DEVELOPING NOVEL OUTCOME MEASURES FOR PERIPHERAL NERVE REGENERATION

A thesis submitted to the University of Manchester
for the degree of Doctor of Philosophy
in the Faculty of Biology, Medicine and Health

2022

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Final word count: 44,319

LIST OF ABBREVIATIONS

2-PD	Two-point discrimination
3D	Three-Dimensional
ADE	Adverse Device Effects
AE	Adverse Events
BAPRAS	British Association of Plastic, Reconstructive and Aesthetic Surgeons
BMRC	British Medical Research Council
CE	Conformitè Europëenne
CI	Chief Investigator
CM	centimetre
COS	Core Outcome Set
CRF	Clinical Research Facility
CRFs	Case Report Forms
DARS	Data Access Request Service
DRG	dorsal root ganglia
ECM	Extra-Cellular Matrix
DASH	Disabilities of Arm, Shoulder and Hand

FDA	Food and Drug Administration (United States of America)
FCE	Finished Consultant Episode
GCP	Good Clinical Practice
GDNF	Glial Cell-Line Derived Neurotrophic Factor
HES	Hospital Episode Statistics
HRA	Health Research Authority
Hf,3D,US	High-frequency, three-dimensional, ultrasound
ICD	International Classification of Disease
IFg	Interferon Gamma
I-HaND	Impact of Hand Nerve Disorders Questionnaire
MFT	Manchester University NHS Foundation Trust
MHRA	Medicines and Health Products Regulatory Agency
MM	millimetres
MRI	Magnetic Resonance Imaging
NGC	Nerve Guidance Conduit
NICE	National Institute of Clinical Excellence
NPRS	Numerical Pain Rating Scale
NRS	Numerical Rating Scale

OCT	Optical Coherence Tomography
PCL	Poly (e-caprolactone)
PGA	Polyglycolic acid
PI	Principal Investigator
PLA	Poly (lactic acid)
PNA	Processed Nerve Allograft
PNI	Peripheral Nerve Injury
PROMS	Patient-Reported Outcome Measures
RCT	Randomised-Control Trial
REC	Research Ethics Committee
s2PD	static two-point discrimination
SAE	Serious Adverse Events
SADE	Serious Adverse Device Effect
SC	Schwann Cell
SDD	Sweat Duct Density
SF-36	Short Form-36
TMG	Trial Management Group
T&O	Trauma & Orthopaedics
tUS	tomographic ultrasound

US	Ultrasound
USA	United States of America
VAS	Visual Analogue Score
WEST	Weinstein Enhanced Sensory Test

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LIST OF PUBLICATIONS

1. R Murphy, A Faroni, A.J Reid, Chapter 23 – Peripheral nervous system responses to biomaterials, Editor(s): Masoud Mozafari, In Woodhead Publishing Series in Biomaterials, Handbook of Biomaterials Biocompatibility, Woodhead Publishing, 2020, Pages 555-572, ISBN 9780081029671, https://doi.org/10.1016/B978-0-08-102967-1.00024-4.

2. Ralph N A Murphy, Heba Elsayed, Sahiba Singh, Jo Dumville, Jason K F Wong, Adam J Reid. A Quantitative Systematic Review of Clinical Outcome Measure Use in Peripheral Nerve Injury of the Upper Limb, *Neurosurgery*, 2021. https://doi.org/10.1093/neuros/nyab060

ABSTRACT

This body of work covers a broad range of unanswered questions on the important clinical problem of peripheral nerve injury (PNI). PNI is a significant health problem that can leave patients with reduced sensation or function of their injured limb and may cause chronic pain that is difficult to treat. Treatments for these injuries have not changed for decades in part due to a lack of data on key aspects of clinical care. Perhaps the most obvious lack of data lies in knowing the scale of the problem, addressed in chapter 2 which uses national data from the National Health Service in England to determine the incidence and epidemiological characteristics of PNI. Chapter 3 describes a single centre Phase 1 clinical trial focussed on the safety of a novel academic designed and manufactured device for nerve gap injury and used in 17 digital nerve injuries with no safety concerns raised. The perceived adequacy of the outcome measures used in this study led to Chapter 4, a systematic review of all outcome measures used in PNI to explore the most frequently used outcome measures and develop the framework for a core outcome set in PNI. In identifying a lack of high quality reproducible and objective outcome measures, novel methods were sought. Firstly, in Chapter 5 which investigates sensory end organ changes, including epidermal thickness and sweat duct density after PNI as a novel biomarker of sensory nerve regeneration. Then in Chapter 6, which explores repair site ultrasound morphometric changes, grey-scale and volumetric measurements and how these relate to current clinical outcomes during nerve regeneration.

Overall, this thesis seeks to define the recent epidemiology of PNI in England in order to demonstrate the scale of the clinical problem. It also seeks to address the need for a clear and uniform approach to outcome measurement of peripheral nerve regeneration whilst developing much needed objective and detailed measurement of the early stages of peripheral nerve regeneration where clinical interventions may be required.

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

COPYRIGHT STATEMENT

ACKNOWLEDGEMENTS

I would like to acknowledge the support of my principal supervisor Mr. Adam Reid for his mentorship and guidance which has been invaluable. I would also like to acknowledge my other supervisors Mr. Jason Wong, Professor Mark Dickinson and Professor Julie Gough for their support and guidance.

My research was awarded:

BAPRAS Pump Priming Fund for Clinical Trials which provided financial support for materials and staff costs.

Royal College of Surgeons (England) Pump Priming Grant which provided financial support for materials and staff costs.

Royal College of Surgeons (England) and Sir John Fisher Foundation Research Fellowship which provided salary costs.

NIHR (Invention for Innovation) i4i provided salary costs for 2 years.

EPSRC/MRC Centre for Doctoral Training (CDT) in Regenerative Medicine (EP/L014904/1) provided salary and PhD support.

Local support from the Manchester University NHS Foundation Trust clinical research facility staff ensured all my studies ran smoothly and on time. Dr. Steven Rogers in the vascular imaging department at Wythenshawe Hospital provided

guidance and great insight into the use of ultrasound imaging in my work. PIUR Imaging © supplied image analysis software prior to market release and provided in-depth technical support, on demand, which was essential in allowing me to complete my work. Dr. Malachy Columb provided invaluable statistical advice and support. I would also like to acknowledge the support I received from my family, without which I would have been unable to complete my research.

Thank you to everyone involved.

JUSTIFICATION FOR JOURNAL FORMAT THESIS

The separate but distinct bodies of work within this thesis represent the investigation of areas of peripheral nerve injury research where knowledge is lacking. Although there is a clear narrative that links all these separate topics, as they are so well-defined it was decided to present them within a journal format. Each results chapter is published or will be submitted for publication as a separate paper, thus the journal format was the most logical choice for this thesis.

CANDIDATE'S DEGREES AND RESEARCH EXPERIENCE

Ralph Murphy undertook his primary medical degree at Imperial College, London obtaining a Bachelor of Medicine and Bachelor of Surgery (MBBS). During his 4th year of undergraduate training Mr. Murphy obtained a Bachelor of Science (BSc) in Gastroenterology and Hepatology. During his BSc he received formal training in statistics, critical appraisal, evidence-based medicine, epidemiology and research methodology. He undertook a clinical research project analysing the effects of HIV on the severity of Liver Disease caused by *Schistosoma* mansoni infection in co-infected adult Ugandans in Kampala, Uganda. He gained considerable exposure to clinical research during this time through preparing consent and planning the supervision of HIV patients undergoing research ultrasound investigations, abdominal examination and stool sample collection.

In 2013, he undertook laboratory-based research during his academic foundation programme which involved an *in-vivo* experiment to analyse the effect of the oral application of Acetyl-*L*-carnitine on sensory neuroprotection. He learnt important basic laboratory techniques and histological analysis of tissue, specifically fixation, staging and staining techniques. He performed the analysis of his samples at the University of Umeå in Sweden and presented his work as an oral presentation at the European Association of Plastic Surgery congress the following year.

Through a competitive national selection process, he was successfully awarded a research fellowship funded by the National Institute of Health Research (NIHR) i4i from 2017 – 2019 as associate principal investigator of a phase I clinical trial of a novel nerve conduit device. He went on to be selected for an Engineering and Physical Sciences Research Council/Medical Research Council Centre of Doctoral Training PhD studentship, the work of which, forms the basis for this thesis.

CHAPTER 1 INTRODUCTION

Elements of this chapter have been adapted from published work:

R Murphy, A Faroni, A.J Reid,
Chapter 23 – Peripheral nervous system responses to biomaterials,
Editor(s): Masoud Mozafari,
In Woodhead Publishing Series in Biomaterials,
Handbook of Biomaterials Biocompatibility,
Woodhead Publishing,
2020,
Pages 555-572,
ISBN 9780081029671,
https://doi.org/10.1016/B978-0-08-102967-1.00024-4.

1.1 Summary

Peripheral nerve injuries (PNI) typically affect the upper limb and are commonly associated with trauma (Grinsell and Keating, 2014); although other causes include tumour resection and iatrogenic injury during surgery for other diseases. Mechanisms of traumatic injury vary and can lead to different injury patterns but those injuries associated with higher forces appear to lead to a greater incidence and severity of PNI (Bekelis, Missios and Spinner, 2015). Traumatic peripheral nerve injuries disproportionately affect young and working members of the population and the most severe injuries, involving transection of the nerve, can lead to functionally devastating consequences with loss of sensation and function, pain and cold intolerance (Irwin *et al.*, 1997) leading to significant socioeconomic problems to both patient and society. As an example, the median cost to society for a working patient with a median nerve injury is estimated as EUR 51,238, which is calculated from rehabilitation costs and loss of productivity from days off work (Rosberg, Carlsson and Dahlin, 2005).

Despite this significant socioeconomic cost, no thorough and systematic national-level epidemiological research has ever been undertaken for PNI in England. There is a clear need for further research into this area to help improve PNI management through guidance of resource allocation and development of prevention strategies and individualised management solutions for this cohort of patients.

In addition to developing a greater understanding of the burden of disease, novel treatments are required to improve the surgical management of PNI, which have not changed for over 30 years (Lundborg, 2000). A greater understanding of the neurobiology of PNI is paving the way for the development of nerve repair solutions that are progressing from animal studies to early stage clinical trials. These novel treatments seek to support and enhance peripheral nerve regeneration to improve outcomes for patients and minimise surgical morbidity. However, the lack of objective and well-defined translatable outcome measures for nerve regeneration can hinder the translation of these novel treatments as current outcome measures are subjective and often not sensitive enough to detect changes as the nerve regenerates, especially early in the regenerative process. Outcome measures for peripheral nerve regeneration need to be sensitive and specific to be able to detect the microarchitectural changes of regeneration and reinnervation that occur. They

must be tolerable to the patient so that they are suitable for repeat use over the complete recovery period of the regenerating nerve and they need to be understandable to the clinician with the ability to infer a change in management should this be required.

1.2 Epidemiology of peripheral nerve injury

In England, the national incidence and epidemiology of PNI has never been systematically analysed despite the significant socioeconomic impact the disease process can cause. This has partly been due to a lack of national level data that is robust and accurate but also because we have previously lacked the technology to analyse such large amounts of data in an efficient and cost-effective way. The last epidemiological-based study of PNI performed in the UK was published over 20 years ago and was limited to a dual-centre retrospective audit of 813 patients over 9 years with incomplete data (McAllister *et al.*, 1996). They found a 3:1 male preponderance in the incidence of PNI with the most commonly injured nerves being the small radial digital nerves of the index finger and ulnar digital nerves of the little finger with the cause attributed to being the most readily exposed areas of the hand. They also found that the 4th webspace common digital nerves were often injured which they believed was most likely due to falls. Unfortunately, they did not analyse individual mechanisms of injury of each nerve, instead they collated grouped data on the level and nerve injured, and mechanism of injury. However, their data suggests that the majority of injuries were due to lacerations from sharp objects due to domestic or industrial accidents.

Single-centre epidemiology studies

Large, retrospective single centre epidemiological studies have been performed in the USA (Missios, Bekelis and Spinner, 2014)(Bekelis, Missios and Spinner, 2015), Iran (Saadat, Eslami and Rahimi-movaghar, 2011), Sweden (Thorsén *et al.*, 2012) and Canada (Noble *et al.*, 1998) (Table 1.1). In Cleveland, Ohio, USA, Missios (Missios, Bekelis and Spinner, 2014) retrospectively explored the epidemiology of PNI in paediatric (<18 years old) trauma patients via the United States National Trauma Data Bank, between 2009 and 2011. The United States National Trauma Data Bank is one of the largest, prospective trauma registries in the world (American College of Surgeons) and is based on coding of trauma similar to the UK Trauma

Audit and Research Network (TARN) database (TARN, 2022). Out of 245,470 patients included in the study, the mechanism of PNI with the highest prevalence was motorcycle crashes (1%). They found that the incidence was associated with an increase of age, severity of injury, an elevated systolic blood pressure on arrival in the emergency room and an increased number of trauma surgeons at the institution. Whereas female sex, rural hospitals and urban non-teaching hospitals were associated with a decreased prevalence of PNI. However, similarly to TARN, data is only collected from trauma/major trauma centres, therefore may exclude less significant injuries such as digital PNI in the hand, which may not necessarily present at trauma centres. In the same region of the USA during the same time period Bekelis *et al.* (Bekelis, Missios and Spinner, 2015) analysed falls associated with PNI in both adults and children. Out of a total of 839,210 fall-injured patients, 3,151 (0.4%) suffered a PNI. The highest incidence occurred in the 3rd decade of life (20-29 years), amongst male patients and was associated with multilevel falls. Female sex and falls with a lower degree of kinetic energy were associated with a lower rate of PNI. Again, more distal, sensory nerve injuries of the hand were excluded and no anatomical breakdown of the types of nerve injury were provided.

Table 1.1: Epidemiological studies of PNI incidence and mechanism

Study	Location	Age-group	PNI Incidence	Leading Mechanism
Missios, Bekelis and Spinner, 2014	Cleveland, Ohio, USA. (Single Centre)	<18 years	0.56 % (1,386 PNIs of 245,570 general trauma patients)	Road-traffic accident
Noble *et al.*, 1998	Toronto, Canada. (Single Centre)	All	2.8% (162 PNIs [brachial plexus and digital nerves excluded] of 5,777 general trauma patients over 10 year period.	Road-traffic accident
Saadat, Eslami and Rahimi-Movaghar, 2011)	Tehran, Iran. (Single Centre)	1-34 years	1.3 % (219 PNIs of 16,753 general trauma patients)	Direct laceration from a sharp object.
Taylor *et al.*, 2008	Insured Patients in USA. (part-National)	All	1.64% (3,617 PNIs of 220,593 limb trauma patients)	Crush Injury
Bekelis, Missios and Spinner, 2015)	Cleveland, Ohio, USA. (Single Centre)	All	0.40% (3,151 PNIs in 839,210 falls patients)	Falls subgroup analysis
Thorsén *et al.*, 2012	Malmo, Sweden (Single Centre)	0-99 years	0.17 % (Digital Nerve Injuries 6.2/100,000 inhabitants/year. General population of Malmo, Sweden)	Knife
Asplund *et al.*, 2009	Sweden (National)	All	0.01% (Total number of PNIs 11,208 over 9-year period in a population of 9 million people. Incidence rate = 13.9/100,000 person-years).	Road-traffic accidents

In Tehran, Iran, Saadat *et al.* (Saadat, Eslami and Rahimi-movaghar, 2011) retrospectively collected data from the Iranian National Trauma Registry Database between August 1999 and February 2004 using the International Classification of Disease, ninth revision (ICD-9) codes to identify PNI. In total 16,753 trauma patients were included in the study, of which only 219 (1.3%) had PNI. 83% of the PNI patients were male and the mean age was 28.6±14.45 years. The most common cause of injury in the PNI patients was direct laceration from a sharp object (61%) followed by road traffic accidents (22%). The most commonly injured nerve was the ulnar nerve at the level of the forearm (15.3%), followed by digital nerve injury. However, the PNI cohort was taken from a major trauma cohort whose mechanism of injury was predominantly significant trauma (road traffic accident (58%) and falls (25%)) therefore the subset of PNI patients is likely to represent mostly major nerve injury. This may explain why the most commonly injured nerve was the ulnar nerve as opposed to distal sensory nerves in the hand.

In Malmo, Sweden, Thorsen *et al*. (Thorsén *et al.*, 2012) examined the epidemiology of digital nerve injury in a single centre study, retrospectively between 1995 – 2005. They found an incidence of 6.2/100,000 inhabitants per year of which 75% were men with a median age of 29 years. Thorsen *et al.* also explored the direct treatment costs for these types of injuries and found that the median cost was 2653 EUR (range 468-6949). A retrospective case-note review was performed of all clinical digital nerve injured patients referred to the only hand surgery department at the only hospital in the city of Malmö, Sweden. Given the population of 275,000 and aforementioned incidence, this equates to around 17 patients per year. Costs were estimated from administrative prices paid by any referring hospital to the Department of Hand Surgery at Malmö University Hospital in year 2009. No further detail is provided, and it is unclear whether these are representative of internal costs, whether these charges are representative of each patient and whether they changed over the study period. Their calculated incidence/100,000 inhabitants per year was in keeping with the estimated digital nerve injury incidence from Asplund et al. (national Swedish epidemiology study below) which was ~ 5/100,000 inhabitants per year (published 3 years prior).

National-level epidemiology studies

Only two national-level studies have been performed to assess the epidemiology of PNI.

Taylor *et al.* (Taylor *et al.*, 2008) explored the incidence of PNI in insured patients only, suffering extremity trauma in the USA during the first 9-months of 1998. Using ICD-9 codes they found 16 million patients with extremity trauma of which 220,593 (1.4%) had a PNI. Eighty-three percent of these were less than 55 years old and fifty percent were male. Crush injury was the most common mechanism of extremity trauma that led to a PNI in this cohort. The authors fail to identify whether their findings are based on patients admitted to level 1/major trauma centres or not however the mechanisms of injury imply significant trauma as the cause and therefore the results are likely to be skewed towards more significant nerve injuries similarly to previously discussed studies. People insured via public health plans such as Medicare/Medicaid and non-insured individuals were excluded from this study which represents at least a quarter of US citizens (Medicaid.gov, 2022), who are most likely to be some of the poorest and therefore undertake manual work which is often associated with PNI (Eser *et al.*, 2009).

Asplund *et al.* (Asplund *et al.*, 2009) from Sweden were the only group to have performed a truly national level retrospective, epidemiology study of PNI. Using the Swedish Hospital Discharge Register (excludes outpatient care) they used ICD-10 codes to classify nerve injuries and found an incidence rate of 13.9 per 100,000 person-years (over 9 years). 63% of nerve injuries occurred at the hand and wrist level however the single most care-consuming injury was brachial plexus injury with a mean of 68 injuries annually involving 960 hospital days as an inpatient. Men were on average nearly three-times more likely to sustain a PNI and the most common age group to sustain a PNI was 20-24 years old. Causes of injury were also explored and the authors stated that their coding system allowed for “very detailed information on causes of injury” and yet they chose to perhaps over-simplify causes in to only 5 categories: traffic accidents. Iatrogenic injuries, self-destructive actions, injuries related to falls and anything else was categorised as “other injuries”. The authors also reported on traumatic amputations alongside PNI in the paper which subsequently meant that neither PNI nor traumatic amputation epidemiology was explored in significant enough detail.

Rosberg and colleagues (Rosberg *et al.*, 2005) later went on to analyse the cost of median and ulnar nerve injuries in the forearm based on the sum of healthcare costs and loss of production costs for each type of injury. They estimated that a median nerve injury in an employed person can cost as much as EUR 51,238 (2005). The cost of these types of injury in the UK has never been explored and could have a significant impact on resource allocation.

No national epidemiological study of PNI has been performed in England to date, despite having one of the most widely available routinely collected data systems in the world. Therefore, this work has been performed and is presented in Chapter 2 of this thesis. Understanding the details of how these injuries are managed remains a gap in knowledge and is not currently routinely collected data. Most nerve injuries are repaired primarily but nerve gap injuries remain a significant problem for which no ideal treatment option exists. The “gold standard” autograft leaves unacceptable donor site morbidity and whilst alternative “bridging” options exist, it is unclear which is the optimal solution.

1.3 Current treatments

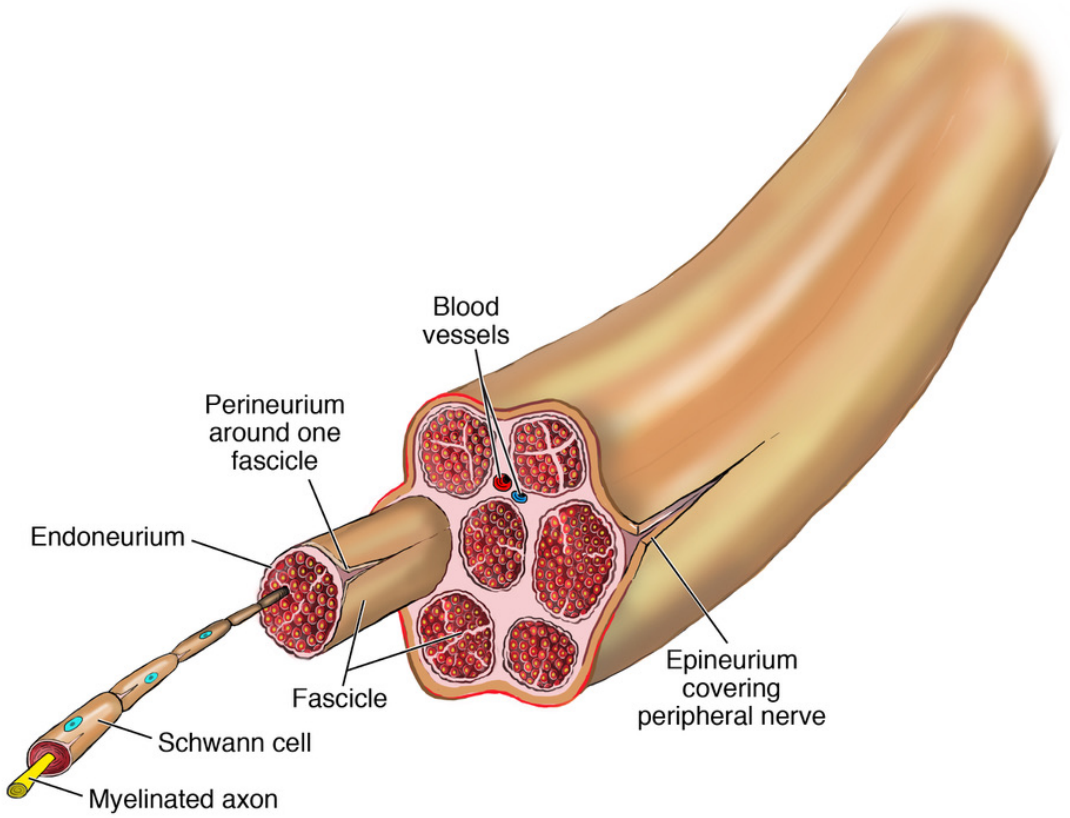
Direct surgical repair

Treatment of a transected nerve (neurotmesis) aims to maximise its regeneration potential through restoration of continuity of the severed ends as quickly as possible (Lundborg and Rosén, 2007; Dahlin, 2008). This helps to reduce the amount of neuronal cell death that occurs after injury (Terenghi, Hart and Wiberg, 2011) by re-establishing transmission of neurotrophic factors from the target-organ to the cell body (Fu and Gordon, 1997; Terenghi, 1999) and also encourages more rapid axonal regrowth to the target organ (Cabaud, Rodkey and Nemeth, 1982).

This involves mobilisation of the cut ends of the nerve, debridement of any interposed scar tissue or debris back to healthy fascicles. Then using meticulous microsurgical repair techniques the nerve stumps are loosely approximated using fine epineurial or fascicular sutures (Kato *et al.*, 1998; Griffin *et al.*, 2013). Direct fascicular opposition involves perineurial (Figure 1.1) suturing aiming to re-align grouped fascicles, improving the accuracy of fascicular orientation. However there

is no evidence that this improves functional outcomes when compared to the epineurial suturing technique (Lundborg, 2004) as the theoretical advantage of better fascicular alignment is outweighed by the increased trauma and scarring that occurs internally in the nerve due to the presence of permanent sutures (Lundborg, 2000)(Cabaud *et al.*, 1976)(Levinthal, Jann Brown and Rand, 1977).

Figure 1.1: A cross-section of a peripheral nerve demonstrating the fascicular arrangement and surgical anatomy.



