to the distribution of the nerve involved (Jain et al. 2009). HRUS also further extends its spectrum through ultrasound-guided fine needle aspiration cytology (FNAC) of nerves for acid-fast bacilli (AFB) detection and histopathological changes of PNL. This minimally invasive method is more advantageous than an open biopsy, which may have sampling error, low sensitivity, and permanent nerve deficit (Jain et al. 2009; Jena et al. 2022). However, only a few studies have evaluated ultrasound-guided FNAC as a diagnostic tool in PNL (Jena et al. 2022). Hence, this study was conducted to determine if combining HRUS of nerves with ultrasound-guided FNAC can assist in the diagnosis of PNL.

2 | Material and Methods

This retrospective study was conducted at the Neurology Outpatient Department of Roy Neuro Care Centre, Ranchi, Jharkhand, India (a specialized neurology referral center) through revisiting charts of suspected cases of PNL from October 2022 to June 2024. We obtained clearance from our institutional ethical committee prior to the study, and all participants had signed informed consent forms. Individuals with symptoms and nerve ultrasound consistent with PNL had their charts reviewed. Symptoms deemed consistent with PNL were numbness, tingling, anhidrosis, painless wounds, anesthesia in any part without skin lesion, paresthesias, hypoesthesia, patchy motor deficit, sensory loss, wasting, or weakness. Those with thickening of nerve(s) by palpation had been noted, which included the greater auricular, ulnar just above the elbow, dorsal cutaneous branch of the ulnar at the wrist, median and superficial radial in the forearm, common fibular near the neck of the fibula, superficial fibular at the distal lateral leg, posterior tibial near the medial malleolus, and sural nerves posterior to the lateral malleolus. Charts of detailed nerve conduction studies were reviewed, including various nerves of the upper and lower limbs, and a diagnosis of mononeuropathy had been considered when a single nerve trunk was affected, multiple mononeuropathy with patchy involvement of more than one nerve territory, and polyneuropathy if symmetric involvement of sensory or motor nerves was present. The type of nerve involvement was classified as sensory, sensory-motor, or motor, while the pattern was classified as axonal, demyelinating, or mixed. Irrespective of results of electrophysiology, HRUS with a Venue Go R4 (GE HealthCare, USA) and a 4–20 MHz linear transducer had been used to scan nerves. This included the brachial plexus, greater auricular, ulnar, median, superficial radial, common fibular, superficial fibular, posterior tibial, and sural nerves.

Different parameters and characteristics of all nerves had been noted by examining in both longitudinal and transverse axes on both sides. A nerve was classified as abnormal based on these parameters:

2.1 | Enlargement of Nerves

After scanning above-mentioned nerves in the whole length, it was determined whether there was enlargement of nerve CSA (in axial plane) at any point in the nerve with reference to a standard normative data (Sindhu et al. 2022; Bae and An 2022; Curcean et al. 2020; Wu et al. 2018; Won et al. 2012) and was noted in the charts.

2.2 | Site of Enlargement

The site/extent of enlargement of each nerve along with the site of maximum enlargement had been noted with reference to fixed anatomical landmarks.

2.3 | Length of Enlargement

The total length of enlargement was noted in centimeters (cm) for all nerves except for the upper trunk of the brachial plexus and the suprascapular nerve (difficult to measure accurately).

2.4 | Pattern of Enlargement and Size of the Nerve

The pattern of nerve enlargement was categorized as monofocal (when a single segment of the nerve less than 5 cm was enlarged), multifocal (when multiple segments of the nerve in its length were enlarged), or diffuse (when enlargement of the nerve was diffuse and length of enlargement was more than 5 cm). CSA had been measured in mm^2 and was traced within the inner margin of the hyperechoic rim on transverse sections and recorded at a point where the value was maximum.

2.5 | Echogenicity

We had analyzed our images with the open-source software ImageJ (National Institutes of Health, Bethesda, MD, version 1.5.1). A gray-scale histogram was used to assess the entire nerve. Nerves were thereby graded as hypoechogenic

(proportion of black above 67%), hyperechogenic (proportion of black below 33%) and normal (Fisse et al. 2019; Gamber et al. 2020).

Vascularity: Increased blood flow (epineural or intra-fascicular blood flow) had been determined in all the nerves scanned using color Doppler.

2.6 | Epineural Thickening/Fibrosis

Presence or absence of epineural thickening and/or fibrosis (hyperechogenicity) was noted.

2.7 | Abscess

Presence or absence of abscess formation had been noted.

Blood investigations had been performed in the department of pathology to rule out possible alternative etiologies of nerve enlargement and included a complete blood count, HbA1c, thyroid function tests, rheumatoid factor, anti-nuclear antibody, and other relevant tests.

2.8 | Exclusion Criteria

We had excluded patients with other conditions that could cause enlargement of one or multiple nerves on the basis of clinical history, symptoms, signs on examination, laboratory investigations, electrophysiology, and ultrasonography characteristics as listed in Figure 1.

According to the charts, 52 patients were identified in the given time frame who had a history and examination consistent with PNL and also had enlargement of at least one nerve out of the 18 nerves examined after excluding the patients with the above disorders (Figure 1). The records revealed that in all cases, an area of skin had been biopsied where paresthesia, hypoesthesia, numbness, or muscle wasting was present and which also matched with electrophysiologic findings and nerve enlargement on HRUS. In cases where there were multiple such areas, biopsy was obtained from the site that had sensory supply from an enlarged nerve on HRUS and/or abnormal results on electrophysiological studies. Hematoxylin and eosin (H & E) and Modified Ziehl Neelsen (ZN) stained sections of all cases were reviewed.

