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Functional outcomes of peripheral nerve repair and regeneration:

clinical and experimental study

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Contents

Abstract

This study investigated pre- and post-operative evaluation of peripheral nerve injuries and functional outcomes of peripheral nerve regeneration after different type of injuries and different type of treatments.

Neuromas/scar neuritis and brachial plexus injuries are specifically addressed; literature and experimental evidence on the outcomes of vascularized nerve grafts are presented.

Summary

Purpose: This thesis wishes to investigate functional outcomes of peripheral nerve repair with vascularized and non-vascularized nerve grafts. Also, it investigates functional outcomes after treatment of neuromas/scar neuritis and brachial plexus injuries.

Methods: This thesis includes six studies: a clinical study on the role of ultrasound in evaluation of peripheral nerve injuries (study I), 2 literature reviews on experimental models of nerve injuries (neuromas and vascularized nerve grafts, studies II and III), an experimental study on vascularized nerve grafts (study IV), a clinical study on functional outcomes after non-vascularized nerve grafts or nerve transfers for brachial plexus injuries (study V) and a literature review on functional outcomes after treatment of neuromas/scar neuritis.

Results: Ultrasounds were a valuable complement to preoperative clinical and electrodiagnostic examination and guided surgical planning in most cases (study I).

Current knowledge on experimental models of neuromas and of vascularized nerve grafts is summarized, which could be useful for future standardization (studies II and III).

Both the literature review and the experimental study suggested that vascularized nerve grafts more effectively promote axonal regeneration and functional recovery compared to non-vascularized nerve grafts, especially in avascular beds. Also, we described a model for prefabricated vascularized nerve grafts based on a venous loop and validated an operative protocol with the MUNE technique in the rat sciatic nerve model (studies III and IV).

Functional outcomes of nerve grafts/nerve transfers after brachial plexus injuries improved in terms of active ROM even after 2 years from surgery, but with limited improvement in daily activities (study V).

No treatment has shown superior short or long-term outcomes in the treatment of scar neuritis, and responses vary significantly and unpredictably among patients (study VI).

Conclusions: Peripheral nerve injuries are often challenging to study and to treat.

Adequate preoperative evaluation is mandatory and we support the use of ultrasounds as first line imaging technique to complement clinical and neurophysiological examination. Vascularized nerve grafts have limited indications but seem to provide a functional advantage in complex cases with an avascular nerve bed.

To improve treatment of neuromas and scar neuritis, further research is desirable to investigate individual differences responsible for the large variability in the therapeutic response.

To better evaluate functional outcomes after brachial plexus injuries, further research investigating patient-reported outcomes measures is desirable.

List of papers

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Toia F., Preface to special issue on "Peripheral nerve repair and regeneration" Plast Aesthet Res, 2015; 2 (4): 147-148. DOI: 10.4103/2347-9264.160876I. 6.

II. Gagliardo A.*, Toia F.*, Maggì F., Mariolo A.V., Cillino M., Moschella F. Clinical neurophysiology and imaging of nerve injuries: preoperative diagnostic work-up and post-operative monitoring. Plast Aesthet Res, 2015;2 (4):149-55. DOI: 10.4103/2347-9264.160877.

III. Toia F.*, Gagliardo A.*, D'Arpa S., Gagliardo C., Gagliardo G., Cordova A. Preoperative evaluation of peripheral nerve injuries: What is the place for ultrasound? J Neurosurg. 2016 125(3):603-14. doi: 10.3171/2015.6.

IV. Toia F., Giesen T., Giovanoli P., Calcagni M. A systematic review of animal models for experimental neuroma. J Plast Reconstr Aesthet Surg, Oct;68(10):1447-63. doi: 10.1016/j.bjps.2015.05.013. Epub 2015 May 28.

V. Tos P., Crosio A., Pugliese P., Adani R., Toia F., Artiaco S. Painful scar neuropathy: principles of diagnosis and treatment. Plast Aesthet Res, 2015; 2(4):156-64. DOI: 10.4103/2347-9264.160878.

Abbreviations

A: artery

AVA: Artery-vein-artery nerve graft

Arterialized venous nerve graft (AVA)

AVV: Artery-vein-vein nerve graft

CAPA: Compound action potential area

CAT: Choline acetyltransferase

CMAP: compound muscle action potentials

CSA: Cross Sectional Area

EMG: electromyography

ENG: electroneurography

FPSMA: vascularized nerve graft based on the femoral popliteal superior muscular artery

GM: gastrocnemius

HPF: high power field

mA: milliampere

mBMRC: modified British Medical Research Council

MNCV: motor nerve conduction velocity

MUNE: motor number unit estimation

NIC: neuroma in continuity

NCV: nerve conduction velocity

NVNGs: non-vascularized nerve grafts

ROM: range of movement

RT-PCR: Reverse transcription polymerase chain reaction

SFI: sciatic functional index

SMUAP: surface detected motor unit action potential

SNAP: sensory nerve action potentials

TA: tibialis anterior

VNGs: vascularized nerve grafts

VVV: Vein-vein-vein nerve graft

CHAPTER 1

1. Introduction

Peripheral nerve surgery has achieved a great improvement in the last century, mainly due to the introduction of microsurgical techniques. Yet, in the last few decades, the scientific progress has slowed down significantly, and surgery is still far from reaching an optimal functional recovery in most cases (I).

This thesis addresses several aspects of nerve regeneration in which research can actively contribute to improve functional outcomes after a peripheral nerve injury. Pre- and post-operative evaluation of peripheral nerve injuries and functional outcomes of peripheral nerve regeneration after different type of injuries and different type of treatments are investigated.

Neuromas/scar neuritis and brachial plexus injuries are specifically addressed; literature and experimental evidence on the outcomes of vascularized nerve grafts are presented.

The first part of the thesis addresses clinical and instrumental evaluation of peripheral nerve injuries, as optimizing functional results requires adequate pre and post-operative work-up (II). A thorough clinical and neurophysiological examination is mandatory, and need to be supplemented by modern imaging technique, as high-resolution nerve ultrasound, whose role in peripheral nerve surgery was investigated in study I (III).

For particular nerve injuries that are challenging to repair, experimental research still plays a major role. This is the case for peripheral neuromas, which can result in an unbearable neuropathic pain and functional impairment. The underlying mechanisms are not perfectly understood and their optimal management is to be defined.

A literature review on experimental neuromas models will be presented in study II, with the aim of taking a step towards standardization of experimental model(s) for neuroma that would help to better compare results, and might ultimately prove of clinical usefulness (IV).

Another topic in which experimental research can contribute to optimization of functional results is the use of vascularized nerve grafts. Although some clinical evidence exists for better nerve regeneration, especially in long nerve defects and severely scarred bed, there is no general agreement on their indications. A literature review on experimental models of vascularized nerve grafts will be presented in study III, to summarize current evidence for superior functional results.

The study of peripheral neuromas and vascularized nerve grafts will be deepened by a clinical review of functional outcomes after surgery for painful neuromas and scar neuropathy, as these conditions are difficult to treat and reported functional outcomes greatly differ among studies (V), and by an experimental study comparing vascularized versus non-vascularized nerve grafts (study IV), in which a recent electrodiagnostic technique (MUNE) will be applied to allow a standardized functional evaluation of muscle reinnervation.

Clinical evidence for functional outcomes after nerve grafts will also be addressed in another difficult to treat type of nerve injury. Brachial plexus injuries yield a devastating degree of impairment, with often unrewarding functional results; literature on long term and patient reported outcomes is limited. A preliminary retrospective study on patients who received non-vascularized nerve grafts or nerve transfers for brachial plexus injuries will be presented (study V).

CHAPTER 2

2. Aims

The aim of this thesis was to investigate several aspects of nerve regeneration and repair. Diagnosis, treatment and functional outcomes following peripheral nerve injuries are specifically addressed. Clinical and experimental evidence from the literature and from original studies is provided.

Pre and post-operative evaluation of peripheral nerve injuries

Study I:

- to investigate the clinical role of ultrasound in the evaluation of peripheral nerve injuries.

Experimental models for the study of peripheral nerve injuries

Study II:

- to review described experimental models for neuromas and investigate experimental evidence for preventing/treating neuromas.

Study III:

- to review described experimental models for vascularized nerve grafts and investigate experimental evidence for their functional outcomes.

Functional outcomes after peripheral nerve repair

Study IV:

- to compare functional outcomes of non-vascularized vs vascularized nerve graft in an experimental rat model.

Study V:

- to evaluate long-term functional outcomes of brachial plexus reconstruction with non-vascularized nerve grafts and nerve transfers.

Study VI:

to investigate literature evidence in functional outcomes of surgical treatment of neuromas/scar neuritis.

CHAPTER 3

3. Methods

3.1. **Pre and post-operative evaluation of peripheral nerve injuries**

3.1.1. Study I: The role of nerve ultrasound (clinical study)

A retrospective study was conducted, evaluating 119 entrapment, tumoral, posttraumatic/postsurgical nerve injuries (subgroups: neuroma, nerve compression, traction neuropathy) of the limbs in 108 patients candidates for limb surgery.

Preoperative evaluation included clinical examination, electrodiagnostic studies (nerve conduction study and electromyography), and ultrasound nerve study.

Electrodiagnostic studies

Sensory and motor conduction velocity, sensory nerve action potentials (SNAP) and compound muscle action potentials (CMAP) were recorded for the injured nerve, the contralateral homologous and other ipsilateral healthy nerves. Eventual denervation potentials and motor unit recruitment patterns were recorded by EMG.

Ultrasound

Instrumental evaluation was always completed by nerve ultrasound on the injured nerve or of all main nerves of the same limb based on clinical and neurophysiological findings.

The nerve fascicular echo-texture, continuity, and surrounding tissues were examined along the whole nerve course, recording eventual anatomic anomalies, dynamic nerve dislocations or compressions. Recorded ultrasonographic characteristics included: epineural hyperechogenicity, intraneural hyper or hypoechogenicity, and maximal Cross Sectional Area (CSA).

Data Analysis

Electrodiagnostic and ultrasonographic data from the three groups "entrapment neuropathies", "post-traumatic or post-surgical neuropathies" and "tumors" underwent an intra and intergroup analysis.

To evaluate the contribution of nerve ultrasound in diagnosis and surgical planning, ultrasonographic findings were classified as "contributive", "confirming", "non-confirming" or "incorrect" with respect to clinical and neurophysiological examination, according to Padua's evaluation scale.

Statistical analysis

Statistical analysis was performed using the SPSS 20.0 software.

The correlation between maximal nerve CSA and neurophysiological severity classification of compression neuropathies was tested through an univariate analysis of variance (ANOVA) test and a post-hoc Fisher's least significant difference (LSD) analysis in carpal tunnel syndrome and through an independent T-test cubital tunnel syndrome. p value ≤ 0.05 were regarded as significant.

3.2. **Experimental models for the study of peripheral nerve injuries**

3.2.1. Study II: Neuroma models (literature review)

A systematic literature review on experimental neuromas was performed. Search was performed in the PubMed database, with "neuroma" and "model" as search

terms (title and/or abstract field). Papers were selected based on abstract or full text review.

Data on the animal and nerve model, the injury type and the methodology study of the neuroma were extrapolated from selected articles and analyzed.

3.2.2. Study III: Vascularized nerve graft models (literature review)

A systematic literature review on vascularized nerve grafts models was performed. Search was performed in the PubMed database, with "vascularized/vascularised nerve graft/grafts" as search term (title and/or abstract field).

Papers were selected based on abstract or full text review. Inclusion criteria were articles in English, Italian, Spanish or French language, introducing an original or a modification of an existing animal model of vascularized nerve graft, or presenting an animal study on vascularized nerve grafts. Exclusion criteria included language other than English, Italian, Spanish or French, studies not concerning experimental vascularized nerve grafts, and anatomical or clinical studies. Further search for relevant articles included references of selected articles (figure 1).

Data on the animal, nerve (donor, recipient, defect length) and vascularization model, on the recipient bed (vascular/avascular), on the evaluation time points and methods, and on results were extrapolated from selected articles and analyzed.

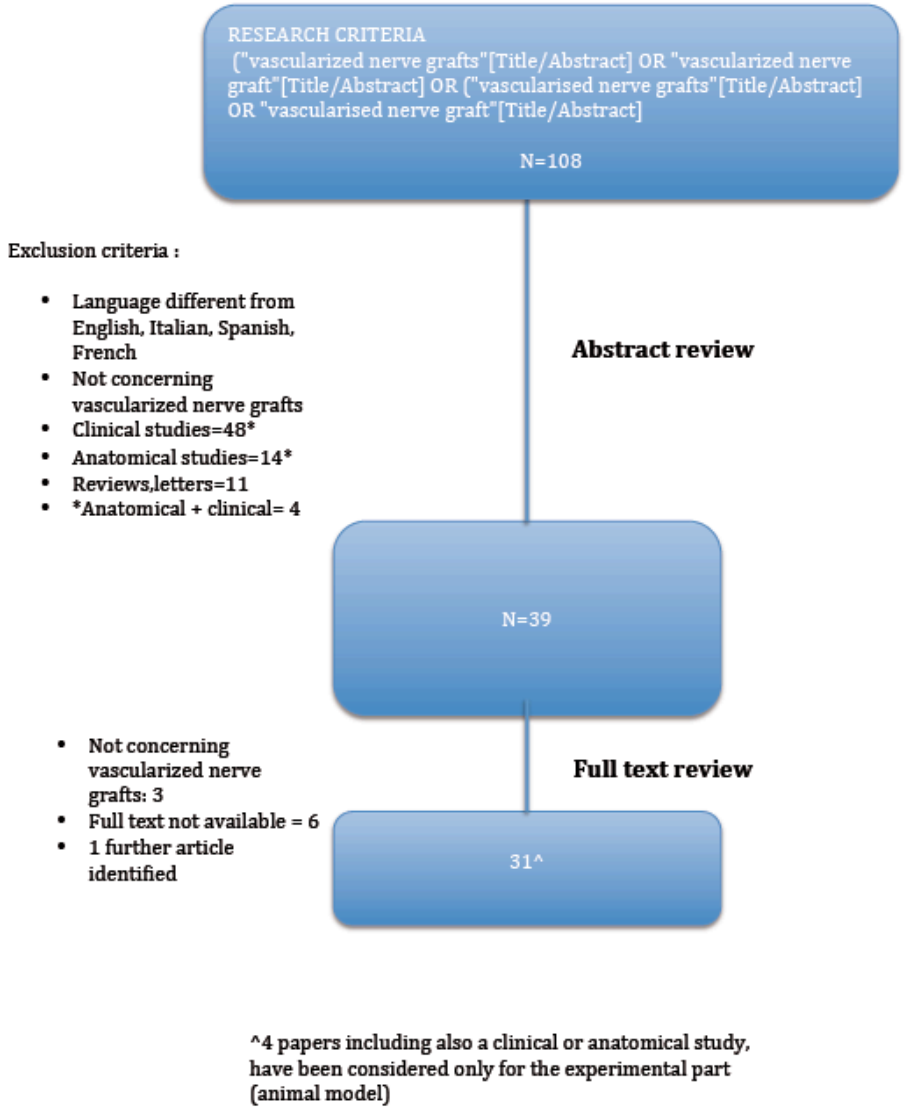


Figure 1: *Systematic literature review strategy*

3.3. **Functional outcomes after peripheral nerve repair**

3.3.1. Study IV: Non-vascularized vs vascularized nerve grafts (experimental study)

The study received prior approval of the Italian Ministry of Health, (n 270/2016-PR del 11-03-2016) and experiments were conducted according to Italian and European legislation.

Sample size was calculate to be 9 animals (using both sciatic nerve to compare VNGs and NVNGs), considering a statistical power of 80% and a significance level (alpha) of 0.05, based on an expected 20% difference in motor units number between the vascularized and non-vascularized nerve grafts. Only 5 animals were included in the experimental group up to date, and preliminary results are presented in this thesis.

Nine adult male Wistar rats (weight 350-400 gr) were used for the study.

Two animals (group 1) were used for defining the experimental model and protocol of the experimental group.

The experimental group (group 2) included five animals, which received a non-vascularized orthotopic sciatic nerve graft on the right side, and a vascularized orthotopic sciatic nerve graft nerve on the left side.

Two animals (group 3) were then used for defining an experimental model and protocol for prefabricated vascularized nerve grafts based on a venous loop.

Group 1:

Animals were anesthetized with urethane (1.2g/kg intraperitoneally). In animal n.1, the sciatic nerve was exposed bilaterally through a combined anterior/posterior approach in the inguinofemoral and gluteal region, while in animal n.2, the sciatic nerve was exposed bilaterally through an anterior approach in the inguinofemoral region. The length of the exposed segment of sciatic nerve

(before bifurcation into the tibial and peroneal nerve) was measured; vessels supplying the nerve were identified and their position recorded. The distance of the nerve from the femoral vessels and their anatomical relationship were also investigated. In animal n 1, ring electrode cuffs of different diameters and length were tested around the nerve, while in animal n 2, a non-implantable stimulating electrode was used, testing different stimulus intensities and durations. Animals were sacrificed at the end of the experiment.

Group 2:

Surgical procedure:

Animals were anesthetized with Xylazine (10 mg/kg) e Ketamine (100 mg/kg). They received antibiotic prophylaxis with enrofloxacin (2.5 mg/kg/die for 7 days) and antalgic prophylaxis with meloxicam (0.3 mg/kg/die).

The sciatic nerve was exposed bilaterally through an anterior approach in the inguinofemoral region, from its emergency beneath the piriformis muscle to its bifurcation into the tibialis and peroneus communis.

Before nerve section and nerve graft, electrophysiologic studies were performed (see below).

In the right side, the nerve was skeletonized, dissecting out its vascular supply and interrupting the ascending branch to the nerve of the caudal femoral artery. A 15 mm graft was then harvested and orthotopically sutured to the proximal and distal stumps of the sciatic nerve with 2 interrupted 10/0 nylon sutures. The graft was enveloped in a 0.12 mm thick silicon sheet (Folioxane, Novatech, France) to prevent revascularization from surrounding tissues. The skin was then closed with interrupted 3/0 silk sutures.

In the left side, the nerve was isolated, respecting its perineural vessels and the ascending branch to the nerve of the caudal femoral artery. A 15 mm graft was

then harvested – pedicled on the ascending branch to the nerve of the caudal femoral artery – and orthotopically sutured to the proximal and distal stumps of the sciatic nerve with 2 interrupted 10/0 nylon sutures. The graft was enveloped in a 0.12 mm thick silicon sheet (Folioxane, Novatech, France) to prevent revascularization from surrounding tissues. The skin was then closed with interrupted 3/0 silk sutures.

Evaluation

Functional outcome following nerve regeneration was evaluated through electrodiagnostic studies, target muscles weight and histomorphology.

Electrodiagnostic studies: estimation of motor unit number with the MUNE technique for the tibialis anterior muscle (for the peroneus communis nerve) and for the gastrocnemius muscle (for the tibialis nerve) was performed at time 0 (after nerve exposure but before surgery on the nerve), 6 weeks after surgery (under general anesthesia but through percutaneous stimulation) and at 12 weeks, after nerve exposure and before animal sacrifice.

A stimulating electrode was positioned on the sciatic nerve in the inguinofemoral region, and its maximum CMAP was evoked by stimulating it at supramaximal intensity (i.e. 10 % above threshold) with a stimulus duration of 0.02 ms.

Then, surface detected motor unit action potential (SMUAP) – which represents the mean value of the amplitudes of the single motor unit – were quantitatively recorded through a pair of monopolar needle electrodes, in a belly tendon montage. Stimulation was performed at 0.5 Hz, with a gradual intensity increase (mA) until a reproducible, "all-or-none" SMUAP was evoked. Fifteen SMUAPs were recorded for each nerve stimulation.

Motor number unit was estimated by the formula: CMAP/mean SMUAP.

Muscle weight

After animal sacrifice (12 weeks), the wet muscle of right and left anterior tibial muscles and gastrocnemius muscles were recorded.

Histomorphology:

After nerve sacrifice (12 weeks), sections of the sciatic nerve were obtained bilaterally:

- 2 mm proximal and 2 mm distal to the proximal nerve suture;
- in the middle part of the nerve graft;
- 2 mm proximal and 2 mm distal to the distal nerve suture (figure 2).

Ematoxilin-eosin and Masson trichromic staining were performed, and the following data were recorded for each of the 5 groups of sections.

- transverse nerve diameter;
- diameter and density of mielinated axons (axons/mm3);
- diameter and density of nerve fibers (fibers/mm3);
- neural/connective tissue area rate;
- number of axons/nerve fibers rate.

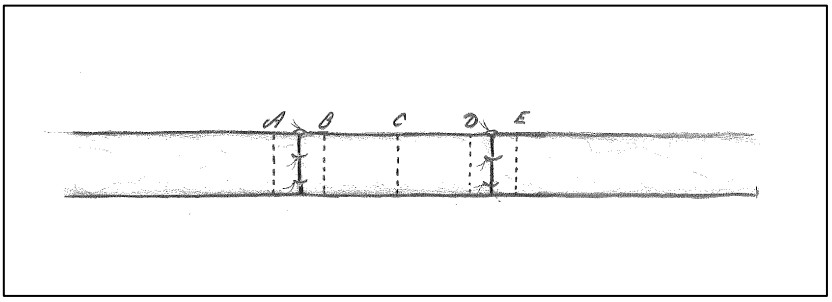


Figure 2: *Sections of the sciatic nerve were obtained 2 mm proximal (A) and 2 mm distal (B) to the proximal nerve suture, in the middle part of the nerve graft (C), 2 mm proximal (D) and 2 mm distal (E) to the distal nerve suture.*

Statistical analysis

Electrophysiological data at time 0, 1 and 2, and histomorphologic data from the different section level were analyzed and compared for each sciatic nerve; electrophysiological, muscles weight and histomorphologic data from the right and the left sciatic nerves were analyzed and compared (repeated measure ANOVA and post-hoc test, Student's T test).

Group 3:

Animals were anesthetized with urethane (1.2 g/kg intraperitoneally). The sciatic nerve was exposed through an anterior approach, taking care not to injure the epigastric vessels.

In animal n.1, the right and the left superficial epigastric veins were dissected for about 2.5 cm, starting from the femoral vein with a cranial direction. The left superior epigastric vein was harvested as a graft. The right epigastric vein was ligated cranially and interrupted. The distal stump was anastomosed to the vein graft, whose distal stump was anastomosed – end-to-end – to the femoral artery.

In animal n.2, the right and the left superficial epigastric veins were dissected for about 3.5 cm, starting from the femoral vein with a cranial direction. The

epigastric vein was ligated cranially and interrupted, and anastomosed – end-to-end on the right side and end-to-side on the left side – to the femoral artery.

For each experiment, easy of juxtaposition among the vascular loop and the sciatic nerve was evaluated, as were technical difficulty and anastomosis patency 1 hour after anastomosis. Animals were sacrificed at the end of the experiment.

3.3.2. Study V: Brachial plexus reconstruction with non-vascularized nerve grafts (clinical study)

A preliminary study consisting of a retrospective review of patients operated by a single senior surgeon for brachial plexus injuries at Sahlgrenska University Hospital during the years 1984 to 2000 was performed. Patients' charts were reviewed; patients who underwent plexus reconstruction with free (non-vascularized) nerve grafts or nerve transfers were selected and data on the level and type of injury, time to surgery, number and type of operations, follow-up length and functional results were extrapolated and analyzed.

3.3.3. Study VI: Painful scar neuropathy (literature review)

A literature review on treatment outcomes of painful scar neuropathy was performed.

Relevant articles on treatment approaches and treatment outcomes for scar neuritis and neuropathic pain, reporting pre- and post-operative pain assessment were selected.

Data on the surgical approach and treatment outcome (pain reduction) were extrapolated from selected articles and analyzed.

CHAPTER 4

4. Results

4.1. **Pre and post-operative evaluation of peripheral nerve injuries**

4.1.1. Study I: The role of nerve ultrasound (clinical study)

With regards to electrodiagnostic findings, ultrasound were "confirmatory" in 36.1%, and "contributive" in 53.8%.

Entrapment neuropathies

With regards to electrodiagnostic studies, ultrasound showed a confirming or contributing role in 91.3% of cases. In carpal tunnel syndrome, there was a positive correlation among the mean maximal CSA, the presence of epineural hyperechogenicity and nerve hypoechogenicity of the median nerve at the wrist and electrodiagnostic severity. In cubital tunnel syndrome, there was no significant correlation among the mean maximal CSA of the ulnar nerve at the elbow and electrodiagnostic severity, and nerve hypoechogenicity was always observed.

Ultrasound showed a contributive role in 43.8% of entrapment syndromes, which included the identification of a concurrent flexor tenosynovitis, perineural scar or anatomical variations such as bifid median nerve and/or a median artery, nerve dislocation at the elbow, uncommon or dynamic sites of compression. It also revealed the cause of the nerve compression (e.g.: ganglion cyst in a Guyon's

canal syndrome), or confirmed suspected clinical diagnosis in the presence of doubtful electrodiagnostic findings.

Post-traumatic and post-surgical neuropathies

Ultrasound contributed even more to diagnosis in traumatic cases (contributive role in 72.2% of cases), mainly due to identification of nerve continuity. It allowed identification of the presence and extension of both terminal neuromas and neuromas in continuity, which typically showed a greatly increased CSA and a typical hypoechoic nerve with an altered echotexture. Diagnosis and localization were confirmed by a Tinel sign elicited by the passage of the probe.

Ultrasound also allowed visualization of nerve continuity and eventual surrounding hyperechoic (fibrous) tissue and identified perineural scar, heterotopic ossification, or foreign bodies, and identification of multiple sites of injury.

In the nerve compression subgroup, ultrasound allowed identification of a multifocal damage in the presence of doubtful electrodiagnostic findings, while in the traction neuropathy subgroup, increased CSA, epineural hyperechogenity, hyper/hypo intraneural echogenity often indicated the need for neurolysis.

Tumors

In the three cases of schwannoma, ultrasound was always diagnostic (CSA greatly increased) in the presence of normal electrodiagnostic findings, and defined the size and the vascular supply of the tumor.

Ultrasound had a contributive role in 100% of tumor cases, allowing visualization and providing anatomical details of the lesions.

4.2. **Experimental models for the study of peripheral nerve injuries**

4.2.1. Study II: Neuroma models (literature review)

Literature review identified 153 papers, of which 13 were selected based on abstract review.

Experimental models of neuroma greatly differ in the animal and the nerve employed, the mechanisms of nerve injury and the evaluation methods. Specific experimental models exist for terminal neuromas and neuromas in continuity (NIC).

The rat is the most widely employed animal, the rabbit being the second most popular model. Research is more active on NIC models, more difficult to generate in a reproducible way. Nerve transection is considered the best method to cause terminal neuromas, while partial transection is the best method to cause NIC. Traditional histomorphology is the historical gold standard evaluation method, but immunolabeling, RT-PCR and proteomics are gaining increasing popularity. Computerised gait analysis is the gold standard for motor recovery evaluation, while mechanical testing of allodynia and hyperalgesia reproducibly assesses sensory recovery.

4.2.2. Study III: Vascularized nerve grafts models (literature review)

The initial search gave back 108 papers proposing a model of vascularized nerve graft and/or comparing vascularized and non-vascularized nerve graft. After abstract/full text review, 31 papers were selected.

Extrapolated data are presented in table 1. In 21 studies, vascularized nerve grafts were studied in a vascular bed, while in 10 studies they were performed in an avascular bed.

The rat was the most frequently used commonly employed animal model (17 studies), mainly Sprague Dawley or Wistar, followed by rabbits (11 studies), dogs (2 studies) and pig (1 study).

In the rat model, the sciatic nerve was most commonly used (10 studies), followed by the femoral nerve (5 studies). Mean nerve graft size was 1.6 cm (range: 0.7- 3).

In the rabbit model, the sciatic or the median nerves were most commonly used (4 studies each). Mean nerve graft size was 2.7 cm (range: 1-5)

In the dog model, the saphenous nerve was used with longer nerve grafts (5 and 10 cm), while in the pig model, the femoral nerve was used.

In most studies, nerve regeneration was evaluated by histomorphology and or electrodiagnostic studies, while only 4/31 (12.9%) of studies included functional tests.