The functional unit of a peripheral nerve is the neuron which has a nerve cell body in communication with the central nervous system and distributes signals to its target organ via a myelinated or unmyelinated axon . Myelin is a predominantly fatty layer with relative electrical resistance and low capacitance that insulates the axon and encourages longitudinal conduction of the action potential (Garbay, 2000). Support cells of the peripheral nervous system, known as Schwann cells (SCs) ensheath the axon and provide a crucial role in the maintenance and regeneration of neurons (Bhatheja and Field, 2006). These axon-Schwann Cell (aSC) complexes are enveloped in a layer of extracellular matrix called the endoneurium which is rich in laminin (Peltonen, Alanne and Peltonen, 2013). These endoneurial tubes are grouped together into nerve fascicles which are surrounded by perineurium. Perineurium acts as the main barrier between the endoneurium and extrafascicular blood supply, with vessels perforating obliquely from the epineurial network to supply the endoneurial structures (Shanthaveerappa and Bourne, 1962)(Piña-Oviedo and Ortiz-Hidalgo, 2008,)(Mizisin and Weerasuriya, 2011)(Lundborg and Goteborg, 1979). Perineurium provides protection to the underlying endoneurial tubes through its ability to modulate external stretching forces due to its composition of fibrillar and microfibrillar collagens and fibronectin (Peltonen, Alanne and Peltonen, 2013). It is surrounded by a basement membrane containing type IV collagen and laminin providing

structural support (Piña-Oviedo and Ortiz-Hidalgo, 2008). Surrounding the groups of perineurial-lined fascicles is the outermost layer of the nerve, the epineurium. This is formed mostly of collagenous extracellular matrix and provides tensile strength to the nerve.

Nerve "gap" management

Sometimes the injury pattern can create a gap (Millesi, 1986) between the severed nerve ends that prevents direct repair. In this instance an alternative to direct repair is required as epineurial approximation under tension leads to poor outcomes (Sunderland *et al.*, 2004). When a nerve gap exists the current "gold standard" treatment is a reversed, autologous nerve graft (Millesi, 2007). This provides a scaffold with readily available neurotrophic factors and Schwann Cells (SCs) to help guide axonal regeneration whilst also preventing the formation of a neuroma and the ingress of scar tissue, which could block axonal growth (Faroni *et al.*, 2015).

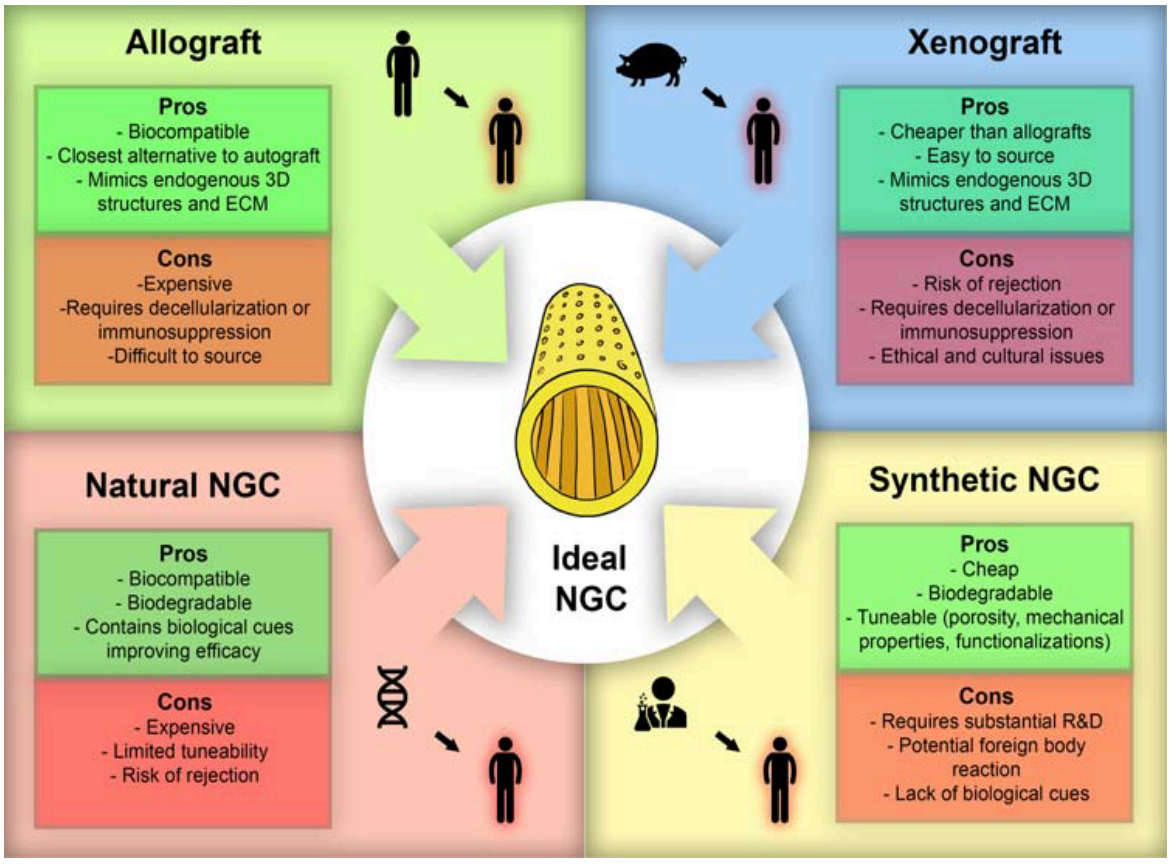
The most commonly used nerve autografts are: sural nerve, medial and lateral cutaneous nerves of the forearm and dorsal cutaneous branch of the ulnar nerve (Ray and Mackinnon, 2010). These small, superficial, sensory nerves are ideal donor nerves as they are relatively easily harvested and do not leave a large functional deficit at the donor site.

Despite the clear benefits of a nerve autograft in peripheral nerve injuries where there is a gap, harvesting of a nerve autograft creates a second incision away from the injury, causes sensory loss in the distribution of the nerve harvested and leads to scarring that may or may not be problematic for the patient. In addition to these problems the nerve ends from where the graft is taken can go on to develop a neuroma, which may or may not require further surgery. These problems have encouraged scientists and nerve surgeons to develop alternatives to the autograft which can provide the optimum environment for axonal regeneration without sacrificing a healthy, functioning nerve. Over the past 80 years, this goal has led to the development of a subset of biomaterial research, often combining the expertise of scientists and nerve surgeons working in collaboration to produce a greater understanding of the requirements of a nerve guidance conduit (NGC) that can replace the need for autograft. The overarching aim is to discover an immunologically inert biosynthetic conduit with regenerative properties comparable to autologous nerve grafts.

Ideally the NGC should be tube-like to allow attachment of either end of the injured nerve and allow axonal growth along the tube while providing protection from external scar tissue which could block the developing growth cones. The walls of the tube need to be porous and permeable to allow the diffusion of oxygen, metabolic substrates, and growth factors required by the regenerating nerve. It must be flexible to allow movement especially if it is to be placed across a joint, while also maintaining enough rigidity to prevent collapse and blockage of the tube. It should be biocompatible and biodegradable in order to reduce the unwanted immunological response from the body when implanted, in addition the internal lumen material should not inhibit axonal growth and instead, should seek to enhance it (Jiang *et al.*, 2010)(Konofaos and Ver Halen, 2013).

Current research has led to the development of a plethora of alternative tubulisation biomaterials which can be divided into: non-synthetic NGCs and synthetic NGCs with subdivisions depending upon the exact material used (Fig. 1.2).

Figure 1.2: Alternative biomaterial approaches to the ideal nerve guidance conduit



The advantages and disadvantages to the four main approaches to the ideal nerve guidance conduit (NGC). (3D = three-dimensional; ECM = Extra-Cellular Matrix; R&D = Research & Development)

NON-SYNTHETIC NERVE GUIDANCE CONDUITS

Blood vessels

Weiss described the use of an arterial segment as a NGC in an experiment (Weiss, 1941) in rats. More recently (Çataltepe *et al.*, 1993; de Castro Rodrigues and Pai Silva, 2001) arterial autografts have demonstrated to have similar regrowth potential when compared with nerve autografts across small segment defects (3 cm) in rats. However, their clinical applicability remains limited owing to the difficulty in safely harvesting an appropriately sized (both diameter and longitudinally) artery for corresponding nerve defects.

Subsequently, interest amongst researchers and clinicians has moved toward the use of veins instead which can be more safely harvested, and veins contain a similarly high level of laminin on their endothelial surface to arteries. They have been validated for clinical application in nerve gaps up to 3 cm (Chiu and Strauch, 1990; Tang, Gu and Song, 1993). The main disadvantage of vein grafts is the presence of valves in the lumen, which may obstruct axonal outgrowth with the potential to form a neuroma. This problem can be avoided by selecting a valve-less section of donor vein or the vein conduit can be inverted to an inside-out orientation as described by Wang et al. (Wang *et al.*, 1993). Another disadvantage to the use of autologous vein grafts is the potential for the tube to collapse thereby impeding axonal regrowth. This is more likely to occur in larger nerve gaps >3 cm and can potentially be avoided by filling the vein with material that acts to splint open the tube and also enhance axonal regeneration. Several different filler materials have been trialed including nerve, muscle, and platelet-rich plasma (Raimondo *et al.*, 2005; Sabongi *et al.*, 2014) which can supply important neurotrophic elements of the extracellular matrix (ECM) and neurotrophic factors which promote SC migration, cell proliferation, and axonal growth-cone guidance.

Allografts:

Peripheral nerve allografts require either pre-processing in order to remove their immunogenic material or immunosuppression of the host in order to maintain graft survival. Commercially available processed nerve allografts (PNA) from deceased

human donors have had immunogenic cellular and non-cellular components removed via a process of chemical decellularization and gamma irradiation (Moore *et al.*, 2011) leaving a sterile and decellularized ECM three-dimensional scaffold with a basal lamina tubular structure. They have been approved by both the United States Department of Food and Drug Administration (FDA) and National Institute of Clinical Excellence (NICE) for use in small (<3cm) sensory nerve gap repairs (NICE, 2017). In a prospective, non-randomized, comparative study from China (He *et al.*, 2015) (n=153) their PNA had significantly better two-point discrimination (p=0.003) than the direct repair group at 6 months, but no difference in Semmes-Weinstein Monofilament testing. PNAs are revascularized after insertion owing to their porous basal lamina structure which provides continued nutritional support to the infiltrating SCs.

Research in animal models has demonstrated a potential target for immunosuppressive therapies in order to allow non-processed allograft survival (Brown *et al.*, 2006), however its transition to human trials has never materialised owing to the success and improved cost efficiency of pre-processing techniques which produce nerve allografts that do not require host immunosuppression.

Commercially produced PNAs are available in the clinic (Axogen, 2019) and with the lack of donor site they are an important clinical option, however they are currently very expensive to produce and are therefore limited to use in adults, in specialist centers in the United Kingdom. Further clinical evaluation for their use is currently ongoing with a large multicentre retrospective study analysing clinical outcomes of PNAs compared with nerve autograft and conduit repairs (Rinker *et al.*, 2017).

Xenografts:

Xenografts have the potential for unlimited supply but have the obvious drawback of immune rejection. In animal studies highly variable outcomes have been reported (Evans, Midha and Mackinnon, 1994; Kim *et al.*, 2014; Deleyto, E and Lasso, 2017) and attempts at human trials around World War II did not show favourable outcomes (Evans, Midha and Mackinnon, 1994). The effects of immune rejection can lead to the deposition of scar tissue which blocks axonal regeneration limiting the gap size for which xenografts can be successfully used to defects less than 10mm (Choi and

Raisman, 2003). Attempts to pre-treat the grafts in order to reduce the antigen burden have been proposed but these have not led to successful breakthroughs in the use of xenografts (Deleyto, E and Lasso, 2017), partly because they significantly reduce the number of available SCs within the graft. Instead researchers in peripheral nerve xenografting have turned their attention towards developing targeted methods of immunosuppression. Although hyeracute rejection does appear to occur in the xenotransplants, graft rejection is thought to be due to a cell-mediated response. Some of the molecular components of this rejection have been identified such as Interferon-gamma (iFg)-producing Th1 cells and IL17-producing Th17 cells (Sun *et al.*, 2009). It is therefore possible that neutralising antibodies targeted towards these molecules may reduce nerve xenograft rejection in the future. Despite the hurdles to the clinical use of peripheral nerve xenografts they have the potential to be a useful resource to nerve surgeons. Whether they develop into a clinically useful tool, however, is yet to be seen.

NATURAL DEGRADABLE NGCs

Collagen:

Collagen constitutes nearly 50% of extracellular peripheral nerve proteins (Deal, Griffin and Hogan, 2012) with a similar ratio of Type I:III (80:20) as the skin (Seyer, Kang and Whitaker, 1977). As a synthetic, biodegradable NGC it has some useful attributes given that it is porous, biocompatible and absorbable (Wangensteen and Kalliainen, 2010) but it's results in in-vivo experiments of nerve regeneration appear to be mixed.

Early clinical experience in humans using a Type I collagen NGCs demonstrated acceptable outcomes in repair of small (10-20mm) sensory nerve gaps in the hand (Bushnell *et al.*, 2008; Lohmeyer *et al.*, 2009; Taras, Jacoby and Lincoski, 2011). In larger diameter forearm motor nerve repairs in humans Dienstknecht et al. (Dienstknecht *et al.*, 2013) and Klein et al. (S. Klein *et al.*, 2016) demonstrated satisfactory sensory and patient-reported outcome measures with the use of collagen conduits. Unfortunately, neither prospective trial compared the use of the collagen conduit with a nerve autograft repair and therefore it remains unclear as to whether collagen conduits can perform as well as the current gold standard in larger diameter nerves and in larger gap sizes. Despite this there are a number of Type I

bovine collagen derived NGCs and wraps that have gained regulatory approval for use in peripheral nerve injury including: NeuraGen® (Integra, FDA 510(k) approval 2001; CE mark 2003), NeuroMatrix®, and NeuroFlex® (Stryker, FDA 510(k) approval 2014) (Kehoe, Zhang and Boyd, 2012). NeuraGen®, the longest standing of these, has demonstrated clinical safety and efficacy in small sensory nerve repair (Bushnell *et al.*, 2008; Lohmeyer *et al.*, 2009; Wangensteen and Kalliainen, 2010) but demonstrated poorer outcomes in mixed motor/sensory nerves (Taras, Nanavati and Steelman, 2005).

SYNTHETIC NGCs

Silicone tubes were used in the first generation of artificial NGCs. They are inert, flexible and readily available however as they are not biodegradable or permeable to large molecules, they were found to have limited use in peripheral nerve regeneration. It soon became apparent that they led to chronic nerve compression and may damage the regenerating nerves (Wang and Cai, 2010). In addition, as they are non-degradable they present a risk of chronic foreign body reaction with excessive scar tissue formation (Johnson and Soucacos, 2008) and require further surgery to remove the device after nerve regeneration is complete.

This has led to a decline in their use and instead researchers have looked to synthetic degradable NGCs instead. The FDA, who have responsibility for regulatory approval of medical devices for use in the USA have stated that the material used in design criteria for NGCs must be biodegradable (Kehoe, Zhang and Boyd, 2012).

SYNTHETIC DEGRADABLE NGCs

Two types of synthetic, degradable peripheral nerve conduit materials have currently been approved for clinical use by the US FDA or European Commission. These are: Polyglycolic acid (PGA) and Poly(lactidecaprolactone) which is a co-polymer of Poly (lactic acid) PLA and Poly (e-caprolactone) (PCL) (Deal and J.W., 2012; Gu, Ding and Williams, 2014).

Polymers:

Synthetic, biodegradable polymers are relatively inexpensive compared to biological compounds and can be engineered with modified physical and mechanical characteristics such as strength, permeability and degradation rate as well as cell attachment and proliferation by using physical or chemical modifications (Nectow, Marra and Kaplan, 2012). Ideally a synthetic conduit needs the mechanical or physical properties that are similar to peripheral nervous tissue in terms of tensile strength, degradation profile and size, in addition it must also avoid swelling and not elicit an inflammatory response during degradation (Nectow, Marra and Kaplan, 2012).

Few NGCs have begun human trials (Table 1.2), as very few pre-clinical, animal studies demonstrate reinnervation of the target organ especially in larger gap injuries and therefore there is minimal evidence of their effectiveness for translation into human trials. Neurotube® (PGA) (Synovis, CE mark 1995; FDA 510(k) 1999) and Neurolac® (PLA and PCL) are the only two in mainstream clinical use.

Table 1.2: Synthetic polymer NGC in-vivo human trials.

Synthetic Materials	**Nerve**	**Defect Size (in mm)**	**Outcomes**	**References**
PGA (Neurotube)	Digital	<8	Results better in NGC group in defects <8mm; 3/46 had extrusion of the device	(Weber *et al.*, 2000)
PGA (Neurotube)	Digital	≤40	>82% patients had S3*/3+ sensory recovery. No extrusions of device.	(Battiston *et al.*, 2005)
	Hand nerves	≤20	Wound healing problems greater with PLCL. No	(Bertleff, Meek and

PCL (Neurolac)			difference in sensory recovery between control (direct neurorrhaphy).	Nicolai, 2005)
PCL (Neurolac)	Digital and wrist sensory nerves	≤25	Mixed	(Costa Serrão de Araújo *et al.*, 2017)

*Mackinnon-Dellon scale for sensory recovery (Mackinnon and Dellon, 1988)

Polyglycolic Acid (PGA)

PGA conduits, although being the first synthetic degradable NGCs, have demonstrated efficacy in animal models with gap defects up to 30mm and similar efficacy to primary repair or nerve graft repair in a randomized clinical trial of small (<8mm) nerve gap injuries (Weber *et al.*, 2000). There is preclinical and clinical safety and efficacy data for Neurotube® (Table 1.2) (Synovis, CE mark 1995; FDA 510(k) 1999) but in larger digital nerve gap injuries (≤40mm) Neurotube did not perform as well as the synthetic NGC market leader, Neurolac® due to its rapid degradation and reduction in mechanical strength which has limited its use (Kehoe, Zhang and Boyd, 2012).

Poly (lactic acid) (PLA) and Poly (e-caprolactone) (PCL)

PLA and PCL co-polymers are biocompatible and are currently being used in NGCs with Neurolac® (Polyganics, FDA 510(k) 2003; CE mark 2004) being made from a poly (65/35(85/15 L/D) lactide e-caprolactone) phospho-ester. Extensive, in vivo, pre-clinical data has been described for the use of Neurolac® and in randomised clinical trials it has been described as having comparable efficacy to autografts in defects up to 20mm (Bertleff, Meek and Nicolai, 2005). Despite the evidence of comparable effect to nerve autograft in randomised trials, clinical experience with the use of Neurolac® has demonstrated some limitations in the adequacy of nerve

regeneration (Table 1.2). Adverse events such as transitory local irritation, infection, allergy and delayed wound healing in addition to failure to provide adequate/complete nerve regeneration have been reported. In addition, the high rigidity and inflexibility of the device leaves room for development of an alternative solution that tackles these issues.

Polynerve

Polynerve is a synthetic, biodegradable PLA/PCL co-polymer NGC made using a solvent casting technique. Its composition is similar to Neurolac® but due to the change in composition of the PCL/PLA blend (Table 1.3) it has greater tensile strength, flexibility and less acidic degradation.

Table 1.3: Key differing features between Neurolac® and Polynerve.

Design Feature	**Neurolac®**	**Polynerve**
Material Composition PCL : PLA	PCL : PLA (85/15 L/D) 35% : 65%	PCL : PLA (50/50 L/D) 80% : 20% (4 : 1)
Internal Lumen Architecture	None	Microgrooves

Furthermore the internal lumen of the Polynerve conduit has been modified to include microgrooves, formed by a solvent casting technique (Sun *et al.*, 2010). Aligned microgrooves have been shown to encourage SC alignment (Mobasseri, Terenghi and Downes, 2014) and this significantly enhances neurite alignment and outgrowth from the proximal regenerating stump (Miller, Jeftinija and Mallapragada, 2001). In-vivo experiments in rats have demonstrated equivocal outcomes in comparison to autograft over 10 mm (Mobasseri *et al.*, 2015) and now requires demonstration of its safety and efficacy in humans.

Clearly, the translation of novel treatments for PNI into clinical practice remains a challenge. One of the reasons that this transition from bench to bedside has not

been rapidly forthcoming is the lack of quality, objective clinical outcome measures, making it difficult to demonstrate the effectiveness of novel treatment modalities. If meaningful outcomes are not possible to record then potentially good interventions will be lost and poor interventions may be allowed to enter clinical use.

In December 2018 the British Association of Plastic, Reconstructive and Aesthetic Surgeons (BAPRAS) highlighted the need for improved outcome measures for surgical interventions when they published the results of a modified Delphi process to define the future research priority of the organisation (Henderson, Reid and Jain, 2018). The second most important research priority was the development of outcome assessments or measures of surgical treatments such as peripheral nerve repair.

1.4 CURRENT CLINICAL OUTCOME MEASURES

A recent systematic review of outcome reporting for brachial plexus reconstruction (Dy *et al.*, 2015) has demonstrated the importance of motor function reporting. This is most commonly assessed using the British Medical Research Council (BMRC) grading system (Compston, 2010), formulated in 1943, with some closely related modifications also in use (Samardžić *et al.*, 2002)(Julia K. Terzis, Marios D. Vekris, 1999)(Kim *et al.*, 2003). Active range of motion and pain were the other two outcomes most commonly reported with patient-reported outcome measures (PROMs) such as Quality of Life: Short-form-36 (Patel, Donegan and Albert, 2007) and the Disabilities of Arm, Shoulder and Hand (DASH) (Hudak, Amadio and Bombardier, 1996) very infrequently reported. Sensory outcomes were not reported at all.

Despite not including all neurobiological regenerative outcomes the review highlights some of the broad categories of outcomes that need to be explored in clinical research of peripheral nerve regeneration. These are: the biology of nerve regeneration (sensory and motor function), patient function and patient symptoms (pain and discomfort) and health-related quality of life. These core domains capture dimensions that are important to a range of stakeholders and begin to allow us to attempt to quantify the very complex nature of peripheral nerve regeneration.

Biology of nerve regeneration

Peripheral nerve regeneration involves complex neurobiology (Lundborg, 2000). Regenerating axons from the proximal end must traverse the site of injury which can be impeded by scar tissue or if there is a gap present they may inadvertently grow into surrounding tissues, creating a painful neuroma (Butí *et al.*, 1996)(Parrinello *et al.*, 2010). Both the target organ, and the proximal nerve cell body can become starved of neurotrophic support from disruption to normal transmission channels at the site of injury during the degeneration phase (Faroni *et al.*, 2015). This can lead to nerve cell death proximally (West *et al.*, 2013) and atrophy of the target organ distally, until the nerve regenerates. Clinicians wanting to monitor these neurobiological changes of the regenerating nerve in order to make clinical decisions and prognostic and diagnostic judgement are limited to measuring end-organ function of the nerve.

Common sensory measures include:

- Two-point discrimination (2-PD) (Dellon, 1981)
- Semmes-Weinstein Monofilament Testing (Semmes, J., Weinstein S., Ghent, L. & Teuber, 1960)
- Locognosia (Weber, as cited by Stevens & Green, 1996)

Common motor measures include:

- Manual muscle testing using the British Medical Research Council muscle strength grading (Compston, 2010)
- Grip strength using dynamometry (Mathiowetz *et al.*, 1984).
- Pinch strength (Mathiowetz *et al.*, 1984)

Patient function

Patient outcomes after treatment for peripheral nerve injury are highly variable with no patient regaining complete pre-injury function (Terzis, Sun and Thanos, 1997). Loss of fine sensory and motor function can significantly reduce a person's ability to undertake pre-injury work activities and routine daily activities such as cooking and dressing. The extent to which their symptoms impact on function can be assessed

through functional scoring assessments such as the Sollerman Hand Function Test (Sollerman and Ejeskär, 1995) and Moberg Pick Up Test (Moberg, 1960) or it can be combined with biological and patient-reported outcomes such as in the Rosen Scoring System (Roseén and Lundborg, 2000).

Patient's symptoms and health-related quality of life

Symptoms of nerve injury, out with the loss of nerve function, commonly include pain and cold intolerance (Rosberg *et al.*, 2013)(Lundborg and Rosén, 2007)(Wojtkiewicz *et al.*, 2015). Current methods to assess pain tend to use well-validated, rudimentary scoring systems such as the Visual Analogue Score (VAS) or the Numerical Rating Scale (NRS) (Breivik *et al.*, 2008) which can help to quantify the objective and subjective aspects of pain reporting. In terms of cold intolerance, the most commonly used measurement tool is the Cold Sensitivity and Severity Scale (Carlsson *et al.*, 2008).

The inability to undertake pre-injury activities whether this is due to loss of nerve function or symptoms as a results of nerve injury can have a significant psychological impact on patients and also reduces their ability to gain future employment. Current methods to measure these outcomes are not necessarily nerve-specific in the case of PROMs (Wang, Sunitha and Chung, 2013) and are usually in the format of a questionnaire. The most commonly used PROMs include:

- Disabilities of the Arm, Shoulder and Hand (DASH) (Institute for Work and Health, 2006)
- Short Form-36 (Novak *et al.*, 2009)
- Michigan Hand Outcomes Questionnaire (Shauver and Chung, 2013)

No current clinical outcome measure can assess or quantify all of the factors involved in the complex process of peripheral nerve regeneration. Current methods to measure these outcomes are often subjective. In addition, there is no clear guidance as to which test to use and when.

The way in which outcomes are reported in clinical peripheral nerve research has yet to be explored and the inconsistencies between studies are unknown. Multiple outcome measures have been reported both short and long-term and are important

at different stages and to different stakeholders, to different degrees. Ultimately a clear consensus on which outcome measures to use and when to use them is needed.