FNAC from an enlarged sensory nerve on HRUS had been obtained and results documented in the hospital chart. These nerves included the dorsal cutaneous branch of ulnar, superficial radial, greater auricular, superficial fibular, and sural. If enlargement was noted in a motor nerve only, the corresponding sensory branch was chosen for FNAC. If the FNAC of the corresponding sensory nerve was negative, FNAC was performed again on the motor nerve. FNAC was performed using real-time visualization by HRUS guidance, and the most enlarged fascicle was chosen for aspiration. FNAC had been performed according to standard procedure (Vijaikumar et al. 2001) and dry and wet smears had been prepared. Dry smears were stained with H & E stain, Leishman Giemsa, and modified ZN stain (to demonstrate AFB leprae), and the wet smear was stained by Papanicolaou as per standard protocol (Sandhu et al. 2021). All these smears had been studied for cytological details in the department of histopathology. Patients were assessed for any loss of motor function after the procedure. Cytological aspirates were classified as proposed by Vijaikumar et al. (2001) into tuberculoid tuberculoid (TT)/borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL), and lepromatous (LL) types, and further, BT/TT aspirates were classified as paucibacillary (PB) or multibacillary (MB) subtypes, as per standard convention (Ridley and Jopling 1966). The records showed that in patients for whom there were inconclusive or negative results on examination for cellular structure and AFB with modified ZN stain in the FNAC aspirate, polymerase chain reaction (PCR) was planned on the same specimen (Figure 1). The specimen was dissolved in normal saline, meticulously sealed and labeled by the technician, and sent overnight under strict cold chain system to The Leprosy Mission Trust India, SBR Research Lab, TML Hospital, New Delhi. Genomic DNA had been extracted and processed using a DNeasy blood and tissue kit (Qiagen, Hiden, Germany) (Qiagen 2019) followed by *M. leprae*-specific repetitive element (RLEP) PCR amplification according to standard protocol (Pathak et al. 2019).

The charts showed that treatment was started based on classification into paucibacillary and multibacillary according to the number of nerves involved on HRUS and histopathology, whichever was higher, and responses were noted at 3 and 6 months based on relief from symptoms. Participants were categorized into satisfactory symptomatic improvement and unsatisfactory response at 6 months. Patients who were lost to follow up were kept under the category "unknown."

3 | Statistical Analyses

The data collected from the above-mentioned charts were organized and tabulated using Microsoft Excel and analyzed using JAMOVI software. The normality of the data was checked using the Shapiro–Wilk test. Categorical variables were presented in frequencies and percentages, while continuous variables were summarized as median and interquartile range (IQR). The correlation between two continuous outcome variables was evaluated using Spearman's rho coefficient (r) whereas the association between a continuous (independent variable-duration of symptom) and a categorical variable (dependent variables-nerve enlargement, hypo echogenicity and Epineural Thickening) was evaluated using binomial logistic regression.

4 | Results

4.1 | Demographic Characteristics

Mean age noted in our study was 38.2 ± 11.3 years and mean duration of symptoms was 13.8 ± 6.4 months. Although sensory symptoms were present in all 52 individuals, motor weakness was only present in 26.9% (Table 1). Single nerve involvement on HRUS (mononeuropathy) was noted in 36.5%, compared to 63.5% cases of mononeuritis multiplex.