In most studies, results were evaluated at an earlier time-point (4-8 weeks) and/or a later time-point (12-36 weeks). Over half of the studies (54.8%) concluded that vascularized nerve grafts perform better than non-vascularized nerve grafts, and 35.3% showed only earlier better results but comparable long-term results to non-vascularized nerve grafts.

REFERENCES	ANIMAL	NERVE GRAFT IMPLANT TYPE	RECIPIENT NERVE	EXPERIMENTAL GROUPS AND VASCULARIZATION MODEL	BED	NERVE DEFECT	TIMEPOINTS	EVALUATION METHOD	CONCLUSIONS
Arakaki et al., 1993	New Zealand White rabbits (25) 2/2.5 kg.	Median nerve (autologue)	1.orthotopic graft 2. median nerve	- Vein-vein-vein nerve graft (VVV) - Artery-vein-vein nerve graft (AVV) - Artery-vein-artery nerve graft (AVA) - Vascularized nerve graft based on brachial vessels	Vascular	3 cm	6 hours	1.histomorphology (Evan's blue dye): fluorescent microscope tracing of tagged albumin injected intravenously (to study microcirculation perfusion and permeability of the endoneurial capillary)	The VVV graft maintained a normal vascular leakage pattern similar to that of the intact sciatic nerve.
Bertelli et al., 1996	Sprague-Dawley rats (F, 84) 220g	Ulnar nerve (autologue)	1.heterotopic graft 2. median nerve	- Vascularized nerve graft - non vascularized nerve graft	Vascular	2 cm	95,120,150, 210,360 days	1.histomorphology (hematoxylin-eosin, true-blue aqueous solution,ATPasi histochemestry): retrograde labeling studies, Flexor carpi radial studies 2. grasping test	Recovery with VNG was 20% faster than with conventional graft, but with no advantage in functional recovery in long term assessment.
Best et al., 1993	Lewis(RT1l) ACI(RT1a) rats (18) 260-310 g	Sciatic nerve (autologous, allogenic, syngenic)	1: orthotopic, autologous 2.sciatic nerve	Vascularized nerve graft based on the femoral popliteal superior muscular artery (FPSMA) Study 1: autologous graft Study 2: 1. syngenic graft 2. allogenic graft 3. allogenic immunosuppressed graft	Vascular	3 cm	14 weeks	Study 1: perfusion assessment: plastic monomer injection and intravascular fluorescence Study 2: 1.histomorphology: toluidine blue,plastic monomer,evans blue labeled albumin ; morphometry(fiber number and diameter) 2. electrophysiology: conduction velocity, motor latency, amplitude. 3. Functionality: walking track analysis	The vascularized immunosuppressed allograft showed similar results to the vascularized syngeneic graft. Both were superior to the vascularized allograft without immunosuppression. This model allows comparison of neural function through grafts between animals of known histocompatibility differences

Cavadas et al., 1994	Albino Wistar rat (M, 15) 200-250g	Sciatic nerve (autologue)	1.orthotopic graft 2.sciatic nerve	-prefabricated graft based on a vascular loop (epigastric veins on femoral vessels)	Avascular (silicon sheet)	2 cm	5 weeks	1. histomorphology (hematoxylin-eosin staining) 2. india ink injection (to show nerve blood supply)	This model allows prefabrication of a VNG. At 5 weeks all nerve were neovascularized and remained viable after free transfer.
Donzelli et al., 2016	New Zealand rabbits (M,20)	Axillary nerve (autologue)	1. heterotopic graft 2.sciatic nerve	- vascularized nerve graft based on axillary artery - non-vascularized nerve graft.	Vascular	-	1, 3 months	1.histomorphology (hematoxilin- eosine, toluidine blue): mean axonal count, axon caliber, myelin thickness, number of myelinated fiber, mean axonal diameter, G ratio 2.EMG and ENG: CMAP , amplitude and latency of signals, NCV 3.Walking track analysis, SFI	VNGs are associated to a more rapid regeneration process and to a faster functional recovery than NVNGs. However the final functional recovery in the long term assessment is not significantly different.

Hatoko et al.. 2003	Wistar Rats (M,50) 250/300 g.	Sciatic nerve (autologue)	1. orthotopic graft 2. sciatic nerve	- vascularized nerve graft based on femoral vessels. - non-vascularized nerve graft	Vascular	1,5 cm	4, 14 weeks	1. histomorphology (hematoxilin-eosine, streptavidin biotin) 2. Western blot analysis: detection of alpha, beta, gamma catenin expression 3. histochemical: biotiny-lated goat anti-mouse IgG and streptavidin conjugated with horseradish peroxidase	The level of beta catenin increased during nerve regeneration in both the VNGs and NVNGs, while the level of alfa and gamma catenins did not increase in both grafts. There was no difference in the levels of the three catenins between the two methods of nerve grafts. This study suggests that beta catenin may play a different role from alpha and gamma for nerve regeneration, and that the expression of these catenins is not influenced by the vascularization of the nerve graft.
Hems et al., 1992	New Zealand white Rabbit (18) 3/3.5 kg	Peroneal nerve (autologue)	1.orthotopic graft 2.peroneal nerve	-free nerve grafts -vascularized nerve grafts based on a gluteal artery branch -freeze-thawed muscle autografts	Vascular	5 cm	36 weeks (250 days)	1. histomorphology: total myelinated nerve fibre counts, fibre and axon diameter) 2. EMG and ENG: sensory receptive area, isometric myogram for the extensor digitorum	Vascularized nerve grafts performed better than free nerve grafts (limited statistical significance)
Iwai et al., 2001	Fischer strain rats (140), 180–220g	Sciatic nerve (syngeneic)	1.orthotopic graft 2.sciatic nerve	-vascularized nerve graft based femoral artery and vein (end-to-end anastomosis) -free nerve graft	Vascular	1.5 cm	2,4,6,8,12,16,24 weeks	1.histomorphology (hematoxylin eosin staining,) 2. EMG: evoked potentials gastrocnemius muscle 3. Other: CAT activity, wet weight of the gastrocnemius muscle	VNG showed better early outcomes, but no significant advantage after 6 weeks.

Kanaya et al., 1992	Sprague-dawley rats (F,75) 250g	Sciatic nerve	1.orthotopic graft 2.sciatic nerve	- vascularized nerve graft based on the caudal femoral vessels - non-vascularized nerve graft - end-to-end repair	Vascular	2.5 cm	4-36 (every 1-2 weeks)	1.histomorphology (tolouidine blue staining) 2. functional assessment and behaviour: gait analysis, sciatic function index. 3.EMG and ENG: contraction force, NCV, peak amplitude of the action potential, CAPA, axonal count.	This study shows that VNG is functionally superior to a NVNG in a normal recipient bed.
Kärcher et al., 1986	Sprague-dawley rats (10)	Femoral nerve(autologue)	1.etherotopic graft 2. sciatic nerve	- vascularized nerve graft based on an arteriovenous fistula (femoral artery to vein) - non-vascularized graft	Vascular	1.5 cm	1,2,3,4,5 months	1.histomorphology (hematoxylin eosin, trichrome-masson, Bodian's silver impregnation)	VNG showed more rapid and better regeneration
Koshima et al., 1985 (Journal of Hand Surgery)	Sprague-dawley rats (75) 250-350g	Sciatic nerve (autologue)	1. orthotopic graft 2. sciatic nerve	- vascularized nerve grafts based on caudal femoral vessels - free nerve grafts	Vascular	1.5 cm	1-24 weeks	1. histomorphology (toluidine blue staining): diameters of myelinated axons, fiber caliber, number of myelinated fibers,number of large myelinated axons) 2. EMG and ENG: evoked potentials gastrocnemius muscle, MNCVs	Vascularized nerve grafts appear to yield better functional outcomes.
Koshima et al., 1985 (Annals of Plastic Surgery)	Wistar strain rats (M,39)	Sciatic nerve	1. orthotopic graft 2. sciatic nerve	- vascularized graft based on the ascending branch of the caudal femoral vessels - non-vascularized nerve graft right sciatic nerve with three different blood supplies vs NVNG	Avascular (silicon tube)	1.5 cm	1-24 weeks	1.histomorphology (toluidine blue): diameter of myelinated axons, number of myelinated nerve fibers, number of myelinated axons larger than 5 microm.	VNG showed more rapid axonal recovery, especially in the earlier postoperative period.

Lux et al., 1988	Mongrel dogs 10/15 kg	Saphenous nerve (autologue)	1.orthotopic graft 2.saphenous nerve	- vascularized saphenous nerve graft based on saphenous vessels - non-vascularized nerve graft	Vascular	5 cm	1,3,7,14 days (histologic study) 0,1,3,6 days (microsphere study)	1. histomorphology (hematoxylin eosine) 2. blood flow study: radioactive microsphere.	This model allows evaluating blood flow qualitatively and quantitatively. Vascularized nerve grafts show an advantage in blood flow during the first post-operative days.
Mani et al., 1992	New Zealand albino rabbit (M/F 76) 3.5 kg	Sciatic nerve (autologue)	1. orthotopic graft 2. sciatic nerve	- Vascularized nerve grafts based on femoral perforators - Free nerve grafts in a vascular and avascular graft bed	Vascular Avascular (silicon sheet)	3-4 cm	Short term evaluation (2,5,9,14 days) Long term evaluation (44 weeks)	1. histomorphology (methylene blue azure II staining): myelinated fiber diameter, frequency distribution, thickness of the myelin sheath, axon diameter) 2.EMG and ENG: conduction distances, latency, amplitude of motor action potentials. 3.angiography: presence or absence of blood vessels, revascularization patterns, rate of longitudinal revascularization	In the long term, the rate, size, and myelination of regenerating nerve fibers through vascularized and non vascularized nerve grafts did not differ significantly, despite the absence of blood supply to the latter in the initial stages.
Matsumine et al., 2014	Lewis rats (7)	Median nerve (autologue)	1.heterotopic graft 2.facial nerve	- vascularized island median nerve based on the median artery and vein - non-vascularized nerve graft	Avascular(silicone tube)	0.7 cm	30 weeks	1.histomorphology (toluidine blue, uranyl acetate, lead stain solution): number of myelinated fibers, regeneration of axon, myelin thickness, axon diameter. 2. ENG: CMAP	This study developed a rat model of vascularized median nerve transplantation to the buccal branch of the facial nerve. It showed that VNGs more effectively promoted axonal regeneration and functional recovery than the NVNGs.

Ozcan et al., 1990	Sprague-Dawley rats (F,30) 250-300 g	Femoral nerve(autologue)	NONE	-1-cm vascularized nerve graft model 4 subgroup: 1.A-V fistula 2.no fistula 3.no-blood flow 4.control -2-cm vascularized nerve graft 1.A-V fistula 2.no fistula	Avascular (silicone tube)	1 cm 2 cm	1 week	1.histomorphology (fluorescein dye) 2.microangiography	This model showed that, when distally ligating the femoral vessels without the creation of an A/V fistula, blood flow into the nerve segment remains uncompromised and can be used as VNG model.
Ozcan et al., 1991	New Zealand rabbits (11) 4/4,5 kg.	Median nerve (autologue)	1.heterotopic graft 2.facial nerve	- vascularized nerve graft model based on brachial vessels with an distal A-V fistula.	Vascular	1 cm	3 months	1.histomorphology (hematoxylin-eosin, tolouidine blue): myelinated axons counts 2. microangiography: micropaque perfusion	This study introduce an heterotopic VNG model, with a graft diameter similar to that of the reconstructed nerve.
Ozcan et al., 1992	New Zealand White rabbits (F,15) 4/4,5 kg.	Median nerve (autologue)	1. heterotopic graft 2. intratemporal facial nerve	- vascularized nerve graft based on an arteriovenous fistula (brachial vessels) - non- vascularized nerve graft - no repair group	Avascular (bony bed)	1 cm	3 months	1.histomorphology (hematoxylin-eosine, toluidine blue,uranyl acetate): muscle fiber diameter, number of myelinated and unmyelinated nerve fibers, total of myelinated axons, myelin sheath thickness 2.EMG and ENG: insertional and spontaneous activity, peak amplitude and latency	Bone as a recipient bed for a nerve graft is far less than optimal Mean axonal counting, nerve conduction and morphometric muscle study results were better in the VNG group, but differences were not statistically significant. Morphometric nerve analysis differences between the two groups were found to be significant.

Ozcan et al., 1993	Lewis rats (31*) 250/300g. * in which only 10 nerves repaired with VNGs and 10 nerves repaired with NVNGs.	Femoral nerve (autologue)	1.orthotopic graft 2. femoral nerve	- prefabricated vascularized amnion tubes Study 1: amnion tubes implant (subcutaneous, nerar femoral or epigastric vessels) Study 2: 1.vascularized amnion conduit based on an inferior epigastric vessel pedicle. 2.non vascularized amnion conduit group 3.vascularized nerve graft group based on femoral vessels 4.non- vascularized nerve graft 5. control group	Avascular(silicone sheet)	1 cm	3 months	1.histomorphology (hematoxilin-eosin, toluidine blue): axonal counting, fiber diameter, myelin sheat thickness. 2.microangiography: micropaque perfusion.	The vascularized amnion conduits showed comparable nerve regeneration to VNGs, and superior nerve regeneration when compared to non-vascularized amnion conduits and NVNGs.
Pho et al., 1985	White rats (18) 200g.	Femoral nerve(autologue)	1.ortothopic graft 2.femoral nerve	- vascularized nerve grafts based on femoral vessels - free nerve grafts	Vascular	2 cm	2,4,6,12 weeks	1. histomorphology (haematoxylin-eosine, phosphotungstate alum haematoxylin, Wilder's, Masson trichrome, Lucol fast blue, Palmgren): vascularization, collapse of reticulum framework, axonal regeneration, remyelination	There was no difference in the degree of vascularization, reticulin framework collaps, rate and extent of axonal regeneration and remyelination between the two groups.
Restrepo et al., 1985	Rabbits (18)	Sciatic nerve (autologue)	1.orthotopic graft 2.sciatic nerve	- vascularized nerve graft based on a proximal vascular pedicle. - non-vascularized nerve graft	Vascular	4.5 cm	5 -15 weeks	1. histomorphology (Blue II): thickness of myelin sheat, regenerating axons, vascularization, diameter of fiber	VNGs showed earlier myelinizaton and better fiber maturation (superior number and great diameter). no functional study)

Saray et al., 2002	New zealand white rabbits (20) 2,5/3 kg.	Sciatic nerve (autologue)	1. etherotopic graft (femoral region) 2. sciatic nerve	- prefabricated vascularized nerve grafts based on femoral vessels - free nerve grafts (femoral vessels ligated proximally and distally)	Avascular (custom-made tube)	3.5 cm	3,7,14 days	1. histomorphology(haematoxylin eosin) 2. microangiography	This model of prefabricated nerve graft does not require microvascular anastomosis for the arteriovenous fistula. The PVNG exhibited neovascularization and preserved viability of the Schwann cells.
Seckel et al., 1985	Sprague-Dawley rats 225/250g	Sciatic nerve (autologue)	1.orthotopic graft 2.sciatic nerve (peroneal fascicle)	- vascularized nerve graft (nerve transected and sutured through an epineurial window) - non-vascularized nerve graft (epineurium dissected away)	Vascular	1 cm	3,4,6 weeks	1.histomorphology (toluidine blue dye): axonal counts, remyelination of the axons, total fiber area, fibrosis and intraneural scarring.	This study failed to show difference in the number of regenerated axons or in the amount of intraneural scarring between the two groups. (
Serel et al., 2010	Wistar albino rats (10) 200/250 g.	Sciatic nerve (autologue)	1.etherotopic graft (femoral region) 2. sciatic nerve	- prefabricated vascularized nerve grafts based on femoral vessels	Avascular (silicon sheet)	1.5 cm	4 weeks	1.histomorphology (haematoxyline-eosine, luxol fast blue staining) 2.EMG: evoked compound action potentials	This model does not allow to build a functional VNG
Shibata et al.,1988	New Zealand white rabbits (43) 2 kg	Median nerve (autologue)	1.orthotopic graft 2. median nerve	- vascularized nerve graft based on brachial artery and vein. - non-vascularized nerve graft	Vascular	3 cm	10, 24 weeks	1. histomorphology (osmic acid): axon numbers, mean axon diameter, muscle weights, 2.EMG and ENG : NCV, CAPA, muscle contraction force.	No statistical difference was indicated in this comparison of VNG with NVNG for most measurements. Muscle strength was superior (20%) for VNGs, but may be clinically not significant.
Tada et al. 2001 (Koshima's model, 1985, Annals of Plastic Surgery)	Wister rats (M,60) 250/300g.	Sciatic nerve (autologue)	1.orthotopic graft 2.sciatic nerve	- vascularized nerve grafts based on caudal femoral vessels - free nerve grafts	Vascular	1.5 cm	20 weeks	1. histomorphology (toluidine blue staining) 2. western blot analysis and level of E caderine expression 3. immunofluorescent staining (E caderine)	The level of E- cadherin expression was significantly higher in VNG than in NVNG, and may affect rapidity of nerve regeneration.

Tark et al., 2001	New Zealand White rabbits (33)	Sciatic nerve (autologue)	1.orthotopic graft 2.sciatic nerve	- vascularized nerve graft based on the inferior gluteal vessels - non-vascularized nerve graft	Vascular	4 cm	2,3,4 months	1. histolomorphology (toluidine blue): myelinized fiber distribution, vascularization, nerve fiber diameter, schwann cells	Morphometric comparisons of axonal regeneration showed better results in VNGs.
Taylor et al., 1976	White pigs 15/20 kg. Patients*	Femoral nerve (autologue)	1.orthotopic/heterotopic graft 2. femoral nerve (homo or contralateral side)	pedicled and free vascularized femoral nerve graft based on femoral vessels (sectioned distally or proximally and distally and reanastomosed for free transfers)	Vascular	6 cm	2 weeks	Macroscopic examination: pulsation and bleeding	They describe a model of island/free vascularized femoral nerve graft
Townsend et al., 1984	Human cadavers (13)* Greyhound dogs (15)	Saphenous nerve	1.orthotopic	- Saphenous artery nerve with/without vein, taken as a unit (vessels anastomosis) - Non-vascularized nerve	Vascular	10 cm	6-12 weeks	1. histomorphology: india ink, fluorescein	The composite vascularized grafts were associated with more rapid axonal regeneration and remyelination.
Vargel et al., 2009	Wistar albino rats (M,40) 250/3000 g.	Femoral nerve (autologue)	1.orthotopic graft 2. saphenous nerve	- Prefabricated venous nerve graft (saphenous nerve and femoral vein) - Flow through venous nerve graft (proximally and distally ligated femoral artery, saphenous nerve and femoral vein transected and repaired) - Arterialized venous nerve graft (proximally and distally ligated femoral vein, saphenous nerve and femoral artery transected and anastomosed through the a femoral vein graft) - Free nerve graft (vessels wrapped)	Avascular (silicon sheet)	1.5 cm	10 weeks	1.historphology: (hematoxyline eosine,masson's thricrome staining): density of myelinated fibers, fiber diametr, axon diameter,myelin thickness 2. EMG and ENG: NCV, amplitude of the negative peak, peak-to-peak amplitude, total area of CNAP	Flow through venous nerve graft and arterialized venous nerve graft showed the better results, with all vascularized nerve grafts performing better results than conventional nerve grafts.

Zhu et al.,2015	New Zealand White rabbits (18) 2.5/3kg.	Central Auricular nerve (autologue)	1. heterotopic graft 2.facial nerve	- vascularized nerve graft based on auricular vessels - non-vascularized nerve graft - vascularized and non-vascularized nerve graft based on auricular vessels	Vascular	2 cm	4 months	1.histomorphology (hematoxylin eosin): myelinated nerve fiber density, mean diameter of regenerated myelinated nerve. 2. immunohistochemistry (mouse anti 200kDA neurofilament heavy antibody, mouse anti CD31 antibody, anti-rabbit policlonal antibody) 3. ENG: NCV, action potential's velocity, amplitude, and latency	This study introduces a new vascularized nerve graft model for facial nerve repair. VNGs showed better functional recovery and more regenerated axons.

Table 1: *Animal models and studies on vascularized nerve grafts.*

4.2.3. Study IV: Non-vascularized vs vascularized nerve grafts (experimental study)

Group 1:

The length of the exposed segment of sciatic nerve (before bifurcation into the tibial and peroneal nerve) was about 2.5 cm; caudal ascending vessels entered the nerve at its bifurcation. The nerve was easily accessed through the anterior approach, at a distance of about 1.5 cm from the femoral vessels.

Ring cuff electrodes were not appropriate for the purpose of the study, as they compressed the small vessels entering the nerve, interfering with its blood supply. Stimulation with non-implantable electrodes was then chosen for subsequent experiments, and the MUNE technique for estimation of motor unit number was standardized (intensity: gradual increase starting at 0.5 Hz, stimulus duration 0.02 ms); repeated measures were recorded and its reproducibility was confirmed.

Group 2:

Electrodiagnostic studies: Mean estimated motor unit number at time 0, 1 and 2 for the gastrocnemius and the tibialis anterior muscles are showed in table 2. Repeated measure ANOVA and Duncan's post-hoc test showed a significant difference in motor unit loss of the left (non-vascularized graft) gastrocnemius muscle at both time 1 and 2 compared to the left (vascularized graft) gastrocnemius muscle, but not of the tibialis anterior muscle. Motor unit loss at time 1 and 2 was significantly higher in the tibialis anterior muscle.

Animal (n)		Right side (mean ± s)			Left side (mean ± s)		
		T0	T1	T2	T0	T1	T2
1	GM*	134±45	39±33	22±13	154±37	28±27	11±6
	TA	118±28	4±7	16±5	97±12	9±9	14±2

Table 2: *Mean estimated motor unit number at time 0, 1 and 2 for the gastrocnemius and the tibialis anterior muscles. * indicates a statistical significant difference in the decrease of motor unit number in the left (non-vascularized graft) compared to the right (vascularized graft) gastrocnemius.*

Muscle weight

There was a minimal difference in the wet muscle of right and left anterior tibial muscles (0.5 vs 0.4 gr) and gastrocnemius muscles (1.2 vs 1.1 gr), which was not statistically significant.

Histomorphology

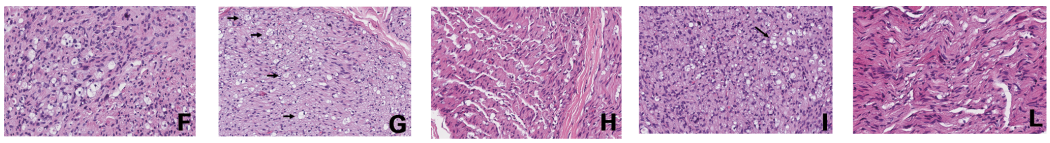
Mean value of nerve diameter, axon diameter and density, fiber diameter and density, neural to connective tissue ratio and axons to fibers ratio are presented in table 3.

Side	Level	Nerve diameter (mm)	Axon diameter (μm)	Axons/5 HPF	Fiber diameter (μm)	Fiber/5 HPF	Neural/connective tissue	Axons/fibers
Right	1	1.01	58.33	2.53	8.43	84.33	3.33	0.59
	2	1.45	61.67	3.27	8.83	105.33	3.33	0.61
	3	1.21	45.67	2.67	8.03	90.33	3	0.54
	4	1.13	28	2.07	7.53	57.33	2.67	0.49
	5	0.89	27.67	3	8.67	49.67	3	0.52
Left	1	1.16	34	2.4	6.97	64.67	2.33	0.48
	2	0.79	35	3.47	8.73	79.33	2	0.49
	3	0.84	27.67	2.7	6.9	55.33	2.33	0.58
	4	0.79	42.33	2.63	8.07	78.67	2.67	0.54
	5	0.65	19.33	2.1	7.27	40.67	2	0.47

Table 3: *Detailed data on histomorphologic nerve characteristics.*

There was a statistically significant drop in the value of all measured parameters of the distal nerve section (n 5) compared to the proximal section (n 1). All considered parameters were slightly higher in the right (vascularized graft) side. Fiber degenerative phenomena from proximal to distal were more evident and connective tissue was more represented in the non-vascularized nerve graft side. Small arterioles were observed in the vascularized side, but only vascular capillaries were observed in the non-vascularized side (figure 3). However, any of the analyzed parameters showed a statistically significant difference between the two sides.

Figure 4: *Series of nerve sections with hematoxylin-eosin staining (x200). From left to right: 2 mm proximal and 2 mm distal to the proximal suture, middle part of the nerve graft, 2 mm proximal and 2 mm distal to the distal suture. A-E: right side, (vascularized nerve graft); F-L: left side (non-vascularized nerve graft). Reduction in number of fibers and fiber degenerative phenomena from proximal to distal are more evident in the non-vascularized nerve graft side. Small arterioles are observed in the right side (C).*



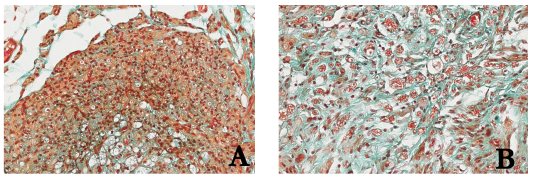


Figure 3: *Nerve section of the nerve grafts with Masson thricrome staining (x200): A: right side, vascularized nerve graft. Neural tissue (red) with some dilated fibers (arrows). B: left side, vascularized nerve graft. Decrease of neural tissue (red) with loose dilated fibers, and collagen deposition (green).*

Group 3:

In all the experiments, the venous loops easily reached the sciatic nerve: in animal 2, (1 anastomosis), despite use of a single epigastric vein, the loop had sufficient length to comfortably reach the nerve (figure 5). End-to-side anastomosis was more challenging than end-to-end due to small vessels' caliber and was found thrombotic 1 hour after surgery.

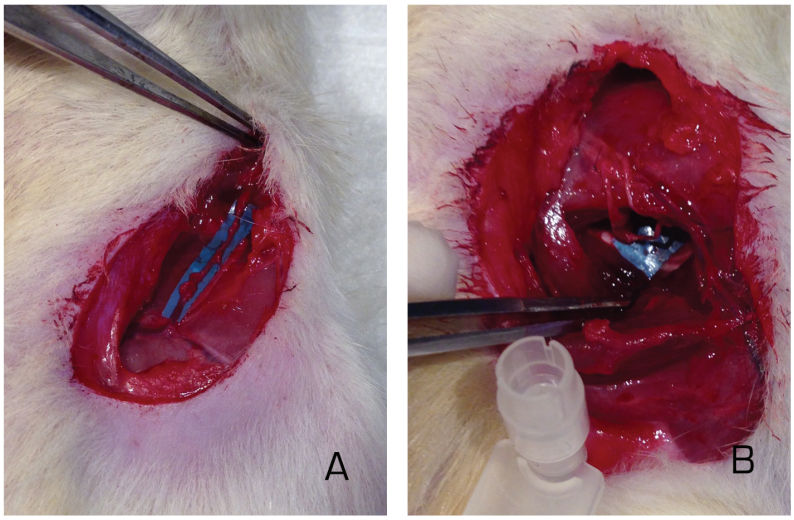


Figure 5: *Venous loop for the vascularized sciatic nerve graft model. A: Epigastric vein dissected (blue contrast). The forceps points out the cranial extremity. B: Venous loop among the epigastric vein and the femoral artery (end-to-end anastomosis, blue contrast), which easily reaches the sciatic nerve (under the blue contrast).*

4.3. **Study V: Brachial plexus reconstruction with non-vascularized nerve grafts (clinical study)**

Thirty-seven patients were operated for brachial plexus injury during the selected period. Thirteen patients underwent brachial plexus reconstruction with free (non-vascularized) nerve grafts or nerve transfers. Complete clinical data were available for 8 patients.

Three patients had a complete brachial plexus injury, 3 had C5-C7 paralysis, 2 had C5-C6 paralysis, and one had an injury of the suprascapularis, axillaris, and musculocutaneous nerves. All but one patient sustained a traffic injury. Mean

time to surgery was 4.8 months. Minimal follow-up was two years and longer follow-up data were available for 3 patients. Two patients were proposed scapulo-humeral arthrodesis (1 refused) and 3 patients received further surgery for restoring hand function.

Data on the level and type of injury, time to surgery, number and type of operations, and follow-up length are summarized in table 4.