Developing a core outcome set

In order to develop this consensus on which outcome measures to use and when, we must first define what outcome measures exist in clinical PNI research in order to define core outcome domains that are relevant to the differing injury types, nerves injured, and stakeholders involved. The Core Outcomes Measures in Effectiveness Trials initiative which began in 2010 in order to develop core outcome sets in clinical trials (Williamson *et al.*, 2017) have published a set of 11 standards required for the development of a core outcome set (Kirkham *et al.*, 2017). The first 4 standards require specification of the scope of the outcomes in the defined clinical area.

Quantification of peripheral nerve regeneration has historically involved assessment of end-organ changes through clinical assessment, such as manual muscle testing using the British Medical Research Council (MRC) scale for individual muscles from the 1970's (Riddoch *et al.*, 1976) or user-dependent tools such as two-point discrimination in the assessment of tactile discrimination (Mackinnon and Dellon, 1985, 1988) or Weinstein-Enhanced Monofilament Testing (Weinstein, 1993) in pressure-threshold testing of sensation. Non-invasive outcome measures such as these have been invaluable in assessing clinical peripheral nerve regeneration for decades however, similarly to peripheral nerve injury treatments (Lundborg, 2000), they have not advanced over this time (Murphy *et al.*, 2021). They are often subjective and user and receiver dependent making accurate objective quantification of nerve regeneration difficult. This prevents accurate, objective quantification of peripheral nerve regeneration and therefore comparison of outcomes between treatment options or nerve-injury centers. We therefore require objective, sensitive measures of the neurobiological processes involved in peripheral nerve regeneration that can provide detailed, quantitative data after injury and during regeneration of the nerve. Alongside other measures of symptomology after peripheral nerve injury, this would provide peripheral nerve surgeons with the neurobiological evidence of the effectiveness of treatment modalities which are currently in development (Faroni *et al.*, 2015).

1.5 TARGETS FOR NOVEL, CLINICALLY APPLICABLE OUTCOME MEASURES OF EARLY PERIPHERAL NERVE REGENERATION

Looking at the biological processes involved in peripheral nerve regeneration we can start to identify areas for the development of non-invasive modalities to accurately quantify the changes that occur along the regenerating axis of a human peripheral nerve.

In the cell body

After axonal transection the neuronal cell body swells, the nucleus is displaced to the periphery and a process of 'chromatolysis' occurs. A process involving dispersal of Nissl granules (aggregates of endoplasmic reticulum) which normally produce neurotransmitters. This is thought to occur due to a lack of neurotrophic support from the target organ (Hanz and Fainzilber, 2006) and represents a shift from normal metabolic activity to a regenerative state (S.K. *et al.*, 2000) needed to support axonal re-growth.

If the severed nerve ends do not re-connect and the re-establishment of the neurotrophic support pathways are not initiated, then the neuronal cell body will fail to continue onto a regenerative pathway. Instead the neuronal cell body follows a "death" pathway in a process akin to apoptosis (Hart, Terenghi and Wiberg, 2008). These changes occurring in the cell body which lies in the dorsal root ganglia in sensory nerves or spinal cord in motor nerves, represent a potential target for measurement.

In animal models the dorsal root ganglia (DRG) can be harvested and histologically examined however in humans this is obviously not possible. Non-invasive imaging has been used such as Magnetic Resonance Imaging (MRI) (West *et al.*, 2007) in order to perform volumetric analysis of the DRG which serves as a proxy measure to direct neuronal cell counting. West et al. (West *et al.*, 2007) have demonstrated the validity of MRI in volumetric analysis of rat DRGs in a sciatic nerve injury model. However, this has yet to be evaluated clinically and instead the latest imaging modalities are being used to explore central nervous system changes during peripheral nerve regeneration as a proxy to changes at the level of the dorsal route

ganglia (Taylor, Anastakis and Davis, 2009; Theuvenet *et al.*, 2011; Goswami *et al.*, 2016).

Longitudinal functional MRI can be used to assess brain activation after sensory stimulus of injured and regenerating peripheral nerves of the upper limb and compared to contralateral controls to assess restoration of somatosensory function (Rath *et al.*, 2011; Yoshikawa *et al.*, 2012; Ma *et al.*, 2017), however it is still early in its development and relies on an intact end-organ-peripheral nerve-central nervous system axis which requires regeneration of peripheral nerves to the end organ first. Therefore, similarly to electrophysiology, it has limited use in the early (depending on location of PNI) stages of peripheral nerve regeneration. This is important as there currently remains a window of clinical uncertainty as to when to intervene in certain peripheral nerve injury types (Sunderland type III-IV) which may benefit from surgical intervention early, if required.

At the site of injury

In animal studies, axonal counting is one of the most commonly used outcome measures for peripheral nerve regeneration (Pfister *et al.*, 2011), often alongside end-organ assessment and/or behavioural changes (Wood *et al.*, 2011). However, it is known that multiple axonal sprouts are emitted from individual axons in the proximal regenerating nerve stump (Aitken, Sharman and Young, 1947)(MacKinnon, Dellon and O'Brien, 1991). Therefore, axon counts in the distal nerve stump may not necessarily reflect the number of neurons that regenerate especially if the experimental condition or surgical procedure encourages axonal sprouting. To tackle this, retrograde labelling with dyes along with axonal counts have been used in animal models to accurately quantify regenerative success however this is not possible in humans (Sulaiman *et al.*, 2002). In the clinical setting, it is still unclear as to how far these regenerating axonal sprouts progress into the distal stump prior to pruning and therefore what quantity/how many truly represent regenerating neurons. However, quantification of the regenerating axons distal to the coaptation site of the nerve ends may still provide useful, objective information as to the outcomes of regeneration.

In humans, non-invasive imaging techniques have been utilised in order to quantify axonal regeneration across the site of injury. Computed Tomography (CT) has the

benefit of being readily available, can be quickly performed and provides good spatial resolution however it has poor contrast and resolution, involves ionising radiation and generally only provides indirect information (Ohana *et al.*, 2014). Instead Magnetic Resonance Imaging (MRI) and Ultrasound (US) imaging have been used more widely in the setting of peripheral nerve injury. MRI has the benefit of visualising nerves and characterising the surrounding soft tissues whilst also providing information on muscle denervation and atrophy (Wasa *et al.*, 2010). Recently, MRI techniques such as diffusion-weighted and diffusion tensor imaging have been used to explore peripheral nerve architecture in greater detail (Y. Zhou *et al.*, 2012)(Zhou *et al.*, 2014). Diffusion tensor imaging has been used to detect average axon diameters using mathematical modelling (Avram *et al.*, 2013) in the central nervous system and may be able to do the same in the peripheral nervous system. High-resolution fascicular MRI of peripheral nerves is able to demonstrate inter-fascicular architectural detail using 3-Tesla MRI (Felisaz *et al.*, 2019) and is widely available. 7-Tesla MRI can demonstrate greater inter-fascicular architectural detail than 3-Tesla machines because of increased signal-to-noise ratio (Yoon *et al.*, 2018), however these devices are not widely available and there is limited literature on its use in clinical peripheral nerve imaging. US in comparison is quicker, more readily available and cheaper to perform. When identifying focal peripheral nerve pathology in sonographically accessible regions US imaging has shown to be more sensitive than MRI (93% vs 67%), with equivalent specificity (86%) in a cohort of patients with mononeuropathies and brachial plexopathies (Zaidman *et al.*, 2013). Despite this neither imaging modality is regularly utilised in the assessment of nerve regeneration after injury. One of the main reasons is the lack of detailed spatial resolution making it difficult to quantify the regenerating axons which are less than half a millimetre in diameter at the wrist level for example (Brill and Tyler, 2017).

In ultrasonography, the advent of high frequency ultrasound probes (>15mHz) and improved software development over the past 30 years (Fornage, 1988)(Stuart, Koh and Breidahl, 2004) has led to the ability to identify much greater neural architectural detail. It is now possible to differentiate between fascicular structures within peripheral nerves at the level of the wrist in vivo (Suk, Walker and Cartwright, 2013) which demonstrates a spatial resolution down to 0.38mm (Brill and Tyler, 2017). High frequency 3D ultrasound (Hf,3D,tUS) has been used to diagnose entrapment of the median nerve in the forearm (Pelz *et al.*, 2017) and in a case report to quantify regeneration through synthetic, polymer nerve conduits at the level of the wrist

(Billakota *et al.*, 2018). However, detailed assessment of peripheral nerve regeneration has never been explored longitudinally in the clinical setting and compared with current clinical outcome measures.

At the target organ

If the regenerating axon does not reach the distal cut-end of the nerve in a timely fashion the chronically denervated SCs will enter a dormant state and down-regulate growth factors leading to an inability to support further axonal progression (Gordon, Tyreman and Raji, 2011). This prevents any further trophic support to the target-organ which soon undergoes atrophy or apoptosis after peripheral nerve injury (~2/3 of muscle units will atrophy by 3 months after axotomy (Fu and Gordon, 1995)). Skin changes also occur after sensory nerve injury and in human studies where hand skin samples underwent histological analysis after ulnar or median nerve injury, atrophy of sweat tubules, ducts and the epidermis correlated with the duration of denervation (Silver, Montagna and Versaci, 1964). Assessment of these trophic changes in the skin during peripheral nerve regeneration after injury has never been assessed and instead nerve fibre density has been used to quantify regeneration, requiring skin biopsies and histological analysis (Polydefkis *et al.*, 2004)(Ebenezer *et al.*, 2007). Non-invasive imaging techniques to explore these trophic skin changes after nerve injury have never been performed and may provide a novel, early, microanatomical measure of peripheral nerve regeneration.

Novel outcome measure of sensory reinnervation

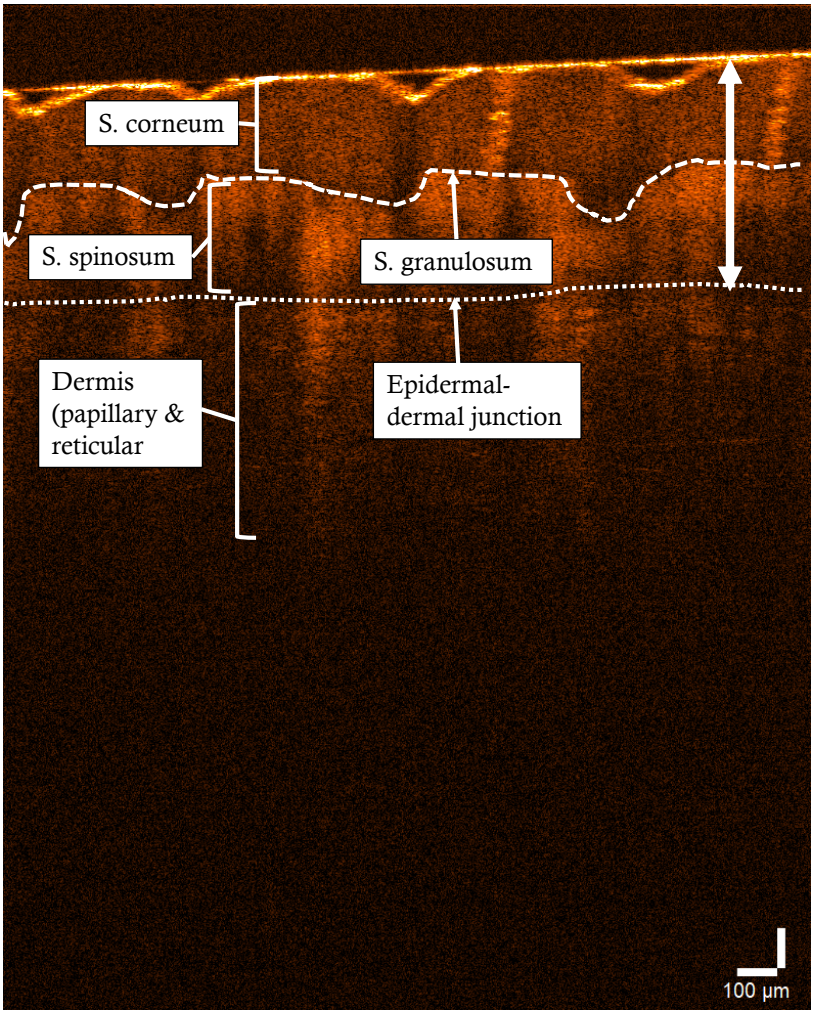
Sensory nerves (mainly C (unmyelinated) and Aδ (myelinated) fibres) innervate the epidermis and dermis through three-dimensional networks transmitting a range of physical and chemical stimuli to the central nervous system (Kelly *et al.*, 2005)(Roosterman *et al.*, 2006). As well as afferent functions, sensory nerves act in an efferent neurosecretory fashion (Paus, Theoharides and Arck, 2006) to regulate blood circulation, lymphatic function and skin appendages (sweat glands, apocrine glands and hair follicles). Substance P (SP), the first neuropeptide to be discovered (V Euler and Gaddum, 1931) has been implicated in cutaneous vasodilation and promotion of cell proliferation (Pernow, 1983) including keratinocytes, fibroblasts and endothelial cells (Tanaka *et al.*, no date). Neuropeptides directly innervating sweat glands include: vasoactive intestinal

polypeptide and peptide histidine methionine. Other neuropeptides released by sensory nerves in the skin include neuropeptide Y and calcitonin gene-related peptide. In peripheral nerve disease and compression states neuropeptide release by sensory cutaneous nerves are often reduced leading to imbalance of neuropeptide levels within the skin (Bove *et al.*, 2013). This leads to reduced target-organ functions such as hypohydrosis and epidermal thinning giving the appearance of shiny, dry and thin skin. Previous rat studies demonstrated that the epidermis of glabrous skin becomes approximately 40% thinner within 1 week following sciatic nerve transection (Li *et al.*, 1997). In human studies where hand skin samples underwent histological analysis after ulnar or median nerve injury, atrophy of sweat tubules, ducts and the epidermis correlated with the duration of denervation (Silver, Montagna and Versaci, 1964). Assessment of trophic changes in the skin during peripheral nerve regeneration after injury has not been assessed and instead nerve fibre density has been used to quantify regeneration, requiring skin biopsies and histological analysis (Polydefkis *et al.*, 2004)(Ebenezer *et al.*, 2007). Clearly there is the need to develop non-invasive methodologies to detect early micro-anatomical and neurobiological evidence of cutaneous reinnervation and explore how these changes correlate with current clinical outcome measures.

Optical coherence tomography

Optical Coherence Tomography (OCT), a light based, non-invasive imaging technique, analogous to ultrasound analyses the light reflectivity of tissues to produce a cross sectional image. In the skin, the image produced is of a resolution of 15μm (normal human red blood cell diameter ~ 7μm (Turgeon, 2004)) to a depth of approximately 2mm (Welzel, 2001) (Figure 1.3). It is used in dermatology practice to measure epidermal thickness: as a biomarker for scleroderma (Abignano *et al.*, 2013), after application of different treatments to quantify their effects (Lu *et al.*, 2013) and also to monitor wound healing (Greaves *et al.*, 2015).

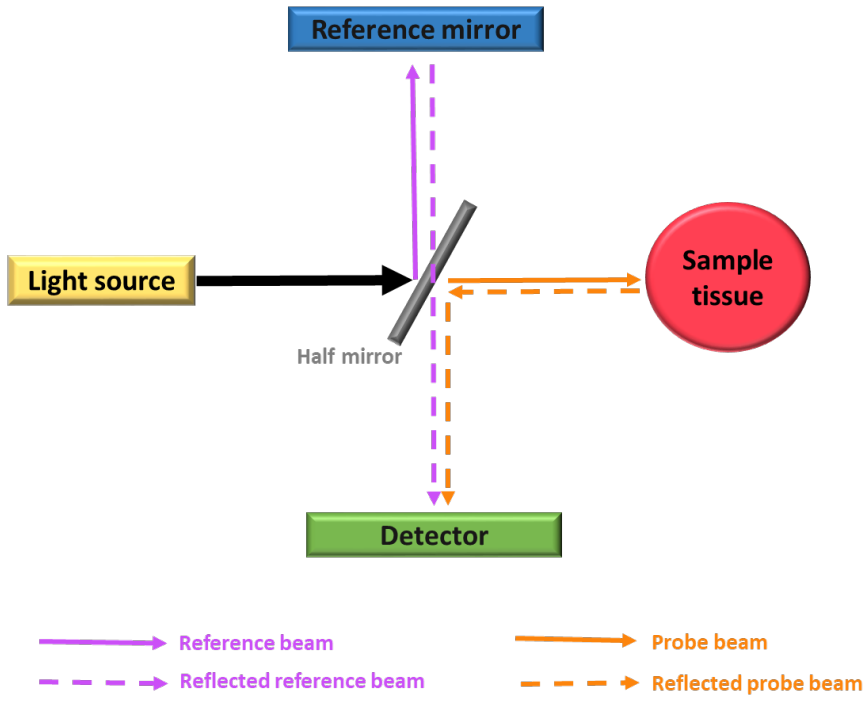
Figure 1.3: Anatomical layers of epidermis as visualised using OCT (Adabi *et al.*, 2017)



Optical coherence tomography cross-section of volar digital skin demonstrating the epidermal and dermal layers to scale.

OCT machines are made up of five components: a light source, a fused fibre-optic coupler, a light detector, a reference mirror and a personal computer that has an analogue-to-digital converter (Matcher, 2011). The light source is a "superluminescent diode", characterised by wavelengths similar to the values of infrared (around 1300 nm) (Welzel, 2001).The principle of OCT is low coherence interferometry whereby an interferogram will be formed when the two beams (reference beam and probe beam) of light have covered the same optical distance.

Figure 1.4: A schematic representation of the principals of interferometry.



A light source is aimed at a half mirror (half-reflective, half-transparent) so that part of the beam of light is reflected to a reference mirror and part of the beam of light travels through to the sample tissue. When the light beam reflects off the reference mirror and sample tissue back to the half mirror, the two beams converge to create a signal in the detector.

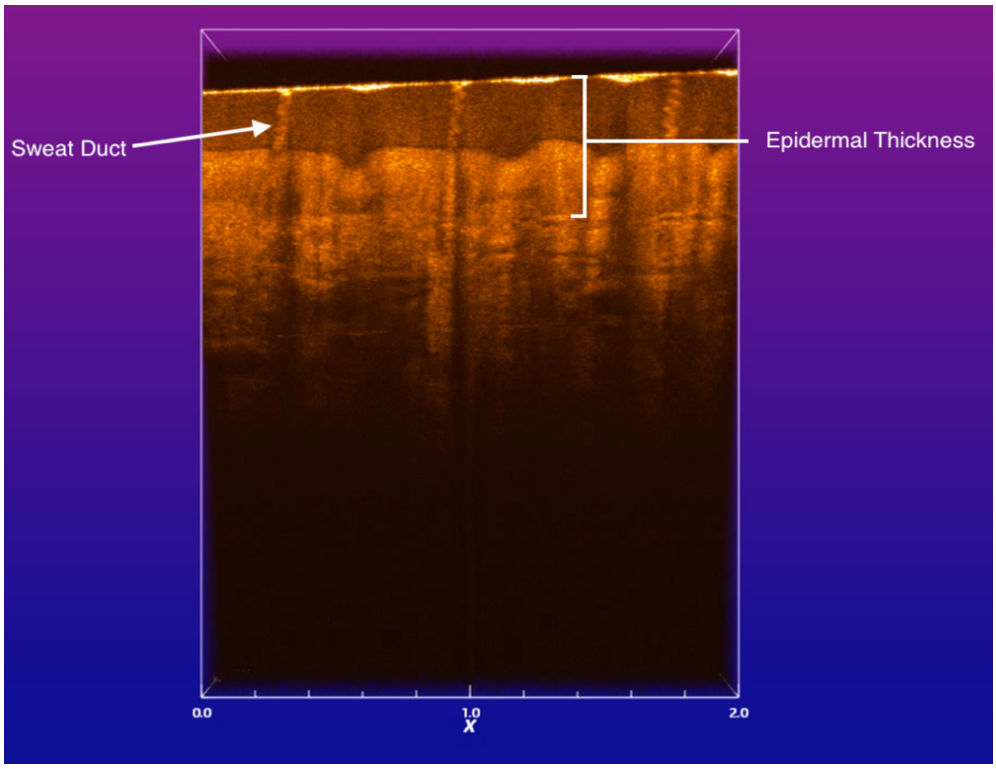
As shown in Figure 1.4, the light beam is split by a half mirror into a reference beam and a probe beam. The reference beam changes its direction towards the reference mirror, a set distance, and then reflects back towards the detector. The probe beam passes straight through the half mirror, continuing its course towards the sample tissue, reflecting back from this to the half mirror where it joins the reference beam towards the detector. When the two beams overlay on the detector, interference signals are produced and transformed into interferograms. The signal is a function of the difference in optical path length between both beams. The sample tissue must be located as far away from the half-mirror as the reference mirror (Matcher, 2011) and the refractive index of the tissue being imaged accounted for, in the case of skin this is 1.43 (Welzel, 2001).

Real time two-dimensional and three-dimensional images are produced through movement of the light beams across the sample in predefined scan settings. The larger the area to be scanned the longer the it takes to produce an image. Detailed

two-dimensional images can be obtained instantly whilst a 1x1x1.5 mm volumetric scan will take up to 10 seconds.

In the setting of peripheral nerve injury, specifically sensory (and autonomic nerves which lie alongside sensory nerves) nerves, epidermal thickness and sweat duct density (Figure 1.5) in the skin appear to change and this change appears to correlate with nerve regeneration (Li *et al.*, 1997). These changes as measured by OCT could be used to assess the regeneration of a peripheral nerve in humans, in a non-invasive, yet objective way and further exploration of its use in this setting is warranted.

Figure 1.5: Human volar digital hand skin as visualised with optical coherence tomography.



This is a two-dimensional, cross-sectional view of volar digital hand skin at the pulp of the index finger down to the dermis. It demonstrates how the epidermis and sweat ducts can easily be visualised.

1.6 Hypothesis and aims

Traumatic peripheral nerve injuries disproportionately affect young and working members of the population and the most severe injuries, involving transection of the nerve, can lead to functionally devastating consequences leading to significant socioeconomic problems to both patient and society. New treatments are required to improve outcomes for patients.

This thesis hypothesises that a novel academic-designed and manufactured polymer nerve conduit (Polynerve) will be safe and effective in repairing sensory nerve gaps.

In undertaking this clinical study, significant other gaps in knowledge were identified. The incidence and epidemiology of nerve injury is often estimated based upon studies examining local populations in major trauma centres; therefore, it was hypothesised that national level statistics from the NHS could provide the epidemiological data necessary to understand the burden of PNI and nerve gap management.

Beyond understanding who PNI affects and how, it became apparent that there was no “gold standard” clinical outcome measure nor guidance on when to use them; therefore it was hypothesised that in systematically reviewing all clinical outcome measures it would be possible to identify the most frequently used and when to use them in order to develop a core outcome set of measures.

In reviewing these clinical outcome measures a clear subjectivity and lack of sensitivity to detect change in the early stages of nerve regeneration was identified. A need for detailed, objective measures of the early stages of regeneration was identified. Unlike in motor nerves, assessment of end-organ changes in sensory nerves, utilising the latest technology, has never been explored. Therefore, this thesis hypothesised that optical coherence tomography could be used to objectively quantify the early end-organ changes in the skin after sensory nerve injury. Further to this, and in order to investigate regeneration at the site of injury/repair, it was hypothesised that high-frequency, three-dimensional, tomographic ultrasound could be used to quantify peripheral nerve regeneration.

Aim 1: Describe the national epidemiology of PNI and identify who it affects, which nerves are most commonly injured, the mechanisms of injury, who (which specialty) most commonly manages these injuries and which operations are most often performed.

Aim 2: Perform a phase I clinical trial of Polynerve and investigate its safety and efficacy of peripheral nerve regeneration.

Aim 3: Systematically review all clinical outcome measures of peripheral nerve regeneration in order to identify the most commonly used outcome measures for upper limb nerve injuries.

Aim 4: Measure epidermal thickness and sweat duct density of volar digital pulp skin using OCT to obtain normative values from a healthy population. Quantify changes of these biomarkers during digital nerve regeneration and compare these to current clinical outcome measures of nerve regeneration.

Aim 5: Identify ultrasound biomarkers of peripheral nerve regeneration at the site of injury/repair and compare them to current clinical outcome measures of nerve regeneration.

CHAPTER 2 The national epidemiology of peripheral nerve injury (2005-2019)

2.1 Background

Peripheral nerve injuries (PNIs) are commonly associated with trauma (Grinsell and Keating, 2014); however, iatrogenic nerve injury may also occur during emergency or elective surgery, for example in fixation of fractures or extirpation of malignant tumours. The commonest site for PNI is in the upper limb (Ciaramitaro *et al.*, 2010), and the consequences to the patient include loss of motor function and / or sensory capacity, pain, and significant impacts on psychosocial wellbeing and employment (Cederlund, Ramel and Rosberg, 2010). Current treatments for nerve transection remain purely surgical but outcomes fail to match the expectations of clinicians and patients whose healthcare needs can be lifelong (Tadjalli *et al.*, 1995a; Julia K. Terzis, Marios D. Vekris, 1999; Kim *et al.*, 2003; Schreuders *et al.*, 2004; Chemnitz, Bjorkman and Dahlin, 2013). Therefore, in a nationalised healthcare system such as in the United Kingdom's National Health Service (NHS), there is a particular need for high quality epidemiological data on PNI in order to identify the populations involved and the current healthcare demands, to help develop risk reducing strategies and to provide decision makers with information with which to ensure resources are distributed to greatest effect.