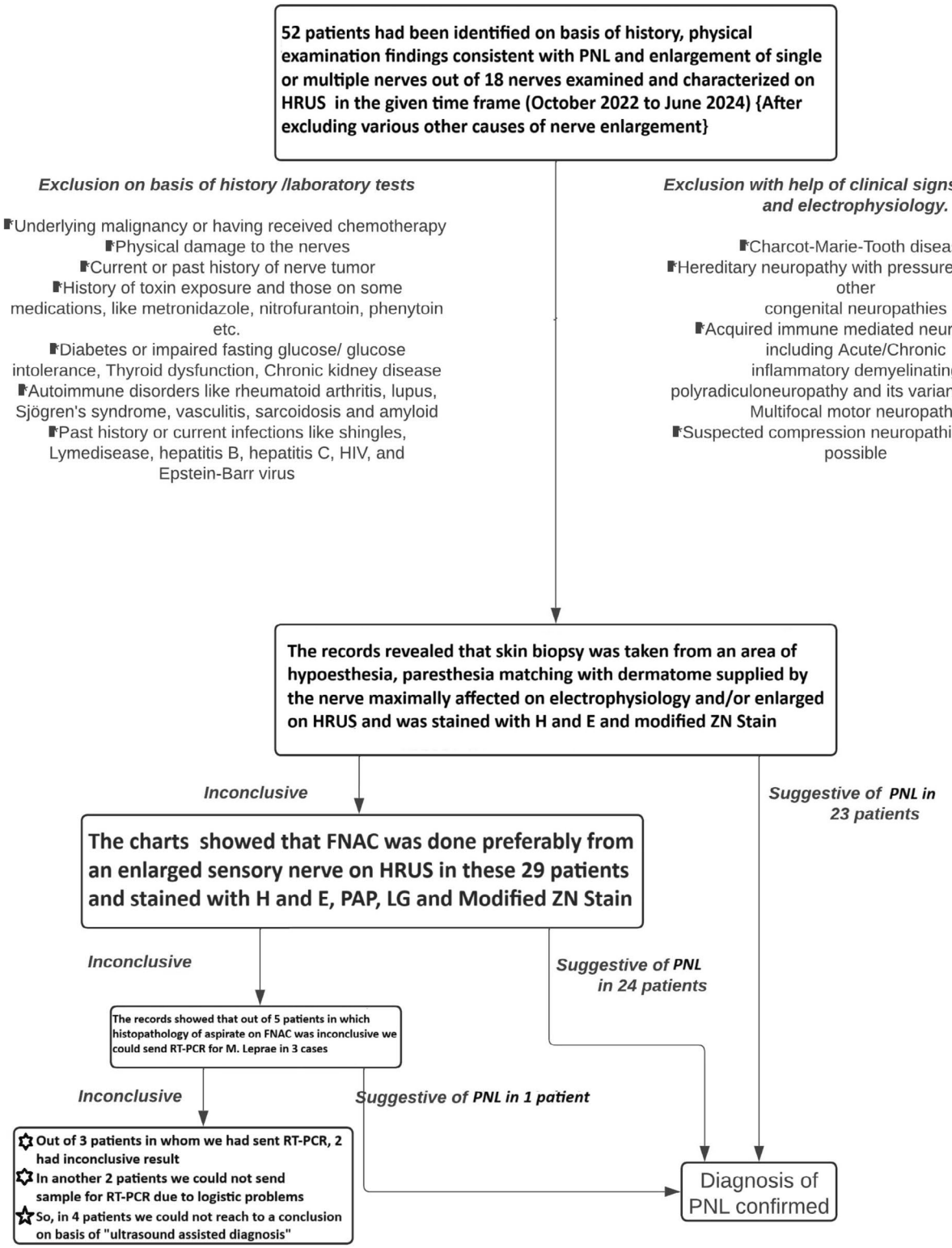


FIGURE 1 | Flow diagram of steps taken to reach a conclusive diagnosis of PNL in 52 suspected cases, which was analyzed retrospectively.

4.2 | Ultrasound Characteristics and Pattern of Nerve Enlargement

We had examined 936 nerves with HRUS in 52 patients, out of which 16.8% nerves were enlarged (Table 2). This enlargement was asymmetric in all nerves throughout the study. Examples of nerve ultrasound of the affected nerves are displayed in Videos 1–5 and examples of FNAC in Video 6.

Out of 104 ulnar nerves examined, 41.4% were enlarged. In 81.4% of cases, enlargement was near the epicondyle followed by near the wrist 18.6% (Table 2). The mean distance of maximum

TABLE 1 | Shows participant demographics in the study.

Variables	Result
Mean age in years ($N=52$)	38.2 (STD 11.3)
Male sex ($N=52$)	61.5%
Mean BMI ($N=52$)	20.6 (STD 3.7)
Mean duration of symptoms in months ($N=52$)	13.8 (STD 6.4)
Paresthesias/Sensory symptoms present ($N=52$)	100%
Motor weakness present ($N=52$)	26.9%
Thickened nerves by palpation ($N=52$)	28.8%
Electrodiagnostic abnormalities ($N=52$)	96.1%
HRUS with multiple nerves involved	63.5%
HRUS with single nerve involved	36.5%
Skin biopsy abnormal ($N=52$)	44.2%
FNAC of nerve is abnormal by cytology + AFB staining ($N=29$)	82.8%
FNAC of nerve is abnormal by PCR ($N=3$)	33.3%
Responsive to treatment ($N=48$)	Satisfactory symptomatic Improvement = 81.3% Unsatisfactory response = 14.6% Not Known = 4.2%

Abbreviations: AFB, acid fast bacilli; BMI, body mass index; FNAC, fine needle aspiration cytology; HRUS, high resolution ultrasound; PCR, polymerase chain reaction; STD, standard deviation.

enlargement of the ulnar nerve from the medial epicondyle was 3.62 ± 1.0 cm. Monofocal enlargement (Video 1) was noted in 55.8% of cases, multifocal (Video 1) in 25.6%, and diffuse in 18.6%. In 1.9% of cases, we also observed an abscess of the ulnar nerve (Video 2). In most cases, median nerve enlargement was near the pronator quadratus muscle (92.9%) (Video 3), followed by near the forearm (7.1%). For all 17 cases of enlargement of the superficial radial nerve, the sole site of enlargement was near the wrist (Video 3). Similarly, the maximum cases of enlargement of the common fibular nerve and posterior tibial nerve were near the fibular neck (93.3%) (Video 4) and the medial malleolus (Video 4), respectively. In all 12 cases where we noted enlargement of the superficial fibular nerve, it was within 10 cm of the lateral malleolus (Video 4). In the cases of multifocal enlargement, the nerve was most commonly noted to be enlarged distal to the area where it emerges from the fascial plane between the fibular and extensor digitorum longus muscles to pierce the crural fascia, and also just 1–2 cm before it bifurcates into medial dorsal cutaneous and intermediate dorsal cutaneous branches. In all 4 cases of enlargement of the greater auricular nerve, it was noted near the mid neck (Video 5). Complete details of characteristics and patterns of enlargement of various nerves are in Table 2.

4.3 | Ultrasound Findings of Intrinsic Nerve Changes and Comparison of These Findings in the MB and PB Subgroups

Increased CSA among ulnar nerves was present in 53.6% of the cases in the MB subtype as opposed to only 26.3% of cases in the PB category (Table 3). Hypoechogenicity was also noted among 51.8% of the cases in the MB and 26.3% of cases in the PB subgroup. Similarly, epineural thickening and increased vascularity (Figure 2) were noted more frequently in the MB subgroup than in the PB category. Other details of HRUS findings of intrinsic nerve changes and their comparison among MB and PB subgroups are listed in Table 3.

4.4 | Skin Biopsy

The charts showed that after skin biopsy, 47.8% of cases had been classified as PB (TT/BT) and 52.2% of cases as MB (BB = 9; BL = 2; LL = 1).

4.5 | Ultrasound Guided FNAC of Nerves in Reaching Diagnosis of PNL

On analysis of the records of histopathological evaluation of the FNAC samples, 82.8% cases had been diagnosed as PNL and were classified into PB (41.7%) and MB (58.3%) subtypes (Table S1). PB was synonymous with BT/TT subtype, and MB comprised 12 cases of BB, 1 case of BL, and 1 case of LL. Histopathological findings on FNAC in various subgroups are enlisted in Table S1.