Patient (n)	Sex/Age	Mechanism of injury	Level of injury	Type of injury	Time to surgery	Surgery	Re-operations	Follow-up
1	M/30	Crush injury	C5-C7	Avulsion	4 months	- Suralis nerve graft to C5 and n. musculocutaneous - Transfer of n. accessories to n. suprascapularis	EDC tenodesis	8 y
2	M/23	Traffic injury	N. suprascapularis, axillaris, musculocutaneous	Rupture	5 months	- Suralis nerve graft to n. axillaris - Neurolysis n. suprascapularis		2 y
3	M/57	Traffic injury	C5-Th1	Rupture	3 months	Suralis nerve graft: superior trunk to n. musculocutaneous and axillaris, C7 to n. radialis, C6 to n. medianus		2 y
4	M/23	Traffic injury, traction	C5-C7 C8-Th1	Rupture Avulsion	3 months	Suralis nerve graft: C7 to posterior and lateral fascicle	(Refused scapulo-humeral arthrodesis)	2 y
5	M/21	Traffic injury	C5 C6-C7	Avulsion	5 months	-Suralis nerve graft to C5	-Transfer of FDS III to ECRB, -Tenodesis EDC and EPL to radius	4 y
6	M/27	Traffic injury	C5-C6 C7	Rupture Avulsion	8 months	- Neurolysis superior trunk - Suralis nerve graft to C5 and C6	- Scapulo-humeral arthrodesis - Transfer FCU to ECRB	4 y
7	M/35	Traffic injury	C5-C6	Rupture	8 months	- Neurolysis C6 - Suralis nerve graft to C5		2 y
8	M/16	Traffic injury	C5 C6-Th1	Rupture Avulsion	2 months	Suralis nerve graft: C5 to n. musculocutaneous and to n.suprascapularis		2 y

Table 4: *Data on the level and type of injury, time to surgery, number and type of operations, and follow-up length for the 8 patients included in the study.*

Functional results

Only 4 patients (50%) returned to work, all to a different job, and 3 patients (38%) complained significant limb pain up to last follow-up visit.

Shoulder reconstruction

At 2 year follow-up, mean active shoulder abduction was 35° (range: 0°-80°), mean active elevation was 48° (range: 0°-140°) and mean active external rotation was 0° in all patients. All patients with a follow-up longer than 2 years showed a further increase in the range of motion, with a mean gain of 20° in abduction and 23° in elevation (not considering patient 6 in which a higher gain was due to further surgery). One patient also gained 10° of external rotation.

Elbow reconstruction

At 2 years follow-up, mean active elbow flexion was 93°. Patients with a follow-up longer than 2 years showed a further 15° increase in the range of motion.

4.4. **Study VI: Painful scar neuropathy (literature review)**

Literature review identified 21 papers, most of which on the treatment of median and ulnar nerve entrapment recurrence. The method most frequently associated with neurolysis was flap coverage (15 articles); the remaining papers described the use of anti-adhesion devices (3 articles), and vein wraps (3 articles).

Mean rate of "positive outcome" (pain resolution/reduction) was high (86%), but pain resolution rate was achieved only in 20% of cases. Outcomes were similar irrespectively of the type of treatment, but greatly differed among studies (range: 57%-100%).

CHAPTER 5

5. Discussion

5.1. **Pre and post-operative evaluation of peripheral nerve injuries (Study I)**

This study demonstrates that ultrasound is a powerful, non-invasive tool for examination of peripheral nerve injuries, which can orient diagnosis and surgical strategy of focal peripheral nerve injuries.

Ultrasound complemented electrodiagnostic studies and showed a contributive or confirming role in most patients within each groups (entrapment neuropathies, post-traumatic/post-surgical neuropathies, tumors). Its contribution was higher for focal masses arising from or compressing the nerve, such as nerve tumors, neuromas, foreign bodies or ganglion cysts, and in complex post-traumatic or post-surgical neuropathies. Also, it allowed diagnosis in all cases with discordant clinical and electrodiagnostic findings (16% of patients in this case series).

Ultrasound also contributed to the planning of the surgical access and strategy, and to a faster and easier surgery for entrapment neuropathies, by showing eventual anatomical anomalies or dynamic nerve dislocations. Some authors also foresee that high-resolution imaging could theoretically replace the electrodiagnostic tests in the diagnosis of some neuropathies.

Ultrasound is a valuable imaging technique for peripheral nerve injuries; besides having a similar accuracy to that of MRI, it allows a better identification of multifocal lesions, a real-time, dynamic and serial examination of a wide anatomical field, and visualization of a long nerve in a single scan; also, it can easily assess flow dynamics and vascular pattern of nerve tumors. It allows to directly visualize the cause and extent of nerve lesion and finds its place between electrodiagnostic tests and exploratory surgery; it can be used at the same time of the clinical examination and electrodiagnostic study, allowing a complete morphological and functional examination of the nerve.

5.2. **Experimental models for the study of peripheral nerve injuries**

Study II

This review points out great differences in all aspects of the published neuroma models (animal, nerve, type of injury, evaluation methods), especially – but not only- between models of terminal neuromas and NIC. Several of these are better with regards to a specific aspect of neuroma physiopathology, prevention or treatment to be studied, making unlikely that a single model could be the gold standard.

Although evidence-based indications for standardisation of neuroma models could not be extrapolated, based on advantages and popularity of available models, we propose our suggestions for standardisation:

1. ANIMAL MODEL: Male Sprague-Dawley rat should be regarded as the rodent reference model, while rabbits could be preferable for special purposes, such as investigation of amputation and muscle reinnervation. The sciatic nerve appears adequate for most studies, while pure sensory nerves (such as the sural and the saphenous nerve) should be preferred for the study of terminal sensory neuromas.

2. MECHANISMS OF INJURY: Nerve transection is the most published technique for terminal neuromas and is easy to reproduce. Resection of a nerve segment is advisable, with a 1 cm gap appearing adequate in the rodent model. Partial ligation, although less popular than nerve crush, should be regarded as the best technique for a reliable and reproducible NIC model.
3. EVALUATION METHODS: Traditional histomorphology is the historical gold standard evaluation method, but needs to be coupled with immunolabeling, RT-PCR and proteomics, promising and reproducible tools which are becoming more and more indispensable in modern research. These tools also allow a more accurate - although indirect - evaluation of pain compared to the autotomy model. Computerized gait analysis is the gold standard for motor recovery evaluation, while mechanical testing of allodynia and hyperalgesia reproducibly assesses sensory recovery.

Study III

Most of the experimental studies on vascularized nerve grafts were done during the 90's, but there has been a new wave interest in the last years.

Their role in the clinical practice is still controversial, and their main indications are scarred beds and need for long or thick grafts. The main drawbacks are variability of their vascular supply, need for sacrifice of a major vascular axis, technical difficulty and length of the procedure. Prefabrication of vascularized nerve grafts could overcome some of these limits, but the few experimental studies available provided limited scientific evidence or failed to build a vascularized nerve graft.

Current literature suggests that vascularized nerve grafts more effectively promote axonal regeneration and functional recovery, especially in avascular beds. Most studies showed an increase in the axonal number and diameter and in

the neural to connective tissue ratio. However, only about 20% of studies showed superior long-term results. This could be partially explained by a limit of the experimental models (mainly rat or rabbits), in which long and thick nerve defects are difficult to reproduce; also, nerve regeneration is known to be faster in rats than in human. This means that the advantage provided by vascularized nerve grafts could be greater in humans, but current knowledge does not support that the differences between vascularized and non-vascularized - although beating statistical significance – yield a clinical advantage that justify the complexity of the procedure.

In most studies, the main evaluation method is histomorphology, while only a few include behavioral/functional tests. Further studies are desirable that not only confirm superiority of vascularized nerve grafts based on evaluation of nerve characteristics, but also provide further evidence for superior functional results, which could be useful in defining clinical indications.

Prefabrication of vascularized nerve grafts has been scantly investigated, also due to its limited clinical implications. However, experimental prefabricated vascular nerve grafts could provide a reference experimental model for providing with blood supply other promising and popular tools in nerve reconstruction, such us nerve conduits or allogeneic grafts.

5.3. **Functional outcomes after peripheral nerve repair (Study IV, V and VI)**

Study IV

The preliminary results of this study showed a trend towards better axonal regeneration and muscle reinnervation in VNGs compared to NVNGs, which did not reach statistical significance. These data could be due to the small sample size, and need to be reanalyzed after reaching the calculated sample size. Also, we applied the MUNE technique to the rat sciatic nerve and defined a reproducible protocol for functional evaluation of muscle reinnervation.

Histomorphology showed preservation of blood supply, less fibrosis and degenerative phenomena in the VNGs. Estimation of motor unit number with the MUNE technique

provided a reliable index of muscle reinnervation, overcoming the problem of alternation (motor units recruited in numerous combinations by stimulation of a motor nerve). It showed a significant difference in motor unit loss of the left (non-vascularized graft) muscles, mainly related to gastrocnemius muscle, but an overall worse recovery for the tibialis anterior muscle.

Also, we described a possible model for prefabricated vascularized nerve grafts based on a venous loop that can be studied through the described evaluation methods, allowing comparison of functional outcomes.

Study V

This preliminary study shows that, in patients with brachial plexus injuries treated with non-vascularized nerve grafts or nerve transfers, shoulder and elbow function continues to improve in terms of active ROM even after 2 years from surgery. However, improvements with regards to employment and daily activities remain questionable. Limited literature exists on long term follow up; several

authors suggested that motor recovery reaches a plateau within 2 to 3 years after surgery and outcome are often reported in terms of muscles' ROM and strength.

Only a few studies evaluated motor function at a longer follow up. Recently, Wang et al. compared shoulder and elbow ROM and mBMRC and showed a significant improvement at 11 years follow up. However, they did not investigate patient-reported outcomes and implications of the reported functional improvement in daily activities.

In our study only 50% of patients were able to return to work, all to a different job, and limb pain continued to significant affect 38% of patients in the long term. Also, further surgery was indicated in 50%, to improve shoulder function or to address hand function. These data suggest that doctor-reported outcomes may overestimate the degree of functional recovery in brachial plexus patients.

This study has several limitations: it is retrospective and has a small size; patients-reported outcome questionnaires were not administered. However, it points out the need for investigation of long-term function based on quality of life evaluation and patient-reported outcomes. Further studies are deserved to provide patients with more information about expected functional outcomes.

Study VI

Painful scar neuropathy is difficult to treat and often only partially solved. There is scant published evidence regarding its diagnostic work-up and treatment.

Different surgical procedures are indicated, from simple external neurolysis in simpler cases to more extensive neurolysis and coverage with a local or free vascularized flap in recurrent cases and in those with a severely injured nerve bed.

Despite active clinical research, no gold standard treatment has been established, as no medical or surgical treatment has shown superiority over the others with regards to the rate and extent of clinical response. If the pain is not alleviated

following the initial procedure, subsequent operations are unlikely to be successful. No treatment among the myriad that have been described assures an effective and/or reliable outcome, and the same treatment can lead to very different outcomes in different patients, from complete resolution to a worsening of symptoms.

Currently, neither surgeons nor pain therapists are able to predict, which patient will respond to treatment and for what duration that response may last.

All these data suggest that the key for improving our approach to neuropathic pain lies in gaining better insight into its underlying mechanisms. A genetic predisposition is likely to exist, and individual differences in biochemical signals involved in nerve pain and their possible modulation for therapeutic purposes deserves further study.

Then, we foresee genetic and biomolecular research as promising fields of future investigation, which could ultimately lead to a better understanding and management of painful scar neuropathy.

CHAPTER 6

6. Conclusion

STUDY I

We advocate routine use of ultrasound nerve imaging in the evaluation of patients candidate to surgery, and support it as first line imaging technique for the study of peripheral neuropathies, as a valuable complement to clinical and electrodiagnostic examination.

STUDY II

A large variety of experimental neuroma models exist. This review summarizes current knowledge and provides suggestions for standardization of experimental model(s) of peripheral neuromas.

STUDY III

Current literature suggests that vascularized nerve grafts more effectively promote axonal regeneration and functional recovery compared to non-vascularized nerve grafts, especially in avascular beds. However, scientific evidence to support a clinically significant superiority is still missing.

STUDY IV

The preliminary results of this study showed a trend towards better axonal regeneration and muscle reinnervation in VNGs compared to NVNGs, which did not reach statistical significance, but need to be reanalyzed when the calculated sample size is achieved.

The MUNE technique provided a reliable and reproducible evaluation of functional outcomes based on muscle reinnervation.

Also, we described a model for prefabricated vascularized nerve grafts based on a venous loop.

STUDY V

These data, although limited in size and evaluation methods, suggest that doctor-reported outcomes may overestimate the degree of functional recovery in brachial plexus patients and that patient-reported outcomes measures are desirable to provide a more realistic picture of expected functional outcomes.

STUDY VI

Diagnosis and treatment of scar neuritis and neuropathic pain are still problematic. No treatment has shown superior short or long-term outcomes, and responses vary significantly and unpredictably among patients.

Further research need to address genetic predisposition and individual differences in biochemical signals involved in nerve pain and their possible modulation for therapeutic purposes.

CHAPTER 7

7. References

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Papers

Preface to special issue on "Peripheral Nerve Repair and Regeneration"

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Peripheral nerve surgery has achieved a great improvement in the last century. The introduction of microsurgery and the technical advances in reparative and reconstructive techniques has been instrumental in advancing nerve surgery techniques. Yet, in the last few decades, the scientific progress has slowed down significantly, and surgery is still far from reaching an optimal functional recovery in most cases.

New surgical approaches are increasingly used for various indications, but current research mainly focuses on the mechanisms of nerve damage and nerve regeneration. It is now clear that nerve regeneration not only relies on surgical reconstruction but also on understanding underlying biomolecular processes that could turn out to be the key for developing novel treatment strategies.

With the present special issue on “nerve regeneration and repair”, we wish to summarize the state of the art of translational and clinical research and present the current trends and future prospects in peripheral nerve surgery. For this purpose, most of the twelve papers in this issue are review papers.

This issue begins with an overview of the current neurophysiologic and imaging tests: preoperative diagnostic work-up and postoperative monitoring, to provide a clinical guide on the assessment of nerve injuries.

Then, we discuss nerve pain and dysfunction following surgery (e.g. in scar neuropathy or in recalcitrant compression neuropathy) and the treatment approaches. Using current literature, we summarize the analysis of reasons for treatment and the current clinical and surgical recommendations. Three papers of this issue focus on different aspects of nerve pain, and suggest promising directions for research on the mechanism of nerve regeneration and nerve guidance (e.g. investigation of genetics and biochemical signaling) and novel therapeutic approaches (e.g. neurostimulation).

We also reviewed modern advances in surgical techniques for complex nerve injuries, such as vascularized nerve grafts, which are indicated for long nerve gaps and scarred beds, and nerve transfers, which are indicated for proximal nerve injuries.

Free vascularized nerve grafts were first described in the 1970s. After initial enthusiasm, their popularity decreased partly due to their technical difficulty, and only few surgeons used the technique. Yet, they perform better than nonvascularized nerve grafts and provide advantage in recovery in selected cases. Their potential could find a greater expression in the next future, as discussed in a review article.

Nerve transfers have opened new horizons in nerve repair strategies: first described in the 19th century, they have revolutionized the 21th century approach to nerve injuries, particularly proximal injuries. They are a valuable tool for otherwise unrepairable nerve lesions candidates to palliative treatment (e.g. tendon transfer) and are finding increasing indications for repair of both motor and sensory nerves. Current indications in the upper limb nerve injuries are reviewed. Also, two of the papers in this issue are focused on “sensory protection” and “babysitting procedures”: local nerves can be redirected to a distal target to prevent the muscle atrophy and the functional loss that follows prolonged denervation.

To complete the tableau of future prospects in nerve regeneration, two papers of this special issue are focused on two novel fields of research: tissue-engineered conduits and robotic-assisted microsurgery.

Ongoing research holds the potential of revolutionizing our approach to nerve repair and regeneration. Tissue-engineering investigates the potential of different biomimetic materials as peripheral nerve scaffold, and

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modifies and directs interactions between cells, growth factors and signaling molecules, and biomaterials, to guide nerve regeneration. Robotic-assisted microsurgery represents a great technological advance, which can be further developed for specific applications in peripheral nerve surgery. It allows a minimally invasive approach, reducing morbidity and perineural adherences and favoring a better nerve regeneration.

Lastly, we looked at composite tissue allotransplantation, where nerve regeneration holds specific features, as the host interaction with allogenic tissues and the need for immunosuppression; the last paper of this issue discusses the role of cortical reorganization, drugs (such as tacrolimus) and adipose-derived stem cells for axonal regeneration and myelination.

I hope that the papers presented in this special issue will serve as a reference and inspiration for students, researchers, and clinicians who have interest in nerve surgery.

Thanks to all the authors and the reviewers for their contributions and to the editorial staff of *PAR*, for working on this special issue and for their precious and continuous support.

I hope you enjoy reading this special issue.

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Clinical neurophysiology and imaging of nerve injuries: preoperative diagnostic work-up and postoperative monitoring

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ABSTRACT

Peripheral nerve injuries are a heterogeneous group of lesions that may occurs secondary to various causes. Several different classifications have been used to describe the pathophysiological mechanisms leading to the clinical deficit, from simple and reversible compression-induced demyelination, to complete transection of nerve axons. Neurophysiological data localize, quantify, and qualify (demyelination *vs*. axonal loss) the clinical and subclinical deficits. High-resolution ultrasound can demonstrate the morphological extent of nerve damage, fascicular echotexture (epineurium *vs*. perineurium, focal alteration of the cross-section of the nerve, any neuromas, *etc*.), and the surrounding tissues. High field magnetic resonance imaging provides high contrast neurography by fat suppression sequences and shows structural connectivity through the use of diffusion-weighted sequences. The aim of this review is to provide clinical guidelines for the diagnosis of nerve injuries, and the rationale for instrumental evaluation in the preoperative and postoperative periods. While history and clinical approach guide neurophysiological examination, nerve conduction and electromyography studies provide functional information on conduction slowing and denervation to assist in monitoring the onset of re-innervation. High-resolution nerve imaging complements neurophysiological data and allows direct visualization of the nerve injury while providing insight into its cause and facilitating surgical treatment planning. Indications and limits of each instrumental examination are discussed.

Key words:

Electromyography, imaging, injury, magnetic resonance imaging, nerve conduction studies, neurodiagnostic, peripheral nerve, ultrasound

INTRODUCTION

Every year more than 5% of patients admitted to a level one trauma center have a concurrent traumatic peripheral nerve injury.[1] These patients are often young adults at the peak of their employment productivity, and therefore, functional decline associated with nerve lesions is particularly significant.[2] Thus, there is a great interest in optimizing both the diagnostic accuracy and early treatment of peripheral nerve injuries.

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The purpose of this review is to discuss peripheral nerve injuries and their diagnostic management and outcomes evaluation with regard to clinical findings and neurodiagnostic studies and imaging.

The goal is to provide a practical guide for general management that is, applicable to all types of nerve injuries. The main classifications and basic principles of a correct clinical approach will be summarized. Next,

the indications and correct timing for each instrumental examination will be reviewed, with a specific focus on innovative methods and future prospects.

CLASSIFICATION OF PERIPHERAL NERVE INJURIES

The most commonly used classification for peripheral nerve injuries is that by Seddon,[3] and Sunderland.[4] The Seddon classification places injuries into three basic types: neurapraxia, axonotmesis, and neurotmesis.

Neurapraxia (praxis: to do, to perform): the nerve axons are intact but cannot transmit impulses. This occurs secondary to ischemic damage with temporary myelin sheath damage. Without myelin, there is an alteration of "saltatory conduction" across the nodes of Ranvier with subsequent slowed or blocked nerve conduction. Neuropraxia is the mildest form of nerve injury; "Saturday night" radial palsy and entrapment neuropathies like carpal tunnel syndrome is good example for this condition.[5,6] Nerve recovery occurs after remyelination and sensory-motor functions can usually completely restored within days to weeks.[7]

Axonotmesis (tmesis: to cut): the axons are damaged or destroyed, but most of the connective scaffold (endoneurium, perineurium, and epineurium) remains intact. Axonotmesis is commonly seen in crush and stretch injuries.[8] After injury, anterograde Wallerian degeneration of the distal axonal fibers is completed within a few days.

Neurotmesis: the nerve trunk is disrupted and loses anatomical continuity. Neurotmesis represents the most severe form of injury with disruption of the axons, myelin sheath, and connective tissues. It may occur following sharp injuries, massive trauma, or severe traction that partially or completely interrupts nerve continuity.[9] In order to enhance the chances for reinnervation after neurotmesis, surgical nerve repair is mandatory.[10] Without surgery, uncontrolled axonal re-growth will generate a neuroma.

The Sunderland classification includes five stages and identifies three types of neurotmesis: (1) stage I corresponds to neuropraxia; (2) stage II corresponds to axonotmesis; and (3) stages III, IV, and V correspond to neurotmesis [Table 1], with impairment of the endoneurium, perineurium, and epineurium.

The distinction between the different types of injury is not always precise. Clinical evaluation benefits from instrumental approaches to discriminate severity at an earlier stage, thus allowing for appropriate and timely treatment.

CLINICAL APPROACH

Patient age, mechanism of injury and associated vascular and soft tissue injuries strongly influence the extent of recovery of the injured nerve. These elements are of great importance and are the primary details collected in the clinical history. A detailed examination includes evaluation of pain and muscular strength and sensory testing in the territory of the injured nerve. The homologous contralateral and other ipsilateral preserved nerves are used for comparison, particularly in polytrauma patients.[11] Appropriate motor and sensory evaluation is mandatory to identify injuries to sensitive, motor, and mixed nerves; early and late signs of autonomic disorders should also be evaluated, including vasomotor disorders and trophic alteration of the skin, nails, and subcutaneous tissue.[11,12] Both negative (e.g. hypoesthesia, muscle weakness, and atrophy) and positive symptoms (e.g. dysesthesia, pain, fasciculations) due to loss of nerve function or inappropriate spontaneous activity, respectively, should be noted.

The simplest standardized clinical evaluation of a cutaneous somatic sensitivity is the test of the pain pathway (the patient's ability to perceive the touch of a sharp object).[13] Clinicians and surgeons generally refer to cutaneous nociception because of less lower overlap of innervating territories when compared to tactile sensation.

Hypoesthesia generally involves all superficial and deep somatosensory systems (tactile, thermal, pain, and proprioception); anatomical charts and diagrams help to

Table 1: Classification of peripheral nerve injuries according to Seddon and Sunderland

Type of injury Seddon classification	Type of Injury Sunderland classification	Major structure involved	Prognosis	Neurodiagnostic findings	Requirement for surgical intervention
Neuropraxia	I	Myelin	Good	Slower conduction velocity or conduction block; EMG with no fibrillation, reduced recruitment and fast firing	None
Axonotmesis	II	Myelin, Axons	Fair (depending on how many fibers are involved)	Reduced CMAP and SNAP amplitudes; EMG with fibrillation, reduced recruitment and fast firing	Depends on extension of the lesion
Neurotmesis	III, IV, V	Myelin, Axons, Endoneurium Perineurium Epineurium	Poor (depending on how many fibers are involved)	Reduced or absent CMAP and SNAP; EMG with fibrillation and motor units loss	Often requires surgical repair

EMG: Electromyography, CMAP: Compound muscle action potential, SNAP: Sensory nerve action potential

identify areas that correspond to specific nerves or to dermatomes (useful for root or spinal level injuries).

Sensory disorders may also include positive (irritative) symptoms which that should be explored: (1) paresthesia (spontaneous feeling of needles, tingling, numbness, and electric shock); (2) dysesthesia and hyperalgesia (inaccurate interpretation of a sensory stimulus which is perceived as different and with an affective unpleasant sensation); and (3) neuropathic pain (spontaneous pain consequent to a lesion in the afferent somatosensory fibers coming from the cutaneous territory of a nerve).

Motor signs and symptoms as a consequence of a reduced number of functional motor units include: (1) hyposthenia: reduced muscle strength as described by the use of the British Medical Research Council scale that recognizes five grades of muscle strength: 0, neither contraction nor movement are visible; 1, minimal contraction visible or flickering (residual functioning motor units) without movement; 2, active movement possible only without gravity (i.e. in a horizontal plane); 3, active movement obtained against gravity; 4, active movement against mild resistance (4-), moderate resistance (4) or strong resistance (4+); and 5, normal strength;[14] (2) muscular hypotrophy or atrophy: reduced volume of the muscle belly for both axonal damage and disuse; it will reach its maximum state in 3-4 months with a potential strength reduction of 80%. If denervation persists, a proliferation of fibroblasts characterizes the histological picture, as new collagen is deposited in both the endo- and perimysium, and atrophied muscle fibers are replaced by thickened connective tissue; (3) absence or reduction of osteotendinous (phasic) reflexes and of muscular tone (tonic reflex) due to involvement of both afferent sensory fibers from muscular spindles and efferent motor neuron axons of the somatic arc reflex; (4) hyposthenia, hypotrophy, and hypotonia configure the picture of partial or total flaccid paralysis of the group of muscles innervated by the affected nervous structures (roots, plexus, nerves); (5) positive symptoms (fasciculations and cramps) are rare in peripheral nerve injuries, but are often seen in radiculopathies; and (6) deformities: in chronic and severe cases, muscle paresis reduced joint movement in conjunction with healthy muscles may lead to deformities (cavus foot, claw-hand) and ankylosis.

No clinical evaluation can distinguish neurapraxia from axonotmesis, and no clinical or neurophysiological examination can distinguish axonotmesis from neurotmesis. To obtain the correct diagnosis and a plan appropriate to treatment, both neurophysiological and imaging studies and clinical re-evaluation over time are often required.

CLINICAL NEUROPHYSIOLOGICAL STUDIES

The neurophysiological or neurodiagnostic study represents an extension of the clinical examination; accordingly, neurodiagnostic tests should always be combined with a directed neurologic examination, in order to identify the clinical abnormalities and establish a differential diagnosis. For this reason, the evaluation is commonly referred as the clinical neurophysiological examination.

Clinical neurophysiological examination is currently the gold standard for diagnosis and determination of prognosis in peripheral nerve injuries,[15,16] in order to localize and quantify clinical and subclinical preoperative damage and postoperative recovery. As such, it yields key information on the type of involved fibers (sensory *vs*. motor), on the underlying pathophysiology (demyelination *vs*. axonal loss), on axonal loss quantification, and consequently on prognosis.

The core neurodiagnostic studies are nerve conduction studies and electromyography (EMG). These tools test the integrity and physiological function of peripheral sensory and motor fibers and the muscles.

In order to reveal axonal loss (presence of denervation potentials), the optimal timing of a neurodiagnostic study is 2-3 weeks after injury.[17,18] Neurodiagnostic studies should be repeated 3 months or more following trauma or surgical repair to assess the ratio of denervation to reinnervation.[19]

Nerve conduction studies

Nerve conduction studies are the first line studies in instrumental evaluation of nerve injuries. They are the most basic and easily performed types of neurodiagnostic studies, and also used for screening prior to any additional testing.[20]

Nerves and muscles are excitable structures and their potentials can be induced and recorded by external electrodes. When the nerve is stimulated, a compound muscle action potential (CMAP) can be recorded from the muscle, and a nerve action potential (NAP) can be recorded from the nerve. Amplitude and latency of the evoked response and conduction velocity are analyzed.[21]

The amplitude of the evoked response estimates the quantity of depolarized motor or sensory fibers, while conduction velocity measures the speed of the fastest (and large caliber) motor or sensory myelinated axons.

Sensory NAPs (SNAPs) are also helpful in differentiating between preganglionic (radiculopathies) and postganglionic lesions; postganglionic lesions produce abnormal SNAP due to Wallerian degeneration of the axons distal to the peripheral injury, whereas in preganglionic lesions axon degeneration occurs in the dorsal root and in the ascending central pathway, leaving peripheral fibers intact and SNAP unmodified, despite anesthesia in the examined cutaneous territory.[21]

Caution should be paid to interpretation of pure or prevalent motor diseases. Although changes in the CMAP are frequently used to preliminarily diagnose peripheral nerve injuries, they are not specific and may reveal, spinal disease of the anterior horn cells (myelopathy, amyotrophic lateral sclerosis, *etc*.), myopathy (muscular

dystrophy, myositis, *etc.*), a myelin-related acquired or congenital disorders (chronic inflammatory demyelinating polyneuropathy, Charcot-Marie-tooth disease)[22] or presynaptic neuromuscular junction disorders (Eaton-Lambert syndrome, botulism).