Sweden have done this well for PNI, estimating the overall incidence to be 13.9/100,000 person-years through analysis of national level inpatient data (Asplund *et al.*, 2009); whilst their incidence of digital nerve injuries was found to be 6.2/100,000 person-years, based on regional level data (Thorsén *et al.*, 2012). Data from Asplund et al. (Asplund *et al.*, 2009) demonstrated that injuries to the brachial plexus consumed 15% of all PNI hospital days and the peak incidence of age for this injury was 25–- 49 years.

No such basic epidemiological data of PNI exists at a national level for the UK; however, McAllister et al. (McAllister *et al.*, 1996) performed a retrospective study of 813 upper limb (excluding brachial plexus) nerve-injured patients from regional (south-east England) case-note data. They found that 57.1% of patients were between 16 – 35 years of age and 74.2% were male. Fifty-two percent of injuries involved nerves distal to the wrist with no bearing on hand dominance. A more recent review of Hospital Episode Statistical (HES) data trends in England between 1998 – 2015 by Manley et al. (Manley, Wormald and Furniss, 2019) has

demonstrated an upward trend in reported cases of PNI however no further details of patient demographics or anatomical data was included.

NHS Hospital Episode Statistics (HES) are freely-accessible, anonymised data describing all hospital admissions, emergency department attendances and outpatient appointments at NHS hospitals in England (NHS, 2019). Hospitals are mandated to submit patient-level data (The Royal College of Physicians Information Laboratory, 2007) to a secondary user service (data warehouse) which then goes through a cleaning process by the HES data quality team to remove duplicates and obvious data quality errors in order to populate the final HES datasets which are updated on an annual basis (HES Data Quality Team, 2014). Individual HES records included in each data set are termed Finished Consultant Episodes (FCE), with each episode representing a period of care for a patient under a single consultant at a single hospital (NHS, 2019). HES records contain codified diagnoses and operations, demographics such as age group and gender and administrative information, including dates of admission and discharge (NHS, 2019). Diagnoses are coded using the World Health Organisations' International Classification of Diseases, 10th Edition (ICD-10) classification, and surgical procedures using the Office of Population Censuses and Surveys Classification of Surgical Operations and Procedures 4th Edition (OPCS-4) codes. HES data provides a large repository of useful clinical information, but it relies on the quality of data inputted from individual hospitals.

This study sought to use HES data to describe the incidence, age, gender and anatomical stratification of PNIs in England, the associated incidence of surgical interventions, trends in outpatient management and relate the anatomical level of injury to the length of stay as a surrogate of healthcare costs.

2.2 Methods

Anonymised HES data was obtained from NHS digital via the Data Access Request Service (DARS)(NHS Digital, 2019). Admitted patient care data was requested for all NHS patients sustaining PNI of all body regions for each year from 2005 to 2020 using PNI-specific 4-character ICD-10 codes (Appendix 1–- Supplementary Table 1) included in each yearly data set. We included the brachial plexus injury code (S14.3) within upper limb PNI groupings in the analysis of anatomical level instead

of neck level trauma as per the ICD-10 coding system (Appendix 1–- Supplementary Table 1). This reflects current clinical practice with brachial plexus injuries being treated by extremity surgeons as opposed to head and neck specialists. Specific data on age, gender, method of admission, total spell length, main speciality, cause of injury and main operation was obtained.

FCEs were used to calculate the total incidence per 100,000 population per year in conjunction with population mid-year estimate data (Statistics, 2020) published by the Office for National Statistics. This methodology has been described in other disease epidemiology studies (Mehta *et al.*, 2019) and traumatic injury epidemiology papers (Tulloch *et al.*, 2021) and is commonly used in PNI epidemiology studies (Asplund *et al.*, 2009; Wiman *et al.*, 2022). Gender and anatomical location of injury were stratified by total number of FCEs/100,000 population per year whilst age was stratified by total number of FCEs.

We further compared admission type of upper limb PNIs by total number of admissions per year, divided into: ordinary admission (> 24-hours stay) or day case (<24 hours) (NHS Digital). We further analysed upper limb PNI length of stay in days to determine changes over the past 15 years.

The total number of FCEs was examined for mechanism of injury and main operation type stratified by anatomical location of injury. Procedures were recorded using standard Office of Population Censuses and Surveys Classification of Surgical Operations and Procedures 4th Edition (OPCS-4) codes (NHS Digital) in relation to the HES record (Appendix 1–- Supplementary Table 2) and combined into clinically-relevant categories.

Statistical analysis

Data are presented as mean (SD), median [interquartiles] and count (%). Incidence rates and incidence rate ratios are presented with 95% confidence intervals (CI). Gaussian normality was assessed using the Kolmogorov-Smirnov omnibus method and normality plots. Incidence rates and incident rate ratios over the period were estimated using random effects models. Trends in incidence rates and episodes over time were analysed using chi-square trend analysis for proportions and robust linear regression. Significance was defined at $p<0.05$ (two-sided). Analyses were

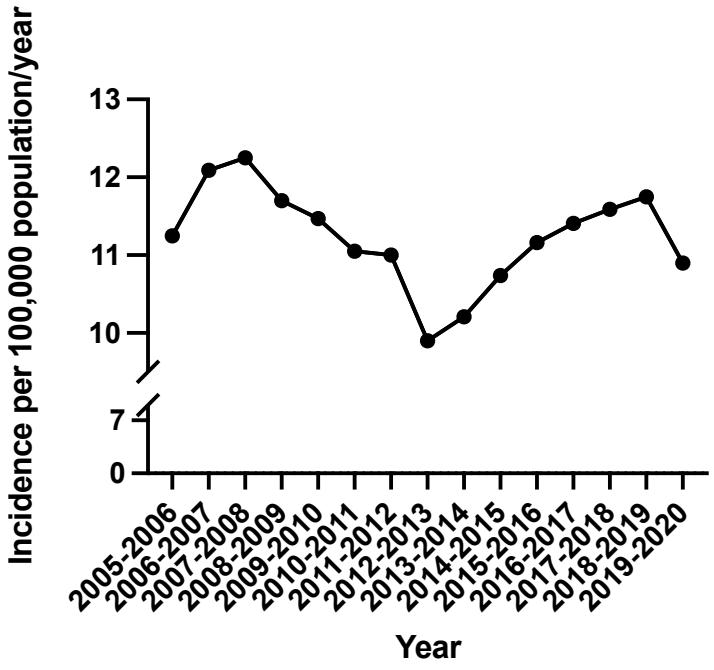
conducted using Number Cruncher Statistical Systems 2020 (NCSS), NCSS Inc., Kaysville, UT; Stata 17.0, Stata Corp, College Station, TX and GraphPad Prism 7.04, La Jolla, CA.

2.3 Results

Overall trends in incidence, gender and age

The incidence of PNI during the study period ranged between 9.9 – 12.3 persons per 100,000 population per year, with an incidence of 11.2 (95%CI 10.9, 11.6) events per 100,000 population per year (Fig 2.1). Over this period there was a significant decrease in the incidence of PNI at 3.8 (95%CI 2.1, 5,5) events per 10 million population per year.

Figure 2.1: Non-birth related total PNI incidence/100,000 population per year.

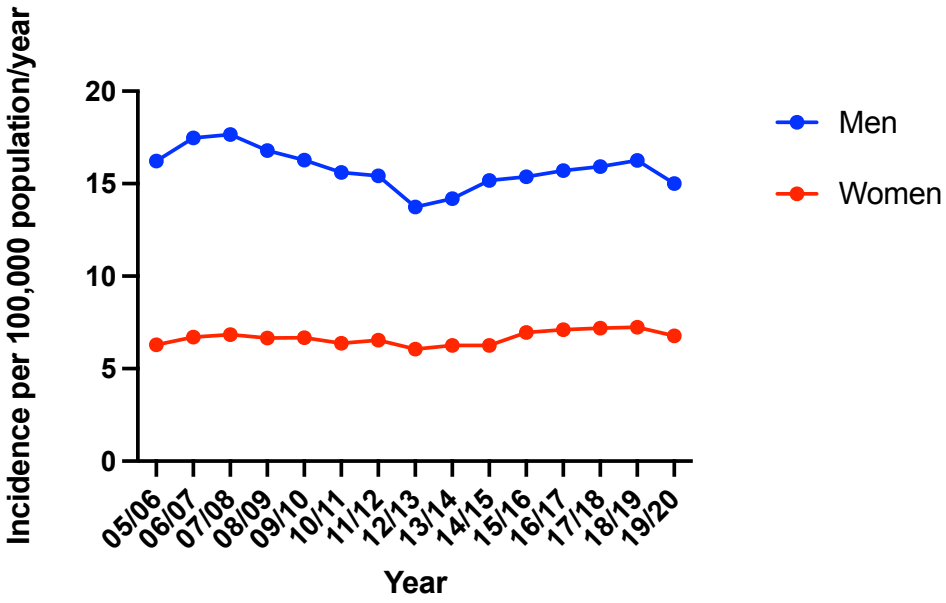


Total number of peripheral nerve injury (PNI) finished consultant episodes (FCEs) per 100,000 population (based on English mid-year population estimates from the Office for National Statistics) per year.

Between 2005-2020, the male incidence of PNI was 15.8 (95%CI 15.2, 16.3) per 100,000 males per year compared to a female incidence of 6.7 (95%CI 6.5, 6.8) per 100,000 females per year (Fig 2.2). Males were significantly (p<0.0001) at least twice as likely to sustain a PNI with an incidence rate ratio of 2.37 (95%CI 2.29,

2.45). Interestingly the incidence of PNI in males significantly decreased by 1.2 (95%CI 0.9, 1.4; *p*<0.0001) events per million males, per year over the period whereas for females, there was a significant smaller increase of 3.9 (95%CI 2.0, 5.7) events per 10 million females, per year) over the same duration. The trends in sex distributions over the period were significantly different (p<0.0001), as shown in Fig 2.2.

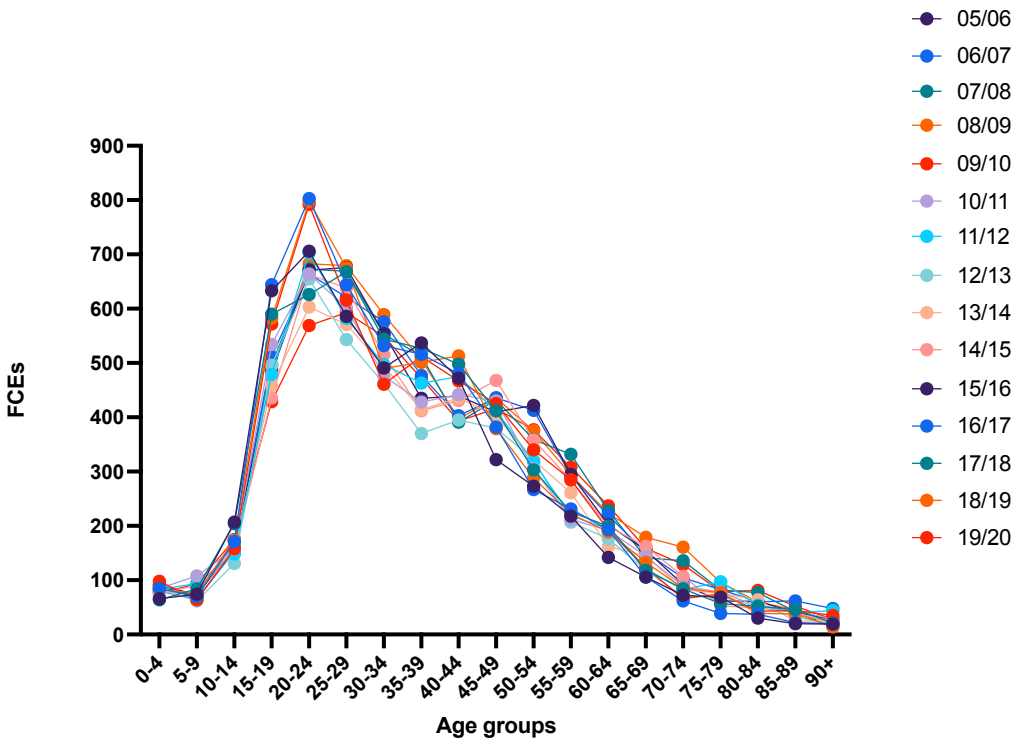
Figure 2.2: Male and female PNI incidence/100,000 population per year.



Total number of male and female peripheral nerve injury (PNI)) finished consultant episodes (FCEs) per 100,000 population (based on English mid-year population estimates from the Office for National Statistics) per year.

Individuals between the ages of 15 – 45 years were most likely to sustain a PNI with a sharp drop in FCEs below this age group. A gradual decline in FCEs was demonstrated from 45 years onwards (Fig 2.3).

Figure 2.3: PNI age-group FCEs per year.



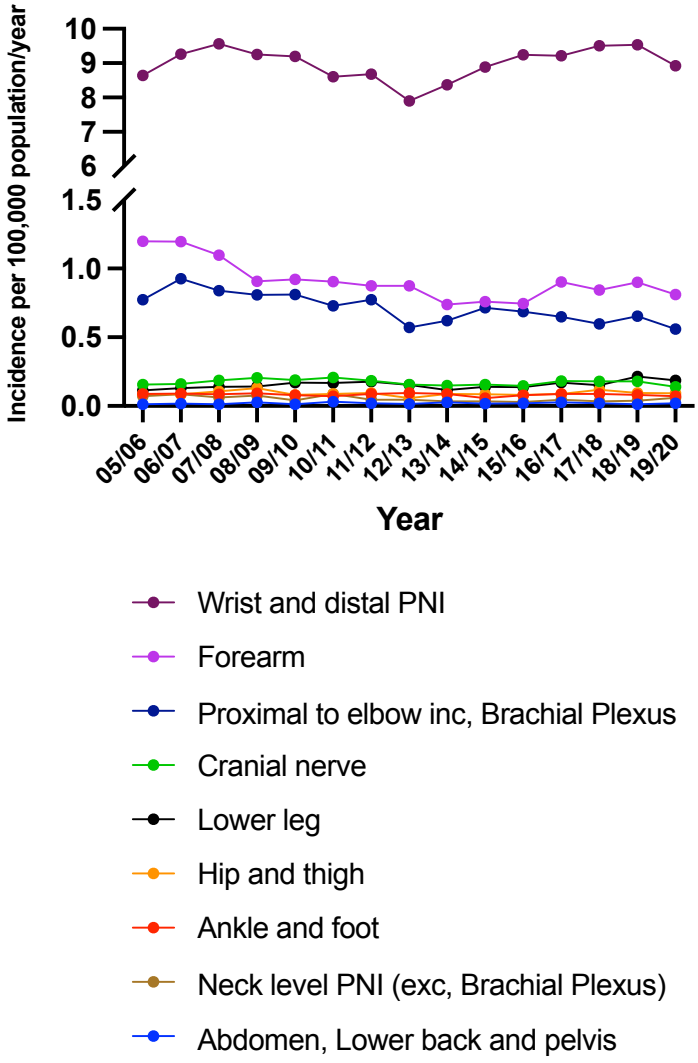
Total number of peripheral nerve injury (PNI) finished consultant episodes (FCEs) per 5-year age grouping per year.

Anatomical stratification of incidence

The most common site of injury was consistently the upper limb (Fig. 2.4) with the majority of injuries occurring to nerves at or distal to the wrist with 9.0 (95%CI 8.7, 9.2) events per 100,000 population per year, forearm 9.1 (95%CI 8.4, 9.8) events per million population per year and proximal to the elbow 7.1 (95%CI 6.6, 7.7) events per million population per year. The next most common were cranial nerve injuries with an incidence of 1.7 (95%CI 1.6, 1.8) events per million population per year.

Over the period there were significant trends to small decreases (p<0.0001) in the incidences of forearm injuries by 2.4 (95%CI 1.9, 2.8) events per million population per year and proximal to elbow injuries by 2.0 (95%CI 1.5, 2.4) events per 10 million population per year. Wrist and distal injury trends were stable (p=0.077) at 1.4 (95%CI -0.2, 2.9) events per 10 million population per year.

Figure 2.4: Anatomical stratification of incidence/100,000 population per year



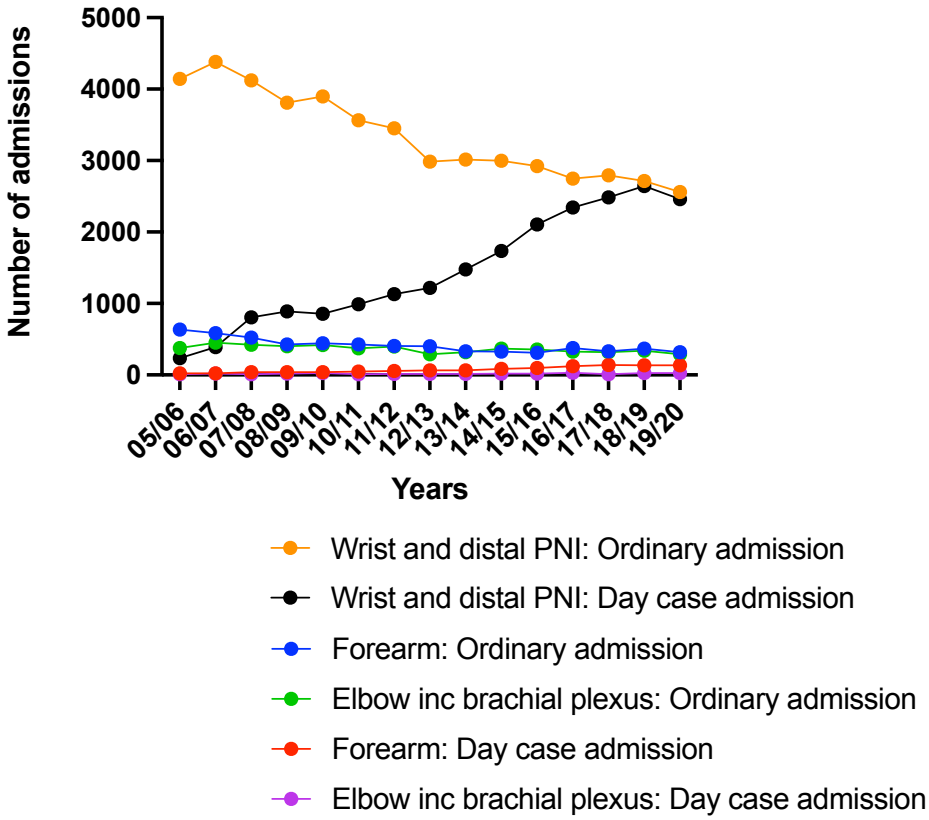
Total number of peripheral nerve injury (PNI) finished consultant episodes (FCEs) based on anatomical location of injury per 100,000 population (based on English mid-year population estimates from the Office for National Statistics) per year.

Admission type and length of stay by anatomical stratification

There were clear significant trends (*P*<0.0001) in inpatient vs outpatient management of PNIs over the study period. Inpatient admissions decreased by 155.4 (95%CI 126.6, 184.2) episodes per year whereas outpatients increased by 184.6 (95%CI 163.3, 206.0) episodes per year (day-case procedures) during the period. These trends were significantly (*p*<0.0001) different (Fig. 2.5). In keeping with this, length of stay for wrist and distal PNIs decreased significantly (*p*<0.0001) by 0.07 (95%CI 0.05, 0.08) days per year; whilst proximal to elbow and plexus injuries have demonstrated a significantly increased length of stay (*p*=0.001) by

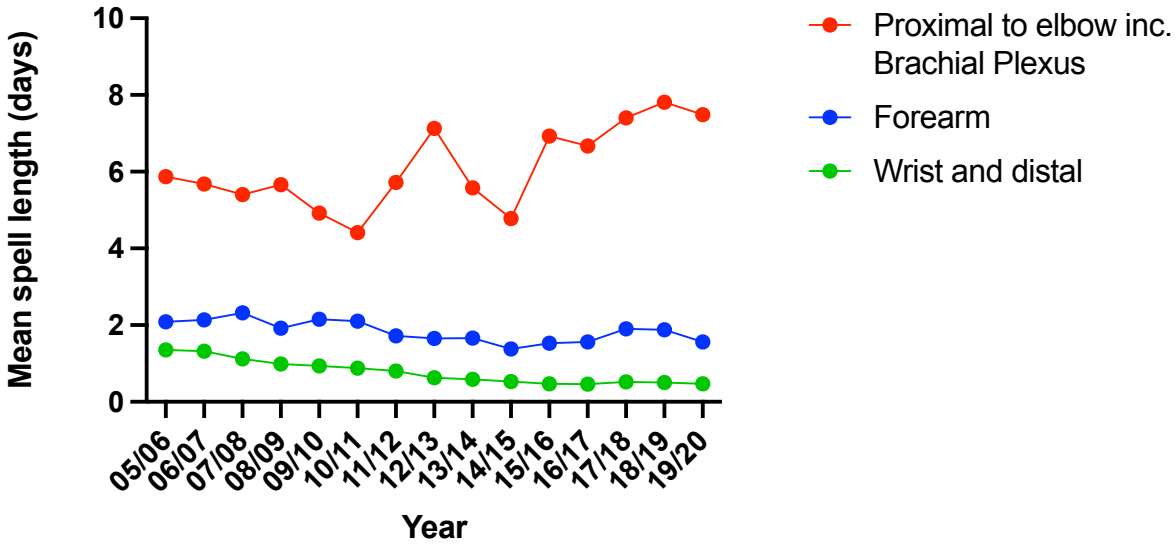
0.16 (95%CI 0.07, 0.25) days per year. Forearm PNI length of stay decreased significantly (p=0.002) by 0.04 (95%CI 0.02, 0.07) days per year (Fig. 2.6).

Figure 2.5: Upper limb stratification of admission type by number of admissions per year.



Total number of admissions based on anatomical location of peripheral nerve injury (PNI) and type of admission (Day Case Admission ≤ 24 hours or Ordinary Admission >24 hours) per year.

Figure 2.6: Upper limb anatomical stratification of mean spell length (days) per year.



Mean length of spell in days of upper limb peripheral nerve injuries per year.

Mechanism of injury and primary speciality by anatomical stratification

There were significant differences in trends (p<0.0001) for mechanisms of injuries to the upper limb during the period. Knife injuries increased significantly (p<0.0001) by 60.9 (95%CI 33.8, 88.0) episodes per year whereas glass injuries decreased significantly (p<0.0001) by 31.0 (95%CI 21.0, 41.0) episodes per year. There were small consistent significant decreases (p<0.0001) in transport injuries and falls (p=0.032) as mechanisms by 3.1 (95%CI 1.7, 4.5) and 4.6 (95%CI 0.5, 8.8) episodes per year, respectively.

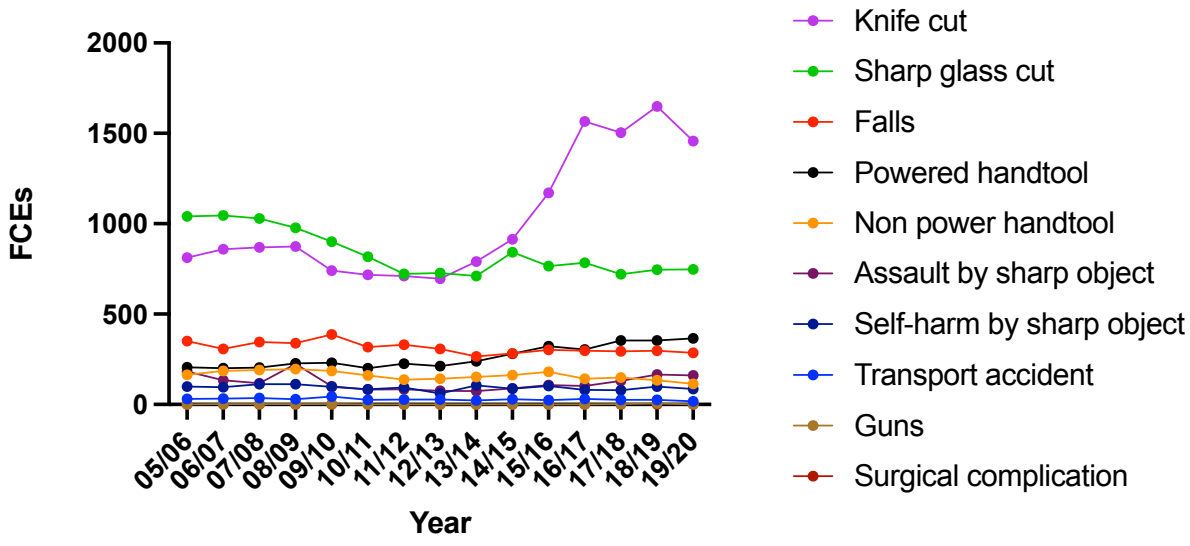
The most common mechanism of wrist and distal PNIs during the study period were knife lacerations with a sharp increase in FCEs from 2014/15 (Fig. 2.7(a)). Glass lacerations were the second most common cause of injury. The most common cause of forearm PNIs were glass lacerations, however there has been a decline in numbers of cases (FCEs/year) from 190 FCE/s/year in 2005/06 to 75 FCEs/year in 2019/20 (Fig. 2.7(b)). Falls were the second most common cause of injury. The most common cause of above elbow PNIs, including the brachial plexus, was consistently falls followed by transport accidents (Fig. 2.7(c)).