4.6 | PCR of FNAC for *M. leprae*

The charts also showed that PCR could be performed on only three samples, out of which we had observed positivity for the repetitive element RLEP in one. Further records revealed that we had performed nerve biopsy in 4 cases where these initial investigative modalities were inconclusive, one of which was consistent with PNL and another with probable vasculitic neuropathy. In 2 cases, no histologic abnormality could be identified.

4.7 | Treatment Response

81.3% of patients had a symptomatic improvement to treatment (Table 1). All the 14.6% cases in which there was an unsatisfactory response were from the MB subgroup.

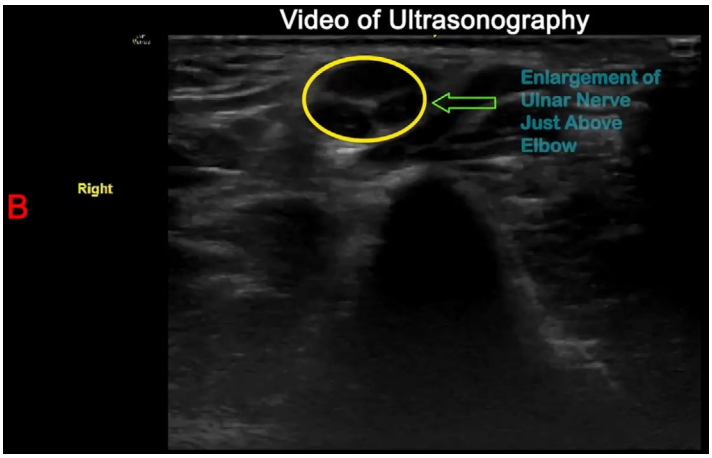
TABLE 2 | Shows ultrasound characteristics of nerve enlargement of various nerves in the study.

Nerve	Number of nerves enlarged	Site of maximal enlargement	Focality	CSA in mm^2	Total length of enlargement in cm
***N* = 104**	***N* (%)**	***N* (%)**	***N* (%)**	**Median Q2 (IQR)**	**Median Q2 (IQR)**
Ulnar	43 (41.4)	Near epicondyle = 35 (81.4) Near wrist = 8 (18.6) Forearm = 0 (0) Arm = 0 (0)	Monofocal = 24 (55.8) Multifocal = 11 (25.6) Diffuse = 8 (18.6)	9.00 (8.25)	10.00 (4.50)
Median	28 (26.9)	Near Pronator quadratus = 26 (92.9) Forearm = 2 (7.1) Carpal tunnel = 0 (0) Arm = 0 (0) Near elbow = 0 (0)	Monofocal = 17 (60.7) Multifocal = 7 (25) Diffuse = 4 (14.3)	9.00 (4.00)	6.50 (8.75)
Superficial Radial	17 (16.4)	Near wrist = 17 (100) Near elbow = 0 (0)	Monofocal = 9 (52.9) Multifocal = 0 (0) Diffuse = 8 (47.1)	2.00 (2.00)	8.00 (2.00)
Common Fibular	30 (28.9)	Near fibular neck = 28 (93.3) Near popliteal fossa = 2 (6.7)	Monofocal = 27 (90) Multifocal = 1 (3.3) Diffuse = 2 (6.7)	12.00 (7.00)	6.00 (2.00)
Posterior Tibial	7 (6.7)	Near medial malleolus = 7 (100) Other = 0 (0)	Monofocal = 7 (100) Multifocal = 0 (0) Diffuse = 0 (0)	11.00 (3.00)	3.50 (1.00)
Sural	16 (15.4)	Near lateral malleolus = 14 (87.5) 5 cm proximal to lateral malleolus = 2 (12.5)	Monofocal = 9 (56.3) Multifocal = 3 (18.8) Diffuse = 4 (25)	2.00 (1.00)	4.00 (1.75)
Greater Auricular	4 (3.9)	Mid Neck = 4 (100) Near Occiput = 0 (0)	Monofocal = 2 (50) Multifocal = 1 (25) Diffuse = 1 (25)	1.00 (1.00)	6.50 (1.75)
Superficial Fibular	12 (11.5)	Within 10 cm of lateral malleolus = 12 (100) Above 10 cm = 0 (0)	Monofocal = 7 (58.3) Multifocal = 4 (33.3) Diffuse = 1 (8.3)	2.00 (1.00)	4.50 (3.50)
Brachial Plexus	5 (4.8) SSN alone = 0 (0) UT alone =4 (80) Both = 1 (20)	N/A	Monofocal = 5 (100) Multifocal = 0 (0) Diffuse = 0 (0)	SSN = 2.00 (1.00) UT = 9.00 (2.00)	N/A

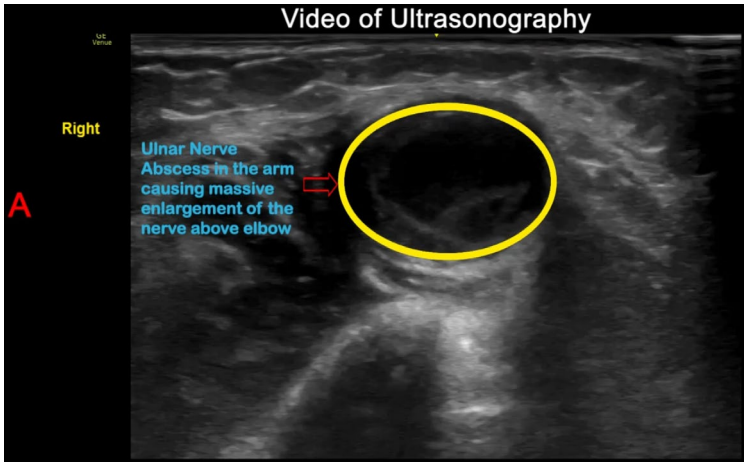
Abbreviations: CSA, cross sectional area; IQR, inter quartile range; Q2, 2nd quartile; SSN, suprascapular nerve; STD, standard deviation; UT, upper trunk.