In neurapraxia, nerve conduction is either slowed or blocked secondary to demyelination. With stimulation proximal to the lesion, the conduction velocity will be reduced (conduction slowing), or the evoked potential amplitude will drop with respect to the normal potential obtained by distal stimulation (conduction block). When nerve remyelination completes, these abnormalities progressively disappear, with eventual complete recovery.

In the case of axonotmesis and neurotmesis, after distal axonal degeneration (which completes in 3-5 days for motor fibers and in 6-10 days for sensory fibers), CMAP and SNAP are reduced in amplitude when stimulating distally to the injury; the ratio between CMAP/SNAP amplitudes on the injured side to the CMAP/SNAP of the normal side is a good estimate of the degree of axonal loss. The higher the axonal loss, the lower the odds of recovery.

For technical reasons, exploration of the proximal peripheral nervous system is more complex; late responses such as F waves and the H reflex can be obtained for further information and somatosensory or motor evoked potentials can be explored.[23,24]

Electromyography

This examination requires the active participation of the patient. Needle EMG provides information on the function of the muscles function and their minimal functional units. It explores both the quantity and quality of motor unit action potentials (MUAP), their spatial-temporal recruitment in order to generate adequate movements, the presence of denervation, and the onset of re-innervation.[18] In partial or gradual denervation, reinnervation occurs early through collateral sprouting by adjacent surviving axons. In nerve transection, the only mechanism available for re-innervation is axonal regrowth from the proximal stump of the injury site. This regrowth is slow (1 mm/day) and may take months to years to reach the target muscles, depends on the distance to be covered.

The first step in EMG of nerve injuries is the evaluation of pathological potentials at rest. Fibrillation potentials and positive sharp waves are the most common potentials and appear 10-21 days after injury, while complex repetitive discharges indicate chronic and ongoing denervation. Although all these potentials are a sign of muscle fiber denervation, they can also be found in myopathies and myositis, which also induce hyposthenia. Fasciculation potentials occur from the spontaneous activation of motor units (all muscle fibers innervated by one neuron), which can be visualized directly as minor muscle twitches. Cramps are a painful involuntary contraction of the muscle which tend to occur when a muscle is in the shortened position and contracting, and can be recorded as a firing of motor unit potentials at high frequency. Many other spontaneous potential can be recorded from muscles, but their discussion is beyond the intent of this review.

The following step in the neurophysiological examination is the analysis of MUAP and their activation and recruitment patterns during voluntary contraction.

In acute axonal loss and pure demyelinating nerve injuries with conduction block, not all motor units can be recruited; the remaining MUAPs have normal morphology but fire with high frequency in order to obtain sufficient contraction, and the recruitment pattern results in incomplete interference. Note that denervation potentials will appear only in case of axonal damage.

In chronic axonal loss and denervation, early collateral sprouting from re-innervation of orphan muscle fibers by surviving axons is recorded on EMG as small satellite potentials of the MUAP's. Later, as the number of muscle fibers per motor unit increases with re-innervation, MUAP's become higher in amplitude, prolonged in duration, and polyphasic; these are the typical neurogenic MUAP's representing the pattern of denervation and reinnervation.

Incomplete nerve transection and in late stages of partial axonal loss, if regrowing axons from the site of injury eventually reach the target, very small low-voltage nascent MUAP potentials will be recorded. As reinnervation occurs, denervation potentials will gradually disappear.

NERVE IMAGING TECHNIQUES

Neurophysiological investigation offers information on the pathophysiology of the nerve deficit, the grade of severity, and prognosis. Although it is a fundamental tool in clinical evaluation, it does not provide precise information on the morphology, etiology or the extent of focal peripheral nerve injuries versus the focal involvement of only few fascicles.

In severe cases with unexcitable nerves and in postoperative patients who do not shows signs of improvement, EMG and conduction velocities cannot provide conclusive information on the presence of neurotmesis, nerve transection, the distance between nerve stumps, and the presence of multiple sites of injury.[25] Imaging assessment, in particular high-resolution ultrasound (HRU) and magnetic resonance imaging (MRI), may overcome these problems by providing information on nerve morphology and its surrounding tissues; these are becoming popular instruments for planning nerve reconstruction and the surgical approach.

High-resolution ultrasound

Although MRI is still more commonly used, based on our experience and on a review of the recent literature, the authors believe that HRU currently represents the most easily available and practical imaging technique for investigation of peripheral nerve pathology [Figures 1 and 2]. These machines are widely available and, when associated with high frequency transducers (7-18 MHz), reach up

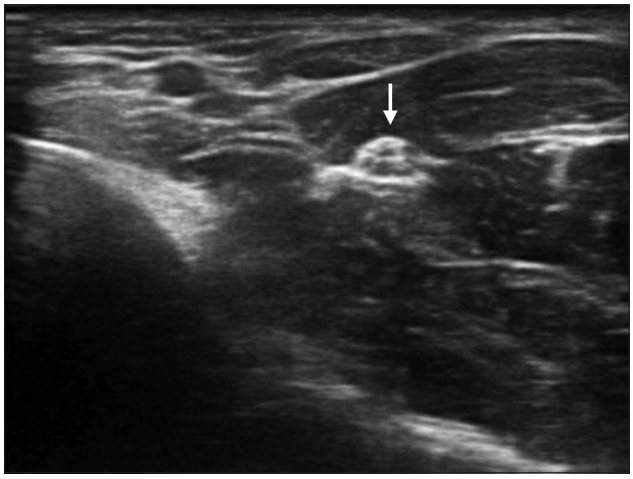

Figure 1: Axial scan of median nerve (arrow) at mid forearm; note the fascicular texture of the nerve and the homogeneous echogenicity of the surrounding muscles

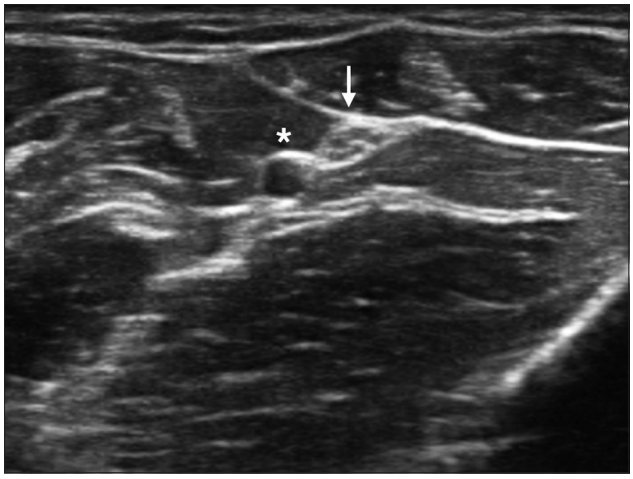

Figure 2: Axial scan of ulnar nerve (arrow) and ulnar artery (*) at forearm; in live scans pulsating arteries are a good landmark to be recognized

to 400 μm in axial resolution, which is higher than that achieved by a common MRI.[26] There is increasing evidence in the literature on the helpfulness of HRU, in particular in cases with equivocal clinical and neurophysiological data;[27] HRU may be diagnostic in a significant percentage of such patients.[28] Its advantages include a bedside, painless study of the nerve along the entire limb, with color-Doppler analysis integration and dynamic scans. In addition, it can be utilized in the presence of metal implants and orthopedic screws, and therefore is preferable to a high-cost, single segment MRI study.

Sonographic criteria for nerve identification are based on fascicular echotexture detection.[26] The cross-sectional area (CSA) of the nerve is one of the most studied parameters and is examined in each nerve along the length of the limb in an axial scan. CSA measurements are performed at the inner border of the thin hyperechoic rim of the nerve,[29] across the site of entrapment or trauma to calculate the distal-proximal CSA ratio. The nerve CSA is significantly related to the neurodiagnostic data and, when performed side by side with a comprehensive neurodiagnostic exam, it increases its diagnostic sensitivity.[30,31]

Echogenicity of the nerve should be reported; an increased CSA of the entire nerve or of a few fascicles, proximal to the site of entrapment or trauma, can be associated with fibrosis of the fascicles or epineurium. A few nerve pathologies, such as Schwannomas, will initially spare the nerve's conduction and sensory-motor functions, manifesting only with inconstant signs of irritation. Fiber sparing and dislocation can be recognized by an experienced HRU examiner.

Finally, nerve continuity can be assessed based on the analysis of the epi-perineurium and on the presence of a partial neuroma or transection.[32]

Imaging will also uncover any predisposing anatomic abnormalities (i.e. bifid median nerve or persistent median artery) or other concurrent diseases in the surrounding tissues which may require a different therapeutic approach. Examples include space-occupying lesions, tumors, tenosynovitis, osteophytes, neurovascular conflicts, abnormal muscles or muscle insertions, synovial cysts, nerve subluxation, postfracture fibrosis, and bone formation.

Neurophysiological and clinical parameters are good predictors of postsurgical recovery, but HRU has also demonstrated its usefulness when correlated with clinical neurophysiology in several nerve pathologies: (1) in patients with a history of trauma, it can reveal neuromas and neurotmesis; (2) in cases of postsurgical neuropathy of an iatrogenic origin, uncommon sites of injury can be localized; (3) in severe diseases with unevocable nerve potentials on neurophysiological examination, the site of injury can be easily showed by ultrasound; (4) in patients with diffuse preexisting (and confounding) neurophysiological alterations and clinical signs of a new neuropathy, the nerve lesions can be delineated; (5) in entrapment neuropathies, for screening purposes (e.g. concomitant tenosynovitis is seen in 21.7% of carpal tunnel syndromes, and dynamic ulnar nerve subluxation is seen at the elbow in 28.5% of cubital grooves); (6) in all brachial plexus pathologies, to identify multiple sites of injury are common; (7) for early selection of surgical candidates;[33,34] and (8) for detection of postsurgical improvement or complications.[35]

HRU does have some limitations, high frequency probes provide optimal spatial resolution for superficial nerve imaging while the deeper nerve course may remain unexplored.[36] The sciatic nerve trunk cannot be investigated over the horizontal gluteal fold, and the tibial and common peroneal nerves cannot be easily examined in the mid leg behind the calf. Both the deep nerve segments and nerve roots emerging from the spine should be explored by MRI. Expert HRU investigation can be used to visualize the cervical roots of the brachial plexus (the anterior branches of the spinal nerves as they emerge from the intervertebral foramen) as well as the trunks in the interscalene area and the cords in the supraclavicular and infraclavicular and axillary regions. A similar guide is helpful in interventional procedures to reach target nerves, such as in regional anesthesia or during steroid infiltrations, thus minimizing the risk of complications.

Ultrasound is already in use for a number of indications in the evaluation of nerves and is likely to find increasing indications in the future.[37-39] However, further clinical and biomedical research is required to further validate its application in preoperative and postoperative monitoring.

Magnetic resonance imaging

MRI is appreciated mainly for its wide overview of the limb with the option of selective volume reconstruction. Direct nerve visualization by MRI has also been optimized;[40,41] "MR neurography" combines fat suppression T2-weighted sequences and diffusion weighting in high magnetic field gradients (1.5T or higher). The nerve's signal increases significantly following traumatic nerve injury, resulting in high contrast of the bright nerve (hyperintense) against the surrounding muscle or fat. The increased nerve signal due to axonal degeneration can be observed both at the site of the injury and distally, and is the single most searched MRI sign for localization of nerve injuries. However, it is not a specific sign, reflecting only endoneurial or perineural edema and slower axoplasmic transport secondary to axonal degeneration.

Diffusion-weighting imaging has the potential to detect structural anisotropy by determination of the main orientation of the axons within the nerves; this method is called diffusion tensor imaging (DTI). From DTI data, a three-dimensional reconstruction of major fascicles can be rendered and is referred to as "tractography".[42] Tractography provides structural information on the nerves, but has low spatial resolution and a low signal-to-noise ratio, adding no additional information to neurophysiological data.

Many techniques including MRI myelography, MR neurography, and DTI can be combined for additional data, for example in root avulsions in patients with brachial plexus injuries,[43] but in order for the higher sequences to be carried out, greater acquisition times are required.

To overcome current limitations of MRI and enable investigation of nerves along a limb with faster image acquisition, widespread upgrade to 3T scanners combined with parallel imaging will be required.

Future application of new technologies for nerve imaging such as very high field magnetic fields (9.4T) MRI,[44] or very high frequency ultrasound probes (55 MHz)[45] will also increase spatial resolution up to a theoretical histological precision of 30 μm.

At this time, HRU provides the highest spatial resolution of direct nerve imaging along the limbs, while MRI provides a high contrast delineation of preselected single segments of the body. Both can assist in the resolution of pitfalls in injury localization, which may arise if only certain proximal nerve fascicles are injured, and others are spared, simulating a distal neuropathy.

CONCLUSION

Evaluation of peripheral nerve injuries remains a challenge for both clinicians and surgeons. A comprehensive clinical and physical examination approach permits formulation of a differential diagnosis to guide the neurophysiological exam and estimate prognosis. Nerve imaging evaluation completes the work-up by visualizing fascicles and continuity of the nerve and its surrounding tissue.

Clinical and instrumental data should be integrated to plan adequate treatment and promote functional recovery. High-resolution nerve imaging, when correlated with neurophysiological data, provides the missing link to clinicians and surgeons, closing the gap between diagnostic and therapeutic approaches. To optimize prognosis, this comprehensive evaluation is mandatory not only during the preoperative stage, but also during follow-up in order to recognize late or non-recovery, thus preventing permanent neurological disability.

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CLINICAL ARTICLE

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Preoperative evaluation of peripheral nerve injuries: What is the place for ultrasound?

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OBJECTIVE The purpose of this study was to evaluate the usefulness of ultrasound in the preoperative workup of peripheral nerve lesions and illustrate how nerve ultrasonography can be integrated in routine clinical and neurophysiological evaluation and in the management of focal peripheral nerve injuries. The diagnostic role and therapeutic implications of ultrasonography for different neuropathies are described.

METHODS The authors analyzed the use of ultrasound in 119 entrapment, tumoral, posttraumatic, or postsurgical nerve injuries of limbs evaluated in 108 patients during 2013 and 2014. All patients were candidates for surgery, and in all cases the evaluation included clinical examination, electrodiagnostic studies (nerve conduction study and electromyography), and ultrasound nerve study.

Ultrasound was used to explore the nerve fascicular echo-texture, continuity, and surrounding tissues. The maximum cross-sectional area (CSA) and the presence of epineurial hyperechogenicity or intraneural hyper- or hypoechogenicity, of anatomical anomalies, dynamic nerve dislocations, or compressions were recorded.

The concordance rate of neurophysiological and ultrasonographic data was analyzed, classifying ultrasound findings as confirming, contributive, or nonconfirming with respect to electrodiagnostic data. The correlation between maximum nerve CSA and neurophysiological severity degree in entrapment syndromes was statistically analyzed.

RESULTS Ultrasonography confirmed electrodiagnostic findings in 36.1% of cases and showed a contributive role in the diagnosis and surgical planning in 53.8% of all cases; the findings were negative ("nonconfirming") in only 10.1% of the patients. In 16% of cases, ultrasound was not only contributive, but had a key diagnostic role in the presence of doubtful electrodiagnostic findings. The contributive role differed according to etiology, being higher for tumors (100%) and for posttraumatic or postsurgical neuropathies (72.2%) than for entrapment neuropathies (43.8%).

CONCLUSIONS Ultrasound is a powerful, noninvasive tool for the examination of peripheral nerve injuries, and can guide diagnosis of and surgical strategy for focal peripheral nerve injuries. It allows direct visualization of the cause and extent of nerve lesions and finds its place between electrodiagnostic tests and exploratory surgery. It can provide invaluable information, such as the presence and extent of a mass, scar compression, or neuromas. The authors recommend it as a complement to routine clinical and neurophysiological evaluation and as the first-line imaging modality for masses of suspected nerve origin.

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KEY WORDS ultrasound; peripheral nerve; nerve surgery; nerve imaging; electrodiagnosis

Since its first description for the assessment of recurrent laryngeal nerve palsy,[30] ultrasound nerve imaging has become an established technique for the study of peripheral neuropathies. Several clinical studies have recently demonstrated its high sensitivity and specificity and validated its usefulness in the diagnostic and therapeutic process for focal and generalized peripheral neuropathies.[1,4,7,15,19,29]

High-resolution ultrasound is a reliable tool for examining both the extraneural and the intraneural morphology of peripheral nerves. It allows evaluation of nerve continuity and shape and detection of nerve enlargements, com-

ABBREVIATIONS CMAP = compound muscle action potential; CSA = cross-sectional area; EMG = electromyography; LSD = least significant difference; MUAP = motor unit action potential; SNAP = sensory nerve action potential.

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plete or partial lacerations, perineural scars, neuromas and nerve tumors, as well as extrinsic nerve compressions from foreign bodies, neoplasms, implants, or heterotopic ossifications.[1,4,13,15,19,21] Furthermore, ultrasound nerve imaging is simple and fast, readily available, cheap, and causes little discomfort to patients; for these reasons it is a valuable complement to clinical and electrophysiological nerve evaluation, which still represent the gold standard for diagnosis of peripheral neuropathies.[11,12]

Nerve ultrasonography is finding increasing indications for diagnosis and surgical planning of peripheral neuropathies, and due to its advantages over other imaging techniques it has also been proposed as the first-line imaging modality for selected cases.[16,33]

The aim of this study is to retrospectively evaluate our experience with ultrasound in peripheral nerve imaging, to analyze the indications and clinical utility for different neuropathies, and to describe how nerve ultrasonography can be integrated into the routine evaluation and management of focal peripheral nerve injuries.

Methods

During 2013 and 2014, 119 entrapment, tumoral, posttraumatic, or postsurgical nerve injuries of the limbs were evaluated in 108 candidates for surgery at the authors' institution. The findings and results were retrospectively analyzed. Candidates for surgery were selected based on clinical examination by a surgeon and a neurologist. All of these patients subsequently underwent both electrodiagnostic and ultrasound nerve studies, with the exception of patients in whom electrodiagnostic studies clearly indicated only a radiculopathy, who did not receive ultrasonographic examination. For all patients in this series, preoperative evaluation included clinical examination, electrodiagnostic studies (nerve conduction study and electromyography), and ultrasound nerve study. Neurophysiological and ultrasound examinations was performed in all cases by one of the authors (A.G.) at the "Clinical Course" Neurophysiology Unit in Palermo.

Study Population

The mean age of the 108 patients was 54.7 years. Most patients with posttraumatic or postsurgical neuropathies were male (75%). The most common pathological condition was entrapment neuropathy (69 patients, 80 nerves). The upper limb was involved more frequently than the lower limb (86% vs 14%). The mean duration of symptoms was 17.6 months (range 2–72 months); no patient with acute nerve injury was evaluated in this case series. Fifteen percent of the patients had diabetes, and all of these patients presented with entrapment neuropathy (Table 1).

Electrodiagnostic Studies

Electrodiagnostic examination included motor and sensory conduction velocity studies of the injured nerve and the contralateral homologous and other ipsilateral healthy nerves (data not shown); needle electromyography (EMG) examination of the target muscles was performed according to international guidelines.[3,6,26,31] Sensory nerve action potentials (SNAPs) and compound muscle action potentials (CMAPs) were classified as normal, pathological, or absent; pathological response included a reduction of the motor or sensory conduction velocity values or a reduction of the SNAP or CMAP voltage. Eventual denervation potentials and motor unit action potential (MUAP) recruitment patterns (interference, intermediate, single MUAPs, absent) were recorded by EMG. A NeMus 2 EBNeuro (Florence, Italy) neurophysiological equipment was used.

Ultrasonography

Ultrasound scanning was performed on the injured nerve when the clinical diagnosis was confirmed by neurophysiological examination; in cases of doubtful or confounding neurophysiological findings, all main nerves of the same limb were explored. Each nerve was visualized along its course throughout the whole limb, exploring the nerve fascicular echotexture, continuity, and surrounding tissues; the presence of epineurial hyperechogenicity or intraneural hyper- or hypoechogenicity was recorded. The maximum cross-sectional area (CSA) was measured at the inner border of the epineurium. For posttraumatic and postsurgical cases—which involved different nerves at different levels—CSA values were normalized as a percentage of the contralateral healthy nerve, which was used as reference to facilitate analysis of the results. For entrapment syndromes—which are often bilateral—CSA was expressed in square millimeters (mm^2) and compared with reference values form the literature.[25] For tumors, CSA was also expressed in square millimeters. Color Doppler ultrasound scans were performed for suspected tumors.

The presence of anatomical anomalies, dynamic nerve dislocations, or compressions was recorded. An Esaote MyLab 25 Gold ultrasound system equipped with a broadband (frequency band 10–18 MHz) linear transducer was used.

Data Analysis

Electrodiagnostic and ultrasonographic data were grouped according to etiological diagnosis into entrapment neuropathies, posttraumatic or postsurgical neuropathies, and tumors, and intra- and intergroup analyses were performed. Posttraumatic or postsurgical neuropathies were further classified as neuromas, nerve compressions, and traction neuropathies, according to clinical history and final clinical and neurophysiological/ultrasonographic diagnosis.

The concordance rate of neurophysiological and ultrasonographic data was analyzed for each group. Ultrasonographic findings were correlated to the electrodiagnostic findings to evaluate their role in diagnosis and development of surgical strategy; results were classified as contributive (influenced diagnostic and therapeutic strategies), confirming (confirmed clinical and neurophysiological diagnosis), nonconfirming (normal ultrasound findings), or incorrect (led to incorrect diagnosis), according to Padua's evaluation scale.[25]

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics, version 20.0 (IBM Corp.). Univariate analysis of

TABLE 1. Characteristics of the study population*

Pathological Condition	No. of Patients	No. of Nerves	Mean Age (yrs)	Sex		Limb		Mean Duration of Sx (mos)	Diabetes
				M	F	Upper	Lower		
Entrapment neuropathies	69	80	56.7 ± 15.6	33 (48)	36 (52)	76 (95)	4 (5)	18.2 ± 19.7	16 (23)
Posttraumatic or postsurgical neuropathies	36	36	50.2 ± 19.2	27 (75)	9 (25)	24 (67)	12 (33)	17.2 ± 20.2	0
Tumors	3	3	63.3 ± 5.7	0 (0)	3 (100)	2 (67)	1 (33)	12 ± 3.4	0
Total	108	119	54.7 ± 17.2	60 (56)	48 (44)	102 (86)	17 (14)	17.6 ± 19.5	16 (15)

Sx = symptoms.

* Values represent numbers of patients or lesions (%) unless otherwise indicated. Mean values are presented with SDs.

variance (ANOVA) was performed for carpal tunnel syndrome, using the maximum nerve CSA as the dependent variable and neurophysiological severity classification as the independent variable; a post hoc Fisher's least significant difference (LSD) analysis was performed. An independent-samples t-test was performed for cubital tunnel syndrome, using maximum nerve CSA as the dependent variable and neurophysiological severity classification as the independent variable. A p value ≤ 0.05 was regarded as significant.

Results

Electrodiagnostic and ultrasonographic findings for the 3 different groups are reported in Tables 2 and 3, and their analysis is reported below.

Entrapment Neuropathies

Carpal tunnel and cubital tunnel syndromes were classified according to the severity of electrodiagnostic alterations.[3,26,31]

In carpal tunnel syndrome, the mean maximum CSA of the median nerve at the wrist significantly increased with the severity of these alterations (ANOVA, p = 0.001; F = 8.866), ranging from a mean of 9 mm in the minimal/mild subgroup—which falls in the range of normal values[25]—to a mean of 20 in the severe/extreme subgroup. Further, a post hoc Fisher's LSD analysis for intergroup analysis showed a significant difference of CSA values between the mild vs moderate (p = 0.02), moderate vs severe (p = 0.01) and severe vs mild group (p = 0.001). The presence of epineurial hyperechogenicity also increased with the severity of electrodiagnostic alterations, and was present in 83.3% of patients with extreme neurophysiological alterations and 25% of those with severe alterations, while it was not found in minimal and mild cases. Nerve hypoechogenicity was never observed in the minimal/mild subgroup, but was always observed in the moderate and severe/extreme subgroups. Also, ultrasound showed anatomical variations predisposing to the entrapment syndrome, namely a bifid median nerve and/or a median artery in 8 patients (17.4%) (Fig. 1).

In cubital tunnel syndrome, the mean CSA of the ulnar nerve at the elbow did not differ significantly between the mild and moderate subgroups (t-test for independent variables), but mean CSA values were always increased relative to normative data in the literature,[25] also in mild cases. Epineurial hyperechogenicity was observed in 40% of mild cases and 100% of moderate cases. Nerve hypoechogenicity was observed in all cases, irrespectively of their severity. Also, ultrasound showed nerve dislocation at the elbow in 14.8% of patients.

In the patient with Guyon's canal syndrome, the electrodiagnostic findings were pathological; ultrasound showed a normal nerve CSA (4.9 mm^2) but revealed the cause of the nerve entrapment, namely a ganglion cyst, guiding the planning of surgical treatment (Fig. 2).

Also, ultrasound complemented the electrodiagnostic diagnosis of anterior interosseous nerve and posterior interosseous nerve entrapments by localizing the site of compression, indicated by an increase of nerve CSA proximally to it (mean values 3 and 4 mm^2, respectively) compared with the healthy contralateral side, and by nerve hypoechogenicity.

Data on the lower limb are limited by the small sample size. In common peroneal nerve entrapment, increased nerve CSA (mean 30 mm^2) and epineurial hyperechogenicity accompanied the pathological electrodiagnostic findings, while in a case of lateral femoral cutaneous nerve entrapment, the only study that confirmed the suspected clinical diagnosis was ultrasonography, which demonstrated a maximum nerve CSA of 6 mm.

Posttraumatic and Postsurgical Neuropathies

Posttraumatic or postsurgical cases were characterized by a great variability of the type and level of injury.

Neuromas always showed a greatly increased CSA (mean 257.8% ± 67.4%), while routine electrodiagnostic findings were normal in 22.2% of cases and pathological in 77.8% (with 22.2% of cases showing a complete axonotmetic/neurotmetic pattern). In all cases, the nerve was hypoechoic, but most importantly, it showed an altered echotexture, diagnostic of neuroma (Fig. 3). Also, in all cases of neuroma, ultrasound allowed measurement of the longitudinal and axial size of the lesion, and a Tinel sign was elicited at the passage of the probe, confirming the diagnosis and localization. The possibility of moving the probe in different planes allows visualization of the whole nerve and assessment of the type of damage (i.e., partial or complete involvement) (Fig. 4).

In the nerve compression subgroup, ultrasound showed an increased CSA (mean 171.3% ± 67.2), a frequent nerve hypoechogenicity (66.7), and an epineurial hyperechogenity in 38.9% of cases; also, multifocal damage, characterized by doubtful, nonconclusive, electrodiagnostic find-

TABLE 2. Ultrasound and electrodiagnostic findings in entrapment neuropathies

Limb & Compressed Nerve	Diagnosis (no. of cases)	Severity (no. of cases)	Ultrasound Findings				Electrodiagnostic Findings			
			Mean Max Nerve CSA (mm^2)	Epineurial Hyperech	Intraneural Hyperech	Nerve Hypoech	SNAP	CMAP	Denervation Potentials	MUAP Recruitment Pattern
Upper limb										
Median n.	Carpal tunnel syndrome (46)	Minimal/mild (5)	9 ± 0.3*	0	0	0	N 0%, P 100%, A 0%	N 100%, P 0%, A 0%	0%	Interference 100%
		Moderate (23)	16.2 ± 4.2†	3 (13.0%)	0	23 (100%)	N 0%, P 100%, A 0%	N 0%, P 100%, A 0%	0%	Intermed 100%
		Severe/extreme (18)	20 ± 3.7‡	8 (44.4%)	0	18 (100%)	N 0%, P 0%, A 100%	N 0%, P 66.7%, A 33.3%	44.4%	Intermed 88.9%, single MUAPs 11.1%
Ulnar n.	Cubital tunnel syndrome (27)	Mild (15)	14 ± 3.5	6 (40.0%)	0	15 (100%)	N 40%, P 60%, A 0%	N 0%, P 100%, A 0%	0%	Interference 66%, intermed 33.3%
		Moderate (12)	15 ± 6.7	12 (100%)	0	12 (100%)	N 0%, P 50%, A 50%	N 0%, P 100%, A 0%	16.6%	Interference 33.3%, intermed 33.3%, single MUAPs 33.3%
	Guyon's canal syndrome (1)	—	4.9	0%	0%	0%	N 0%, P 100%, A 0%	N 0%, P 100%, A 0%	100%	Intermed 100%
Ant interosseous n.	Kiloh-Nevin syndrome (2)	—	3 ± 0	0%	0%	100%	—	—	50%	Intermed 50%, single MUAPs 50%
Pst interosseus n.	Nerve entrapment (1)	—	4	100%	0%	100%	—	P 100%	100%	Single MUAPs 100%
Lower limb										
Common peroneal n.	Nerve entrapment (3)	—	30 ± 3.5	100%	0%	0%	P 100%	P 100%	100%	Single MUAPs 100%
Lat femoral cutaneous n.	Nerve entrapment (1)	—	6	0%	0%	0%	—	—	—	—

A = absent; Ant = anterior; hyperech = hyperechogenicity; CMAP = compound muscle action potential; CSA = cross-sectional area; hypoech = hypoechogenicity; intermed = intermediate; MUAP = motor unit action potential; n. = nerve; N = normal; P = pathological; pst = posterior; SNAP = sensory nerve action potential.