During the period there was a significant (p=0.002) increasing trend to plastic surgery by 40.0 (95%CI 18.0, 62.0) episodes per year, with significant decreasing trends in orthopaedic surgery (p=0.006) and neurosurgery (p=0.001) specialities by 19.5 (95%CI 6.7, 32.3) and 0.7 (95%CI 0.4, 1.0) episodes per year, respectively.

The primary specialty for wrist and distal PNI was most commonly plastic surgery with an average of 3,822 (+/- 310) FCEs per year compared to trauma and orthopaedic surgeons with an average of 822 (+/- 76) FCEs per year (Fig. 2.8(a)). Again, forearm PNIs were most commonly managed by plastic surgeons with an average of 322 (+/- 49) FCEs per year compared to trauma and orthopaedic surgeons who managed an average of 115 (+/- 27) FCEs per year (Fig. 2.8(b)). In contrast, above elbow PNIs, including the brachial plexus, were most commonly managed by trauma and orthopaedic surgeons however there was a sharp decline in numbers over the study period with 211 FCEs managed in 2005/06 to 80 FCEs in 2019/20 (Fig. 8(c)). There was a gradual increase in other specialities managing these types of PNI over the study period with plastic surgeons managing similar numbers of these types of PNI by 2019/20 (68 FCEs) (Fig. 8(c)).

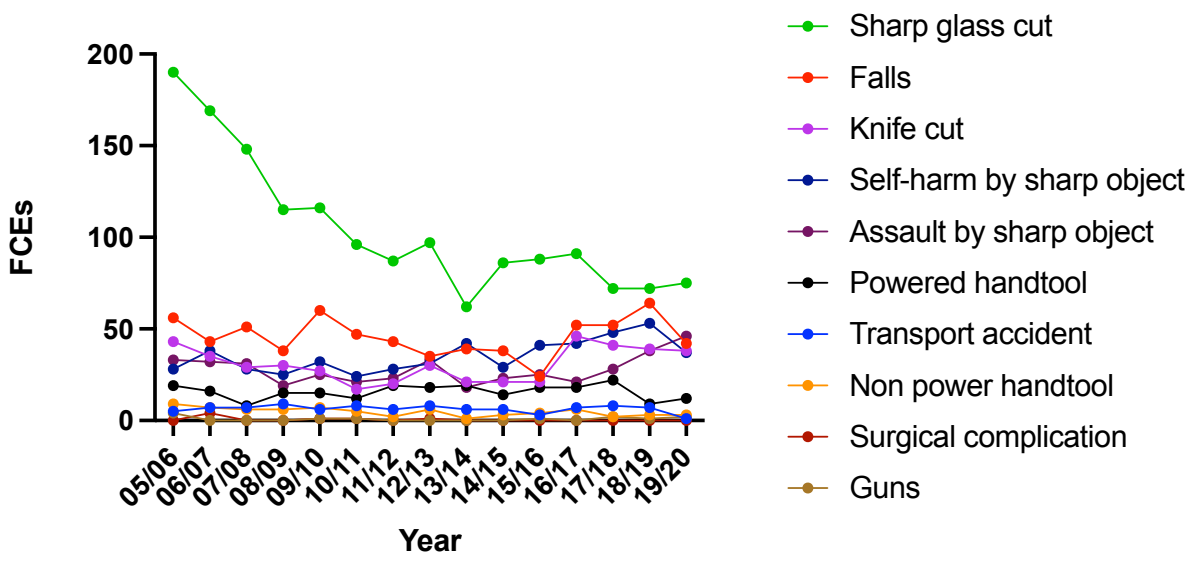
Figure 2.7: Upper limb anatomical stratification of PNI by mechanism of injury:

(a) Wrist and distal PNI;



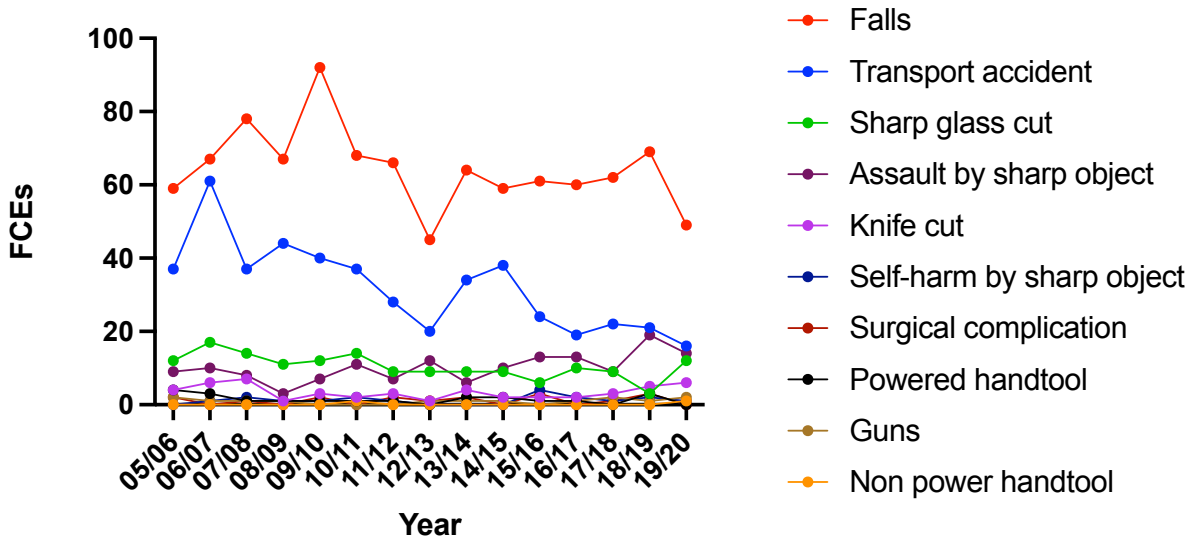
Total number of wrist and distal level peripheral nerve injury finished consultant episodes by mechanism of injury per year.

(b) Forearm PNI;



Total number of forearm level peripheral nerve injury finished consultant episodes by mechanism of injury per year.

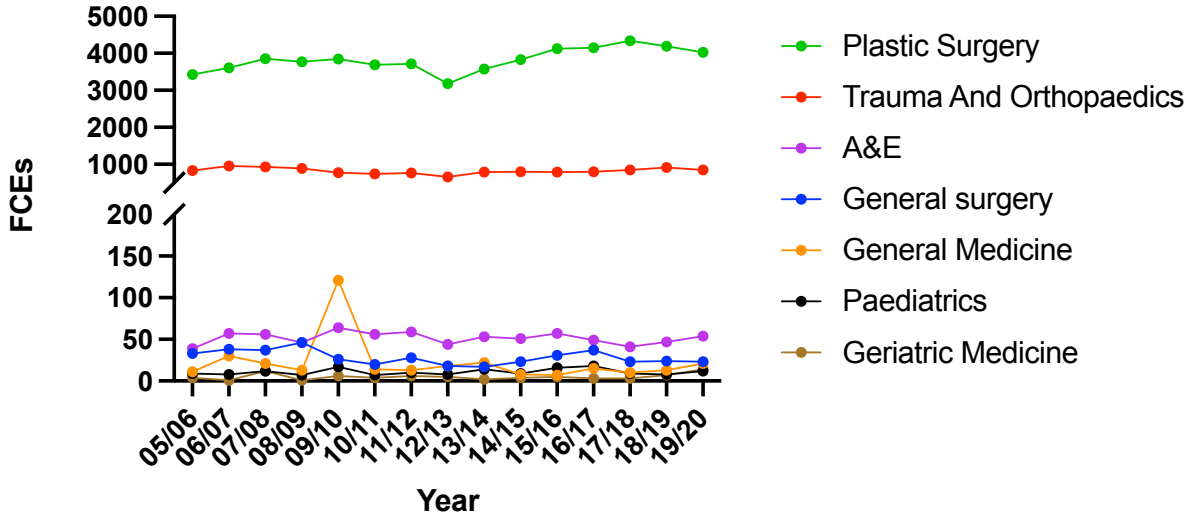
(c) Elbow and proximal including Brachial Plexus.



Total number of elbow level and proximal, including Brachial Plexus, peripheral nerve injury finished consultant episodes by mechanism of injury per year.

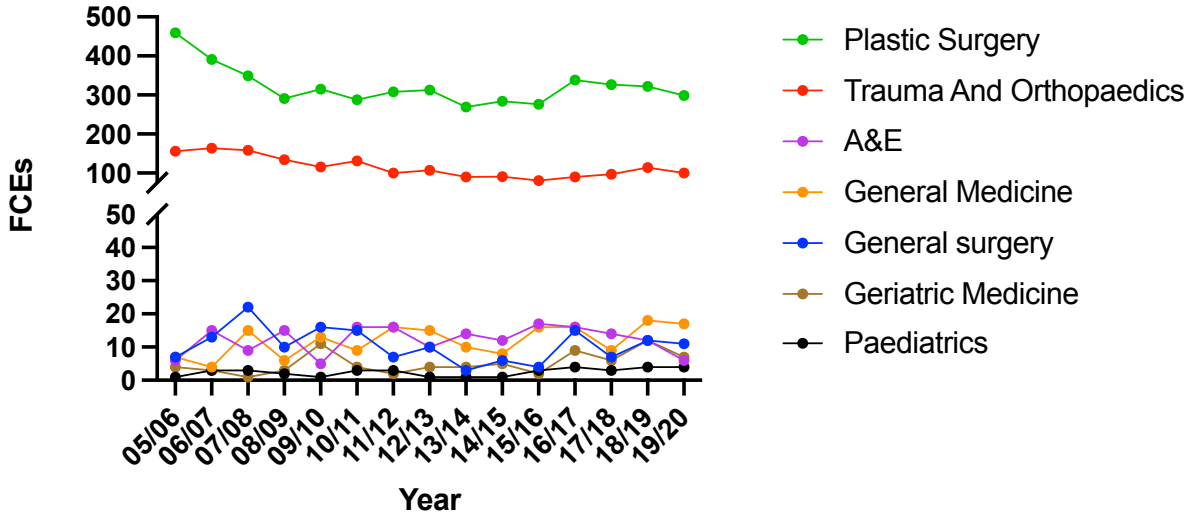
Figure 2.8: Upper limb anatomical stratification of PNI by primary speciality of lead consultant:

(a) Wrist and distal PNI;



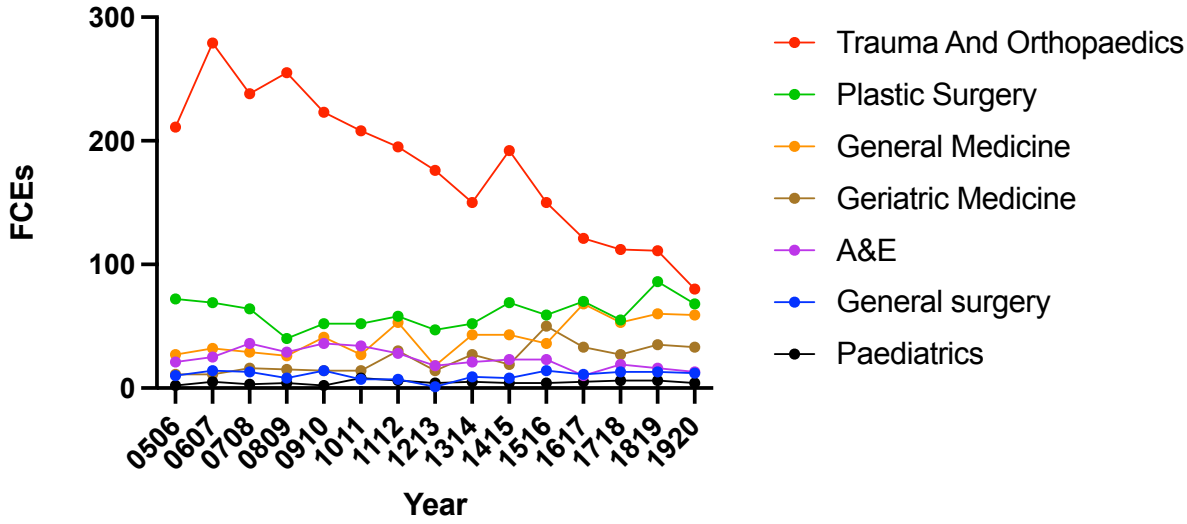
Total number of wrist and distal level peripheral nerve injury finished consultant episodes by primary speciality of lead consultant per year.

(b) Forearm PNI;



Total number of forearm level peripheral nerve injury finished consultant episodes by primary speciality of lead consultant per year.

(c) Elbow and proximal including Brachial Plexus.



Total number of elbow and proximal level, including Brachial Plexus, peripheral nerve injury finished consultant episodes by primary speciality of lead consultant per year.

Surgical procedures stratified by anatomical location of PNI

During the period there were significant increasing trends for neurosynthesis (p=0.022) and graft procedures (p<0.0001) by 27.6 (95%CI 4.6, 50.6) and 5.6 (95%CI 4.5, 6.7) episodes per year, respectively. There was a significant decreasing trend (p=0.009) for explorations by 3.4 (95%CI 1.0, 5.9) episodes per year whilst the rates of transfer procedures remained stable during the period.

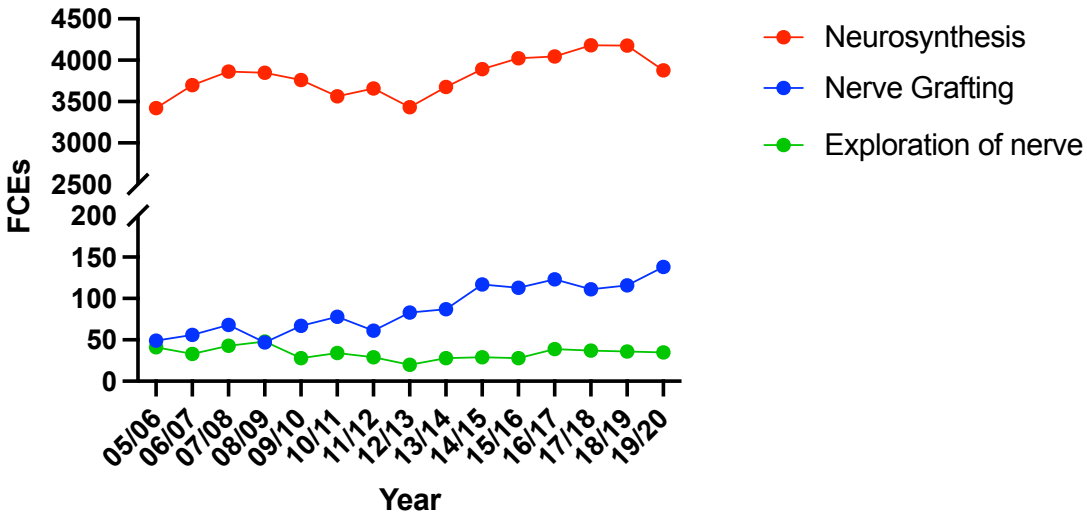
There was an average of 3808 (+/- 239) neurosynthesis FCEs per year and an average of 88 (+/- 30) nerve grafting FCEs per year performed for wrist and distal PNIs (Fig. 2.9(a)).

For forearm PNIs there were an average of 302 (+/- 53) neurosynthesis FCEs per year and an average of 19 (+/- 4) nerve grafting FCEs per year (Fig. 9(b)).

For elbow and proximal including Brachial Plexus PNIs there were an average of 40 (+/- 9) neurosynthesis FCEs per year and an average of 20 (+/- 6) nerve grafting FCEs per year. In addition there were an average of 18 (+/- 16) nerve exploration FCEs per year and 10 (+/- 6) nerve transfer FCEs per year (Fig. 9(c)).

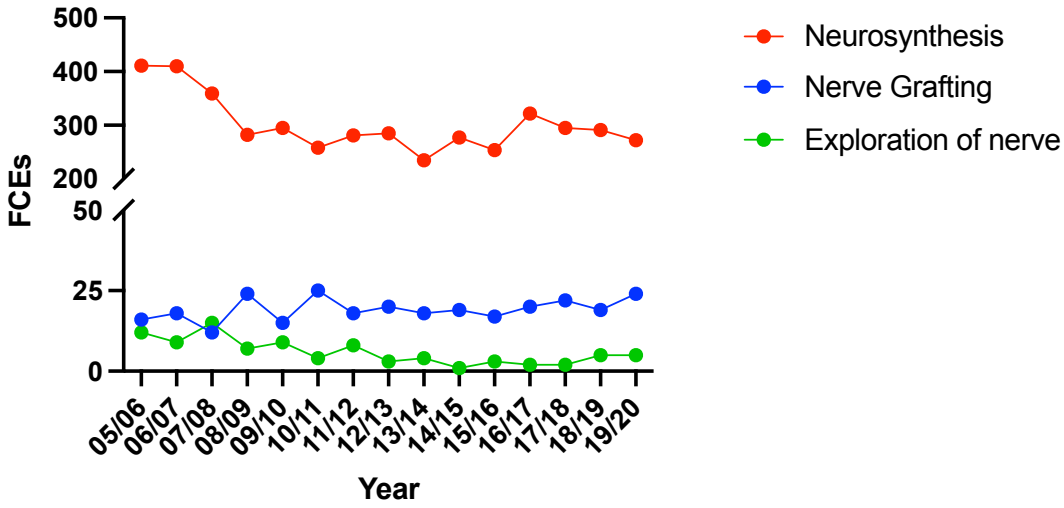
Figure 2.9: Upper limb anatomical stratification of PNI by operations:

(a) Wrist and distal PNI;



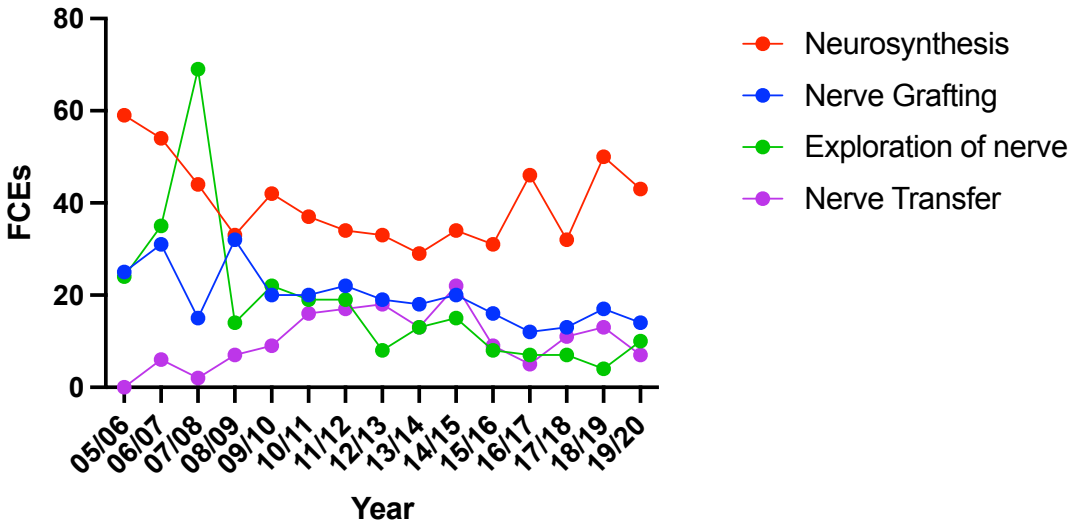
Total number of wrist and distal level peripheral nerve injury finished consultant episodes by operations per year.

(b) Forearm PNI;



Total number of forearm level peripheral nerve injury finished consultant episodes by operations per year.

(c) Elbow and proximal including Brachial Plexus.



Total number of elbow and proximal level, including Brachial Plexus, peripheral nerve injury finished consultant episodes by operations per year.

2.4 Discussion

We present the first national epidemiological study of PNI in England utilising hospital admission data from NHS Digital. It is the first study to date that utilises a national health service-based dataset to explore the incidence of PNI with a focus on upper limb nerves, mechanisms of injury, lead specialty, length of stay and operations performed. Clearly PNI remains a significant problem on a national level with an incidence over the past 15 years of over 11 persons per 100,000 population per year. This incidence is similar to ovarian (11.35 per 100,000) or stomach (9.77 per 100,000) cancers which although have significant mortality risk (45% and 20% 5-year survival respectively) (CRUK, 2019), may not lead to the same morbidity of PNI which can lead to significantly reduced upper limb function and pain (Irwin *et al.*, 1997; Rosberg, Carlsson and Dahlin, 2005). This incidence is much greater than that of Sweden (1.54 per 100,000 population) or Finland (1.01 per 100,000 population in men and 0.45 per 100,000 population in women), being the only other European nations to publish their PNI incidence (Asplund *et al.*, 2009; Wiman *et al.*, 2022). Similar to these scandinavian PNI epidemiology studies, and others, we have found that men are more than twice as likely as women to sustain a PNI and the most common age groups affected are between 15 – 45 years (Asplund *et al.*, 2009; Saadat, Eslami and Rahimi-movaghar, 2011; Missios, Bekelis and Spinner, 2014;

Bekelis, Missios and Spinner, 2015; Wiman *et al.*, 2022). Furthermore, our national level data supports previous findings on the high relative proportion of distally based upper limb nerve injuries in comparison to other anatomical sites. Around 77% of all PNIs affect wrist and distal level nerves.

Patients with more distally based PNI injuries are more frequently being treated on an outpatient basis, saving on inpatient resources and maximising day-case treatment pathways. Length of stay for the most significant, or proximally based upper limb nerve injuries has demonstrated a significantly increasing trend over the past 15-years with patients now spending on average, over a week in hospital. This may be better coded in the future through the advance of major trauma organisation via major trauma centres, in order to identify the cause. It is likely that other significant injuries are associated with more proximal upper limb nerve injuries given that falls and transport accidents are the two leading causes. Further work is needed to understand this increasing length of stay and its effect on ongoing therapy and rehabilitation costs.

Although the number of more proximal PNIs are low (7.1 (95%CI 6.6, 7.7) events per million population per year) they lead to significant disablement and are associated with high therapy and rehabilitation costs (Dy *et al.*, 2020). In central nervous system disorders, specialist inpatient rehabilitation services are well recognised for their ability to cost-effectively rehabilitate working-age patients (Turner-Stokes *et al.*, 2016) yet it remains uncertain what rehabilitation is available nationwide for PNI patients. National standards on the management of these injuries contain no guidance on length of stay or rehabilitation (BOAST, 2021).

A significantly increasing trend in knife injuries, and as the predominant cause of more distally based upper limb PNI injuries, prompts the need for greater injury-prevention strategies and awareness of the dangers of knife handling. Understanding the mechanisms of injury in future prospective studies may allow for more targeted prevention strategies for these injury types. In more proximal injuries, falls and transport accidents remain the most common cause, indicating that significant trauma was involved in the mechanism of injury. Injuries of this nature are therefore likely to be managed at major trauma centres. Clearly there is a need for on-site specialist teams that are able to manage these injury types or adequate referral pathways to centres that are.

Plastic surgeons and trauma and orthopaedic (T&O) surgeons were the two groups most commonly managing PNI patients. However there has been a significantly increasing trend to plastic surgery management of PNI patients over the study period. Conversely there has been a significant decreasing trend to T&O management of PNI patients. In addition, general medicine and geriatric medicine have demonstrated increasing trends in the management of more proximal PNIs. This may indicate that operative treatment is becoming less common for these PNIs.

There was a significant increasing trend for neurosynthesis and graft procedures during the study period with a significant decreasing trend for explorations. This may indicate improved assessment or imaging of certain PNIs allowing surgeons to determine whether nerves are more likely to be in-continuity or not prior to making management decisions. Graft procedures include all procedures for nerve gap management including nerve guidance conduits, which have increased significantly for the most common types of PNI.

The limitations of this study are due to the nature of HES data. HES data is reliant on national coding rules and individual hospital coding inputs and may therefore not be entirely representative of each hospital or region from where the data comes from. Data may be missing or may have been included incorrectly due to inaccurately coded data, unfortunately it is not possible to accurately reduce this methodological error. However, our data does not deviate significantly from other PNI epidemiological studies utilising different data sets. HES data does not include patients that have been treated within the private sector or patients that sustained an injury that had not sought medical help. It is unclear how many patients this may represent however we believe this is not likely to represent a significant number.

Future research into the epidemiology of PNI is needed to understand the cause of the increased length of stay for more proximal upper limb PNIs with an associated cost-analysis of both inpatient and outpatient rehabilitation and therapy costs. Mechanism of injury could be further explored to understand the location of injury for example whether the injury was at home or in the workplace which would help in the development of prevention strategies. Prospectively collected outcomes of surgery are required, especially in more disabling proximal injuries, in order to

understand the treatment effect of different surgeries. These need to be uniform and objective so that they can be collated and compared between nerve-injury centres.

CHAPTER 3 Translation of a novel treatment for peripheral nerve gap injuries: The UMANC Trial

Elements of this chapter have been adapted from published work:

Murphy R, Faroni A, Wong J and Reid A. Protocol for a phase I trial of a novel synthetic polymer nerve conduit ''Polynerve'' in participants with sensory digital nerve injury (UMANC). *F1000Research* 2019, **8**:959 (https://doi.org/10.12688/f1000research.19497.1)

Translation of this novel treatment, produced through the University of Manchester, was funded by the NIHR. This afforded me the opportunity to run a clinical trial as co-principal investigator and undertake a PhD. The rates of nerve grafting have shown significant increase over the last 15-years as seen in the previous chapter. Outcomes are known to be poor and there is the added cost of significant donor site morbidity. An alternative to peripheral nerve autograft developed through the University of Manchester has been translated into humans which is discussed in more detail in the following chapter.