4.8 | Correlation Between Different Parameters of the Study

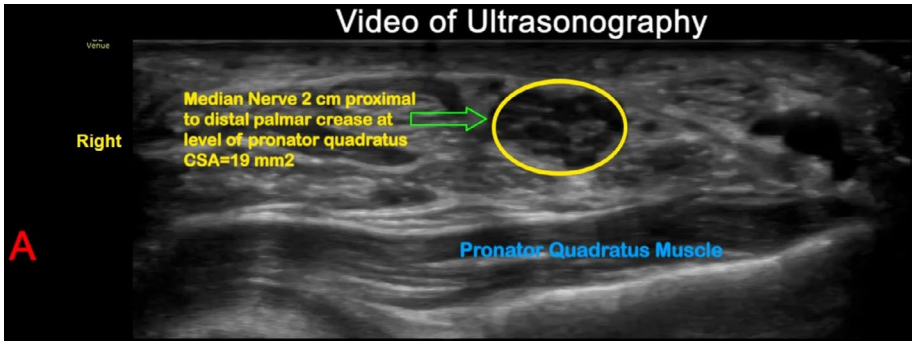
A strong positive correlation ($r = 0.8$, $p < 0.001$) was observed between the duration of symptoms and the number of nerves involved, indicating that longer symptom durations were associated with greater nerve involvement (Table 4). Significant associations were identified for nerve enlargement in most nerves, including the ulnar (OR 1.5, $p < 0.001$) and median (OR 1.20, $p = 0.002$) among others. Hypoechogenicity was significantly associated with the duration of symptoms in the ulnar (OR 1.48, $p < 0.001$), median (OR 1.17, $p = 0.005$), and several other nerves. Similarly, epineural thickening was significantly correlated with symptom duration for most nerves. These findings emphasize the impact



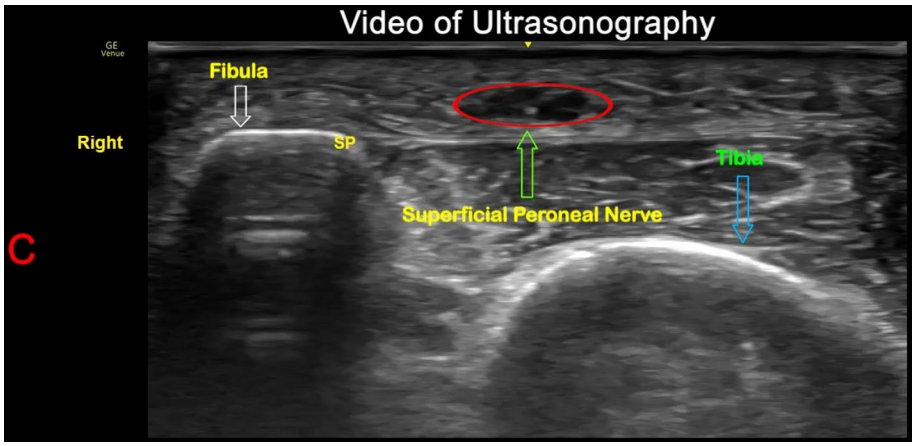
VIDEO 1 | (A) Ultrasonography of the ulnar nerve in a patient with multifocal enlargement: an enlarged hypoechogenic ulnar nerve is visualized near the distal forearm and near the medial epicondyle. (B) Ultrasonography of the ulnar nerve in a patient with monofocal enlargement: a gradually enlarging ulnar nerve near the medial epicondyle attains maximum enlargement 4 cm above the epicondyle, followed by gradual normalization in the arm. Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/jcu.24081



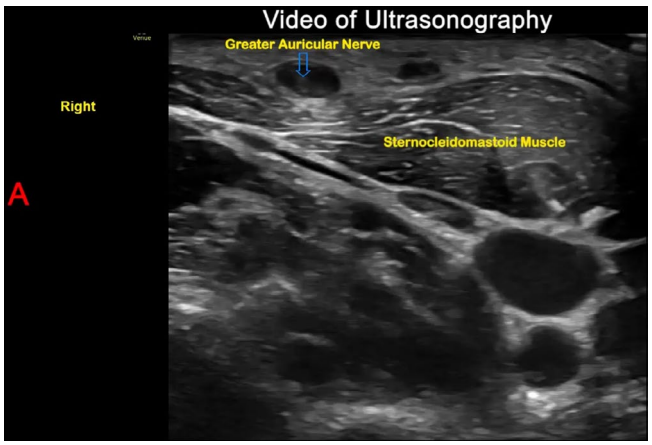
VIDEO 2 | (A) Ultrasonography of the ulnar nerve in the axial plane in a patient with abscess: Just above the medial epicondyle, massive enlargement of the nerve is noted with hypoechogenicity, thickening of epineurium, disorganization of the fascicular pattern, and a round anechoic area suggestive of intraneural abscess. (B) Ultrasonography of the ulnar nerve in the same patient in the longitudinal plane. Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/jcu.24081



VIDEO 3 | (A) Ultrasonography of the median nerve in a patient with monofocal enlargement: an enlarged median nerve just proximal to the carpal tunnel with hypoechogenicity of the nerve as it lies superficial to the pronator quadratus muscle. (B) Ultrasonography of the superficial radial nerve in a patient with diffuse enlargement: the nerve is diffusely enlarged in the distal forearm up to the wrist joint. Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/jcu.24081