* $p < 0.05$ minimal/mild versus moderate (ANOVA and post hoc LSD).

† $p < 0.05$ moderate versus severe/extreme (ANOVA and post hoc LSD).

‡ $p < 0.05$ severe/extreme versus minimal/mild (ANOVA and post hoc LSD).

TABLE 3. Ultrasound and electrodiagnostic findings in posttraumatic and postsurgical neuropathies

Diagnosis (no. of cases)	Nerve (no. of cases)	Ultrasound Findings					Electrodiagnostic Findings			
		Mean Max Nerve CSA (% of contralat)	Epineurial Hyperech	Intraneural Hyperech	Nerve Hypoech	Multifocal Damage	SNAP	CMAP	Denervation Potentials	MUAP Recruitment Pattern
Neuroma (9)	Median n. (3), ulnar n. (3), superficial radial n. (1), lat digital n. (1), proper palmar digital n. (1)	257.8 ± 67.4	0 (0%)	0 (0%)	9 (100%)		N 33%, P 33%, A 33%	N 55.6%, P 22.2%, A 22.2%	66.7%	Interference 44.4%, intermed 22.2%, single MUAPs 11.1%, absent 22.2%
Nerve compression (18)*	Median n. (4), ulnar n. (8), sciatic n. (1), femoral n. & saphenous n. (1), common peroneal n. (2), superficial peroneal n. (2)	171.3 ± 67.2	7 (38.9%)	0 (0%)	12 (66.7%)	4 (22.2%)	N 22.2%, P 50%, A 27.8%	N 28.8, P 61.1, A 11.1	33.3%	Interference 27.8%, intermed 38.9%, single MUAPs 27.8%, absent 5.6%
Traction neuropathy (9)	Brachial plexus (4), common peroneal n. (4), tibial n. (1)	137.3 ± 67.1	4 (44.4%)	3 (33.3%)	3 (33.3%)	4 (44.4%)	N 66.7%, P 33.3%, A 0%	N 33.3%, P 66.7%, A 0%	66.7%	Interference 44.4%, intermed 44.4%, single MUAPs 11.1%

* Due to scar, recurrent entrapment, fracture complications, or foreign bodies.

FIG. 1. Ultrasound axial scan of the median nerve at the wrist, showing 2 anatomical variants associated with carpal tunnel syndrome. **Upper:** Bifid median nerve in axial scan at the wrist. The nerve is divided by a fibrous hyperechoic septum *(asterisk)* into 2 compartments *(arrows)*. **Lower:** A persistent median artery (A) can be found within this septum.

ings, was identified in 22.2% of cases. Ultrasound also revealed morphological details that guided not only the etiologic diagnosis, but also surgical strategy, such as the presence of perineural scarring, heterotopic ossification (Fig. 5), or foreign bodies (Fig. 6).

In the traction neuropathy subgroup, all patients showed pathological electrodiagnostic findings. Ultrasound showed an increased CSA (mean 137.3% ± 67.1%), an epineurial hyperechogenicity in almost half of the cases (44.4%), and an alteration of intraneural echogenicity in 66.7% of cases (hyperechogenicity in 33.3%, hypoechogenicity in 33.3%). Individual analysis of CSA and nerve echogenicity guided diagnosis and surgical strategy (Fig. 7).

Tumors

In the 3 cases of schwannoma, routine electrodiagnostic findings were always normal, and diagnosis was achieved with ultrasound, which also provided information on the size and the vascular supply of the tumor (Figs. 8–10, Video 1).

VIDEO 1. Video clip of a dynamic ultrasound axial scan of the median nerve along the arm. Moving the probe along the limb, maintaining the nerve in the center of the screen, long tracts of nerves are easily and quickly explored. In this case, details on which nerve and on how many fascicles are intact could also be achieved (also see Figs. 8–10). Copyright Andrea Gagliardo. Published with permission. Click here to view.

The CSA was greatly increased (mean 105 ± 84.3 mm^2), and in 1 case an intratumoral calcification was identified. In all 3 cases, diagnosis was confirmed by subsequent MRI.

Role of Ultrasound

Ultrasound confirmed electrodiagnostic findings in 36.1% of the patients of this case series (47.5% of entrapment neuropathies and 13.9% of posttraumatic or postsur-

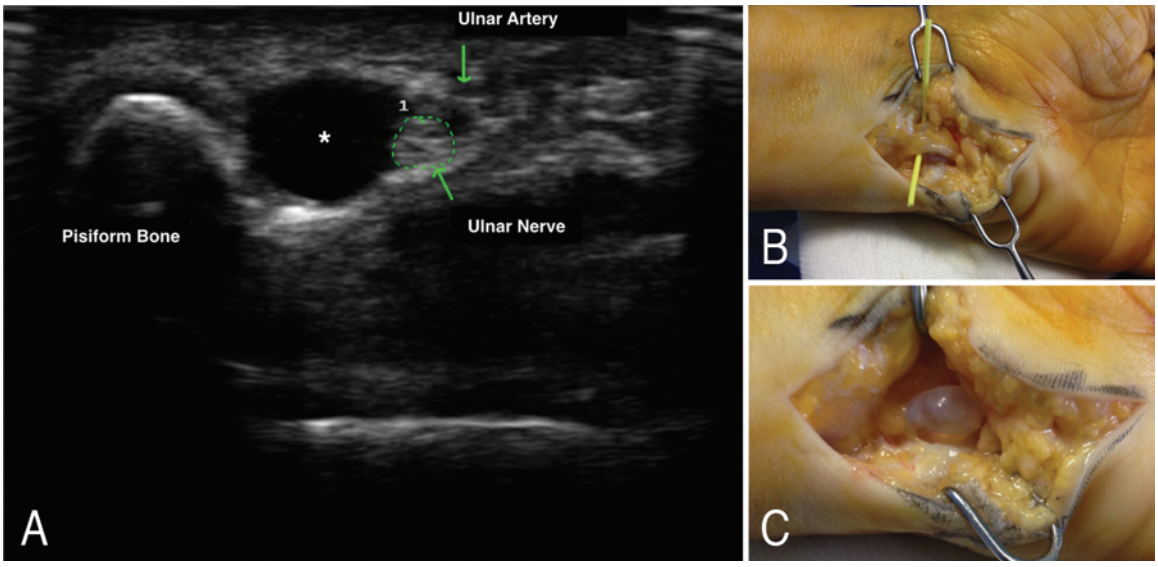

FIG. 2. A: Ultrasound axial scan of the Guyon canal at the wrist. The ulnar nerve is generally located between the pisiform bone and the ulnar artery. In this case, ultrasound revealed a ganglion cyst *(asterisk)* occupying the canal and causing nerve compression. **B:** Intraoperative photograph obtained after nerve exposure and decompression but before removal of the ganglion cyst. **C:** Intraoperative photograph showing the cyst. The presence of the cyst was known beforehand and the risk of missing it was avoided. Figure is available in color online only.

gical neuropathies), and showed a contributive role in diagnosis and surgical strategy in 53.8% of cases. This rate was higher for tumors (100%) and posttraumatic or postsurgical neuropathies (72.2%) (Fig. 11). In 16% of cases, ultrasound was not only contributive, but had a key diagnostic role in the presence of doubtful electrodiagnostic findings.

Ultrasound showed a contributive role in 43.8% of the cases of entrapment syndrome, which included the identification of a concurrent flexor tenosynovitis (in 8.8% of cases), perineural scar (3.8%), or anatomical variations, such as a bifid median nerve and/or a median artery (10%),

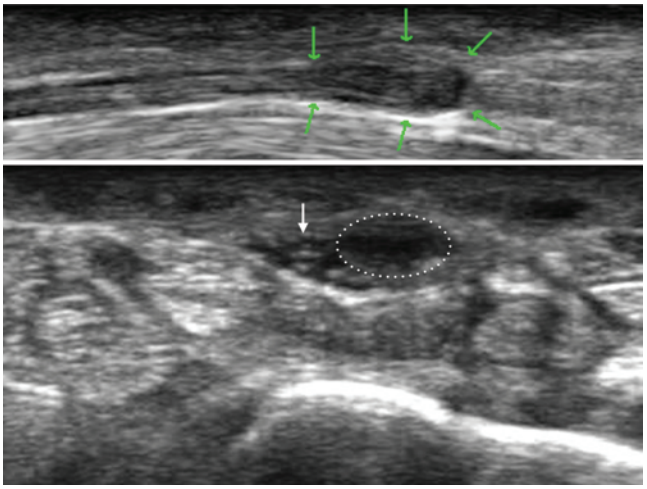

FIG. 3. Ultrasound scans of the forearm showing the typical shape of a neuroma in longitudinal **(upper)** and axial **(lower)** views of the median nerve. The axial scan shows a partial neurotmesis of the nerve with preservation of a significant percentage of nerve fascicles *(arrow)* in continuity below the neuroma. Figure is available in color online only.

and nerve dislocation at the elbow in 11.3% of cases. Also, ultrasound contributed by the identifying a compressive mass in the Guyon canal (1.3%) and uncommon sites of compression (5%) or dynamic compressions (2.6%) not identified by electrodiagnostic findings. Lastly, it contributed to the diagnosis of Parsonage-Turner syndrome in a patient with ulnar nerve palsy, by excluding the presence of focal lesions along the whole limb (1.3%).

Ultrasound had a contributive role in 72.2% of traumatic cases, mainly due to identification of nerve continuity. It identified the presence and extension of both terminal neuromas and neuromas-in-continuity in 25% of cases (100% of suspected neuromas). In compression and traction neuropathies, ultrasound allowed a direct visualization of nerve continuity and eventual surrounding hyperechoic (fibrous) tissue that contributed to the neuropathy, leading to diagnosis in 13.9% of posttraumatic/postsurgical cases. For instance, a suspected ulnar nerve injury in a patient who had sustained an elbow fracture was diagnosed as a brachial plexopathy, while in 2 postsurgical cases of fibula fracture, a concurrent scar neuropathy of the superficial peroneal nerve (in 1 case) or of the common and superficial peroneal nerves (in 1 case) was identified. In compression neuropathies, ultrasound also allowed the distinguishing of different anatomical pictures in patients with similar clinical and electrodiagnostic findings, contributing to diagnosis and surgical planning in 11.1% of cases. For instance, in 2 extreme cases in which sensory and motor ulnar potentials could not be evoked subsequent to fractures, ultrasound showed a greatly increased nerve CSA at the elbow without other significant findings in 1 case, and excessive bone deposition (Fig. 5) that surrounded and compressed the nerve in the second case. In another case, ultrasound showed a mild reduction of the space within the condylar groove, resulting in a superficial position of the ulnar nerve

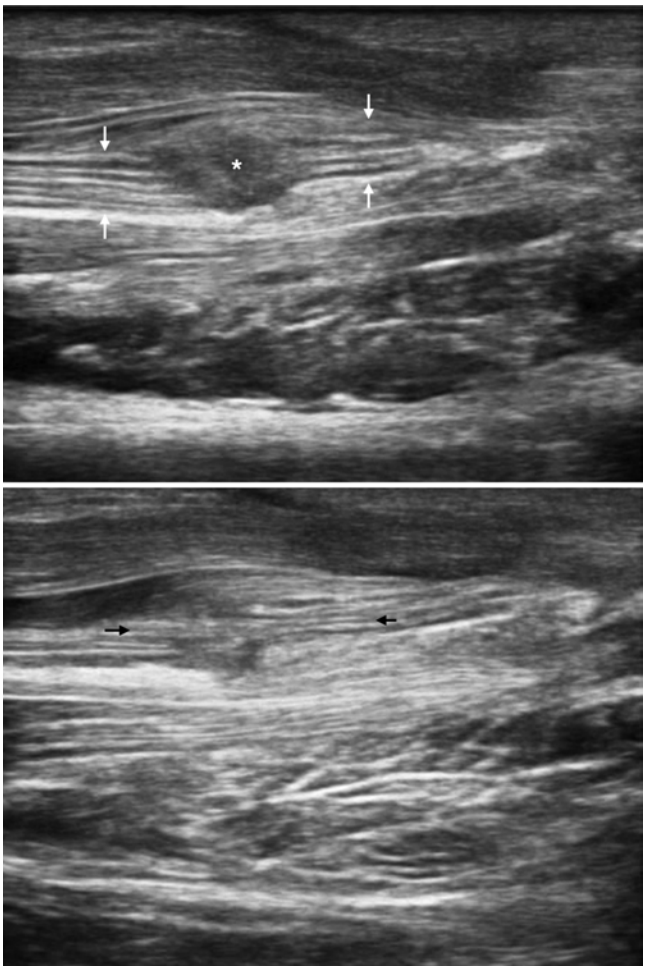

FIG. 4. Longitudinal ultrasound scans of the median nerve. Note the interruption of many fascicles *(white arrows)* for the presence of a neuroma *(asterisk)*. Slightly moving the probe, different planes of the nerve can be explored and a small number of fascicles *(black arrows)* show their continuity across the lesion. These are the advantages of a dynamic real-time high-resolution ultrasound examination.

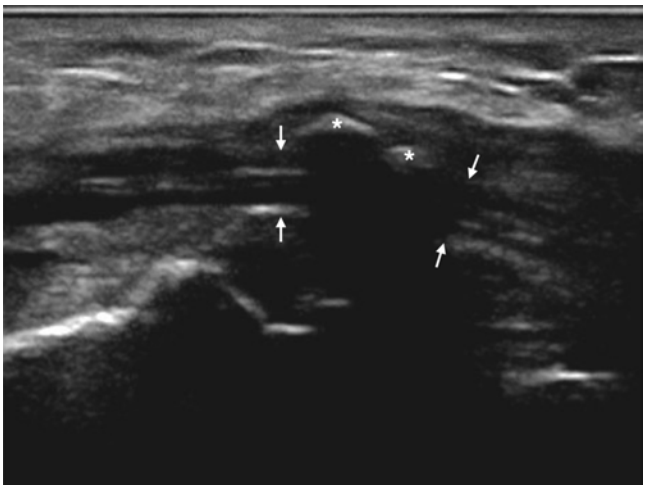

FIG. 5. Ulnar nerve *(arrows)* in longitudinal ultrasound scan at the elbow. After a displaced fracture of the elbow, the patient did not recover any motor or sensory ulnar function. Ultrasound showed deposits of bone *(asterisk)* over the nerve fascicles, as a "fatal embrace" with the ulnar nerve. Note the shadow cone of the ultrasound that does not pass through the bone and obscures the underlying nerve.

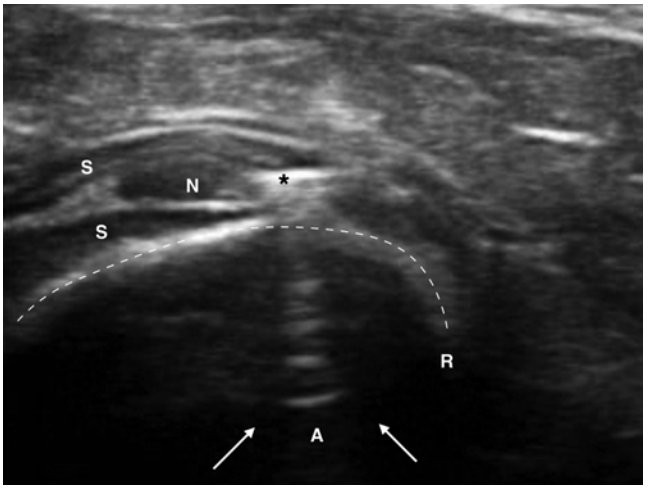

FIG. 6. Ultrasound axial scan of the posterior interosseus nerve (N) between the superficial and deep heads of the supinator muscle (S) in a patient who had suffered a hunting accident. The nerve has a focal huge increase of its CSA. A small hyperechoic metallic bullet *(asterisk)*, easy to identify because of the prominent ultrasound artifact that it generates *(arrows)*, pushes laterally and compresses the nerve, causing a deficit of the finger extension. The bullet is located on the superficial profile of the cortical bone of the radius (R). With an exact preoperative diagnosis surgery can be targeted without the need for wide exploration and dissection.

and dynamic subluxation. Ultrasound also identified multiple sites of injury caused by the same trauma and not immediately recognized by neurophysiological examination or helped diagnosis in patients with a previous nerve injury before trauma in 22.2% of cases; for instance, 2 patients with a brachial plexopathy had a concurrent ulnar or radial nerve injury, and a patient with a history of hip surgery and L-5 radiculopathy had a challenging common peroneal nerve injury after a mild knee sprain.

Ultrasound had a contributive role in 100% of tumor cases, allowing visualization and providing anatomical details of the lesions.

The results of ultrasound examination were negative ("nonconfirming" with respect to electrodiagnostic findings) in only 10.1% of cases—all minimal-grade carpal tunnel syndromes, 1 median nerve posttraumatic compression, 1 common peroneal nerve traction injury, and 2 mild cases of brachial plexus traction neuropathy. The results of ultrasound examination were also negative in 1 case of mild cubital tunnel syndrome, 1 case of suspected ulnar nerve entrapment that resulted in a diagnosis of cervical myeloradiculopathy, and 1 case of foot drop due to partial proximal sciatic nerve impairment.

Discussion

The purpose of this study was to evaluate the usefulness of ultrasound in the preoperative workup of peripheral nerve lesions. In our clinical series, routine ultrasound

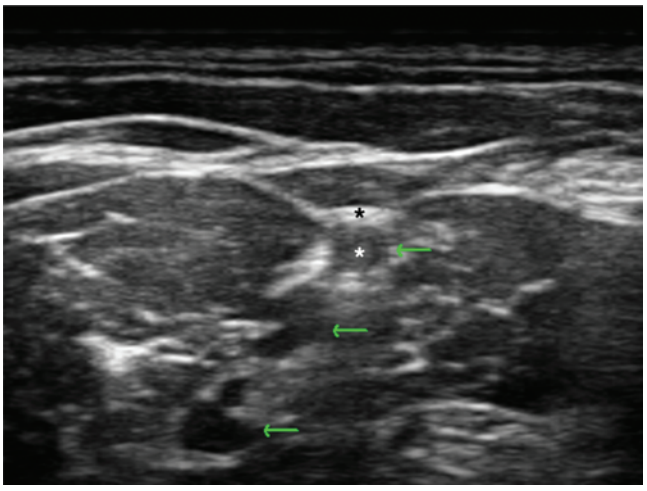

FIG. 7. Superior trunk brachial plexopathy. Ventral branches of the C-5 *(upper arrow)*, C-6 *(middle arrow)*, and C-7 *(lower arrow)* spinal nerves in the interscalenic area. Note that the superior trunk shows an inner hyperechogenicity *(white asterisk)* and is surrounded by a thick hyperechoic fibrous tissue *(black asterisk)*. This pattern suggests a stretching injury of the nerve trunk. Figure is available in color online only.

assessment was always added to standard clinical and neurophysiological examination of patients with peripheral neuropathies who were candidates for surgery and proved useful both as a diagnostic tool and in surgical planning. Our decision to introduce ultrasound as a routine examination for surgical candidates was driven by the expectation of clinical utility: an easier surgery in entrapment injuries due to the anatomical details provided and a lower need for MRI in posttraumatic/postsurgical injuries.

Ultrasound allows real-time evaluation of lesions. Within our team, there is a close cooperation between the surgeons, the neurophysiologist, and the neuroradiologist: this means that unexpected findings can be immediately discussed and further assessment immediately performed based on the surgeon's indications, thus tailoring the examination to each patient to provide accurate information useful for surgical treatment.

The diagnostic contribution of ultrasound was higher for focal masses arising from or compressing the nerve, such as nerve tumors, neuromas, foreign bodies or ganglion cysts, and in complex posttraumatic or postsurgical neuropathies; in our series it allowed diagnosis in 16% of cases with discordant clinical and electrodiagnostic findings. Its practical contribution in the planning of the surgical access and strategy was even higher; besides being the reference instrumental evaluation in the above-mentioned cases, it seems to contribute to a faster and easier surgery for entrapment neuropathies. Thanks to the anatomical details provided, the surgeon knows in advance about anatomical anomalies or dynamic nerve dislocations; also, we hypothesize that the presence of hyperechoic fibrous tissue could indicate epineurial fibrosis and the need for neurolysis. Surgeons perceived these data as facilitating and speeding surgery; however, we did not measure surgical times or correlate ultrasonographic and surgical findings, which we are currently investigating in a prospective study.

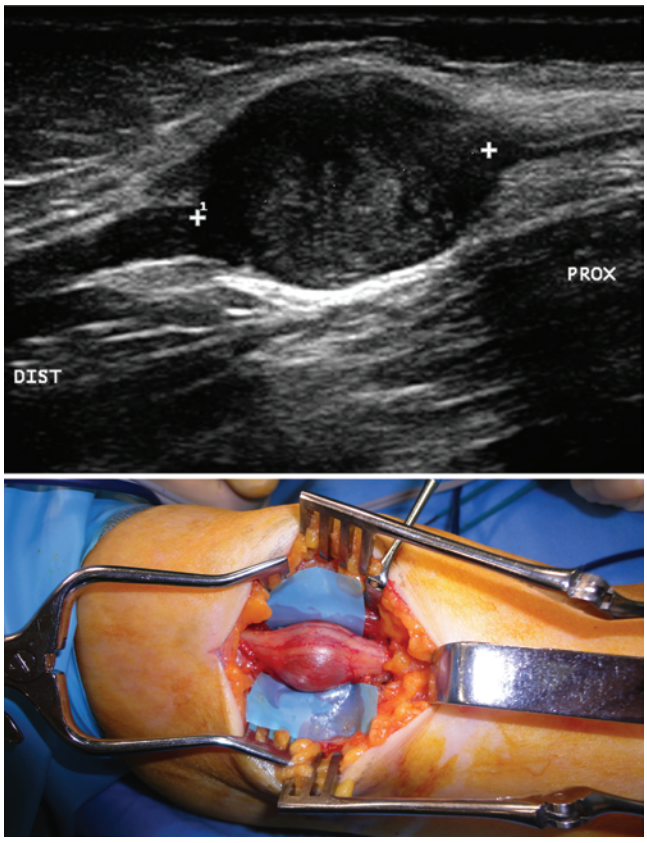

FIG. 8. Upper: Schwannoma of the median nerve at the mid-arm in a longitudinal ultrasound scan. Following the median nerve by ultrasound, from the distal carpal tunnel to the axilla, we clearly show a mass, which originates from within this nerve and which displaces and does not infiltrate most of nerve fascicles. **Lower:** Intraoperative photograph showing the lesion. The diagnosis of schwannoma was confirmed by surgical exploration and histological examination. This case beautifully illustrates how close the ultrasound image is to reality. In this particular case, already conscious of the diagnosis and knowing that the nerve conduction and continuity are intact, thanks to the unparalleled high-resolution details, ultrasound imaging allowed for improvements in patient information, surgical planning, and decision making. Figure is available in color online only.

Ultrasound is a reliable, cheap, and readily available diagnostic tool. Its accuracy is similar to that of MRI. Although MRI is generally recognized as having a better contrast, and is still preferable for the study of deep nerves, ultrasound has been reported to have a greater sensitivity and an equal specificity, and to allow a better identification of multifocal lesions for peripheral nerve lesions.[33] MRI for nerve visualization is better performed in preselected regions and by high magnetic field scanners (1.5 T or higher). Although T1-weighted spin-echo sequences offer good morphological evaluation, and the "MR neurography" technique[10,18] provides a high-contrast nerve signal by the use of highly weighted T2 sequences with long echo times for muscle suppression, selective fat suppression, and spatial radiofrequency pulses outside the imaging volume for vessel suppression, the presence of some metallic devices could contraindicate or severely hamper the MR image acquisition.

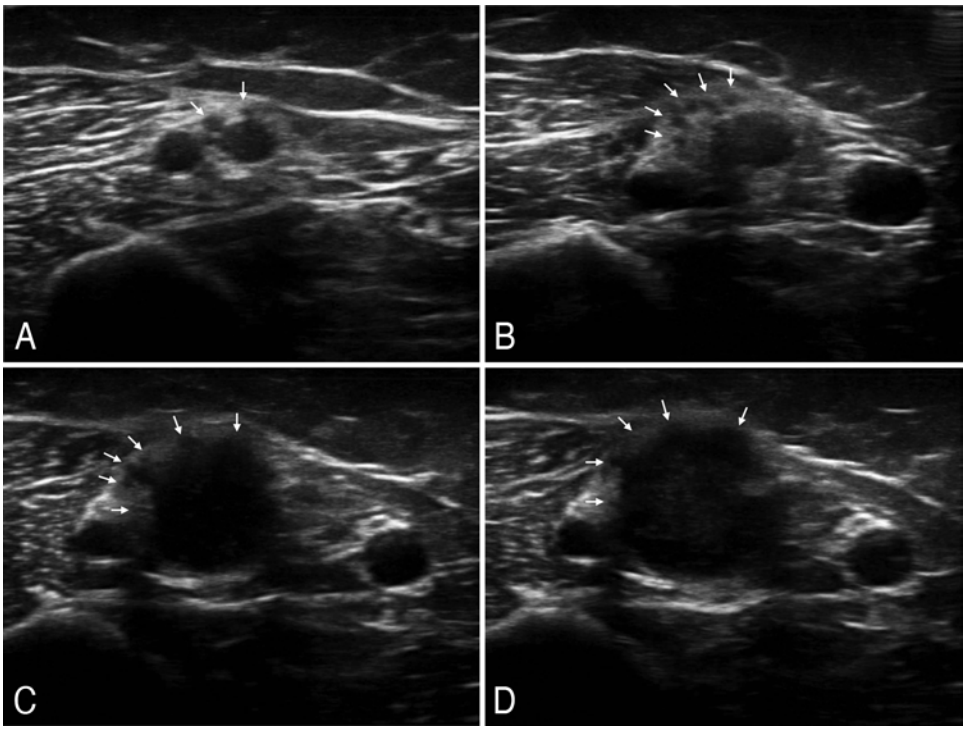

FIG. 9. A–D: Axial ultrasound images showing the same schwannoma as in Fig. 8. Schwannomas usually displace most axon fibers, which can be spared during tumor enucleation. In axial live scans, the fascicles surrounding the tumor could be followed along the nerve *(arrows)* (also see Video 1).

Ultrasound has no absolute contraindications in common practice, and its advantages over MRI include being less time-consuming and allowing real-time, dynamic, and serial examination of a wide anatomical field as well as visualization of a long nerve in a single scan.[17] Also, it can easily assess flow dynamics and vascular patterns, which provide further anatomical details that can be especially useful in the diagnosis of nerve tumors (Fig. 10). Furthermore, ultrasonography can be performed at the same time as the clinical examination and electrodiagnostic study, allowing a complete morphological and functional examination of the nerve.

Based on these findings, we believe that ultrasound is likely to replace MRI as the first-line imaging technique for focal lesions arising from or compressing a nerve; however, a limited number of cases were analyzed in this series, and this assumption need validation on a larger scale.