3.1 Introduction

Peripheral nerve injuries most frequently present with nerve stumps that can be approximated for surgical repair: direct, end-to-end suture repair of the epineurium (neurorrhaphy). Excessive tension over the suture line leads to poor results (Terzis, Faibisoff and Williams, 1975); therefore, when the nerve stumps cannot be approximated without tension, an alternative surgical method is required.

Where the nerve gap exceeds more than 5 mm, there are two fundamental options, either 'autologous nerve grafting' or use of a bridging material or 'conduit' (Daly *et al.*, 2013). This study examines the first-in-man use of a new nerve conduit device 'Polynerve' to repair small nerve gaps in digital sensory nerves of the hand. Polynerve is a degradable co-polymer of poly-ε-caprolactone (PCL) and Poly-L-lactic acid (PLA) which is shaped as a cylinder with a novel internal lumen consisting of a specific micro-grooved architecture.

Poly-lactic acid (PLA) and PCL

PLA and PCL are biocompatible and approved for use in numerous biomedical applications. Polylactide (L- and DL- forms) copolymerised with PCL have found utility in nerve guide conduits and Neurolac® (Polyganics, FDA 510 (k) 2003; CE mark 2004), consists of a poly ((85/15 L/D) lactide ε-caprolactone). Neurolac® has extensive pre-clinical *in vivo* data, and in randomised clinical trials Neurolac® is reported to have comparable efficacy to gold standard autograft in defects up to 20 mm (Bertleff, Meek and Nicolai, 2005). Known adverse events associated with the use of a Neurolac® nerve guide include but are not limited to: failure to provide adequate nerve regeneration at sites where too much tension or compression occurs; failure to provide adequate/complete nerve regeneration; transitory local irritation; infection; allergy and delayed wound healing. Limitations are reported to be the high rigidity and inflexibility of the device. In comparison to Neurolac®, Polynerve has greater tensile strength, flexibility and less acidic degradation due to modifications in the PCL:PLA ratio (Polynerve = 4:1, Neurolac® = 1:1). The microgrooves on the internal lumen provide a protected environment for ingress of Schwann cells which align on the micro-patterned grooves (Mobasseri, Terenghi and Downes, 2014) and aid subsequent nerve regeneration (Mobasseri *et al.*,

2015). *In vivo* studies on rat sciatic nerve gaps of 10 mm demonstrated comparable efficacy of regeneration of Polynerve repairs to nerve graft repairs (current clinical gold standard) in both short (3 week) and long (16 week) timepoints (Mobasseri *et al.*, 2015).

We sought to build on the background *in vitro* and *in vivo* evidence for Polynerve by conducting a fist in man clinical investigation to assess safety of the device. It was decided that the most appropriate clinical model was sensory nerve gap in the hand. Secondary objectives of this study included evaluation of the effectiveness of repair by measuring appropriate sensory outcome measures and assessing device resorption using high-frequency, three-dimensional, tomographic ultrasound scanning (Hf,3D,tUS).

3.2 Methods:

This study was a UK-based, prospective, single-centre, unblinded, phase I clinical trial of a novel nerve conduit device. The study registered eligible participants to undergoing repair of a transection of a sensory nerve of the hand using a novel, synthetic nerve conduit polymer. All participants that received the nerve conduit device were followed for a period of 12 months post-surgery to undergo clinical assessment and sensory testing. Where practical, those eligible were followed up past 12-months and up to a period of 36 months after surgery to assess resorption of the device using Hf,3D,tUS.

The trial was conducted in accordance with the principles of GCP and the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments. The UK Health Research Authority (HRA) and the Medicine and Healthcare products Regulatory Agency (MHRA) provided ethical permissions for the clinical investigation through the South Manchester Research Ethics Committee (REC reference: 17/NW/0111).

Eligible participants were identified by the Principal Investigator and Co-Investigators within the Department of Burns, Plastics and Reconstructive Surgery at Manchester University NHS Foundation Trust (MFT) outpatient department and trauma database.

Participants deemed eligible for consideration and potential entry into the study (Table 3.1) were provided with a verbal and written explanation of the study. The participant was given at least 4 hours and, in most cases, greater than 24 hours to consider participation. The minimum of 4 hours was stated because these were participants with traumatic injuries and occasionally required or were offered an operation within this time period. After all queries had been addressed and the clinical team was confident that the participant understood the study and all its requirements, participants were consented onto the study.

Table 3.1: UMANC Participant eligibility criteria.

Inclusion criteria	**Exclusion criteria**
Provision of informed consent prior to any study specific procedures.	Concomitant injuries requiring surgical treatment from other specialists out with the hand injury.
Traumatic injury/injuries to the hand with clinical suspicion of sensory nerve transection mandating surgical exploration.	Specified co-morbidities that would increase a participant's risk of infection including diabetes, renal/liver disease, autoimmune diseases, primary or secondary immunocompromised participants (including immunosuppressive drugs or known disease resulting in suppressed immunity).
Male and females aged 18-80.	A stated hypersensitivity or allergy to the polymers PCL/PLA.
	Any other significant co-morbidity impacting on the risk of surgery (to be determined by the surgical team).
	Known to have participated in a clinical trial of an investigational agent or device in the previous 30 days.

Intervention

Participants recruited onto the trial were operated on by way of routine surgical procedure in an operating theatre at MFT. An experienced consultant plastic

surgeon and plastic surgery registrar with microsurgical expertise operated on all patients. Patients underwent local or general anaesthesia as determined by the clinical need.

In standard operating theatre sterile conditions, the wound was debrided and irrigated as necessary. The digital nerve was examined under loupe or microscope magnification and a decision made as to the most appropriate surgery method. If the nerve gap was less than 5 mm and the stumps could be co-apted in a tension-free manner, then the nerve was repaired primarily (end-to-end). If the nerve gap was greater than 20 mm it was repaired with a nerve graft. In the instance of a nerve gap between 5 and 20 mm, the Polynerve biomaterial nerve conduit was used. Three diameters of Polynerve were available sterilised and packaged: 1.5 mm, 2 mm and 3 mm diameter. All Polynerve conduits were 24mm in length and were cut with a scalpel on a metal surface to fit the gap and allow for 2mm insertion of the nerve stumps. The nerve was sutured into the Polynerve conduit with an 9/0 Ethilon suture (Ethicon, UK) on a reverse-cutting needle. We used saline to repeatedly rinse the conduit to prevent blood pooling during insertion of the nerve stumps.

Skin was repaired with standard treatment of Ethilon suture (Ethicon, UK). The wound was covered with standard treatment of a barrier dressing such as Mepitel (Mölnlycke Health Care, Sweden), and a secure gauze-based dressing overlying. If concomitant injuries existed such as tendon injuries, then the hand was dressed using standard treatment including post-operative splinting, otherwise the fingers were not routinely immobilised.

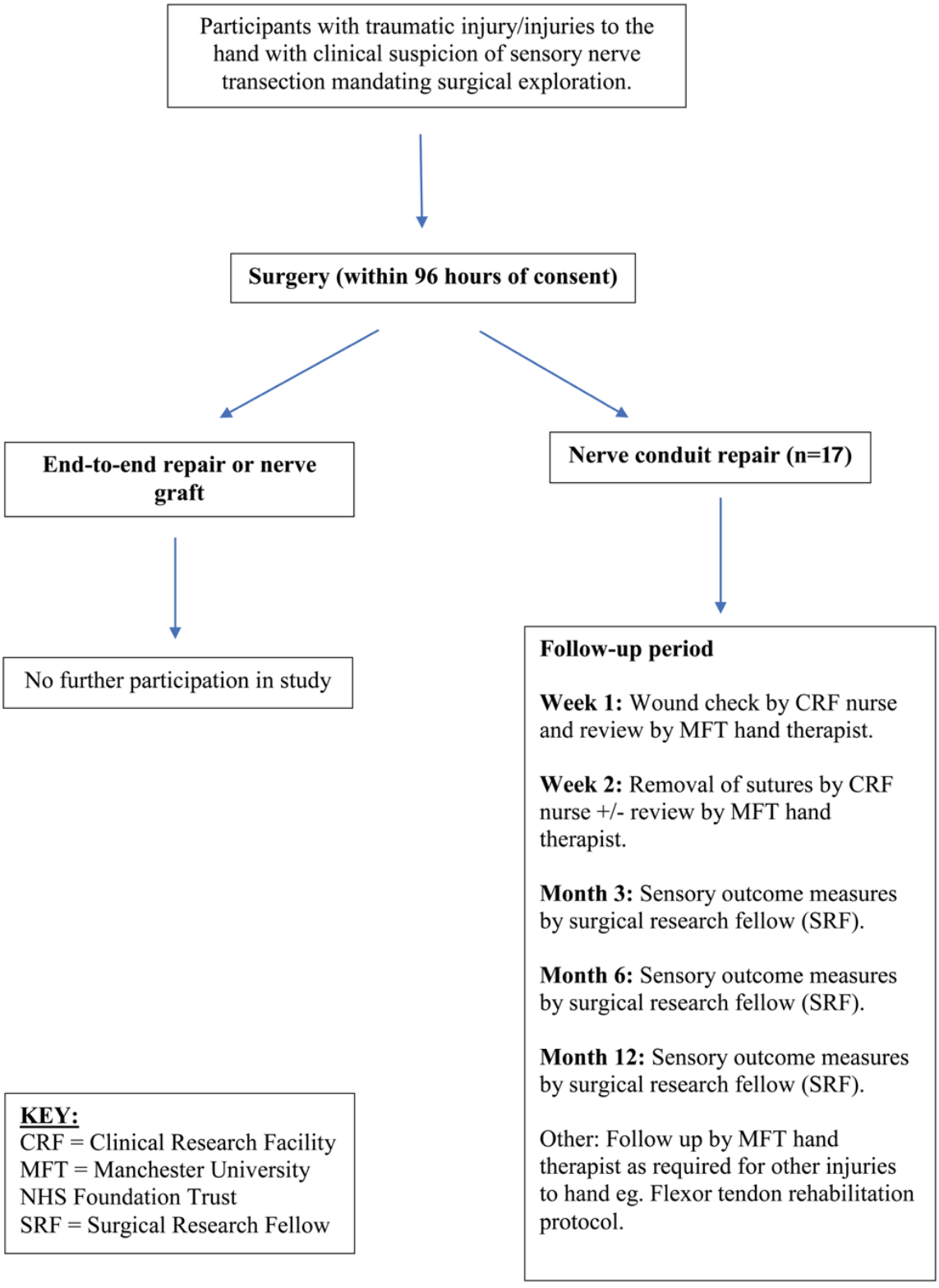
All participants received antibiotics at induction of anaesthetic and for 1 week post-operatively. This was co-amoxiclav 625 mg three times daily, or if penicillin allergic clarithromycin 500 mg twice daily. This is standard treatment following nerve graft surgery.

Standard post-surgical follow-up was conducted 1 week and 2 weeks post-surgery, and additional follow up at 3 months, 6 months and 12 months post-surgery was scheduled (Figure 3.1). Where practical, and for those patients eligible and available to attend for Hf,3D,tUS, they were followed up at 6 monthly intervals depending on which stage of follow-up they were at when the Hf,3D,tUS became available for use.

The COVID-19 pandemic and subsequent national lockdowns lead to fewer patients followed up, reduced follow up time and missed appointments.

Further methodological detail on the use of the Polynerve device can be found in Appendix 3 and on adjuvant care, in the study protocol (Murphy *et al.*, 2019). All patients were reviewed in hand therapy 1 week and 2 weeks after surgery and provided with sensory re-education advice at their 2 week appointment and then discharged if there were no wound healing concerns. Patients with concomitant injuries were followed-up for different periods depending upon the other injuries as per standard hand therapy regimes.

Figure 3.1: A flowchart for UMANC participant timeline.



Participants who qualified for enrolment were operated on within 96 hours of consent and either had end-to-end repair/nerve graft (standard treatment) or nerve conduit repair (experimental treatment). Those having standard treatment were discontinued from the study, whilst those having the experimental treatment went on to have 12-month study follow-up.

Sample size

As this was a phase I study, primarily assessing safety of the device, a pragmatic decision on achievable recruitment numbers at a single centre who would be able to complete 12-months follow-up was made, and no formal power calculations were performed. The trial aimed to recruit 17 participants over 12 months at an intended rate of approximately 1 to 2 participants per month. All participants fitting the eligibility criteria were recruited.

Primary outcome: Safety was assessed based on the number and degree (based on the Clavien-Dindo classification of surgical complications (Clavien *et al.*, 2009)) of adverse device effects (ADE) that occurred during the study period ("Safety Reporting - Health Research Authority"). Any ADE occurring within the defined 12-month follow-up period was included in the ADE summaries.

Secondary outcome: To measure degree of efficacy of the nerve conduit device, standard sensory outcome measures were used:

- two-point discrimination (2PD) (Dellon, 1981),
- the Weinstein Enhanced Sensory Test (WEST) (Weinstein, 1993),
- Locognosia test (adapted methodology from Jerosch-Herold *et al.* JBJSB 2006 (Jerosch-Herold, 2006))

Testing was performed by a plastic surgery registrar trained in the described sensory testing techniques and overseen by a senior occupational therapist, who was also a co-investigator, specialising in peripheral nerve injury.

All tests were performed in a quiet room with the patient sat opposite the examiner with the arms rested on a pillow with elbows at ~45-90 degrees of flexion and predominant (not complete) supination of the wrists. This position was chosen as the most comfortable position for most patients to sit through the sensory testing without movement of the hands. The hands were then placed through a JAMAR sensory testing shield (Performance Health, IL, USA) so as to blind the patient to the testing instrument during the examination. Before the start of each test, and as needed, patients were reminded to keep their hands rested into the pillow in order to prevent lifting of the fingers towards the testing instrument.

The WEST monofilament tool (Fabrication Enterprises Inc., NY, USA) developed by Sidney Weinstein (Weinstein, 1993) was used for pressure threshold testing. This tool includes five standard filaments which apply a standard calibrated force of 0.07g, 0.2g, 2.0g, 4.0g and 200g. For the purposes of this study a corresponding integer from 5 (0.07g) to 1 (200g) represented the incremental monofilaments with 0 representing an inability to detect any force. A random monofilament test was employed whereby each filament was applied in random order to the test sites with the lowest detectable stimulus recorded in terms of the corresponding integer.

The s2PD test was performed incrementally using a blunt-tipped discriminator (Baseline DISCRIM-A-GON, Fabrication Enterprises Inc., NY, USA) as described by Mackinnon and Dellon (Mackinnon and Dellon, 1985) starting with the smallest width and just sufficient pressure for the subject to detect the stimulus. Each pin set distance was performed 10 times and a score of 7 positive responses out of 10 was deemed a positive response.

The locognosia test was adapted from Jerosch-Herold et al. JBJS 2006 (Jerosch-Herold, 2006) for use in digital nerve injury patients. A diagram of the hand with a superimposed grid of zones, numbered is presented to the patient. The patient is asked to identify the zone where a suprathreshold stimulus has been perceived. The stimulus is delivered using a WEST monofilament (Fabrication Enterprises Inc., NY, USA), which upon contact with the skin bends, providing a repeatable peak force of 200g.

Standardisation of the testing was achieved by following the well-established protocols and comparison to a contralateral control to demonstrate regenerative change over time towards the control.

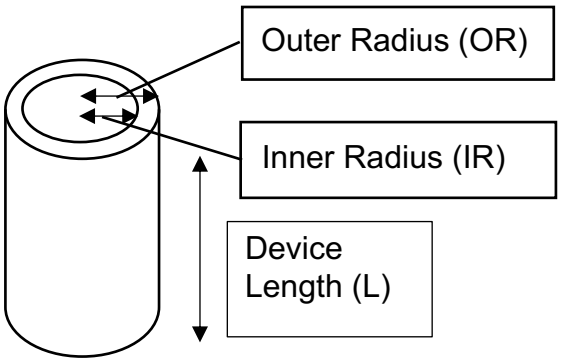
Hf,3D,US was performed using a Mindray™ Resona 7 (Mindray™, Shenzhen, China) ultrasound machine with a 20mHz probe and PIUR imaging (Vienna, Austria) 3D, tomographic ultrasound system (tUS) software was used for 3D image acquisition and analysis (Downey, Fenster and Williams, 2000). A fully trained ultrasound technician performed the ultrasound scans and a clinician trained in ultrasound image analysis performed the volumetric analysis of the Polynerve device. Fused tUS scans were used to measure device volume using a standard

technique to measure digital arterial wall volumes (Hughes *et al.*, 2021). As the device is resorbed by a process of hydrolysis, volumetric measurements of the remaining device volume were calculated using the same method to calculate arterial plaque volumes (Casella *et al.*, 2015).

Pre-operative device volume was calculated based on the device length, device radius and thickness of the Polynerve device as per the standard calculation for a hollow cylinder volume:

Figure 3.2: A schematic for the calculation of the volume of the Polynerve device.

Volume of Polynerve device = $\pi L (OR^2 - IR^2)$



OR = device diameter/2

IR = (diameter/2) – device thickness (0.14 mm +/- 0.01)

L = nerve gap (mm) + 4mm (to allow 2mm either end to insert the cut nerve ends).

Polynerve device volume was estimated from the outer lumen volume minus the internal lumen volume.

Statistical methods

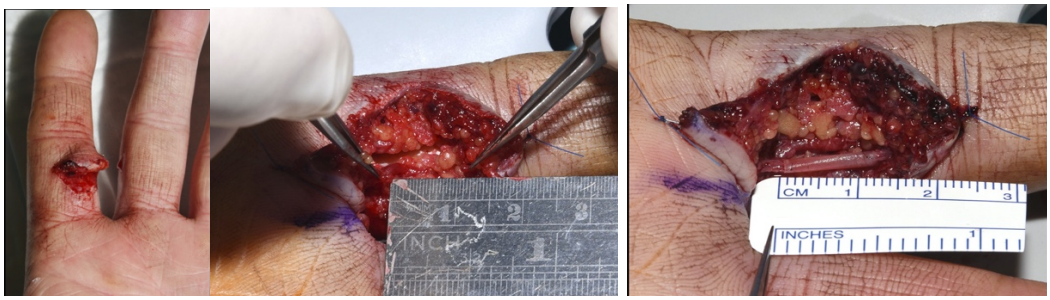
Demographic data was tabulated. All primary outcome data was described and summarised. Secondary outcome measurement data was tabulated and median ± range of static 2PD, WEST and Locognosia compared to contralateral control nerves were presented with Wilcoxon signed rank tests between groups. Levels of significance were set as p-values (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

3.3 Results:

A total of 22 patients were deemed eligible and consented into the study between 04-Dec-2017 to 21-Aug-2018 (8.5-month period). There were 3 females and 19 males. There were 21 white British and 1 Asian British people. The age range was: 20 – 70 and the most common mechanism of injury was circular saw injury (Table 3.3). The mean nerve gap size was 10mm.

Figure 3.3: Surgical exploration and installation of Polynerve device

(a) (b) (c)



Intra-operative photos of (a) circular saw injury with suspected nerve gap to the left index finger ulnar digital nerve: (b) a nerve gap of 12 mm identified; (c) a 1.5mm diameter and 14mm length Polynerve device fitted in the nerve gap.

A total of 17 patients had a Polynerve device fitted (Table 3.2) whilst the remaining 5 patients deemed eligible were excluded intraoperatively as they did not have a 5-20mm nerve gap. Fourteen patients completed the study follow-up. Three patients did not complete 12-months follow-up and were removed from the study. Two of these patients were lost to follow-up and one patient with poor soft tissue coverage interfered with the wound during their second post-operative week causing the device to fall out into their dressings. Once healed they chose not to undergo further surgery.

Table 3.2: Demographics of nerve-injured patients with Polynerve device fitted.

Trial Number	Age	Gender	Mechanism of Nerve Injury	Concomitant Injuries	Nerve Gap Size (mm) (Mean = 10mm)	Level of Nerve Injury	Digit Injured
001	70	M	Trauma–- circular saw	FPL, RCL IPJ, UDN injury	10	Inter-Phalangeal Joint	Left thumb
002	53	M	Trauma–- circular saw		10	Base/Mid. Proximal Phalanx	Left index finger
003	28	M	Trauma–- glass laceration		8	Proximal phalanx	Right index finger
007	41	M	Trauma–- ceramic toothbrush holder	Zone I FDP	6	Mid. Middle Phalanx	Right index finger
008	20	M	Trauma–- slipped climbing serrated metal fence		18	Mid. Middle Phalanx	Left middle finger
010	29	M	Trauma–- circular saw		6	Proximal Inter-Phalangeal Joint	Right index finger
011	49	F	Trauma–- glass laceration		5	Base of middle phalanx	Right little finger
013	35	F	**Neuroma**–- previous glass laceration		8	Base of middle phalanx	Left middle finger
016	25	F	Trauma–- opening metal scourer packet		6	Proximal Inter-Phalangeal Joint	Right middle finger
017	47	M	**Neuroma**–- previous knife laceration		15	Mid-proximal phalanx	Left thumb

018	24	M	**Neuroma**–- previous glass laceration		18.5	Metacarpal Phalangeal Joint	Right thumb
019	20	M	Trauma–- Stanley knife		5	Base of Proximal Phalanx	Left thumb
020	47	M	Trauma–- circular saw		5	Base of Middle Phalanx	Left index finger
021	37	M	Trauma–- circular saw	DIPJ UCL repair and k-wire	7	Just proximal to distal interphalangeal joint	Left little finger
022	34	M	Trauma–- circular saw	Zone I FDP	12	Mid. Middle Phalanx	Left index finger
023	56	M	Trauma–- circular saw	LMF Zone II FDP, UDN injury	6	Mid. Proximal Phalanx	Left middle finger
024	35	M	**Neuroma** – previous Stanley knife injury		18	Metacarpal Phalangeal Joint	Left index finger

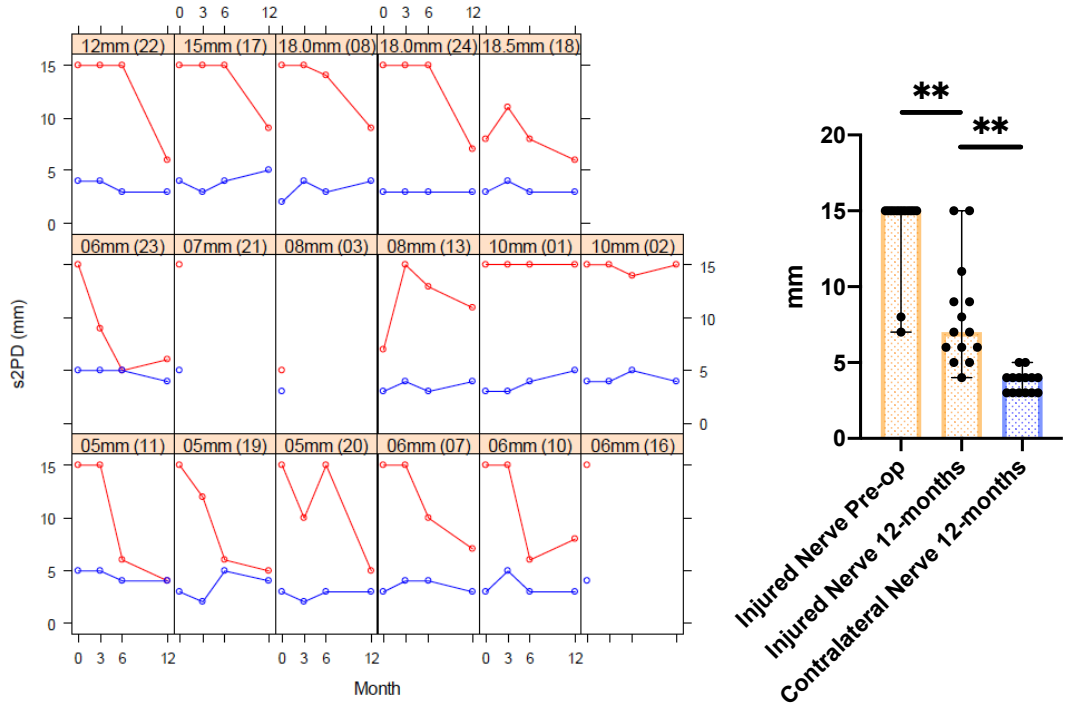
Outcomes Data; There were six recorded adverse events (fall in bath, pain from position of finger splint, vomiting due to antibiotic, post-operative bleeding, myocardial infarction, wound dehiscence and device fall out). Only the myocardial infarction event in participant 020 was classified as serious but was not related to surgery or the device. There were no reported adverse device events.

No increased risk of infection or abnormal wound healing was reported. All wounds were fully healed between week 2 and 3 months, except in one patient who suffered significant soft tissue damage and self-inflicted wound dehiscence. After the wound was fully healed and in discussion with the patient, they chose not to undergo further surgery.

Sensory outcomes (median values (ranges)):

Figure 3.4: Static two-point discrimination (s2PD) outcomes of Polynerve-repaired digital nerve and contralateral (control) digital nerve.