VIDEO 4 | (A) Ultrasonography of the common fibular nerve in a patient with monofocal enlargement: Near the neck of the fibula, the common fibular nerve is noted to be enlarged with swelling of individual fascicles and hypoechogenicity. (B) Ultrasonography of the posterior tibial nerve in a patient with monofocal enlargement: The medial malleolus is visualized along with other structures near the tarsal tunnel, including the posterior tibial nerve, which is noted to be enlarged and hypoechogenic. (C) Ultrasonography of the superficial fibular nerve in a patient with diffuse enlargement: An enlarged superficial fibular nerve is visualized in the subcutaneous plane, superficial to the extensor digitorum longus tendon and further in the groove between the lateral and anterior compartments. (D) Ultrasonography of the sural nerve in a patient with diffuse enlargement: A diffusely enlarged sural nerve is seen in the subcutaneous plane, superficial to the Achilles tendon and soleus muscle. Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/jcu.24081



VIDEO 5 | (A) Ultrasonography of the greater auricular nerve in a patient with monofocal enlargement: roots of C5, C6, and C7 are visualized between the anterior and middle scalene muscles, and an enlarged greater auricular nerve lying over the sternocleidomastoid muscle. (B) Ultrasonography of the brachial plexus with enlargement of the upper trunk: enlarged roots of C5, C6, and C7 are visualized between the anterior and middle scalene muscles followed by an enlarged upper trunk. Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/jcu.24081

of symptom duration on nerve pathology, particularly in terms of nerve enlargement, hypoechogenicity, and epineural thickening.

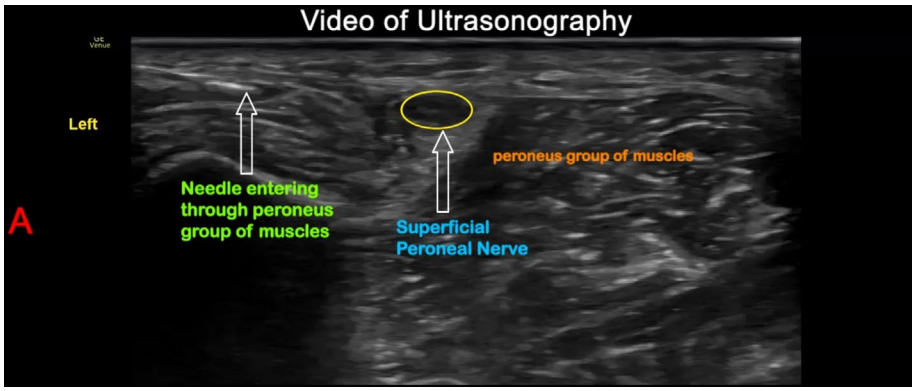
4.9 | True Positivity Rate

After combination of all the steps in which ultrasound had played a vital role toward confirmation of PNL including ultrasound directed skin biopsy and ultrasound guided FNAC ("ultrasound assisted diagnosis"), the true positivity rate noted was as high as 92.3%.

5 | Discussion

A delay in diagnosis of PNL increases ongoing silent transmission in the family and society and augments further disease progression and propagation, potentially resulting in increased risk of disability, stigmatization, and discrimination (Pitta et al. 2022) (Jardim et al. 2003). The present data suggest that the mean duration of symptoms prior to diagnosis (13.8 ± 6.4 months) was shorter than previous studies where it was 38.2 months (Pitta et al. 2022), which also correlates with the motor dysfunction being present in only a minority (26.9%) as compared to previous studies (81%) (Jardim et al. 2003). In fact, in the previous reports, the majority of patients had visited 3–5 health care providers before receiving a conclusive diagnosis (Jardim et al. 2003) (Urgesa et al. 2022), which may be attributed to a lack of efficient tools to suspect and subsequently confidently diagnose PNL. Therefore, knowledge of patterns and characteristics of nerve involvement on HRUS is of utmost importance for a strong suspicion of this disease by clinicians.

The most commonly reported pattern of nerve involvement in PNL is mononeuritis multiplex, followed by mononeuropathy (Razdan et al. 2024; Pitta et al. 2022), similar to what we noted. The mean distance of maximum enlargement of the ulnar nerve (the most common nerve involved) from the medial epicondyle (3.62 ± 1.0 cm) was comparable to data of previous researchers (4 cm) (Nagappa et al. 2021). Also, the pattern of enlargement was diffuse (more than 5 cm) in the majority, which is characteristic of ulnar nerve involvement in PNL as opposed to cubital tunnel syndrome in which there is short segment enlargement (Mezian et al. 2021). Like previous



VIDEO 6 | FNAC of various nerves: Initially the needle is visualized entering the nerve element; subsequently, there is to and fro motion of the needle along with negative suction to obtain sufficient aspirate, followed by release of suction before removing the needle from the tissue. (A) Axial section of superficial fibular nerve (B) Longitudinal section of superficial fibular nerve (C) Sural nerve (D) Dorsal ulnar cutaneous nerve (E) Superficial radial nerve (F) Greater auricular nerve. Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/jcu.24081

researchers (Reddy et al. 2021), we also noted that the median nerve was diffusely enlarged above the wrist at a point proximal to the distal wrist crease at the level of the pronator quadratus muscle, as opposed to carpal tunnel syndrome where the enlargement is distal and a relatively short segment. It is important to note that as this cannot be palpated, ultrasound helps immensely in detecting enlargement. In general, patients with PNL seem to have both greater extent as well as length of enlargement, as compared to other causes of nerve enlargement (Grant et al. 2015). Enlargement of the brachial plexus noted in this study is consistent with available literature (Verma et al. 2022). Epineural thickening and hypoechogenicity in various nerves could be an additional finding for augmenting early suspicion; however, it was noted more frequently in the MB than the PB subgroup, which was probably because the majority of the patients in the MB cohort were in the BB subset which has a propensity for diffuse nerve involvement (Visser et al. 2012). Overall, we also noted a significant positive correlation between the duration of illness and the number of nerves involved, CSA of nerves, percentage of nerves with hypoechogenicity, and epineural thickening indicating that long-standing disease may lead to progressive destruction, changes in nerve morphology, and a poor response to treatment (Leon et al. 2016).