Entrapment Neuropathies

Our results suggest that, for entrapment neuropathies, ultrasound is a sensitive diagnostic tool which correlates with electrodiagnostic findings: in carpal tunnel syndrome, nerve CSA and nerve hypoechogenicity were always normal in minimal or mild cases and always altered in moderate to extreme cases. Also, the presence of epineurial hyperechogenicity indicated a more advanced degree of severity, and could represent a good indicator of epineurial fibrosis and need for surgical neurolysis. In cubital tunnel syndrome, ultrasound was even more sensitive, nerve CSA and nerve echogenicity being altered also in mild cases. From a surgical point of view, ultrasound facilitated surgical approach by showing anatomical or dynamic alterations such as a bifid median nerve or nerve dislocation that would have not be identified preoperatively otherwise. If it is true that these alterations would not have altered the surgical plan, it is also true that this thorough preoperative evaluation allowed for a better discussion with the patient and for faster and easier surgery, at the expense of only the small amount of extra time required for the preoperative ultrasound evaluation.

Our findings are supported by the data in the literature, which show an emerging diagnostic role for ultrasound in entrapment neuropathies. Simon et al.[29] reported that changes in the CSA and echogenicity correlate with the severity of the lesion. Filippou et al.,[9] Yoon et al.,[32] and Simon et al.[29] found high-resolution ultrasonography more useful than electrodiagnostic tests for ulnar neuropathy; also, Beekmann et al.[2] showed that the sensitivity of electrodiagnostic tests can be increased from 78% to 98% by adding ultrasonography, while Pardal-Fernandez[27] even proposed substituting ultrasonography for EMG and velocities conduction studies as a first-line examination in selected cases. Cesmebasi et al.[5] also reported on the usefulness of dynamic ultrasound in identification of a palmaris profundus tendon, a feature that, determining a dynamic compression, cannot be shown by MRI.

Posttraumatic and Postsurgical Neuropathies

In posttraumatic or postsurgical cases, the main contribution of ultrasound was visualization of nerve continu-

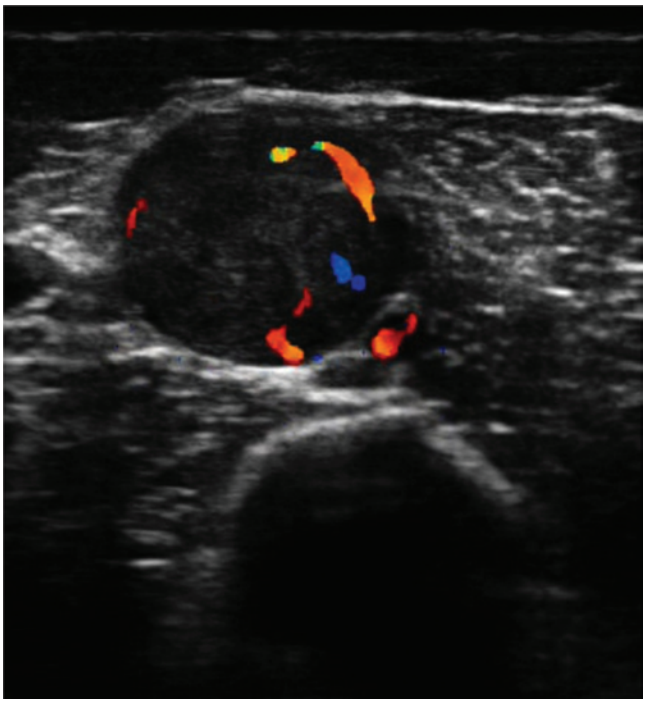

FIG. 10. Same schwannoma as in Figs. 8 and 9 in an axial and color Doppler ultrasound scan, which shows tumor neovascularization. The feeding artery, the plexiform veins, and eventual hypervascularity can be visualized with this technique. Figure is available in color online only.

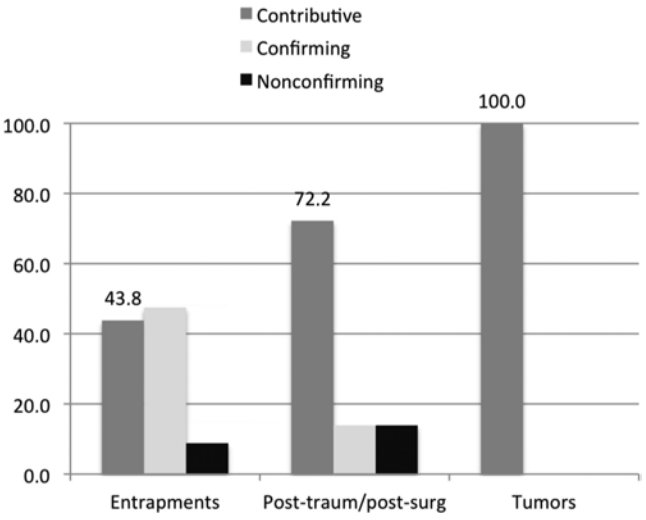

FIG. 11. Role of ultrasound with respect to neurophysiological examination in diagnosis and surgical planning in the 3 different groups of peripheral neuropathies. post-traum/post-surg = posttraumatic/postsurgical.

ity. It allowed not only the identification of an amputation neuroma of larger nerves (i.e. median or ulnar) but also of small terminal branches (common palmar and proper palmar digital nerves) or neuromas-in-continuity, distinguishing interrupted fascicles from those in continuity.

Ultrasound was particularly useful in cases with atypical clinical or neurophysiological characteristics, as already reported by Padua et al.,[24] and in all cases in which no neural response could be evoked. Also, it was useful in identifying multifocal damage or uncommon sites of injury, which cannot be achieved by electrodiagnostic testing alone. As reported by other authors,[8,14,21] ultrasound facilitated surgical planning in patients with neuromas, foreign bodies, and postfracture complications, allowing for targeted surgery and avoiding wide accesses for exploration because of accurate localization and estimation of nerve damage.

Tumors

The contributive role of ultrasound was more significant for tumors, in which it was diagnostic in 100% of cases, all of which had negative results on routine electrodiagnostic examinations. These findings were in accordance with the literature: Gruber et al.[16] proposed ultrasound as the first imaging technique for a superficial mass of suspected nervous origin; Simon et al.[28] reported a close correlation between ultrasound and MR tractography findings and considered ultrasound a valuable preoperative investigation for evaluating the risk of iatrogenic injury and for planning the optimal surgical strategy.

In our study, we observed a higher contributive role of ultrasound than was reported in the study by Padua et al.[25] (53.8% vs 42.3%) and a lower rate of negative (not confirming) role (10.1% vs 17.7%), which could be explained by a selection bias of our study population, as all patients in our series were candidate to surgery.

Based on the results of our study, we advocate the routine use of ultrasound nerve imaging in the evaluation of surgical candidates and support it as first-line imaging technique for the study of peripheral neuropathies, as a valuable complement to clinical and electrodiagnostic examination. Electrodiagnostic testing still represents the standard diagnostic tool for peripheral neuropathies as it provides unique information on the type of fibers involved (sensory or motor) and on the pathophysiology of the lesion (demyelination and/or axonal damage), but it may be uncomfortable for patients. We believe that ultrasound should be considered as a useful complement—not an alternative—to electrodiagnostic testing, although, as the diagnostic accuracy of imaging techniques increases, some authors foresee that high-resolution imaging could theoretically replace the electrodiagnostic tests in the diagnosis of some neuropathies.[20,27]

Ultrasound yielded negative results in cases of myeloradiculopathy (cervical or lumbar), in which motor/somatosensory evoked potential testing and MRI allowed final diagnosis. Thus, if proximal nerve impairment is suspected to be the main cause or to contribute to the clinical picture, ultrasound should not be recommended as the first-line imaging technique, and MRI shows a higher diagnostic value.

Our study has limitations: being a retrospective study, it is less reliable than a prospective study with regard to patient selection, and it did not include a control group. However, patient selection followed clearly defined inclusion and exclusion criteria based on our routine clinical protocol.

The study was not blinded, as the same clinician per-

forming the electrodiagnostic tests performed also the ultrasound examination. This could introduce a bias in the evaluation of their correlation; however, the aim of the study was not to evaluate the role of ultrasound alone but its role as a complementary evaluation in a practical clinical setting, where all diagnostic tests are performed during the same medical examination.

Our patient population was very heterogeneous, thus the numbers for each single nerve and type and level of lesion are limited; statistical analysis was performed only on a part of the data, as some subgroups (e.g., tumors) included only a small number of patients. However, this clinical series reflects the variability encountered in clinical practice and shows how nerve ultrasound contributes to the daily evaluation and management of focal peripheral nerve injuries. The influence of diabetes and other predisposing factors on nerve ultrasound characteristics was not evaluated. The duration of symptoms in the posttraumatic or postsurgical groups showed a wide range (2–72 months), and differences in nerve ultrasound findings were not evaluated with respect to this variable. Only a few patients in this subgroup received an early evaluation, and the role of ultrasound in this subset of patients was not evaluated, but could prove even more crucial: it is often difficult, with clinical and neurophysiological examination, to determine the type of lesion and the timing of surgery in the early phases, while ultrasound could allow early differentiation of neurotmetic from axonotmetic injuries, avoiding unnecessary delay in treatment.

Conclusions

Ultrasound is a powerful, noninvasive tool for examination of peripheral nerve injuries, which can guide diagnosis and surgical strategy of focal peripheral nerve injuries. It allows direct visualization of the cause and extent of nerve lesions and finds its place between electrodiagnostic tests and exploratory surgery. It can be used to complement a doubtful electrodiagnostic test, providing invaluable information, such as the presence and extent of a mass, scar compression, or neuromas. We recommend it as a complement to routine clinical and neurophysiological routine evaluation and as a first-line imaging modality for masses of suspected nerve origin.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: A Gagliardo, Toia, Cordova. Acquisition of data: A Gagliardo, D'Arpa, C Gagliardo. Analysis and interpretation of data: A Gagliardo, Toia, C Gagliardo. Drafting the article: A Gagliardo, Toia. Critically revising the article: A Gagliardo, D'Arpa, Cordova. Reviewed submitted version of manuscript: A Gagliardo, Toia. Approved the final version of the manuscript on behalf of all authors: A Gagliardo. Statistical analysis: A Gagliardo. Administrative/technical/material support: G Gagliardo. Study supervision: A Gagliardo, G Gagliardo, Cordova.

Supplemental Information

Videos

Video 1. https://vimeo.com/140429696.

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REVIEW

A systematic review of animal models for experimental neuroma

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Peripheral neuroma

Summary Peripheral neuromas can result in an unbearable neuropathic pain and functional impairment. Their treatment is still challenging, and their optimal management is to be defined. Experimental research still plays a major role, but — although numerous neuroma models have been proposed on different animals — there is still no single model recognised as being the reference.

Several models show advantages over the others in specific aspects of neuroma physiopathology, prevention or treatment, making it unlikely that a single model could be of reference. A reproducible and standardised model of peripheral neuroma would allow better comparison of results from different studies.

We present a systematic review of the literature on experimental in vivo models, analysing advantages and disadvantages, specific features and indications, with the goal of providing suggestions to help their standardisation.

Published models greatly differ in the animal and the nerve employed, the mechanisms of nerve injury and the evaluation methods. Specific experimental models exist for terminal neuromas and neuromas in continuity (NIC).

The rat is the most widely employed animal, the rabbit being the second most popular model. NIC models are more actively researched, but it is more difficult to generate such studies in a reproducible manner. Nerve transection is considered the best method to cause terminal neuromas, whereas partial transection is the best method to cause NIC. Traditional histomorphology is the historical gold-standard evaluation method, but immunolabelling, reverse transcriptase-polymerase chain reaction (RT-PCR) and proteomics are gaining increasing popularity. Computerised gait analysis is the gold standard for motor-recovery evaluation, whereas mechanical testing of allodynia and hyperalgesia reproducibly assesses sensory recovery.

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This review summarises current knowledge on experimental neuroma models, and it provides a useful tool for defining experimental protocols. Furthermore, it could help future research to define standard experimental model(s) of peripheral neuromas, allowing better comparison of results and improvement of our understanding of such a complex disease.

Introduction

Peripheral neuromas can severely impact the quality of life: both terminal neuromas and neuromas in continuity (NIC) can result in unbearable neuropathic pain, functional impairment and psychological distress.

A huge number of techniques for neuroma prevention and treatment have been proposed, including electrocoagulation,[1] transposition of the proximal stump into the muscle, bone or nerve,[2–6] lipofilling[7] and neural capping with synthetic or biological materials.[8–11] The large number of techniques in use reflects the poor clinical results and high failure rates: up to date, their treatment is still challenging, and their optimal management needs to be defined. This is probably due to their complex aetiology and to the high numbers of clinical variables that influence their appearance and their treatment's outcome. Moreover, histological nerve evaluation, performed in many experimental studies, cannot be reproduced in the clinical setting, limiting the direct investigation of how a human neuroma develops and how it can be best prevented and treated.

These are the reasons why experimental in vivo research still plays a major role. A large number of neuroma models have been proposed in different animals, although none has proved to be comprehensive, and it is used as a universal reference in this field.

Standardisation of an experimental model for neuroma would help to better compare results, and it might eventually help in proving the clinical usefulness in the prevention and treatment of neuromas. Our aim was to review the literature on experimental in vivo neuroma models, and to analyse their advantages and disadvantages, specific features and indications.

Materials and methods

A systematic electronic search was performed in the PubMed database combining 'neuroma' and 'model' as search terms (title and/or abstract field). Studies in languages other than English, Italian, German, Spanish or French, and clinical studies and/or studies not concerning experimental neuromas were excluded based on abstract review. Studies proposing an original animal model or a modification of an existing animal model of experimental terminal neuromas or NIC were then selected based on a full-text article review, whereas those using an already-described animal model were excluded. References of selected articles were evaluated to identify further relevant articles. Selected articles were reviewed, analysing the animal and nerve model, the injury type and the methodology study of the neuroma.

Results and discussion

An initial search returned a total of 153 papers, published between 1975 and 2013. After abstract and reference review, a total of 36 articles were selected.

Many experimental models of neuroma have been described. Most models are similar to those on nerve regeneration[12] and on neuropathic pain,[13–15] because these two aspects of nerve injuries also play a key role in neuroma physiopathology. However, neuromas show several peculiar aspects, and they attract increasing interest in defining specific experimental (preclinical) models to improve the prevention and treatment of peripheral neuromas.

These models greatly differ in the animal and nerve model, the injury type and the study methodology. In the following section, each aspect is discussed separately, and an overview on the main models for terminal neuromas and NIC is provided. Data on terminal neuromas and NIC are summarised in Tables 1 and 2, respectively.

Animal model

Animal

The rat is by far the most commonly employed animal model of experimental neuroma[10,16–41]; mouse[35,42–44] with the rabbit[45–49] being the second most popular models. However, although the anatomy of rat nerves is well established and similar to human anatomy,[12] peripheral nerve regeneration is much faster in rodents than in humans, and this limitation is accentuated by the fact that relative short nerve gaps can be obtained. Thus, it is difficult to compare this in vivo model with humans and to translate results into clinical practice. Some authors[45–49] prefer rabbit models to overcome the limits of the short regeneration time and short gaps in smaller animal nerve models[50]: nerve regeneration is less effective in rabbits, making this model closer to humans.[51] Yet, their nerve anatomy and limb muscle function are still far from those of the human being.[52] Primates, despite the advantage of being more similar to humans, are rarely employed because of practical and ethical limitations.[53]

The popularity of rat models for the study of peripheral nerve injuries probably relies on several advantages[12]:

Table 1 Animal models and studies on terminal neuroma.

References	Animal model			Evaluation methods			Aim(s)
	Animal	Nerve	Type of injury	Histology	Behavioural study	Other	
Yan et al., 2014[10]	Male Sprague—Dawley rat	Sciatic nerve	- Transection (15-mm gap) ± - Proximal stump capping with a nanofibrous scaffold	- Trichrome Masson's staining - Immunolabeling with antineurofilament (NF200) - Weight ratio of neuroma/normal nerve segment - Transmission electron microscopy	Autotomy	- Quantitative RT-PCR (gene expression of MAG, MBP, PMP22, NCAM-1 and RhoA) - Western-blot analysis (substance P, NGF, TGF-b1, collagen I and III, c-fos)	To investigate the structural and morphologic mechanisms by which the nerve capping technique prevents the formation of painful neuromas after neurectomy
Chim et al., 2013[16]	Sprague—Dawley rat	Sural nerve	- Avulsion - Transection - Folding into itself - Burying into muscle	- Haematoxylin and eosin, Masson trichrome staining - S-100 immunostaining - Evaluation of nerve cross-sectional area, ratio of neural to connective tissue **(widest part of the nerve three slides)** - Evaluation of mRNA expression of ciliary neurotrophic factor (CNF) and calcitonin gene-related peptide (CGRP) - Light microscopy			To compare the incidence of neuroma formation and neuropathic pain following different techniques of nerve ablation
Koplovitch et al., 2012[17]	Male and female HA selection rats	Saphenous and sural nerves	Two-stage injury: - Spared nerve injury (SNI) + - Ligation and transection/crush		- Autotomy - Sensation evaluation (pinching) - Tactile allodynia (von Frey test) - Residual sensation test with pinch with Kelly forcep (foot withdrawal)		To propose an alternative model to neuroma model of neuropathic pain for analgesic drug testing
Miyazaki et al.,	Male Sprague—Dawley	Tibial nerve	Transection + transposition				To compare the efficacy of

(*continued on next page*)

Table 1 (*continued*)

References	Animal model			Evaluation methods			Aim(s)
	Animal	Nerve	Type of injury	Histology	Behavioural study	Other	
2012[18] (Dorsi et al.'s model, 2008)	rat		(subcutaneously, superiorly to the lateral malleolus) + oral administration of morphine, pregabalin, gabapentin and duloxetine		- Neuroma tenderness assessment (von Frey test, withdrawal score) - Hind paw mechanical hyperalgesia (von Frey test)		pregabalin, gabapentin, morphine, and duloxetine on neuroma pain with their efficacy on mechanical allodynia
Kim et al., 2012[45] (Kim et al.'s model, 2010)	Female New Zealand white rabbit	Median, radial and ulnar nerve	Transection	- Toluidine blue staining - Polarised light microscopy		Electromyography	To test the management of a mixed nerve end neuroma by target muscle reinnervation
Ko et al., 2011[47] (Kim et al.'s model, 2010)	Female New Zealand white rabbit	Median, radial and ulnar nerve	Transection	Histomorphology (nerve cross-sectional area, myelinated axon count, myelinated axon cross-sectional area)			To investigate changes in the retrograde zone of injury in an amputation model
Marcol et al., 2011[20]	Male Wistar C rat	Sciatic nerve	- Transection (1-cm gap) - Transection (1-cm gap) + microcrystallic chitosan gel application to the proximal stump	- Light microscopy - Haematoxylin and eosin, Masson trichrome staining - Immunohistochemical labelling of macrophages (ED-1) and nerve cones (GAP-43) - Histomorphology (neuroma size, scar and regenerating nerve thickness) - Light and laser scanning microscopy	Autotomy		To examine the influence of the microcrystallic chitosan application to the proximal end of totally transected peripheral nerves on the development of painful post-traumatic neuroma
Kim et al., 2010[46]	Female New Zealand white rabbit	Median, radial and ulnar nerve	- Transection and transposition into anterior chest + shoulder disarticulation	- Toluidine blue staining - Histomorphology (nerve cross-sectional area, myelinated fibre count,			To create a reproducible end neuroma model subsequent to

				myelinated fibre cross-sectional area) - Polarised light microscopy			forelimb amputation
Ziv-Sefer et al., 2009[21] (Kim and Chung's model, 1992; Wall et al.'s model, 1979)	- Male and female HA—LA selection rats - F1 hybrids of reciprocal HA × LA crosses - Sabra rat (Wistar-derived strain) - Inbred Lewis rat	- L5 spinal nerve - Sciatic and saphenous nerves	Ligation + transection (2-mm gap)		- Autotomy - Tactile hyperalgesia (pinprick test) - Tactile allodynia (von Frey test) - Heat allodynia (Hargreaves test)		To investigate if pain phenotype in the neuroma and the SNL models share common pathophysiological mechanisms
Dorsi et al., 2008[22]	Male Sprague—Dawley rat	Tibial nerve	Transection ± transposition (subcutaneously, superiorly to the lateral malleolus)	- Toluidine blue staining - Light microscopy	- Neuroma tenderness assessment (von Frey test, withdrawal score) - Hind paw mechanical hyperalgesia (von Frey test) - Lidocaine block test	- Electrophysiological procedures	To produce a neuroma accessible for mechanical testing and outside of the innervation territory of the injured nerve
Huang et al., 2008[23]	C56BL Mouse — Sprague—Dawley rat	Saphenous nerve	Transection + Ligation + insertion into a tube			- Proteomic profiling (2D-difference gel electrophoresis and mass spectrometry) - Immunoblotting - S35 methionine labelling	To examine changes in protein expression associated with the formation of hyperexcitable neuromas
Minert et al., 2007[42] (Kim and Chung's model, 1992)	Male mouse (several strains)	- L4, L5, L6 spinal nerves - Sciatic and saphenous nerves	Transection (3—4-mm gap)		- Autotomy - Tactile hyperalgesia (pinprick test) - Tactile allodynia (von Frey test) - Heat allodynia (Hargreaves test)		To propose a spinal nerve neuroma (SNN) model of neuropathic pain
Lago et al., 2007[24]	Female Sprague—Dawley rat	Sciatic nerve	Transection + repair with a silicone guide + - Amputation model (capped silicone chamber) or - distal tibial branch implanted into the	- Toluidine blue staining - Histomorphology (cross-sectional area, myelinated fibre count) - Immunohistochemistry (antibodies anti-ChAT-choline acetyltransferase-,		Nerve conduction tests (functional evaluation of target muscle reinnervation)	To assess the long-term maintenance of regenerating axons in an amputee peripheral nerve.

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Table 1 (*continued*)

References	Animal model			Evaluation methods			Aim(s)
	Animal	Nerve	Type of injury	Histology	Behavioural study	Other	
			gastrocnemius muscle and peroneal nerve apposed to skin	CGRP, GAP-43, growth-related peptides) - Light microscopy			
Marcol et al., 2007[19]	Male Wistar C rat	Sciatic nerve	Transection + connective tissue chambers (containing fibrin, fibrin + BDNF, fibrin + Ab anti-BDNF)	- Toluidine blue, Van Gieson and Masson's trichrome staining - Light and fluorescent microscopy	Autotomy		To establish the role of BDNF applied locally in autotomy behaviour after sciatic nerve transection
Tyner et al., 2007[25]	Male Sprague—Dawley rat	Sciatic nerve	- Transection (2-cm gap) ± biosynthetic collagen nerve guides	- Toluidine blue staining - Immunolabeling (NF200- antineurofilament or S100- anti-Schwann cell monoclonal antibodies)	Autotomy (modified Wall scale)		To examine the use of biosynthetic collagen nerve guides to prevent the development of post-traumatic neuromas
Sinis et al., 2007[26]	Inbred Lewis rat	Median nerve	- Transection (1—2-cm gap) - Transection (gap) ± implantation into pectoral muscle	- Histomorphology (cross-sectional area of the neuroma, neuro-connective tissue ratio) - S-100 immunostaining - Light microscopy			To analyse the distal stump influence on neuroma formation in cases of transection of the median nerve
Elwakil et al., 2007[48]	Red rabbit	Facial nerve	Nd:YAG Laser coagulation/ transection	- Haematoxylin and eosin, TEM (toluidine blue and basic fuchsine) staining - Light microscopy and transmission electron microscopy			To evaluate the neodymium:yttrium aluminium garnet (Nd:YAG) laser (1064 nm) nerve transection technique for prevention of neuroma formation.
Okuda et al., 2006[27]	Male Sprague—Dawley rat	Sciatic nerve	- Transection (1.5-cm gap) ± insertion of the proximal stump into a silicon tube	- Toluidine blue staining Immunolabeling (polyclonal antibody against S-100, monoclonal antibody against ED-1, polyclonal rabbit anti-mouse NGF	Autotomy (Wall scale)		To verify the pain-like behaviour inhibitory effect of covering the proximal stump of the sciatic nerve with a silicone tube

				IgG, polyclonal rabbit anti-mouse TrkA IgG) - Light microscopy			
Turgut et al., 2005[29]	Male Wistar rat	Sciatic nerve	- Pinealectomy or pinealectomy + given melatonin + - Transection (1-cm gap)	- Toluidine blue staining - Stereological analysis - Light microscopy			To test the role of melatonin on neuroma formation and peripheral nerve regeneration
Xu et al., 2002[30]	Male Sprague—Dawley rat	Sciatic nerve	Transection (2—3-cm gap)	- Toluidine blue staining - Immunolabeling (anti-CGRP rabbit polyclonal antibody; anti-NF-200 — heavy neurofilament subunit — mouse monoclonal antibody, anti-GFAP — glial fibrillary acidic protein — rabbit polyclonal antibody) - Fluorescent microscopy		Local blood flow evaluation (laser Doppler flowmetry, microelectrode hydrogen clearance polarography)	To examine local perfusion, axon penetration and other characteristics of long-term (6 month) experimental neuromas created by sciatic nerve transection and resection of the distal sciatic nerve and its branches
Zeltser et al., 2000[31]	Sabra rat (Wistar-derived local strain)	Saphenous nerve	Section (few mm gap): - Laser neurectomy - Electrocut - Cryosurgery - Iridectomy scissors - Nerve ligation		Autotomy		To propose the autotomy model to promote research on better neurectomy methods for neuroma prevention
Menovski et al., 1999[32]	Female Wistar rat	Sciatic nerve	- Transection (5-mm gap) or - Diffuse coagulation (7 mm) and transection by nd:YAG laser	- Toluidine blue and basic fuchsine staining - Neuroma size, adhesions and outgrowth of nervous tissue into the distal stump - Light microscopy and transmission electron microscopy (TEM)	Autotomy		To evaluate whether the neodymium: yttrium aluminium garnet (Nd:YAG) laser could prevent neuroma formation after neurectomy
Macias et al., 1998[33]	Sprague—Dawley rat	Sciatic and median-ulnar nerves	Transection and isolation with silicone tubes	- Carbonic anhydrase and cholinesterase staining for motor-sensory labelling			To address the relationship between afferent and efferent axons within developing neuroma
Gonzales	Male	Sciatic nerve	- Transection (gap)	- 'Routine' histology	Autotomy (Wall scale)		To investigate the

(*continued on next page*)

Table 1 (*continued*)

References	Animal model			Evaluation methods			Aim(s)
	Animal	Nerve	Type of injury	Histology	Behavioural study	Other	
et al., 1985[34]	Sprague—Dawley rat		- Transection + centrocentral anastomosis - Transection + centrocentral anastomosis (delayed) - Transection (gap) + neuroma resection (delayed)	- Light microscopy			role of centrocentral anastomosis on the formation of terminal neuromas and on the time course of autotomy following experimental transection of the sciatic nerve
Mackinnon et al., 1985[53]	Monkeys (*Macaca fascicularis*)	Palmar cutaneous nerve median nerve, superficial branch radial nerve, dorsal branch ulnar nerve	- Transection (2-cm gap) - Transection + transposition into muscle - Transection and subcutaneous transposition	- Toluidine blue staining - Histomorphology (neural to connective tissue ratio, fibre density and fascicular diameter) - Light and electron microscopy			To evaluate the histologic changes of implantation of a transected sensory nerve into muscle
Wall et al., 1979[35]	Male Sabra rat and female CBA mouse	Sciatic and saphenous nerves	- Ligation and transection - Transection and encapsulation by perineurium suture - Transection and resuture - Crush - Ligation - Crush + ligation - Transection (5-mm gap)		Autotomy		- To describe the time course and degree of autotomy following various types of nerve injuries - To propose a neuroma model of neuropathic pain

Table 2 Animal models and studies on NIC (neuroma in continuity).