(a) (b)

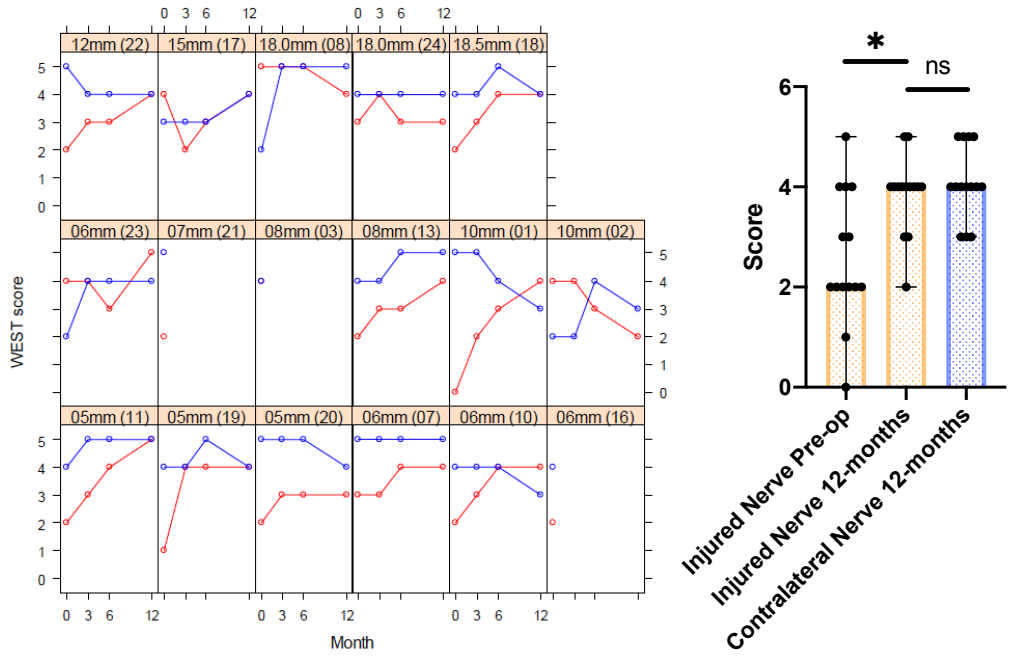


(a) Panel plots of individual patient s2PD values (blue=contralateral control nerve; red=injured/Polynerve-repaired nerve) from smallest to largest gap size (bottom left to top right). Panel plot titles include gap size in millimetres and (participant number). (b) Bar chart of median s2PD values and ranges, of the Polynerve-repaired digit pre-op = 15mm (7-15) versus 7mm (4-15) by 12-months (p=0.003) and versus a contralateral control digital nerve score of 4mm (3-5) at 12-months (p=0.001).

Four patients 011, 019, 020 and 018 demonstrated a complete return to normal s2PD (≤5mm) in the injured/Polynerve repaired territory. Seven patients (007, 008, 010, 017, 022, 023, 024) achieved a s2PD <10mm, whilst patient 013 achieved a 12-month s2PD of 12mm. Two patients (001 and 002) did not demonstrate any improvement in s2PD yet their WEST scores and locognosia scores (below) demonstrated a return to normal. Observationally, it was felt that the s2PD test did not work for patients 001 and 002. There was a significant difference between the s2PD results in the injured/Polynerve-repaired territory compared to the normal, contralateral control territory by 12-months.

Figure 3.5: Weinstein-Enhanced Sensory Testing (WEST) outcomes of Polynerve-repaired digital nerve and contralateral (control) digital nerve.

(a) (b)

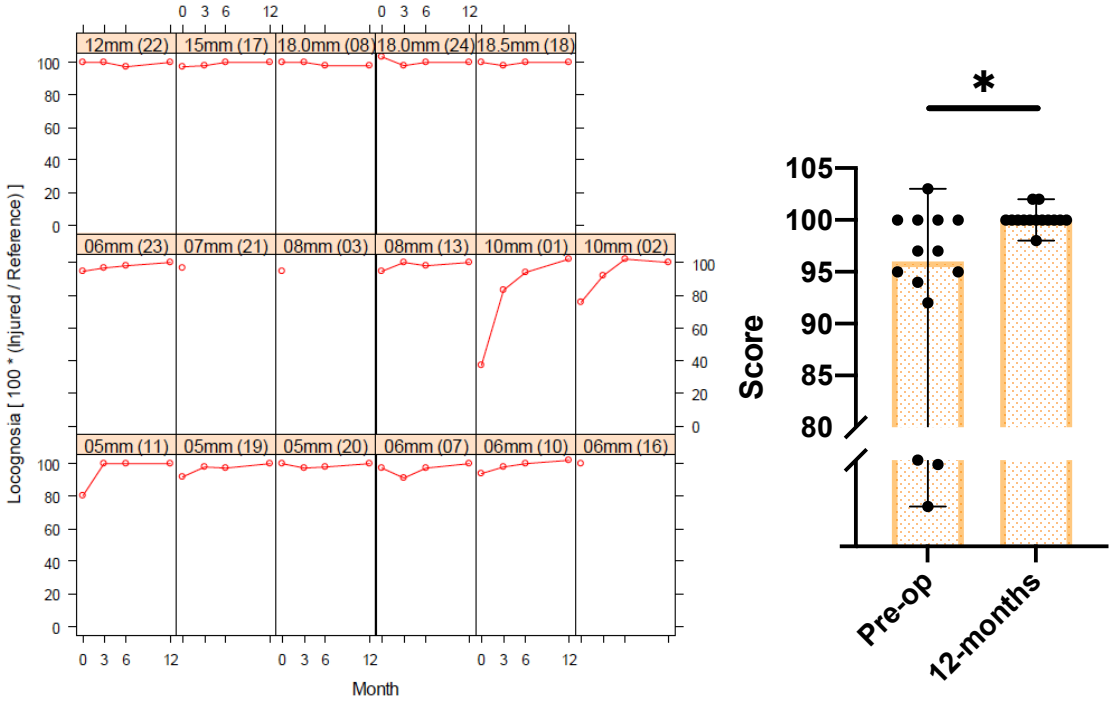


(a) Panel plots of individual patient WEST scores (0-5, where 5 represents the smallest 0.07g monofilament and 0 represents >200g filament/undetectable force) (blue=contralateral control nerve; red=injured/Polynerve-repaired nerve) from smallest to largest gap size (bottom left to top right). (b) Bar chart of median WEST values and ranges of the Polynerve-repaired digit pre-op = 2 (0-5) versus 4 (2-5) by 12-months (p=0.017) and versus a contralateral control digital nerve score of 4 (3-5) at 12-months (p=0.32).

Eight patients (001, 010, 011, 017,018, 019, 022, 023) returned to normal WEST scores in the injured/Polynerve repaired territory. The remaining 6 patients all returned to within 1 WEST score of their contralateral control score. There was no significant difference across the patient cohort between the injured/Polynerve-repaired territory to the contralateral, normal territory by 12-months.

Figure 3.6: Locognosia outcomes of Polynerve-repaired digital nerve and contralateral (control) digital nerve.

(a) (b)



(a) Panel plots of individual patient locognosia scores from smallest to largest gap size (bottom left to top right). (b) Bar chart of median locognosia scores and ranges which demonstrated an improvement from 96 (37-103) pre-op to 100 (98-102) by 12-months (p=0.012).

All patients demonstrated either normal or near-normal locognosia scores by 12-months. However there was also minimal change from pre-operative values demonstrating a low sensitivity of the test for measurement of digital nerve regeneration.

Degradation of the device visualised through high-frequency, three-dimensional, ultrasound scanning (Hf,3D,tUS),

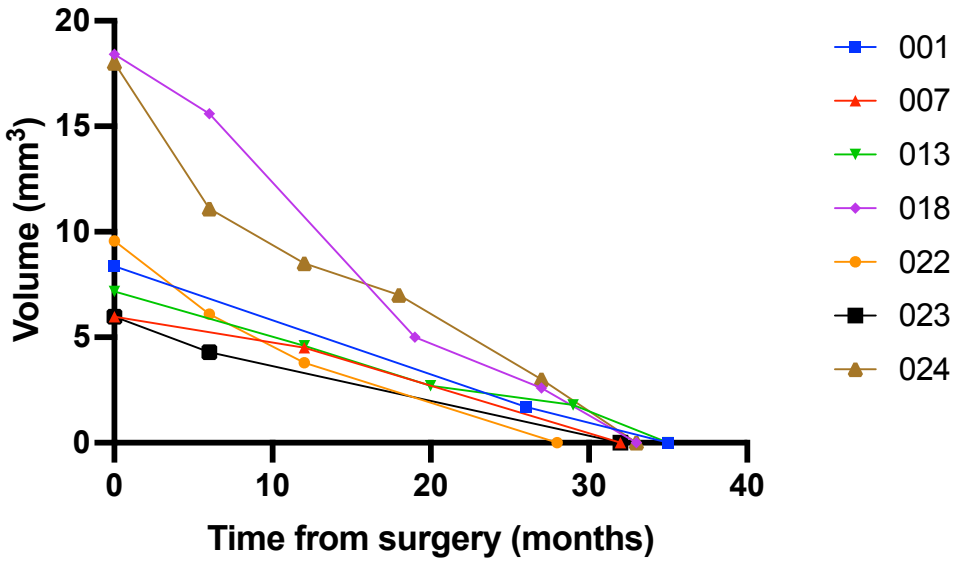
A total of 7 participants were eligible (only those without an intracardiac device were able to take part due to the electromagnetic radiation created by the three-dimensional ultrasound hardware) and available (willing to return to hospital for research visits during the COVID-19 pandemic) to take part in the extended follow-up study to evaluate the degradation of the Polynerve device in-vivo. Table 3.3

details the participants who took part in this follow-on study. Figure 3.7 details the volume measurements of the Polynerve device using Hf,3D,tUS at the various timepoints for assessment up to 35 months after surgery.

Table 3.3: UMANC trial participants eligible for Hf,3D,tUS and their nerve gap size and initial Polynerve device volume at time of surgery.

Trial I.D. Number	**Age**	**Gender**	**Date of surgery**	**Nerve Gap Size (mm)**	**Volume of implanted Polynerve device pre-op (mm^3)**
001	70	M	05/12/2017	10	8.37
007	41	M	06/03/2018	6	5.98
013	35	F	20/06/2018	8	7.18
018	24	M	16/07/2018	18.5	18.41
022	34	M	27/07/2018	12	9.57
023	56	M	22/08/2018	6	5.98
024	35	M	21/08/2018	18	18

Figure 3.7: Volumetric change of Polynerve device fitted in Phase I trial participants as measured by Hf,3D,tUS.



Volume (mm^3) change since device installation for each participant in high-frequency, three-dimensional, ultrasound scan (Hf,3D,tUS) follow-up.

Regardless of volume of the device fitted at time of surgery, all devices were fully resorbed by 35 months after surgery.

3.4 Discussion

Polynerve is a material composite of PCL and PLA polymers which are already widely used in clinical biomaterials (Manavitehrani *et al.*, 2016). Previous problems associated with their use in hollow tube nerve guidance conduits (NGCs) with similar composition, include device extrusion and abnormal wound healing (Bertleff, Meek and Nicolai, 2005). Polynerve however has an increased PCL:PLA ratio (4:1) compared to the commercially available hollow tube constructs (1:1). This confers less acidic degradation, greater flexibility and improved tensile strength. *In vivo* rat models have not demonstrated adverse reactions with its use and instead have shown comparable regenerative results to autograft (Mobasseri *et al.*, 2015) however the specific 4:1 PCL:PLA ratio has never previously been used in a biomaterial implanted into humans.

Here we have demonstrated that it is safe to use in this ratio in humans with no reported adverse events related to the device in our study population. No increased risk of infection or abnormal wound healing was reported. In addition, we have

shown that the device is fully resorbed by the body by 35 months allowing time for complete nerve regeneration to occur.

In comparison to other synthetic, biodegradable polymer nerve conduits that are simple hollow tube constructs, Polynerve contains a novel topography that enhances nerve regeneration *in vitro* and *in vivo* (Mobasseri, Terenghi and Downes, 2014)(Mobasseri *et al.*, 2015). The micropatterned grooves improve neurite outgrowth (Li, McNally and Shi, 2008) and can be further enhanced through functionalisation with extracellular matrix (ECM) proteins to improve cellular adhesion (Yu, Leipzig and Shoichet, 2008). Optimising the ECM substrate may improve Schwann cell adhesion and help to improve proliferation and maintenance of a regenerative phenotype (Xu *et al.*, 2020). Further work is needed however to improve the viability of implanted Schwann cells across NGCs which can be limited by lack of vascularisation (Balakrishnan *et al.*, 2021). Pre-vascularisation techniques or structure-based angiogenic approaches may improve vascularisation across NGCs and thus increase Schwann cell survival (Wong *et al.*, 2022).

In this first-in-man trial we have demonstrated excellent sensory recovery in our study population with 5/14 patients (011, 018, 019, 020, 022) with gap sizes ranging from 5-18.5mm achieving near complete recovery of sensation. A further 7 patients (007, 008, 010, 017, 023, 024, 013) achieved some recovery of sensation although not complete. No patient failed to improve sensory function from pre-operative levels; however, in patients 001 and 002, their s2PD scores did not improve towards the control but their locognosia scores did. Both of these patients were unable to differentiate between the two points of the discriminator on the injured side despite both insistent that their sensation was improved and locognosia testing (which also tests tactile discrimination) improving as expected. This raises the question as to the validity of s2PD testing and reliability to detect change between individuals and over time. In addition, given any significant change in locognosia scoring from pre-operative to the 12-month time point in 11/14 patients it raises doubts about the validity of this test for tactile discrimination. In patient 001, their WEST score actually deteriorated in the control digit whilst the injured side improved and in 002 their WEST score was worse on the control than the injured side, eventually improving by 6 months and deteriorating again by 12-months. Patients 019 and 020 control digit WEST scores also deteriorated over the course of the 12-month follow up period whilst patients 008, 017 and 023 had worse WEST scores in the control digit

than the injured digit prior to surgery further demonstrating the subjectivity and lack of reliability of these clinical outcome measures. However, despite these discrepancies the overall grouped median values of sensory test scores (s2PD, WEST and locognosia) from our Polynerve-repaired cohort demonstrated comparable outcomes to the those in the literature undergoing autograft (gold-standard) repair of similar nerve injuries and gap sizes (Table 3.4).

Table 3.4: Polynerve vs Autograft (text in green represents the best performing device, yellow highlighted text represents methodological differences between studies).

PAPER./STUDY TYPE/N NUMBER	NERVES REPAIRED/GAP SIZE	SENSORY OUTCOME DATA	POLYNERVE OUTCOME DATA
Weber et al. (2000). RCT. N=8	Digital Nerves/>8mm gaps	(mean) s2PD = 13.1 mm	10-20mm gaps: (mean) s2PD = 8.6mm (12 months)
Wang et al. (1996). Cohort. N=6	Digital Nerves/15-20mm gaps	(mean) s2PD = 6.7 mm (minimum 12 months)	10-20mm gaps: (mean) s2PD = 8.6mm (12 months)
Pilanci O et al. (2014). Cohort. N=12	Digital Nerves/≤20mm gaps	(mean) s2PD = 7.2 mm (mean follow-up 20.7 months (S.D. 9.3-41months)); (mean) threshold detection force ~ 0.10g	<20mm gaps (mean) s2PD = 8.1mm (12 months); (mean) threshold detection force ~ 0.45g
Stang F et al. (2013). Cohort. (cadaveric donor). N=28	Digital Nerves/(average) 22mm gaps	(mean) s2PD = 9.5 mm (average follow-up 15-16months)	<20mm gaps (mean) s2PD = 8.1mm (12 months)
Unal M et al. (2017). Cohort. N=11	Digital Nerves/(mean) 18.3mm gaps	(mean) s2PD = 5.9mm (mean follow-up 35 months); (mean) threshold detection force ~0.38g	10-20mm gaps: (mean) s2PD = 8.6mm (12 months); (mean) threshold detection force ~0.50g

In comparison to the available literature reporting on the clinical use of hollow tube synthetic conduits, Polynerve has demonstrated improved sensory outcomes in similar types of nerve injury and gap sizes with a much lower complication profile (Table 3.5). In comparison to cohort studies utilising Neurolac® to repair digital nerve gap injuries <20mm Bertleff et al. (2005) (Bertleff, Meek and Nicolai, 2005) (n=17) reported that 3 patients had "wound healing problems", whilst removal of the device was required in 1 patient. s2PD results were similar to Polynerve patients with gaps <10mm however pressure threshold testing was much worse than Polynerve by 12 months (20g/mm^2 vs 0.4g/mm^2). Chiriac *et al*. (2012) (Chiriac *et al.*, 2011) (n=23 patients) reported 2 patients with device fistulation/extrusion and 17/28 nerves injured having no recovery of sensation at all. Costa Serrão de Araújo et al. (Costa Serrão de Araújo *et al.*, 2017) (n=12) reported 3 patients with worsening pain requiring revision surgery, however in the remaining patients the mean s2PD = 8.1mm by 12-months which was equivalent to Polynerve.

Table 3.5 Polynerve vs Synthetic Conduits (text in green represents the best performing device).

PAPER/CONDUIT MATERIAL/STUDY/N NUMBER	NERVES REPAIRED/GAP SIZE	SAFETY DATA	OUTCOME DATA	POLYNERVE OUTCOME DATA
Weber et al. (2000): hollow PGA conduit. RCT. N=62 (number of nerves)	Digital Nerves/mean 7mm (+/-5.6) gaps	3 patients had device extrusion.	(mean) s2PD = 10.3mm (9 months)	<10mm gaps: (mean) s2PD = 7.6mm (12 months)
Battiston et al. (2005): hollow PGA conduit. Cohort. N=16	Digital nerve gaps/mean 2.0 mm	-	(mean) m2PD = 9.58mm (30 months)	<10mm gaps: (mean) s2PD = 7.6mm (12 months)
Bertleff et al. (2005): hollow PCL/PLA (Neurolac) conduit. RCT. N=17	Digital nerve gaps/average 6-8mm	3 patients had "wound healing problems", removal of device required in 1 patient.	average s2PD = 8mm (12months), m2PD = 9.5mm (12 months) and about	<10mm gaps: (mean) s2PD = 7.6mm (12 months) ~0.4g/mm^2 force threshold (12 months)

			20g/mm^2 force threshold (12 months)	
Chiriac et al. (2012): hollow PCL/PLA (Neurolac) conduit. Cohort. N=23 (11 digital nerves)	Digital nerve gaps/mean 12mm	2 patients had device fistulation/extrusion. 17/28 nerves injured had no recovery of sensation (Weber >30 and Semmes-Weinstein ≥75)	In digital nerve patients: (mean) s2PD = 19.8mm (mean-19months), (mean) WEST = 31.9g	10-20mm gaps: (mean) s2PD = 8.6mm (12 months), (mean) WEST = 0.50g
Costa Serrão de Araújo et al. (2017): hollow PCL/PLA (Neurolac) conduit. Cohort. N=12 (9 digital nerves)	Digital nerve and wrist sensory nerve gaps <20mm (no specification of the treated gap sizes)	3 patients "worsening pain" requiring revision surgery.	In digital nerve patients (not requiring revision surgery): mean s2PD 8.1mm (at 12 months) and (median) WEST score of 4	<20mm gaps (mean) s2PD = 8.1mm (12 months) and (median) WEST score of 4

Although Polynerve appears to have a better safety and regenerative outcome profile than other synthetic hollow tube conduits trialled in digital sensory nerve gap cohorts, we did not directly compare Polynerve to autograft, allograft or other nerve conduits which would provide a better comparison of its efficacy in nerve regeneration. In addition, further phase II/III clinical trials are needed to investigate Polynerve's safety profile in a much larger patient cohort.

No clinical study to date, utilising synthetic NGCs, has demonstrated the length of time taken for device resorption. An important consideration for clinicians and patients, especially if complications occur at a later timepoint after surgery and given these devices are inserted into palmar tissues which may be more regularly

noticeable to patients. We utilised a novel imaging technique in Hf,3D,tUS in order to visualise the remnant Polynerve material at sequential timepoints after implantation. We found that the Polynerve device was fully resorbed by 35 months after surgery which was expected given the long degradation time of PCL of 2-3 years (Middleton and Tipton, 2000; Nair and Laurencin, 2007). It remains to be investigated as to whether this slow degradation rate of the Polynerve device would cause any functional impairment for patients during their recovery.

We have demonstrated that Polynerve is safe to use in humans, with no evidence of adverse events or adverse device effects demonstrated in our patient cohort (n=17; <20mm sensory nerve gaps). Further phase II/III trials are needed to evaluate safety further in addition to further preclinical studies to assess Polynerve's utility in mixed nerve gap injuries or longer nerve gaps. Although sensory outcomes appear to be good compared to other hollow synthetic NGCs a randomised trial comparing Polynerve, Neurolac® and autograft would provide much greater insight into the comparisons between Polynerve and Neurolac® and how they both compared to the "gold standard" autograft. One concern highlighted by this study however is the poor validity and reliability of commonly used sensory outcome measures. Without robust measures of peripheral nerve regeneration, we cannot effectively compare outcomes between different NGCs/treatments. A consensus on which outcome measures to use and when is needed so clearer comparisons can be made between nerve injury centres. In addition, more objective measures of peripheral nerve regeneration that remove subjectivity and are valid and reliable are needed to improve reporting of outcomes in PNI research.

CHAPTER 4 Systematic review of outcome measures used in clinical peripheral nerve injury research

This chapter has been published in Neurosurgery:

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https://doi.org/10.1093/neuros/nyab060

4.1 Introduction

PNI is a significant health problem, and despite targeted microsurgical interventions, no patient with a major nerve injury ever regains full, preinjury levels of function (Altissimi, Mancini and Azzarà, 1991; Al-Ghazal *et al.*, 1994; Dahlin, 2008; Kachramanoglou *et al.*, 2017). This clinical unmet need requires best practices adopted as widely as possible and ultimately new interventions to improve functional outcomes for patients. Several groups worldwide report on a variety of surgical interventions and new discoveries in nerve biology and materials science lend itself to translating new technologies; however, in order to adopt or develop the best of these approaches, clinicians, scientists, and other researchers must be able to compare treatments between patients and between distinct surgical units to allow accurate assessment of their treatment effect. Currently, very few novel treatments are reaching the clinical arena at least in part due to the inconsistent reporting of outcomes following nerve repair surgery.

Multiple outcome measures of nerve regeneration in humans exist, and in previous research these have been inconsistently categorized into several broad domains: sensory, motor, function, PROMs, pain, and finally, neurotrophic measures, a term used to describe measures that indirectly examine nerve regeneration at the repair site, end organ, or centrally within the brain. Within each domain several distinct outcome measures exist, but there is no collective agreement across researchers and clinicians in deciding which to use and in what circumstances. Literature reviews have highlighted the inconsistencies in reporting of functional outcomes (Wood *et al.*, 2011; Wang, Sunitha and Chung, 2013; Rayner *et al.*, 2020), and a recent systematic review of outcome reporting in brachial plexus injury has demonstrated the variety in use (Dy *et al.*, 2015). The latter study focused on motor outcome reporting following plexus injury; therefore, the landscape of outcome measure reporting in each of the other critical domains and following PNIs distal to the brachial plexus remains largely unknown.