Traditionally, the diagnosis of this disorder has been based on nerve biopsy, which has multiple practical limitations, including sampling error, low sensitivity, and risk of permanent nerve damage (Sandhu et al. 2021). Hence, clinicians tend not to perform this procedure initially, probably resulting in prolongation of the illness. In addition, NCS, the first typical investigation for this disease, can be normal in some cases, as noted in our study. In previous times, evidence of nerve enlargement clinically, such as a palpable tender thickening of the ulnar nerve near the medial epicondyle, was an important marker to point to a probable diagnosis of PNL, especially in endemic regions. Similarly, ultrasound is an important tool in that it picks up nerve enlargement before the nerve is palpable, thereby helping in early suspicion of the disease. In the absence of ultrasound, these patients presenting as mononeuritis multiplex or mononeuropathy were previously scheduled for nerve biopsy, which either resulted in unnecessary biopsies or the disease remained undiagnosed for a long period for those reluctant to undergo biopsy. If HRUS is combined with clinical history, examination, and electrophysiology findings, it is quite possible to narrow down the differential further; for example, a demyelinating congenital neuropathy would also have enlarged nerves but with symmetric, diffuse findings. Compressive neuropathies may have enlargement of the nerve at the point of compression, but this tends to be very focal. Overall, if we combine the clinical findings, laboratory investigations, electrophysiology, and HRUS, there are only a few possible diagnoses left to be considered in this scenario, in which PNL is the most important owing to the endemicity of the disease in this part of the world.

Additionally, HRUS guided FNAC, a relatively "nerve sparing" procedure, which also allows the examination of motor nerves safely (Vijaikumar et al. 2001; Sandhu et al. 2021) when sensory nerves are not involved or cannot be sampled, provides an additional advantage when it comes to confirmatory diagnosis of this disorder. This is in contrast to a traditional nerve biopsy, which is not appropriate for motor nerves because it results in permanent weakness. Therefore, if we combine all the steps in which ultrasound had played a vital role toward confirmation of PNL (excluding benefits toward suspicion), the true positivity rate of ultrasound-assisted diagnosis was as high as 92.3%. This is even higher than the 80.85% (CI: 69.60%, 92.10%) sensitivity for nerve biopsy, as shown by Kulshreshtha et al., who mandated nerve biopsy for diagnosis of PNL (Kulshreshtha et al. 2018), which has also been suggested to be the gold standard in diagnosis of PNL. The reason behind such a high true positivity may be the fact that all the steps of the diagnostic paradigm were based on clinical-electrophysiological-radiological correlation and diagnostic procedures such as FNAC (or even the skin biopsy) were guided by HRUS in the form of direct visualization of nerve, which allowed perfect sampling of the pathological part with high accuracy.

Nevertheless, if nerve biopsy is indispensable, HRUS can also guide in selecting a pathological site for biopsy, which could be much less invasive compared to standard biopsies that involve a larger incision. This has also been tried by researchers in the

TABLE 3 | Shows comparison of changes in intrinsic characteristics of nerves in paucibacillary (PB) and multibacillary (MB) subgroups.

Site	Variable	Multibacillary (28 cases; 56 nerves examined)	Paucibacillary (19 cases; 38 nerves examined)	Inconclusive (5 cases; 10 nerves examined)
Ulnar[a]	Increased CSA	30 (53.6%)	10 (26.3%)	3 (30.0%)
	Hypoechogenicity[a]	29 (51.8%)	10 (26.3%)	4 (40.0%)
	Epineural thickening	28 (50.0%)	9 (23.7%)	4 (40.0%)
	Increased vascularity	7 (12.5%)	2 (5.3%)	3 (30.0%)
Median	Increased CSA	21 (37.5%)	2 (5.3%)	5 (50.0%)
	Hypoechogenicity	21 (37.5%)	5 (13.2%)	5 (50.0%)
	Epineural thickening	18 (32.1%)	3 (7.9%)	5 (50.0%)
	Increased vascularity	12 (21.4%)	0 (0.0%)	4 (40.0%)
Superficial radial	Increased CSA	10 (17.9%)	4 (10.5%)	3 (30.0%)
	Hypoechogenicity	12 (21.4%)	4 (10.5%)	3 (30.0%)
	Epineural thickening	10 (17.9%)	3 (7.9%)	2 (20.0%)
	Increased vascularity	5 (8.9%)	2 (5.3%)	2 (20.0%)
Common fibular	Increased CSA	20 (35.7%)	9 (23.7%)	1 (10.0%)
	Hypoechogenicity	21 (37.5%)	7 (18.4%)	1 (10.0%)
	Epineural thickening	21 (37.5%)	7 (18.4%)	1 (10.0%)
	Increased vascularity	3 (5.4%)	1 (10.0%)	1 (10.0%)
Superficial fibular	Increased CSA	11 (19.6%)	0 (00.0%)	1 (10.0%)
	Hypoechogenicity	10 (17.9%)	1 (2.6%)	2 (20.0%)
	Epineural thickening	9 (16.1%)	1 (2.6%)	1 (10.0%)
	Increased vascularity	0 (0.0%)	0 (0.0%)	0 (0.0%)
Sural	Increased CSA	14 (25.0%)	0 (0.0%)	2 (20.0%)
	Hypoechogenicity	15 (26.8%)	1 (2.6%)	2 (20.0%)
	Epineural thickening	13 (23.2%)	1 (2.6%)	1 (10.0%)
	Increased vascularity	0 (0.0%)	0 (0.0%)	0 (0.0%)
Posterior tibial	Increased CSA	5 (8.9%)	0 (0.0%)	2 (20.0%)
	Hypoechogenicity	6 (10.7%)	1 (2.6%)	2 (20.0%)
	Epineural thickening	5 (8.9%)	0 (0.0%)	2 (20.0%)
	Increased vascularity	0 (0.0%)	0 (0.0%)	0 (0.0%)
Greater auricular	Increased CSA	2 (3.6%)	0 (0%)	2 (20.0%)
	Hypoechogenicity	2 (3.6%)	0 (0%)	2 (20.0%)
	Epineural thickening	2 (3.6%)	0 (0.0%)	0 (0.0%)
	Increased vascularity	0 (0.0%)	0 (0.0%)	0 (0.0%)
Brachial plexus	Increased CSA	4 (7.2%)	1 (2.6%)	0 (0.0%)
	Hypoechogenicity	1 (1.7%)	1 (2.6%)	0 (0.0%)
	Epineural thickening	4 (7.2%)	1 (2.6%)	0 (0.0%)
	Increased vascularity	0 (0.0%)	0 (0.0%)	0 (0.0%)