References	Animal model			Evaluation methods			Aim(s)
	Animal	Nerve	Type of injury	Histology	Behavioural study	Other	
Mavrogenis et al., 2013[36]	Male rat	Sciatic nerve	Crush and distal transection ± OX7-saporin microinjection	- Haematoxylin and eosin staining - Fluorescent labelling with black—gold or fluoro-ruby - Measurement of neuroma's diameter - Light and fluorescence microscopy			To establish an NIC model To evaluate the effect of the OX7-saporin axonally transported immunotoxin conjugate on neuroma-in-continuity formation
Alant et al., 2013[38]	Male Lewis rat	Sciatic and femoral nerves	- Crush - Focal compression ± traction - Transection and repair	- Toluidine blue staining - Staining for rhodamine phalloidin and neurofilament - Axon and myelin measurement (axon diameter/fibre diameter, fibre area/intrafascicular area) - Light and fluorescence microscopy	- Skilled locomotion test: horizontal ladder rung (video recording and later analysis)		To evaluate the correlation between axonal misdirection and behavioural deficit and between functional recovery and degree of attrition of motor nerve
Alant et al., 2012[37]	Male Lewis rat	Sciatic nerve	- Crush - Focal compression ± traction	- Haematoxylin and eosin, Masson's trichrome staining - Double staining for laminin and neurofilament - Fluorescence microscopy	- Skilled locomotion tests: tapered/ledged beam apparatus, horizontal ladder rung (video recording and later analysis) - Ground reaction force		To define an NIC model for preclinical evaluation of treatments
Kotulska et al., 2011[43] (Kotulska et al.'s model, 2006)	APP/SOD1 overexpressing mice	Sciatic nerve	Transection + suture	- Toluidine blue staining - Immunolabeling (anti-GAP-43, S-100,anti-ED-1) - Nerve thickness - Light microscopy, confocal laser scanning microscopy	- Autotomy - Walking pattern (SFI)	Muscle mass and limb circumference	To examine neuropathic pain-related behaviour and peripheral nerve regeneration in mouse model of Down syndrome
Tomita et al., 2007[39]	Male Sprague—Dawley rat	Peroneal nerve	- Transection - Transection + crush (three sequential applications of needle forceps,	- Double retrograde neurons labelling (true blue, diadimino yellow) - Histological analyses (10 mm distal to the	Walking-track analysis (peroneal functional index)	- Electrophysiological tests (sciatic stimulation and MAPs from tibialis anterior)	- To establish a new NIC model - To analyse the

(*continued on next page*)

Table 2 (*continued*)

References	Animal model			Evaluation methods			Aim(s)
	Animal	Nerve	Type of injury	Histology	Behavioural study	Other	
			followed by transection at the same location) + interposition of the aponeurosis of the spinal muscles between the transected stumps + bypass graft with the ulnar nerve	transection site and from the bypass graft)		- Muscle weighing (tibialis anterior)	effects of the bypass graft in the treatment of NIC
Ma et al., 2006[28] (Seltzer et al.'s model, 1990)	Male Sprague—Dawley rat	Sciatic nerve	Partial ligation Perineural, intraperitoneal or intramuscular injection of anti-IL-6 antibody or CGRP antagonists	- Immunostaining (double staining of CGRP, CRLR and RAMP1 with invading macrophage marker ED1) - Confocal microscopy	- Mechanical allodynia (von Frey test) - Thermal hyperalgesia (hot plate)		- To determine whether the overproduction of IL-6 by invading macrophages in injured nerves is involved in neuropathic pain - To explore if CGRP in neuroma and invading macrophages contributes to the maintenance of neuropathic pain - To determine whether CGRP is involved in the up-regulation of IL-6 in invading macrophages
Kotulska et al., 2006[44]	Wild-type and heterozygous trkB-deficient mouse	Sciatic nerve	- Transection (3-mm removed) - Transection + suture - Transection + gap injury model (5-mm graft)	- Toluidine blue staining - Immunolabeling with anti-GAP-43 - Light microscopy, confocal laser scanning microscopy	Autotomy		To examine the role of trkB in post-traumatic neuroma formation
Song et al., 2006[49]	Male New Zealand rabbit	Lateral fascicule of the peroneal nerve	Transection	- Haematoxylin—eosin, Luxol fast blue, Van Gieson staining - Light microscopy		- Electrophysiologic tests (motor nerve conduction velocity and compound motor action potential) - Molecular	To establish a neuroma-in-continuity model

						markers' expression (quantification of ciliary neurotrophic factor (CNTF) in nerve and of calcitonin gene-related peptide (CGRP) in mRNA (real-time QPCR)	
Adelson et al., 2004[40]	Sprague—Dawley rat	Tibial nerve	- Crush (haemostat applied for 30 s) - Transection/repair	- Toluidine blue staining (sections 5 mm above and distal to the neurorrhaphy) - Retrograde dual labelling with fluoro-ruby or fluoro-emerald - Electron, standard light and fluorescent microscopy		- Evoked electromyography	To evaluate treatment of NIC with terminolateral neurorrhaphy (jump grafts)
Malushte et al., 2004[41]	Female Fisher rat	Tibial nerve	- Partial transection Control groups: - Crush - Neurectomy (transection-resection 1 cm)		Walking-track analysis (TFI), Print Length Index (PLI), Toe Spread Index (TSI). Intermediate Toe Spread Index (ITSI)	Wet muscle mass (gastrocnemius)	To assess functional recovery following partial tibial nerve lesions

compared with mice, rats are more resilient, and they have larger nerves, which simplifies microsurgical procedures; compared with any other animal models, a higher number of standardised functional tests exist,[54–58] making the evaluation of results easier.

Sprague–Dawley rat is the most commonly used strain,[10,16,18,22–25,27,28,30,33,34,39,40] but Lewis,[21,26,37,38] Fisher[41] and Wistar rats[19–21,29,31] have also been used. Most studies use males,[10,17–22,25,27–30,34–39,42] and only a minority use females.[24,31,32,41,45–47] These differences hamper the comparison of results, because nerve anatomy and the tendency to develop neuropathic pain vary between strains and genders as follows[21,59–62]:

1. **Strain differences**: The anatomy of the sciatic nerve is similar in Sprague–Dawley, Wistar and Lewis rats, but not in Fisher rats, in which the tibial and peroneal divisions are surrounded by separate epineuria at the femoral level, and not by the same epineuria as in the other strains.[63] In addition, Rigaud et al. showed that, whereas the sciatic nerve receives an equal contribution by L4 and L5 spinal nerves in Wistar rats, it mainly derives from the L5 nerve in Sprague–Dawley and in Brown Norway rats.[59] Regarding neuropathic pain, Carr et al. showed that Lewis rats do not develop autotomy following sciatic nerve injury,[64] whereas Ziv-sefer et al. showed that neuropathic pain also varies between different lines from the same strain, and it is related to autotomy: high-autotomy lines from Wistar-derived Sabra strain rats showed not only a higher spontaneous neuropathic pain following hindlimb denervation but also a significantly higher tactile allodynia and hyperalgesia with respect to low-autotomy lines from the same strain.[21]
2. **Gender differences**: The degree of spontaneous pain, allodynia and hyperalgesia also varies with sex. Ziv-sefer et al. showed that females of the low-autotomy line develop a heat allodynia, hypersensibility and pinprick hyperalgesia higher than the male counterpart and comparable to the high-autotomy line[21]; Devor et al. showed that the male pain phenotype presents a greater within-strain variability than the female counterpart, being more influenced by environmental factors.[65] Furthermore, nerve regeneration is more efficient in females due to hormonal influences[61,63,64]; Kovavic et al. reported a larger number of myelinated axons in the anastomosed nerve segment in females, which could be related to a higher sprouting capacity of thin myelinated sensory axons.[66]

Nerve

Despite the fact that neuromas and nerve injuries in general are far more common in the upper limb, most of the experimental models are designed on the hindlimb. The sciatic nerve, due to its accessible position and easy dissection, is the most widespread model for neuroma investigation.[10,19,20,24,25,27–30,32,34,36,37,43,44] Savastano et al.[67] recently reviewed the rat sciatic nerve model, and they supported it as a 'simple and subtle model for investigating many aspects of nervous system damage and recovery'. Other popular models are the tibial,[18,22,40,41] sural,[16] peroneal[39,49] or saphenous nerves.[23,31]

The forelimb has been less often used and mostly for terminal neuromas. This is probably due to the small size of the rat forelimb and its nerves. The use of forelimb models in rabbits is more frequent.[45–47]

Some models use more than one nerve of the forelimb,[45–47,53] the hindlimb,[17,21,35,38,42] or both.[33] A neuroma model on the facial nerve has also been described, but it is far less popular.[48]

Mechanism of injury

Peripheral neuromas result from different mechanisms of injury. The variety of mechanical lesion models used also reflects this. The choice mainly depends on the type (terminal neuromas and NIC) and aspect of the neuroma to be investigated (neuroma prevention, motor recovery or neuropathic pain).

Models of terminal neuroma often use nerve transection[10,18–20,22,25–27,29,30,32,33,42,45–47] to reproduce a Sunderland's grade 5 nerve injury,[68] with sciatic nerve neurotmesis being the most popular model.[10,19,20,24,25,27,29,30,32–34] Transection is usually associated with nerve resection to prevent regeneration through the distal stump, as transection alone would not allow to reproduce a terminal neuroma due to the efficient regeneration potential of the rat. The length of the gap ranges between 2 and 30 mm. As pointed out by Lindenlaub et al.,[69] the gap length influences neuroma formation; Sinis et al.[26] also showed that shorter gaps are associated with larger neuromas, and they postulated that it may be due to the influence of growth factors released from the distal stump. Elwakil et al.[48] and Menovski et al.[32] reported the use of neodymium-doped yttrium aluminium garnet (nd:YAG) laser to reproduce a terminal neuroma. Some authors combined ligation and transection in a single-[21,23,35] or two-stage model.[17] Ligation alone or crush injuries have been rarely reported[17,28,35] for terminal neuromas, due to their low potential for nerve damage, and to the high nerve regeneration potential of rats. Others reported multiple injuries in different subgroups of animals to investigate the physiopathology of terminal neuromas,[24] or to compare methods for neuroma prevention.[16,31,34,51]

Models of NIC are less numerous, and they have received attention only in the last 10 years. They aim at reproducing a Sunderland's grade 3 and 4 nerve injuries[68] through crush injuries,[40] crush and distal transection,[36,39] partial transection,[41] transection and suture[40,43] or partial ligation.[28] Some authors also compare different mechanisms of injury in the same animal model.[37,38,40,41,44] Although other types of injuries are well reproducible, crush injuries are difficult to standardise in terms of pressure applied and subsequent response, making comparison of results difficult. Moreover, nerve crush injuries in rats always correspond to Sunderland's grades 2 and 3, and they are likely to undergo spontaneous recovery; thus, crush should not be considered as the method of choice for NIC models.

Evaluation methods

Histology

Morphological analysis is the most commonly used method, usually performed in light,[18,22,24,26,27,29,45,46,32,34,50,36,38,43,44,49,19,20,47,39,49] electron,[10,30,32,50,36,40] fluorescence[19,37,38,40] or confocal laser microscopy.[20,43,44]

Data on the level where biopsies of the nerve and the neuroma are taken and analysed are heterogeneous and often missing, preventing appropriate comparison of results: Chim et al.[16] cut three sections from the 'widest part of the nerve'; Dorsi et al.[22] sections proximal and distal to the site of ligation, and from the neuroma itself; Tomita et al.[39] sections 10 mm distal to the transection site and from the bypass graft; and Adelson et al.[40] sections 5 mm proximal and distal to neurorrhaphy.

Haematoxylin and eosin[16,18–20,36,50,37,40,49] and toluidine blue[10,18,22,32,45,46,24,19,25,27,30,38,43,44] are the most commonly used stains; some studies also use Masson's thricome,[10,20,37] which also allows the labelling of the connective tissue.[70]

This is sometimes combined with immunolabelling, the most common targets being S100 protein,[16,25–27,43] neurofilament 200 (NF200),[10,25,30,37,38] neuronal growth-associated protein (GAP)-43,[20,24,43,44] macrophages (ED-1),[20,27,28,43] nerve growth factor (NGF)[27] and calcitonin gene-related peptide (CGRP).[24,28,30] Double staining has also been reported to identify axons of different origin.[36,39]

The most studied morphologic parameters are nerve cross-sectional area,[16,24,26,43–47] the ratio of neural to connective tissue,[16,26,51] myelinated axon count,[24,45–47] neuroma cross-sectional area,[45–47] neuroma size,[20,32,36] scar and regenerative nerve thickness,[19,20] axon and myelin fibre diameter and area.[38] In a recent paper, Yan et al.[10] proposed the weight ratio of neuroma/normal nerve segment, which allows to normalise the results, making their comparison easier.

Other less common methods include quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) — which is used to evaluate the gene expression of several proteins, such as ciliary neurotrophic factor (CNTF) and CGRP[10,16,49] — proteomic profiling and immunoblotting,[23] laser Doppler fluorometry and microelectrode hydrogen clearance polarography.[30]

Interest in CNTF and CGRP has been increasing. They are evaluated through immunolabelling and RT-PCR, and they give an indirect evaluation of nerve regeneration and neuropathic pain, respectively.[71–73] Ma et al. also showed that increased levels of CGRP in neuroma play a role in the maintenance of thermal hyperalgesia.[28]

Behavioural tests

Behavioural tests provide a direct measure of functional recovery (of both motor and sensory functions), and they are commonly used in experimental neuroma models.[10,17–21,25,27,28,31,32,34,35,37–39,41–44] Numerous tests exist, with not one being the absolute reference.

The sciatic nerve model is the most widely employed for behavioural tests. Most tests are based on walking-track analysis,[39,41] because spontaneous locomotion in rats is 'very consistent, symmetric, and replicable'.[74] Alant et al. proposed the ladder-rung task, as a sensitive locomotion test for the evaluation of both motor and sensory recovery.[38] In a previous study, they also measured ground reaction forces (GRFs) 'to determine the contribution of each limb to weight support, propulsion, braking, and balance during locomotion'. The sciatic functional index (SFI) described by de Medinaceli in 1982[73] is an easy, inexpensive and reliable method still in use,[43] which evaluates the print marks of both feet. Its assessment can be hindered by the dragging of the tail and by flexion contracture or autotomy. Similarly, Tomita et al.[39] reported the use of the peroneal functional index (PFI) described by Bain et al.[75] for walking-track analysis. Although these traditional methods are still in use, video-assisted gait analysis is gaining increasing popularity,[37,38] because it can provide a more accurate kinematic description, and it holds the potential for a quantitative comparison of the results.

'Autotomy' (the tendency to attack the anaesthetic limb[35]) is a common evaluation method for terminal neuromas,[10,17,19–21,25,27,31,32,34,35,42–44] and it is less used for NIC,[43,44] probably because it occurs more frequently in protocols using ligation or transection (as in most NIC models) than in the crush injury (as in most terminal neuroma models) (Ref. 42; Obata et al., 2003). The existence of strain differences in autotomy has been demonstrated in rat[64] and mouse,[77] and it contributes to limit its potential as a standard evaluation tool. Moreover, Kryger et al.[76] have shown that the morphologic alterations typical of neuroma formation do not always correlate with high autotomy scores.

Sensibility recovery is commonly evaluated by the assessment of tactile mechanical allodynia,[17–21,28,42] heat allodynia,[21,42] sensation,[17] tactile hyperalgesia[19–22,42] and neuroma tenderness.[18,22]

Electrophysiological evaluation

Electrophysiological tests provide a quantitative measure of nerve activity, are minimally invasive and allow serial evaluation of the animal. In addition, similar tests can be applied to different animal models and in the clinical practice.[78]

Despite this, they are not very popular,[22,24,39,45,49] probably because they poorly correlate with functional tests of locomotion and functional recovery.[79]

Terminal neuromas

In 1979, Wall described the neuroma model of neuropathic pain,[35] in which a terminal neuroma is reproduced by the transection of the sciatic nerve or ligation of both the sciatic and saphenous nerves. To date, the most popular model for terminal neuroma continues to be sciatic nerve neurotmesis in the rat,[10,19,20,24,25,27,29,30,32,34,35,38] but recently, a number of alternative models have been developed, which focus on the investigation of specific aspects of peripheral neuromas.

Some of the reviewed studies aim at evaluating the best method for neuroma prevention, and they compare different techniques for nerve injury: transection is associated with subcutaneous[22,53] or intramuscular transposition,[16,34,51] laser neurectomy[31,32,48] or capping.[10,24] Chim et al.[16] also evaluated the role of avulsion and folding the nerve into itself.

Chim et al.[16] proposed an interesting and simple model in the sural nerve of Sprague—Dawley rats, which allows evaluating different ablation techniques with regard to the incidence of neuroma formation. Their findings are of clinical interest for both the prevention and treatment of peripheral neuromas, and they suggest that avulsion and muscle burying are better than folding or transection in preventing neuroma. Although their model is well reproducible for transection, folding and burying into muscle procedures, the traction forces applied are not standardised, and they could be better defined to guide further research.

In the study of sensory terminal neuromas, models using the sural and/or saphenous nerve[16,17,23,31] are preferable to the mixed sciatic nerve.

The model described by Kim et al.,[45,46] instead, addresses the investigation of motor reinnervation potential of terminal neuromas. They first described an amputation model[46] in the rabbit, and later they applied it to targeted muscle reinnervation using a pedicled rectus abdominis flap.[45] Kim's model is well reproducible, adequately mimics human upper limb amputation and — by studying three nerves per animal — allows optimisation of resources; one limitation of this study is that no behavioural test has been performed to measure muscle functional recovery. This model was initially designed for improving signalling for myoelectric prostheses, but it could be used to study other aspects of terminal neuromas.[47] It has also been used by Mcclintic et al.[78] to study a non-invasive evocative test for subcutaneous, painful tissue with intense focused ultrasounds (iFUs), and to investigate if focal stimulation provided by iFU can differentiate focal, subcutaneous neuropathic tissue from nearby tissue.

Another novel model for terminal neuroma is that described by Dorsi et al.[22]: the tibial nerve transposition (TNT) model. This model specifically addresses sensory dysfunction due to a terminal neuroma. The posterior tibial nerve is ligated and transected in the foot just proximal to the plantar bifurcation, and the proximal stump is tunnelled proximal to the lateral malleolus: by doing so, the neuroma is accessible for direct pain testing in response to mechanical stimulation, and it is located outside the innervation territory of the injured nerve. Thus, this model allows independent evaluation of neuroma tenderness and hyperalgesia, which — as shown in their study — depend on different neural mechanisms. Mikayazi et al.[18] successfully used this model to evaluate the efficacy of multiple drugs on neuroma pain compared to their efficacy on mechanical allodynia.

A different neuroma model of neuropathic pain was described by Koplovitch et al.[17]: they proposed a two-stage injury model on saphenous and sural rat nerves, combining a 'spared nerve injury' (SNI), with a ligation and transection or crush. Different from the classic SNI model,[15] in which autotomy is rare, their model showed that spontaneous dysaesthesias and pain are nonetheless present after an SNI: the SNI has a priming effect, and autotomy appears when sensory sensibility is removed by the second-stage nerve injury. As suggested by the authors, this model could be useful for testing drugs for spontaneous neuropathic pain.

Although the autotomy model continues to be popular,[10,17,19—21,25,27,31,32,34,35,42—44] several more objective methods for the indirect evaluation of pain are emerging, such as immunoblotting, RT-PCR and proteomics.

Yan et al.[10] recently reported a novel approach for neuroma pain investigation in the rat sciatic neurotmesis model. They divided animals into two groups, and in one of them the proximal stump was capped with a nanofibrous scaffold. Besides autotomy, they investigated several indirect pain-related markers through Western blotting and RT-PCR analysis: the expression of C-fos and substance P, of myelin-specific genes associated with mature Schwann cells, of neural cell adhesion molecule (NCAM)-1 (associated with immature Schwann cells, and of neurotrophic and growth factors (NGF and TGF-b1)) was compared in the two groups. Their results suggest that this model is effective in evaluating the pain level, and that the capping technique yields a pain relief effect. Other indirect pain markers used in neuroma studies are CNTF and CGRP.[16,24]

Proteomics plays a promising role in the study of peripheral neuromas. Huang et al.[23] used both proteomic and immunoblotting approaches in a rodent model to catalogue altered protein expression in hyperexcitable neuroma, and they compared it to a normal nerve. They found around 200 proteins, which displayed a $>$1.75-fold change in expression, and they identified 55 of these proteins using mass spectrometry. They also used immunoblotting to examine the expression of some ion channels previously associated with neuropathic pain, and S35 methionine in vitro labelling to demonstrate local protein synthesis of neuron-specific genes.

Neuromas in continuity

NIC corresponds to grade 3 and 4 nerve injuries according to Sunderland's system,[66] which are more difficult to replicate in a reproducible experimental model, and they are more difficult to evaluate in the clinical setting.

Recently, several new models for NIC have been proposed, in the attempt to identify a nerve injury that could reproduce an NIC in a predictable and reproducible manner. Traction and crush are the most common types of injuries.[36—39,41]

Alant et al.[37] proposed a combination of traction and crush forces on a rat sciatic nerve to reproduce Sunderland's grade 3 and 4 nerve injuries.[68] A reproducible compression force is applied with a malleus nipper, but the threshold forces needed to recreate an NIC are still to be defined. They speculate that the combination with traction may not be necessary when using higher compression forces, which would make this model even more reproducible. This injury model, designed on the sciatic nerve, may be adapted to nerves of different calibre and location. In a subsequent study,[38] the same authors combined this model with the established axonal misdirection model in

the femoral nerve,[80] to correlate the degree of motor-axon misdirection with behavioural deficit in an NIC model.

Tomita and colleagues[39] introduced an NIC model on the rat peroneal nerve: they induced a crush and transection injury, followed by repair with the interposition of muscle aponeurosis to cause intraneural scarring and to create a 'real' NIC. They also studied nerve regeneration through an ulnar nerve graft bypassing the neuroma, and they showed that it was enhanced by the nerve bypass grafting technique. Their NIC model is reliable and reproducible, as shown by histological, behavioural and electrophysiological analysis. It also confirms the role of bypass grafts in improving nerve regeneration following an NIC, and it could be a useful model for further investigation of the timing, the long-term outcomes and the effect on the sensibility of bypass grafts.

Others proposed NIC experimental models based on a partial nerve injury.[28,41,48] Song et al.[49] excised a 15-mm segment of the lateral peroneal fascicle in the rabbit peroneal nerve, keeping the medial fascicle intact. Malushte et al.[41] described a partial tibial lesion (previously described by Seltzer et al.[14]), and they compared it to crush and neurectomy lesions. All these partial nerve injury models proved to efficiently determine an NIC, demonstrating a high reliability and reproducibility of this injury technique.

Towards a standardised model

A reproducible and standardised model of peripheral neuroma is desirable, as it would allow a better comparison of results from different studies. However, this review points out great differences in published models, especially — but not only — between models of terminal neuromas and NIC.

Several of these are better with regard to a specific aspect of neuroma physiopathology, prevention or treatment to be studied, making it unlikely that a single model could be of reference for all that we still have to learn on peripheral neuromas.

Furthermore, due to the great differences in all aspects of the different neuroma models (animal, nerve, type of injury and evaluation methods), it was not possible to statistically compare the reviewed studies and their results; thus, evidence-based indications for the standardisation of neuroma models cannot be extrapolated from this review. Nevertheless, based on advantages and popularity of available models, we propose our suggestions for standardisation:

1. **Animal model**: Male Sprague—Dawley rats should be regarded as the rodent reference model, whereas rabbits could be preferable for special purposes, such as investigation of amputation and muscle reinnervation. The sciatic nerve appears adequate for most studies, whereas pure sensory nerves (such as the sural and the saphenous nerves) should be preferred for the study of terminal sensory neuromas. Moreover, even more complex models can be kept within the hindlimb, in which functional tests are easier to compare.
2. **Mechanisms of injury**: Nerve transection is the most published technique for terminal neuromas, and it is easy to reproduce. Resection of a nerve segment is advisable, with a 1-cm gap appearing adequate in the rodent model. Partial ligation, although less popular than nerve crush, should be regarded as the best technique for a reliable and reproducible NIC model.
3. **Evaluation methods**: Traditional histomorphology is the historical gold-standard evaluation method, but it needs to be coupled with immunolabelling, RT-PCR and proteomics, and promising and reproducible tools, which are becoming more and more indispensable in modern research. These tools also allow a more accurate — although indirect — evaluation of pain compared with the autotomy model. Computerised gait analysis is the gold standard for motor-recovery evaluation, whereas mechanical testing of allodynia and hyperalgesia reproducibly assesses sensory recovery.

We hope that the data provided and our suggestions can help future research to define standard experimental model(s) of peripheral neuromas, allowing researchers to better compare the results and to move one step further in our understanding of such a complex disease.

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Painful scar neuropathy: principles of diagnosis and treatment

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ABSTRACT

Nerve-tissue interactions are critical. Peripheral nerve injuries may involve intraneural and extraneural scar formation and affect nerve gliding planes, sometimes leading to complex clinical presentations. All of these pathological entities involve pain as the main clinical symptom and can be subsumed under the term "painful scar neuropathy". The authors review the literature on treatment approaches to peripheral nerve scar neuropathy and the outcomes of neurolysis-associated procedures and propose a simple classification and a therapeutic approach to scar neuropathy. The search retrieved twenty-one papers, twenty of which reported pain reduction or resolution with various techniques. There is no consensus on the best therapeutic approach to neuropathic pain due to scar tethering. Most authors report good or excellent results with different techniques, from nerve wrapping with anti-adhesion devices to nerve coverage or wrapping with vascularized tissue. The authors' classification of and therapeutic approach to peripheral nerve scar lesions aims at promoting a logical approach based on the analysis of lesion type (perineural, or endoneural and perineural), pain type (due to traction or external trauma, pain at rest), and number of previous operations. Patients need to be informed that multiple procedures may be required, that outcomes may be partial, and that surgery can potentially worsen preoperative conditions. The review found no evidence for the best therapeutic approach to scar neuropathy, but there is consensus on a multidisciplinary approach.

Key words:

Complex regional pain syndrome type II, painful neuropathy, painful scar neuropathy, scar neuritis, traction neuropathy

INTRODUCTION

Peripheral nerves have the ability to adapt to different positions during limb and joint movements. Such flexibility is enabled by a gliding apparatus around the nerve that provides for elongation during movement. Small nutritional vessels entering the epineurium from surrounding muscles are among the principal connections between nerves and soft tissue.

A peripheral nerve subjected to elongation stress can extend a few millimeters compared to its length at rest. Elongation is enabled by a conjunctiva-like structure[1] constituting the outermost layer of the nerve trunk that Millesi *et al*.[2] designated paraneurium. The inner nerve structure can also undergo elongation, and gliding planes have been detected between deep epineurium and perineurium[3] as well as between individual fascicles. Joint excursion, therefore, involves complete epineurial and intraneural movement, where nerve elongation

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compensates for the tension generated by movement and requires an intact gliding surface between the nerve and its surrounding tissue.

Clearly, the movement also stretches perineural and intraneural vascular structures, inducing vessel strain and reducing blood flow. A healthy gliding system prevents excessive stress from being exerted on vessel walls and ensures a sufficient blood supply to axons and Schwann cells. Preclinical studies have demonstrated that an 8% increase in nerve tension induces a 50% reduction in intraneural blood flow, whereas tension exceeding 15% of the baseline value induces an 80% reduction.[4] In a study of rat sciatic nerves subjected to crush lesions, Boyd *et al*.[5] documented nerve tension exceeding the intraneural microvessel compression threshold due to physiological movements, and found that it resulted in perineural scar formation and reduced intraneural vascularization.

Similarly, in the clinical settings formation of a perineural scar for any reason increases the tension on the nerve and may lead to prolonged ischemia. Wilgis and Murphy[6] described an association between reduced longitudinal gliding of the peripheral nerve and symptom recurrence following surgical decompression. In 1979, McLellan and Swash[7] reported that impaired linear gliding can induce a nerve lesion at a distance from the compression area, thus introducing the notion of traction neuropathy. The term indicates a condition related to impaired nerve gliding, whereas in Hunter 1991 description,[8] it designates neurological symptoms due predominantly to the movement of the affected nerve. However, traction neuropathy may be too narrow a definition, given that some patients with extensive perineural fibrotic reactions experience constant pain both at rest and in the absence of movement. The condition is likely due to a fibrotic response that is, initially perineural and eventually becomes intraneural due to compression secondary to chronic scarring. Perineural fibrosis can induce ischemic stress in the involved fascicles, followed by degeneration of distressed axons, the repair process may subsequently lead to formation of an in-continuity neuroma with residual nerve function whose symptoms also involve pain at rest.[9] Pain at rest may also be related to a perineural scar associated with intraneural scarring due to a traumatic Grade III or IV injury or to a Grade V lesion (nerve transection) according to Sunderland's classification.[10] A painful neuroma at the suture site has been described in nearly 5% of repaired nerves.[11] We, therefore, agree with Elliot[9] that "traction neuropathy" is a somewhat limited definition, whereas "scarring neuritis" or "scar neuropathy" encompass all the conditions related to formation of perineural and intraneural fibrotic tissue involving neurological symptoms and induced by a nerve injury (intraoperative lesion, cut injury, stretching, or extrinsic compression due to fracture or hematoma).[12]

Based on our experience and the pathophysiology of nerve injuries, both fibrosis around a nerve (traction neuropathy) and inside/outside it (as in neuroma-in-continuity) can be classified as scarring neuritis/scar neuropathy, whose distinctive symptom is pain due to the pathological condition affecting the nerve.