Abbreviation: CSA, cross sectional area.
[a]Hyperechongenicity was also noted exclusively in 2 (3.6%) of the ulnar nerves out of 56 nerves examined.

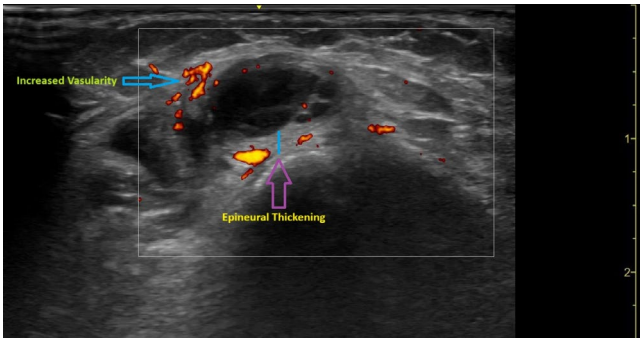


FIGURE 2 | Ultrasonography with power doppler mode showing an enlarged ulnar nerve just above the medial epicondyle with increased vascularity and epineural thickening.

past for other pathologies (Forney et al. 2019). Overall, a quick and non-invasive diagnostic modality as ultrasound not only augments the suspicion of diagnosis of PNL but also opens paths toward minimal invasive methods in confirmation of this disease which in turn reduces non-compliance and dropouts from treatment. In a nutshell, HRUS can be a third eye for clinicians working in this field, especially neurologists. However, it is not widely available at neurology centers/clinics and there is operator dependence; hence, formal training is prudent prior to imaging the nerves and guiding FNAC/Biopsy. Further larger studies of ultrasound-guided FNAC and biopsy involving multiple cohorts of different subgroups of PNL may be required to validate these findings so that the invasive standard biopsy could be avoided.

5.1 | Study Limitations

As we could not perform PCR in all five cases, the true positivity rate of ultrasound-assisted diagnosis may have been underestimated. Out of all four patients in which the diagnosis could not be established in our study, some had a different diagnosis such as vasculitis, as revealed later in the biopsy; hence, the true positivity rate of ultrasound-assisted diagnosis may have been underestimated in our study. Additionally, a larger study with all potential diagnoses included in the denominator could replicate a real-world practice even more accurately than this study.

6 | Conclusion

Point of care neuromuscular ultrasound can serve as a powerful diagnostic tool for individuals with suspected PNL. HRUS can screen nerve segments to assess for enlargement, which can then be used to guide skin biopsies. Additionally, for those with negative skin biopsies, ultrasound-guided nerve FNAC can be used as a minimally invasive procedure to gather tissue for histology and PCR for the diagnosis of PNL. It is therefore recommended that physicians who frequently assess patients for suspected PNL consider learning this highly effective ultrasound technique.

TABLE 4 | Shows correlation and association between Duration of Symptoms and various characteristics of nerves.

	p
Duration of symptoms (months) vs. number of nerves involved ($r = 0.8$)[b]	**< 0.001**
Duration of symptoms (months) vs. response to treatment[a]	0.994
Association between duration of symptoms vs. nerve enlargement[a]	
Ulnar	**< 0.001**
Median	**0.002**
Superficial radial	**0.004**
Common fibular	0.137
Posterior tibial	**0.019**
Sural	**0.005**
Greater auricular	0.464
Superficial fibular	**0.046**
Brachial plexus	0.093
Association between duration of symptoms vs. hypo echogenicity[a]	
Ulnar	**< 0.001**
Median	**0.005**
Superficial radial	**< 0.001**
Common fibular	**0.015**
Posterior tibial	**0.031**
Sural	**0.009**
Greater auricular	0.464
Superficial fibular	0.637
Brachial plexus	0.536
Association between duration of symptoms vs. epineural thickening[a]	
Ulnar	**< 0.001**
Median	**0.003**
Superficial radial	**< 0.001**
Common fibular	**0.016**
Posterior tibial	**0.044**
Sural	**0.005**
Greater auricular	0.104
Superficial fibular	0.091
Brachial plexus	0.093

Note: < 0.05 is the significance level. Singificance of bold is that those are statistically significant.
[a]Binomial logistic regression.
[b]Spearman's rho coefficient.

Author Contributions

U.R. conceptualization, data curation, investigation, writing the first draft, review and critique. A.P. conceptualization, review and critique, validation. M.S.C. conceptualization, review and critique, validation. A.K.S. conceptualization, review and critique, validation. B.M. review and critique, methodology, validation. H.V. review and critique, methodology, validation. S.B.S. formal statistical analysis, software, supervision. R.S. review and critique, validation. N.R. review and critique, validation. I.S. review and critique, validation. C.R. review and critique, project administration, supervision.

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Ethics Statement

We hereby confirm that the present study conforms to the ethical standards and guidelines of the journal. We confirm that the ethical principles for medical research involving human subjects (Declaration of Helsinki, WMA, 1975, revised 2000) have been followed. The study was approved by the institutional ethics committee. The patients have given written and informed consent for the online publication of their ultrasound videos. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and its Supporting Information. Raw data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.