End-neuromas, which are associated with similar symptoms, and neuromas-in-continuity without residual function, are not addressed in the present review, because their management is fairly well established: the former may benefit from relocation to deep, protected areas, whereas for the latter the initial treatment of choice is reconstruction with nerve grafts or conduits.

This review describes and discusses the main diagnostic and therapeutic approaches to neuropathic pain due to neuroma-in-continuity and peripheral nerve compression in scar tissue based on the literature and the authors' personal experience. The condition is complex and difficult to treat, and there is no consensus on the most appropriate surgical approach.

Different surgical procedures and products that limit scar formation and reduce pain are also reviewed, and a treatment algorithm based on the type of pain, lesion type, number of previous operations, and imaging data is proposed. Finally a review of the literature for treatment outcomes, with emphasis on the resolution of pain symptoms, is presented.

EPIDEMIOLOGY

Perineural scarring and consequently traction neuropathy have traditionally been considered to be complications of nerve decompression surgery. Nerve tethering in the surgical scar is still the main cause of symptoms related to perineural scarring. For instance, 7-20% of patients subjected to primary median nerve release report pain and symptom recurrence.[13] The condition is difficult to manage, so much so that according to different reports compression symptoms persist after 40-90% of revision procedures,[14] and 20% of patients actually require a third operation.[14] Clinical failure rates of 25% have been reported after ulnar nerve release at the cubital tunnel,[15] and a review of 50 studies found symptom recurrence in approximately 75% of treated patients.[16] As noted above, 5% of nerve sutures have been estimated to induce a pain syndrome.[11]

However, the problem is not confined to peripheral nerves. Indeed, one of the most common complications of microdiscectomy and laminectomy, found in 15-20% of patients, is failed back syndrome, which seems to be related to the formation of scars entrapping the released nerve roots.[17] These patients often undergo additional procedures for the new symptoms.

Besides compression syndrome recurrence, neurogenic pain may be related to the formation of a neuroma-in-continuity associated with a partial lesion or severance of the peripheral nerve. This condition is found in 60-70% of traumatic injuries involving a peripheral nerve.[18]

CLASSIFICATION OF SCARRING NEURITIS

Millesi *et al*.[19] have extensively investigated peripheral nerve gliding and devoted considerable effort to describing

the role of the nerve-muscle tissue interface in normal nerve function.

Millesi *et al.*[19] vast surgical experience with peripheral neurolysis led to the publication of a seminal paper describing a new anatomo-surgical classification of perineural and intraneural scar lesions. The classification is a useful approach to perineural and intraneural scar injury because it couples each subgroup of fibrotic lesions to specific types of surgical neurolysis based on scar severity. However, although intraneural lesions are described in excessive detail, the clinical outcomes do not seem to correlate with preoperative pain measurement.

Here we describe a simplification of Millesi *et al.*[19] original classification and propose an approach that, by correlating the pathological findings to clinical and imaging data, has the potential to improve surgical treatment. The revised classification encompasses two injury types, extraneural and intraneural/extraneural scar lesions, based on the perineural tissue changes that impair nerve gliding and the intraneural problems that give rise to pain and hypersensitivity. Type I injuries are related to compression due to causes such as prior surgery, hematoma, and bone fragments, with involvement of the gliding surface (conjunctiva-nervorum) and formation of extensive scar tissue around the nerve, as depicted in Figure 1. These lesions are generally amenable to simple external neurolysis, with additional surgical procedures as required to avoid recurrence of perineural fibrosis (i.e. restoration of the gliding plane by anti-adhesion gel, vein conduit or other wrapping material). Pain is often related to joint movement and is less frequent at rest. On ultrasound (US) examination, the nerve has a normal fascicle structure. Type II injuries affect the entire nerve structure, from the epineurium to the endoneurium, and are usually secondary to significant nerve trauma such as a partial lesion or a transection of the nerve trunk treated by neurorrhaphy (neuroma-in-continuity). These injuries require procedures that may involve nerve fascicles and the epineurium, from epineurectomy and epineurotomy up to partial resection and grafting as described by Millesi *et al.*[19] In type II lesions additional surgical procedures are directed not only at avoiding recurrence of perineural fibrosis, but also at protecting the nerve against external mechanical insults. Outcomes are less predictable than in type I lesions. Pain at rest is common and is exacerbated by external trauma. US examination provides useful information on the intraneural pathology.

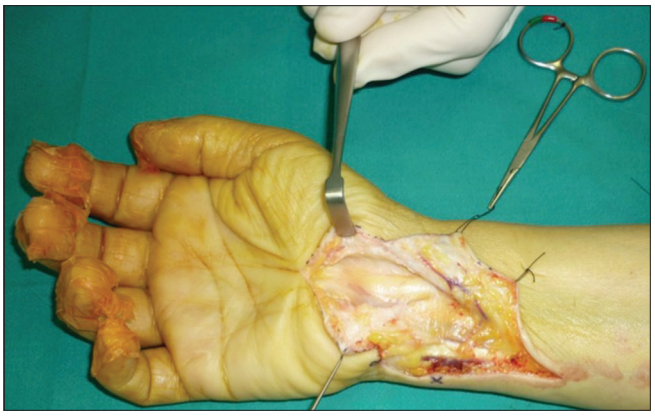

Figure 1: Median nerve entrapped in scar tissue

Type II lesions, with the exception of partial lesions due to a laceration or the sequelae of a nerve suture, correspond to Sunderland's third-degree lesions, which from the pathological standpoint include painful neuroma-in-continuity with residual function, one of the most challenging therapeutic problems. Fourth- and fifth-degree lesions are outside the scope of this review, as they lack residual nerve function and are managed by resection and reconstruction.

CLINICAL SYMPTOMS AND SIGNS

Patients typically report pain of four types, as described by Elliot[9]: spontaneous pain, pressure pain, movement pain, and hypersensitivity or unpleasant skin sensation to light touch, including hyperesthesia, hyperpathia, and allodynia.

The causal association is most obvious for pressure pain and movement pain elicited by the motion of adjacent tendons and joints. At present, hypersensitivity usually involves the skin overlying the affected nerve portion. The most poorly understood and unpleasant of these pain types is spontaneous pain, which is found in the majority of patients; it is most often a continuous or basal pain with spikes of increased intensity, or spiking pain that is often severe, has a variable frequency, and may be associated with reflex motor activity, example, jerking of the entire upper limb.[9]

These symptoms, presenting singly or combined, are compounded by complex regional pain syndrome type II (CRPS II) or causalgia,[20,21] due to fiber disorganization within the neuroma-in-continuity. Typical CRPS II features are onset after a nerve injury and continuous pain or allodynia-hyperalgesia that is usually, but not invariably confined to the territory of the injured nerve. Edema, skin blood flow abnormalities, or abnormal sudomotor activity may be detected in the area affected by pain since the time of injury. Timely management appears to be critical.[22]

DIAGNOSIS

History is crucial to establish the cause of symptoms, be it related to simple nerve decompression, reconstruction, direct trauma, or posttraumatic scarring.

Physical examination and pain type, at rest or elicited by movement or mechanical stimuli, may provide information on the lesion type. Pain at rest commonly entails that the scar involves the deep nerve structure. Perineural scarring usually induces nerve tethering, which is exacerbated by movement, that is, a loss of peripheral nerve gliding. Tinel's sign is invariably positive, and the patient often has hyperalgesia and/or allodynia in the territory of the involved nerve.[9,23]

As regards diagnostic imaging, US provides reliable information on the actual extent of the nerve injury (due for instance to a previous procedure), the amount of scarring, and the state of the outer and inner connective tissue layers of the nerve trunk. It thus provides an indication for surgery by demonstrating, before the operation, the various degrees of scarring described by Millesi *et al*.[19]

Moreover, according to a paper of the European Society of Musculoskeletal Radiology, musculoskeletal US seems to be the imaging technique of choice for peripheral nerve structure evaluation.[24]

Most studies use US to investigate the intraneural structures and changes due to chronic compression or trauma.[25] In these patients, US has proven to be even more effective than electrophysiological tests in depicting intraneural distress.[25] Some studies compare US findings, including signs of edema, loss of echogenicity, and fascicular echostructure before and after tunnel syndrome surgery.[26]

Padua *et al*.[27] group has advanced an interesting proposal that agrees with our classification of scar lesions, highlighting that valuable US features include depiction of very small nerves and dynamic imaging, which can document how the nerve interacts with surrounding tissue. Indeed, key diagnostic features of scarring neuropathy are an assessment of the nerve's relationships with surrounding tissue and depiction of any gliding impairment.

A critical advantage of US is that it affords direct visualization of the nerve injury, thus providing information on its cause and enabling treatment selection.[27] We thus feel that US scanning of the nerve and surrounding tissue entails a dual benefit for both patient and surgeon: it identifies the site of the nerve injury and depicts its relationships with scar tissue, documenting any obstacles to gliding. Combining anatomo-sonographic findings, electromyography data, and clinical information can help the surgeon select the most appropriate treatment approach.

Magnetic resonance imaging (MRI) enhances diagnosis and surgical planning; conventional MRI may depict indirect signs of nerve damage such as edema whereas high-resolution MRI provides direct visualization of injured and scar-tethered nerves, including the smaller peripheral branches.[28,29]

In experienced hands, MRI and US can provide crucial information in preoperative planning of revision nerve release surgery by documenting residual or recurrent pathology or the sequelae of previous surgery.

Electromyography examination is also important because it documents the degree of peripheral nerve distress, and findings can be compared over time (preoperative, postoperative, follow-up examination).

However, it is still unclear why similar pathological conditions induce pain in some patients but are painless in others, including patients with in-continuity neuromas and end-neuromas.

SURGICAL OPTIONS

Surgical exploration, neurolysis under magnification, and procedures aimed at preventing new scar formation such as flap coverage and application of anti-adhesion devices must be preceded by appropriate medical treatment and pharmacological and physical therapy with dedicated operators for at least six months. Although there is no consensus on surgery timing,[30] surgery is generally indicated when medical and physical therapy have failed to bring benefit.

Some authors have achieved pain reduction in a large number of patients using pulsed radiofrequency before surgery or following a recurrence.[31]

Surgical treatment of these conditions begins with neurolysis. External neurolysis is performed in cases with external compression, to free the nerve from the extrinsic compression. This may involve either accessing only the epineurium (epineurotomy) or removing part or all of it (partial or total epineurectomy) as shown in Figure 2a. Only in very selected cases is internal neurolysis performed, to treat an intraoperative iatrogenic injury or postoperative scar recurrence between fascicles. The procedure begins with identification of the normal proximal and distal nerve portions; the nerve is then mobilized above and below the injury site and its course toward the injury site is carefully dissected free of external scarring, points of tethering, or abnormalities.

The second step involves the relocation of the nerve tract involved by neurolysis to a "soft" vascularized bed enabling gliding.[30] Other procedures use vascularized or nonvascularized autologous tissue or an anti-adhesion gel. However, anti-adhesion devices, flaps, or other autologous tissues are not unequivocally recommended.

Here we propose a management strategy of posttraumatic scar lesions based on two mainstays, including (1) lesion categorization into extraneural and intraneural as described above, and (2) clinical information in terms of pain symptoms.

A combination of history data and US findings, which document the intraneural injury in a very early phase, supplies critical work-up information and provides an indication for external neurolysis versus a more extensive

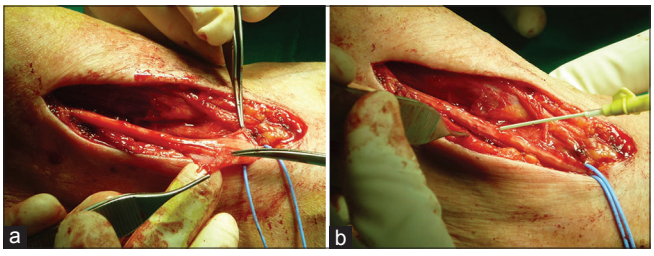

Figure 2: (a) External neurolysis and epineurectomy on median nerve at the elbow; (b) application of carboxy-methylcellulose/phosphatidylethanolamine gel on median nerve after neurolysis

neurolysis involving the epineurium and if necessary the perineurium.

Another key factor is the number of previous operations, simple external neurolysis is indicated after the first recurrence while a vascularized flap with a more extensive neurolysis is indicated following multiple failed surgical treatments.

Type I injuries, where scar tissue hampers gliding, should be managed by external neurolysis if the intraneural echostructure is normal, anti-adhesion gel, vein-wrapping, or thin flap coverage may be sufficient.

In type II lesions (neuromas-in-continuity), where US depicts a lack of structural homogeneity inside the nerve, more extensive neurolysis may be required, with epineurectomy and rarely, internal neurolysis under magnification. These patients also require deep nerve transposition, coverage with thick vascularized flaps, and restoration of a suitable gliding bed.

Patients with continuous pain due to an earlier traumatic injury to superficial nerves triggered by external stimuli, and those undergoing revision of a failed prior revision procedure, require deep nerve transposition and coverage with thick vascularized flaps providing both biological and mass effects.[32]

Relevant clinical data, including pain type (due to external compression, continuous, or movement-related) and cause of the lesion, can indicate the most appropriate management strategy. Patients with pain due to direct trauma may benefit from the bulk effect of a flap or from nerve relocation to a deep, protected area, whereas simple neurolysis with application of anti-adhesion devices is preferable in simple traction neuropathy, where pain is more often secondary to external traction.

Early active movement after surgery is indicated to prevent adhesion recurrence.

The next section describes the main techniques used in the treatment of scarring neuropathy and painful neuroma-in-continuity with residual nerve function after neurolysis.

SURGICAL MANAGEMENT AFTER NEUROLYSIS

Commercial gels and anti-adhesion devices

These devices are used to restore the lost gliding surface. Since 1970, when intraperitoneal anti-adhesion devices were first introduced, a number of products characterized by different shapes and chemical compositions have been developed to limit perineural scar formation. Gels developed specifically for peripheral nerve-tissue began to be produced in 2000. Early anti-adhesion gels were based on collagen-dextran (ADCON-T/N) and were initially used in spinal surgery. Preclinical application to rat peripheral nerve achieved a satisfactory reduction of perineural scarring. These gels were, however, abandoned after reports of wound dehiscence and dural fistula formation.[33]

Products based on hyaluronic acid (HA) have proved to be more effective. Initial preclinical studies have documented their anti-adhesion properties and safety.[34] HA is marketed alone as Hyaloglide(R)[35] or associated to carboxy-methylcellulose (CMC, Seprafilm(R)).[36]

However, there is no consensus on the actual effect of anti-adhesion devices. According to some researchers they reduce collagen deposition by interfering with granulocyte diapedesis and blocking the synthesis of interleukin-1, which is crucial for fibroblast activation,[37] whereas others deny an effect on cytokines and admit only to a physical barrier action.[38]

CMC has subsequently been associated with other molecules, including phosphatidylethanolamine a nonionic molecule whose tensioactive properties provide tissue lubrication and a mechanical barrier to restore gliding.[39] CMC-PE has also been shown to reduce perineural adhesions; it is already available on the market and has proven to be highly effective in preventing the formation of abdominal, spinal and tendon adhesions.[40]

In 2005, another macromolecule, polyethylene glycol oxyde (PEO), was associated with CMC to enhance its anti-adhesion effect. Preclinical studies have documented its ability to reduce protein, hence collagen, deposition on tissue.[40,41] However, there is no conclusive evidence for its effectiveness in the peripheral nervous system. A single paper has demonstrated its safety and effectiveness in an animal model (Tos *et al.*, paper submitted). A representative image of gel application after neurolysis is shown in Figure 2b.

Collagen-based products have recently been developed for wrapping around injured nerves.[42,43] These products are theorized to form a microenvironment within the compressed nerve, which keeps nerve growth factors within the epineurium to enhance nerve gliding, and which are subsequently slowly absorbed.

A recent study of a small sample with a short follow-up describes a novel nerve-wrapping technique for the upper extremities using a type I collagen conduit wrap. Its effectiveness is similar to that of other anti-adhesion devices, but it entails a lower fewer risk of complications compared to wrapping the nerve in autologous tissue such as vein (Neura Wrap; Integra LifeSciences, Plainsboro, NJ, USA).[43]

There are therefore several different types of anti-adhesion devices, but scant information as to which is the most effective at the clinical and preclinical level, even though all seem to limit perineural scarring formation without any particular side effects. A major advantage is their fast application and less invasive surgical dissection, without the need for further procedures (and possible attendant injury), which considerably reduces operating time compared to the surgical approaches described above.[34] Notably, there are no clinical trials comparing the effectiveness of the two approaches. A recent case review has advanced the proposal to apply anti-adhesion devices in cases where the nerve, released from the scar, appears

healthy or only moderately injured, and to use local or free flaps for clearly distressed nerves in the presence of a strong inflammatory reaction.[44]

Vein conduits

Masaer *et al*.[45] was the first to describe nerve-wrapping in an "opened" vein segment, which provided satisfactory results both in terms of sensitivity improvement and of reduction of recurrences.[46] Elliot[9] reported poor outcomes in neuromas-in-continuity of the palm and the fingers, describing pain recurrence at the site of treatment due especially to repeated trauma, because the thin vein wall does not adequately protect the nerve against external insults.

Some authors suggest covering sutures with a vein, as earlier for collagen-gel, to prevent end-neuroma formation at direct suture sites.[47]

Flaps

A variety of flaps, pedicled (local) or free, are used for coverage after neurolysis: synovial, fascial, adipofascial, muscle and skin with subcutaneous tissue flaps.

Compared to vein wraps, gels, and other anti-adhesion devices, flaps have a dual function: to envelop the injured nerve in a highly vascularized tissue to maximize nutrient supply, and to provide a bulk effect, for example, protection against external mechanical insults. This approach is often used in patients in whom revision surgery has had poor outcomes or when the quality of local tissue does not allow a simpler procedure.

Typical local flaps raised in patients with recurrences or sequelae of carpal tunnel syndrome (CTS) include the hypothenar fat pad flap first described by Cramer[48] and improved by Strickland, and the palmaris brevis flap described by Rose *et al*.[49] Their main advantage is that they provide a buffer of highly vascularized adipofascial or muscle tissue above the treated nerve. The synovial flap from the flexor tendons described by Wulle is still a very good option for recalcitrant CTS.[50]

Thicker flaps can be raised from the volar forearm: the dorsal ulnar artery adipofascial flap described by Becker and Gilbert[51] can be used as an adipofascial flap to wrap the nerve [Figure 3a and b] or as a fasciocutaneous flap to provide greater protection, the adipofascial radial artery perforator flap[32] and the adipofascial variant of the posterior interosseous flap raised from the dorsal portion of the forearm[52] can be employed in the same way; and the pronator quadratus muscle flap[53] may be a useful solution when the injury is proximal to the wrist.

Numerous free vascularized flaps, described for coverage of freed nerves, are however, rarely used. The free omental flap,[54] lateral arm flap, scapular flap, and groin flap[44] seem to be more effective than local flaps, yet the approach is recommended only for use in patients with severe conditions who have already been treated and in those with hand and forearm lesions where a local flap would impair hand use. Yamamoto *et al*.[20] have gone further, and they raised an anterolateral vascularized thigh flap that included the lateral cutaneous nerve of the thigh

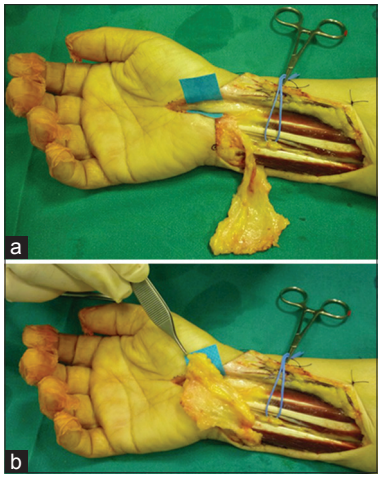

Figure 3: (a) Adipofascial dorsoulnar Becker flap covering and wrapping a median nerve; (b) the bulk effect of the flap protects the nerve from external trauma

to reconstruct the median nerve, and described early pain resolution and full recovery of wrist and hand mobility five months from the procedure. We recommend such complex procedures only in patients with severe nerve injury and failure of multiple surgical procedures, where another local flap could result in local tissue damage.

Pain neuromodulation

Multiple surgical failures may provide an indication for direct peripheral nerve stimulation, to relieve chronic pain through preferential activation of myelinated fibers, inducing long-term depression of synaptic efficacy.[55,56]

Spinal cord stimulation, which is applied more often to treat CRPS I, may also be beneficial.[57]

SCAR NEURITIS AND OUTCOMES: LITERATURE REVIEW

PubMed was reviewed for papers reporting treatment approaches and patient outcomes of scar neuritis and neuropathic pain, in particular studies of recurrent median and ulnar nerve compression, postsurgical fibrosis of lower and upper limb nerves, CRPS II, and application of HA acid and gels that also described pre- and post-operative pain assessment by the visual analogue scale (VAS) or numerical rating scale. Case reports and animal studies were excluded. Papers were sorted by the treatment approach to neurolysis.

Overall, 21 papers were retrieved; the majority described the treatment of median and ulnar nerve entrapment recurrence. The method most frequently associated with neurolysis was flap coverage (15 articles); the remaining papers described the use of anti-adhesion devices (3 articles) to reduce pain and prevent recurrences, and vein wraps (3 articles).

All approaches provided good outcomes, although most studies involved small samples, from 4 patients to 65 patients. All methods achieved a postoperative reduction of at least four VAS points. All but one study described complete or satisfactory pain reduction. These

data are summarized in Table 1. No alternative options are mentioned for patients reporting no improvement.

Despite published reports of highly satisfactory outcomes and success rates close to 100% with a range of techniques, clinical practice demonstrates that such conditions are difficult to treat and at times are only partially solved.

There is scant published evidence regarding the diagnostic work-up and treatment of scar neuropathy. Patients should be warned that their condition is not easy to address and that surgical treatment may have to be followed by a more aggressive approach if symptoms persist.

Patients with pain due to nerve entrapment in scar tissue require careful evaluation through history, assessment of pain type, and accurate US scanning, to establish the site of the scar tissue injury and whether the nerve contains internal damage. In patients for whom surgery will be straightforward local tissues provide a suitable bed, barrier devices may be applied first to attempt to treat the problem by a less invasive approach. Patients subjected to multiple procedures due to recurrences and those with a severely injured gliding bed require more extensive neurolysis and coverage with a local or free vascularized flap.

If symptoms are due exclusively to external trauma and the patient has pain at rest, wraps or thick adipofascial flaps are the treatment of choice to avoid external trauma and protect the nerve. If the lesion is external to the nerve and pain is due to scar tethering the prognosis is more favorable and the risk of recurrence lower, whereas pain due to intraneural injury is more difficult to treat because the outcome of internal neurolysis is unpredictable and may itself induce formation of even worse scarring.

Data on the timing of a recurrence varies widely, from twenty days to thirty days to months, the mechanism of recurrence is also unclear.

Helping patients with these conditions requires a multidisciplinary approach and close collaboration of the surgeon, pain clinician, physiotherapist, and psychologist, because for reasons that are still unclear the patient is often the very cause of the problem. The risk of persistent or even worsening pain symptoms should be clearly stated prior to surgery, as any intervention may induce symptom worsening in patients with complex pain syndromes.

If the pain is not alleviated following the initial procedure, subsequent operations are unlikely to be successful, and further attempts may involve diminishing returns.[30,76]

Table 1: List of the 21 papers describing peripheral nerve neurolysis, associated procedures, and pain outcomes retrieved by the PubMed search, sorted by the technique used for neurolysis

Author	Surgical approach	Nerve	Pain alleviation. Number of patients and percentage (%) of pain reduction
Reisman and Dellon[58]	Abductor digiti minimi	Median	Pain reduction in 11/12 patients (91)
Strickland *et al.*[59]	Hypothenar fat pad flap	Median	Excellent results in alleviating recalcitrant idiopathic CTS (95 satisfaction in 62 patients)
Rose[60]	Palmaris brevis muscle flap	Median	Complete pain relief in all patients (13 hands) (100)
Jones[61]	Pedicled or free flaps	Median/ulnar	Pain reduction in 7/9 patients (78)
Giunta *et al.*[62]	Hypothenar fat pad flap	Median	Pain reduction in 8/9 patients (89)
Frank *et al.*[63]	Hypothenar fat pad flap	Median	Pain reduction in 8/9 patients (89)
Guillemot *et al.*[64]	Fat graft	Median	No pain reduction in 4 patients
Mathoulin *et al.*[65]	Hypothenar fat pad flap	Median	Pain resolution in 41/45 patients (98)
De Smet and Vandeputte[66]	Hypothenar/ulnar fat pad flap	Median	Pain reduction in 9/14 patients (64)
Dahlin *et al.*[67]	Pedicled ulnar, dorsal forearm flaps Free groin, scapular, lateral arm flaps	Median	Pain reduction in 10/14 patients (71)
Goitz and Steichen[54]	Free omental flaps	Median	Pain reduction in 7/11 patients (63)
Luchetti *et al.*[68]	Fascial and fasciocutaneous island flaps (hypothenar fat pad, forearm radial artery, forearm ulnar artery, ulnar fascial fat, and posterior interosseous)	Median	Four point VAS score reduction in 23/25 patients (92)
Craft *et al.*[69]	Hypothenar fat pad flap	Median	Pain resolution in 83% of 28 patients
Fusetti *et al.*[70]	Hypothenar fat pad flap	Median	Pain reduction in 18/20 patients (90)
Elliot *et al.*[71]	Vascularized forearm fascial flap	Median/ulnar	Pain resolution in 8/14 patients (57)
Soltani *et al.*[43]	Collagen: neurolysis + collagen wrap	Median/ulnar	Resolution/improvement in 4 patients (median) Resolution in 3/4 patients (cubital tunnel syndrome)
Espinoza *et al.*[72]	Microneurolysis alone versus ADCON/TN	Median/ulnar	Pain reduction in 80% of 54 patients
Atzei *et al.*[35]	Neurolysis or nerve repair with Hyaloglide(R)	Hand nerves	Pain reduction quicker with Hyaloglide(R) 14 patients treated with HA versus 16 treated without gel
Varitimidis *et al.*[73]	Autologous vein	Median	Pain reduction in 14/15 patients (93)
Masear[74]	Vein: autologous+allograft	Median and various peripheral nerves	Good/excellent results in 94/119 patients (79); no pain relief in 9/119 patients
Kokkalis *et al.*[75]	Vein wrap	Ulnar	Pain reduction in 100% of 17 patients

CTS: Carpal tunnel syndrome, VAS: Visual analogue scale

FUTURE DIRECTIONS

Overall, the diagnosis and treatment of scar neuritis and neuropathic pain still present significant problem areas. A clear lesion classification correlating injury with the clinical problem and convincing evidence of the effectiveness of one treatment above the others would improve both diagnosis and clinical outcomes.

Despite active clinical research, no gold standard treatment has been established, as no medical or surgical treatment has shown superiority over the others with regards to the rate and extent of clinical response. No treatment among the myriad that have been described assures an effective and/or reliable outcome, and the same treatment can lead to very different outcomes in different patients, from complete resolution to a worsening of symptoms. Currently, neither surgeons nor pain therapists are able to predict, which patient will respond to treatment and for what duration that response may last.

All these data suggest that the key for improving our approach to neuropathic pain lies in gaining better insight into its underlying mechanisms. A genetic predisposition is likely to exist, and individual differences in biochemical signals involved in nerve pain and their possible modulation for therapeutic purposes deserves further study.

Then, we foresee genetic and biomolecular research as promising fields of future investigation, which could ultimately lead to a better understanding and management of painful scar neuropathy.

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