Objectives

The aims of this study were to classify outcome measures used in PNI of the upper limb into clinically relevant domains, determine the frequency of use by anatomical

site of injury, describe the range of time points after injury where the outcome measures are used, and identify common areas of inconsistencies in their reporting

4.2 Methods

Protocol and registration

This systematic review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (Liberati *et al.*, 2009). The protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO), study CRD42018103001, and is available from https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42018103001

Search and eligibility criteria

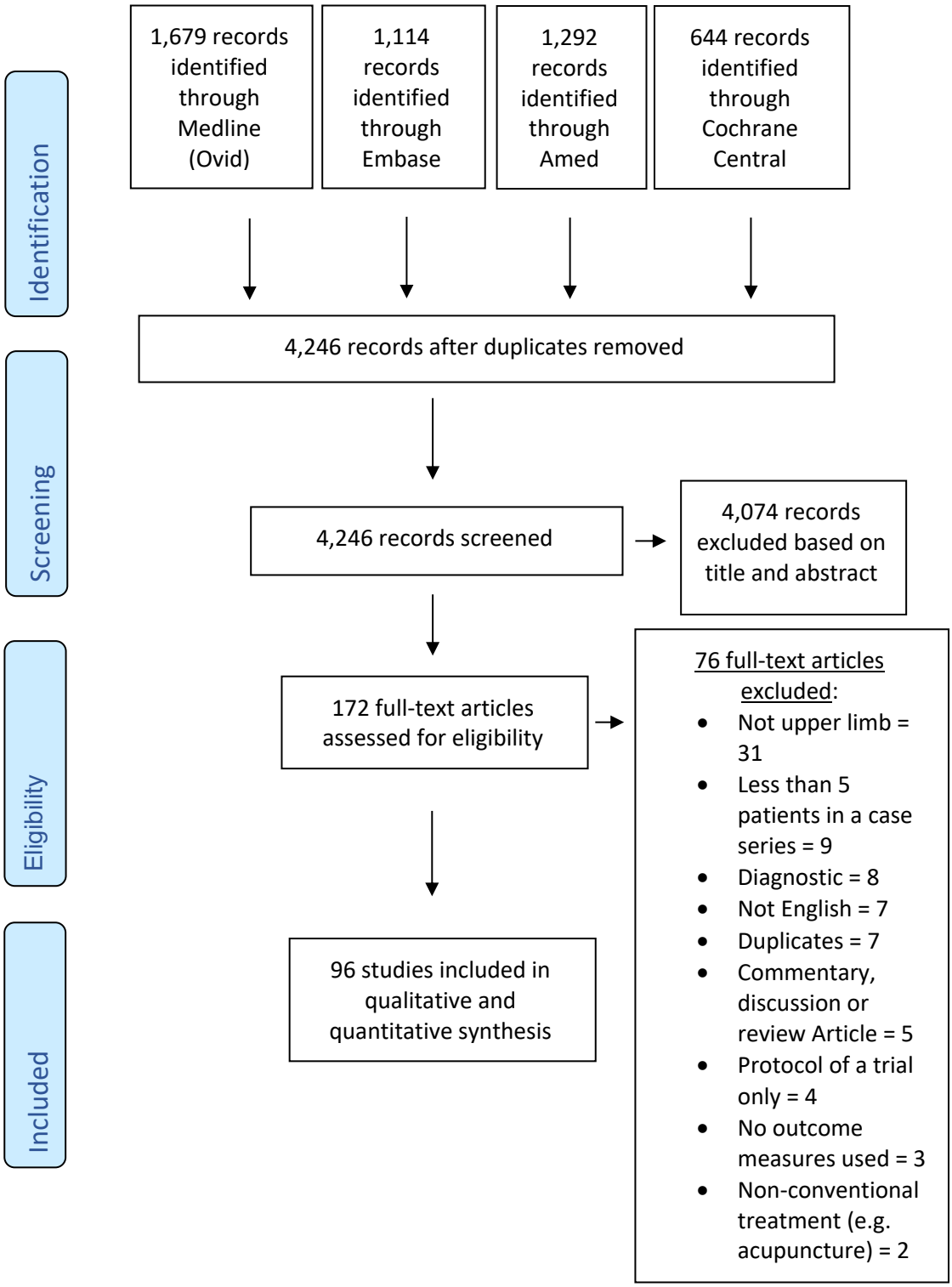
We performed a specific search of the English-language literature on January 8, 2019, using MEDLINE, EMBASE, AMED and Cochrane Central Register of Controlled Trials databases (all years considered up to the date of the searches). We searched these databases using the following keywords: “peripheral nerve inj*” (key word) or “nerve regeneration” AND “peripheral nerves” (subject headings). The searches were conducted by the principal author, who expanded the key words into corresponding Medical Subject Heading terms. The broad nature of the search was chosen to be as inclusive as possible for potentially relevant studies.

Inclusion and exclusion criteria

We included articles that were (1) in English; (2) whose full text was available; and (3) involved adults (>18 yr old) (4) with a PNI (5) of the upper limb, (6) where a measurement of outcome was reported. Exclusion criteria were studies that (1) described cadaveric or non-human studies; (2) were only conference abstracts; (3) did not primarily involve the upper limb (minimum 5 patients with upper-limb injuries); (4) were case series with less than 5 patients; (5) were diagnostic studies; (6) commentaries, discussions, or literature reviews; (7) trial protocols; (8) studies not involving any outcome measures; and (9) those studies employing purely nonconventional treatments for PNI (eg, acupuncture) (Figure 4.1). Lower limb

nerve injuries were excluded, as the functional demands of a lower limb are different and would require a dedicated suite of outcome measures, some of which will overlap with upper limb.

Figure 4.1: Flowchart demonstrating study selection for inclusion in systematic review.



Study selection and data extraction

All articles underwent a title/abstract screen for inclusion eligibility by 2 independent reviewers (Ralph Murphy and Sahiba Singh). Those deemed potentially relevant were obtained as full-length papers and screened for eligibility by the same 2 reviewers. After each screening round, the 2 reviewers met to consolidate inclusion/exclusion decisions. Disagreements were arbitrated by the senior author (Adam Reid).

A data extraction form was produced a priori to collect the following from each article: authors; journal; year of publication; title; geographical location; number of patients; nerves injured; anatomical location of nerves (if available); injury type; intervention; study type; outcome/outcome domain; outcome measurement, eg, technique/instrument; specific metric/format of outcome data from each participant used for analysis and the specific time points used for analysis. Each identified outcome was classified into 1 of 10 outcome domains. Data were extracted independently by 2 reviewers (Ralph Murphy and Heba Elsayed) and results were consolidated.

Appraisal and synthesis of results

Outcomes were extracted verbatim from source papers and then grouped within a domain based on their given or implied definition using a “best-fit” approach (Table 4.1) (Williamson *et al.*, 2017). Sensory and motor domains were further subclassified into objective and subjective subdomains based on the subjective or objective nature of the outcome measure. All authors were involved in this process encompassing a multidisciplinary group of researchers and clinicians. The data extracted from each study using our data extraction proforma was tabulated by domain and accompanied by a narrative review. We calculated how frequently each outcome measure was reported within these domains, how often a named specific instrument was used (eg, scale, clinical test, or piece of equipment) to measure the outcome, how often a specific metric or format of recorded outcome data from each participant was used to record the measurement (eg, force (in g or g/mm^2)), and the frequency of time-point specification from injury or surgery was recorded.

Table 4.1: Outcome Measure Domains

Outcome Measure Domain	Definition	Examples
Sensory Objective	Objective assessment of sensory receptor reinnervation.	Tactile gnosis (static two-point discrimination)
Sensory Subjective	Subjective assessment of sensory receptor reinnervation.	Medical Research Council Sensory Scale
Motor Objective	Objective assessment of muscle reinnervation.	Dynamometry of grip or pinch strength
Motor Subjective	Subjective assessment of muscle reinnervation.	Muscle strength (British Medical Research Council Grading)
Sensorimotor Function	Objective assessment of composite functions (combined sensory and motor reinnervation)	Moberg's Pick-up Test
Psychology & Wellbeing	Assessment of the psychological progress during regeneration.	Hospital Anxiety and Depression Scale (HADS)
Disability	Assessment of disability caused by peripheral nerve injury	Disabilities of the Arm, Shoulder and Hand (DASH) score; Groningen Activity Restriction Scale
Quality of Life	Assessment of quality of life after peripheral nerve injury.	The sense of coherence 13-item scale
Pain and Discomfort	Assessment of the pain or discomfort felt by the patient after peripheral nerve injury.	The numeric pain rating scale
Neurotrophic Measures (End-organ) (At the repair site) (Central Nervous System (CNS))	Assessment of regeneration along the anatomical axis of the peripheral nervous system after injury.	End-organ: Computed Tomography cross sectional area of muscle. Repair site: Tinel's test CNS: functional magnetic resonance imaging

We subsequently grouped outcome measures by anatomical site of injury in order to identify the most commonly used (in 3 or more studies) outcome measures for

hand sensory nerves, mixed (motor and sensory) upper limb nerves, and nerves of the brachial plexus.

4.3 Results

Study selection

The electronic database search yielded 4246 articles, of which 96 remained eligible for inclusion (Figure 4.1). Of the 96 studies included in the final analysis, there were 15 randomized control trials (RCTs), 8 case-control studies, 18 cohort studies, 5 observational studies, and the remainder were case-series or retrospective reviews. A total of 56 individual outcome measures were utilized across 28 different countries with 7097 patients included. A total of 16 studies involved injury to the brachial plexus, 59 studies involved injury to mixed (motor/sensory) upper limb nerves (distal to the brachial plexus and proximal to the hand), and 17 studies involved sensory nerve injuries of the hand.

Domain Categorization

Ten domains were used to categorize the 56 outcome measures identified through our search (Table 4.1). No study included all 56 outcome measures nor did any study utilize an outcome measure from all 10 domains. The most widely utilized outcome measures were 2-point discrimination, static (30 studies) or moving (16 studies) to assess tactile discrimination and the MRC Scale for assessment of motor function (30 studies).

A more detailed review of outcome measures used within each domain is included in supplementary tables 1-8 (Appendix 3).

Specific instrument

Only one study did not specify the instrument being used for quantification of results. Gordh et al (Gordh *et al.*, 2008) used a cold metal roller to assess cold at baseline but did not perform any further measurements and did not specify the method of quantitation of the cold threshold. Sensory and motor outcomes (Appendix 3: Supplementary Tables 1 and 2) were most often assessed using well-known

instruments. Sensorimotor function and psychology and well-being (Appendix 3: Supplementary Tables 3 and 4) were frequently assessed by a range of different instruments or scoring systems with no commonly (used in 3 or more studies) used instruments. Disability (Appendix 3: Supplementary Table 5) was most commonly assessed using the Disabilities of the Arm, Shoulder and Hand (DASH) PROM and was used in 8/11 studies assessing disability. Quality of life (Appendix 3: Supplementary Table 6) was commonly assessed using the Short Form-36 (SF-36) PROM and was used in 4/7 studies assessing quality-of-life outcomes. Pain and discomfort was measured using a variety of instruments (Appendix 3: Supplementary Table 7) but pain intensity scales (visual analogue scale [VAS] and Numeric Pain Rating Scale [NPRS]) were the most commonly used in 17/18 studies assessing pain and discomfort. Electrophysiology was the most commonly used instrument to assess neurotrophic outcomes (Appendix 3: Supplementary Table 8) in 14/17 studies.

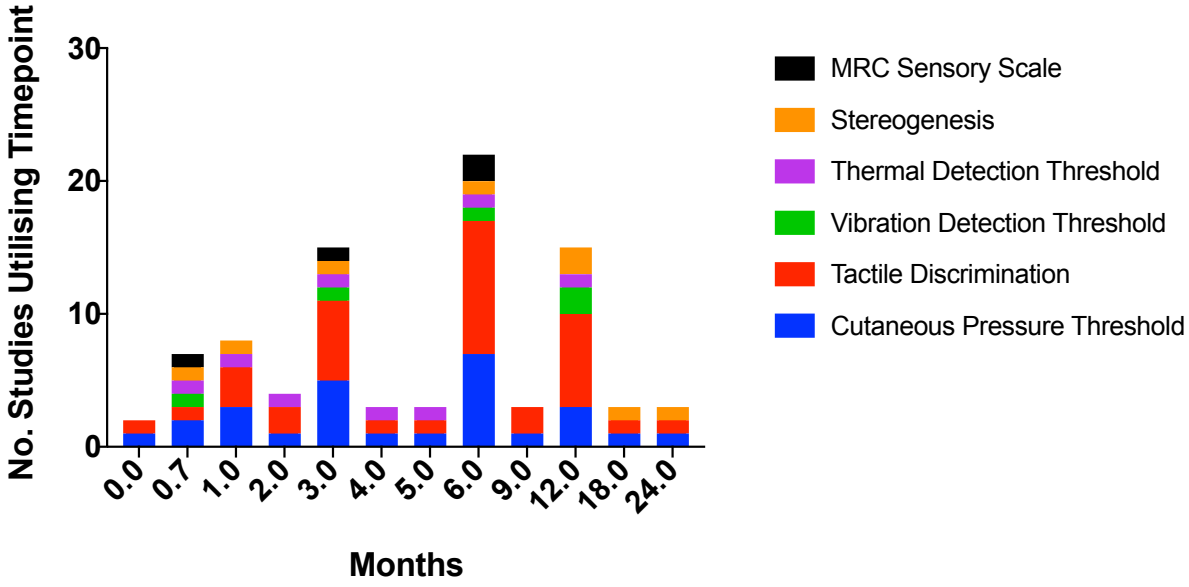
Specific metric usage

The specific metric or unit of outcome measurement was uniformly well described in all domains (Appendix 3: Supplementary Tables 1-8).

Time points for assessment

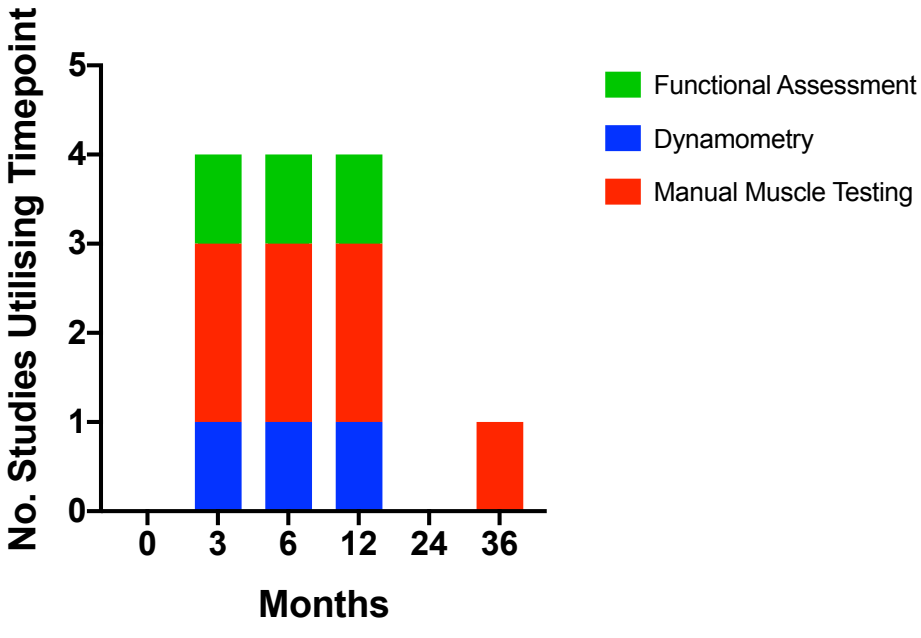
The time points from injury or surgery used for assessment of outcomes were highly variable with the majority of studies defining a range as opposed to specific time points. For sensory and motor outcome measurements, most studies specified time points for assessment since injury/surgery and thus a more detailed analysis was performed (Figures 4.2 and 4.3). Most studies performed sensory and motor assessments at 3, 6, and 12 months after injury/surgery, with only 4 studies continuing sensory or motor measurements after 12 months of follow-up.

Figure 4.2: Cumulative bar chart of time points for sensory outcome measure use.



Time points for the usage of sensory outcome measures varied widely; however, there was a clear trend in their use (Figure 4.2). Most studies obtained a baseline or early estimate of sensation at time 0, 3 weeks, or 1 month after surgery (or after injury in observational studies). After this, the majority of studies utilized sensory outcome measures at 3 and 6 months with the final assessment at 12 months. A small minority of studies continued sensory assessments of any modality past 12 months, with no study making a sensory assessment after 24 months.

Figure 4.3: Cumulative bar chart of time points for motor outcome measure use.



Time points for the usage of motor outcome measures followed a common trend (Figure 4.3). No study undertook a baseline assessment up to 1 month after surgery or injury. Instead, all studies measured outcomes at 3, 6, and 12 months. Similarly, to sensory outcomes, the end point for motor outcome assessment was 12 months with only 1 study making an assessment after this at 36 months.

Disability outcome reporting time points were specified in 3/8 studies (Appendix 3: Supplementary Table 5) utilizing the DASH which was used monthly post-injury/surgery up to 6 months in hand sensory nerve injured patients (Wong *et al.*, 2015); at 3, 6, and 12 months postoperatively in mixed (sensory/motor) upper limb nerve injuries (Bai *et al.*, 2015) and at 12 months postoperatively in patients undergoing nerve transfers for upper root brachial plexus injuries (Ferreira, Martins and Siqueira, 2017). The SF-36 PROMs' usage time points for the assessment of quality of life were specified in 2/4 studies, at baseline, and then at 6 months post-surgery (Colini Baldeschi *et al.*, 2017) and at 7 time points over a 15-wk period in a drug trial to treat neuropathic pain (Gordh *et al.*, 2008). The time points for the use of pain intensity scales VAS and NPRS since PNI or surgery was often specified (10/12 studies utilizing VAS and 5/5 studies utilizing NPRS). VAS was used preoperatively, monthly in the first 6 months, then every 6 months for 2 years (Eisenberg, Waisbrod and Gerbershagen, 2004) and preop then 1, 3, and 6 months after surgery (Colini Baldeschi *et al.*, 2017). The NPRS was used 1 week preop and 1, 3, and 6 months postoperatively (Sumitani *et al.*, 2008). Neurotrophic measures were used at wide-ranging time points after injury/surgery (Appendix 3: Supplementary Table 8) with only 4/17 studies specifying time points for assessment of which 3 utilized electrophysiology. This was used at 1, 3, 6, 12, 18, and 24 months (Krarup *et al.*, 2017); 8 and 12 months (Mackel, 1985); and 12, 18, and 24 months postoperatively (Becker *et al.*, 2002).

Summary of Outcome Measure Use at Different Anatomical Sites of Injury Brachial Plexus (Total = 16 Studies)

In 16 studies of brachial plexus injures, outcome measures were reported across 6 domains with a clear focus on motor assessment: motor objective (3/16 studies) and motor subjective (8/16 studies), disability (DASH, 3/16 studies), quality of life (SF-36, 3/16 studies), pain and discomfort (VAS, 3/16 studies), and neurotrophic measures (electrophysiology, 3/16 studies) (Figure 4.4).

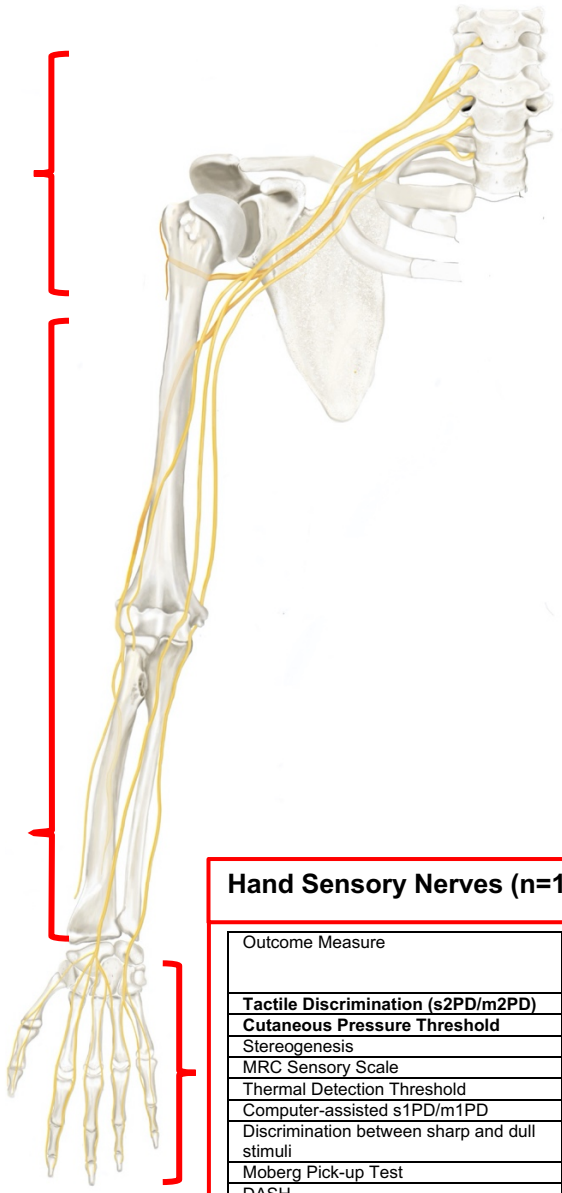
Figure 4.4: Frequency of outcome measure use by anatomical location of peripheral nerve injury (brachial plexus, motor/sensory upper limb nerves, purely sensory nerves of the hand) (4 studies did not specify anatomical location of nerve injuries).

Brachial Plexus (n=16)

Outcome Measure	Number of studies
Manual Muscle Testing (MRC Motor Scale)	**5**
DASH	**3**
Dynamometry	**3**
Range of Motion	**3**
SF-36	**3**
Pain VAS	**3**
Electrophysiology	**3**
Cutaneous Pressure Threshold	2
Tactile Discrimination (s2PD/m2PD)	2
Manual Muscle Testing (Louisiana State Scale)	2
Clinician administered DN4 Questionnaire	2
Vibration Detection Threshold	1
Thermal Detection Threshold	1
MRC Sensory Scale	1
PGIC	1
Modified Rankin Scale	1
Overall Neuropathy Limitations Scale (OLNS)	1
Short-Form McGill Pain Questionnaire (SF-MPQ)	1
Pain Catastrophizing Scale	1
Cold Intolerance Severity Score	1
Hospital Anxiety and Depression Scale (HADS)	1
Posttraumatic Stress Disorder Checklist-Civilian Version (PCL-C)	1
Beck Depression Inventory (BDI)	1
MR Neurography	1

Mixed (Motor/Sensory) Upper Limb Nerves (n=59)

Outcome Measure	Number of Studies
Manual Muscle Testing (MRC Motor Scale)	**21**
Tactile Discrimination (s2PD/m2PD)	**13**
Pain VAS	**12**
Numeric Pain Rating Scale	**11**
MRC Sensory Scale	**10**
Electrophysiology	**9**
Cutaneous Pressure Threshold	**8**
Thermal Detection Threshold	**8**
Stereogenesis	**8**
Mechanical/Dynamic Allodynia	**8**
Vibration Detection Threshold	**6**
Dynamometry	**6**
Imaging (fMRI, MRI-muscular cross-sectional area; Near-nerve stimulation, CT and USS-cross sectional area of innervated muscle, neuroimaging, 3D cortical mapping)	**5**
DASH	**4**
Sensorimotor Function	**4**
Thermal &/Mechanical Pain Threshold	**4**
(Motor) Functional Assessment	**3**
SF-36	**3**
Cold Intolerance Severity Score	**3**
Patient Global Impression of Change (PGIC)	2
Wind-up ratio	2
Pain Catastrophizing Scale	2
Mechanical Pain Scale	2
Neuroticism, Extraversion, and Openness (NEO) Five Factory Inventory	2
Cognitive Capacity Test	2
Ninhydrin Sweat Test	1
Mayo Elbow Score	1
Sense of Coherence Scale (autogenetic theory)	1
Clinical Global Impression of Change (CGIC). Used by investigator	1
qDASH	
Questionnaire to assess consequences of nerve injury	1
Groningen Activity Restriction Scale	1
Instrumental Activities of Daily Life (IADL)	1
Overall Neuropathy Limitations Scale (OLNS)	1
Hospital Anxiety and Depression Scale (HADS)	1
Tinel's	1



Hand Sensory Nerves (n=17)

Outcome Measure	Number of Studies
Tactile Discrimination (s2PD/m2PD)	**14**
Cutaneous Pressure Threshold	**12**
Stereogenesis	2
MRC Sensory Scale	2
Thermal Detection Threshold	1
Computer-assisted s1PD/m1PD	1
Discrimination between sharp and dull stimuli	1
Moberg Pick-up Test	1
DASH	1
Occupational Performance Model	1
Cold Intolerance Severity Score	1
Tinel's	1
Electrophysiology	1

Image courtesy of Georgia Savvides, University of Manchester: georgia.savvides@student.manchester.ac.uk

Mixed (Motor and Sensory) Upper Limb Nerve Injury (Total = 59 Studies)

In mixed (motor and sensory) upper limb (distal to brachial plexus and proximal to wrist) nerve injury studies, there was a much broader spread of outcome measure use with 41 outcome measures used across 59 studies (Figure 4.4). Subjective motor assessment, the MRC Motor Scale, was the most commonly used single outcome measure (21/59 studies). However, sensory outcome measures were chosen in 54/59 studies compared to motor outcome measures in only 32/59 studies. Pain and discomfort (44/59 studies) most commonly assessed using VAS (12/59 studies) or NPRS (11/59 studies). Novel neurotrophic measures were utilized in 5/59 studies. Studies examined the central nervous system response to PNI using functional magnetic resonance imaging (MRI), 3D cortical mapping/thickness (MRI), or cerebral blood flow (positron emission tomography scanning) or they examined end-organ changes, most commonly muscle cross-sectional area using either MRI, computerized tomography, or ultrasound scanning (Appendix 3: Supplementary Table 8).

Hand Sensory Nerve Injury (Total = 17 Studies)

In 17 studies of hand sensory nerve injuries, objective sensory measures were most commonly utilized with tactile discrimination (14/17 studies) and cutaneous pressure threshold (12/17 studies), the 2 most commonly used (Figure 4.4). Only 2/17 studies assessed disability after hand sensory nerve injury with one study using the DASH questionnaire and another using the occupational performance model (Townsend, Brintnell and Staisey, 1990).

4.4 Discussion

This systematic review highlights inconsistencies in use and reporting of outcome measures and their results within the PNI literature. This is important because a lack of clarity and standardization of assessment in these life changing injuries precludes meaningful comparisons between patients and across patient cohorts subject to differing interventions. Furthermore, this has a negative impact on the translation of novel treatments or outcome methodologies into clinical trials. To date, there have

been no systematic reviews of outcome measure use across the large and varied spectrum of clinical upper limb PNI research.

Systematic reviews of outcome measure use in clinical brachial plexus research have demonstrated a focus on measures of motor recovery with a lack of data collected on patient-centred outcomes such as quality of life or the effect on mental health, specifically anxiety, and depression (Dy *et al.*, 2015)(Miller *et al.*, 2017). We have found similar results in our systematic review, which has highlighted a clear trend toward motor outcome reporting in brachial plexus studies, with a focus on subjective measures versus objective measures of motor function (Appendix 3: Supplementary Table 2 and Figure 4). This may be due to a lack of PROMs available for use after PNI that effectively assess patient symptoms and function (Fonseca *et al.*, 2018). In our systematic review, we have also demonstrated a widely heterogeneous use of outcome measures in mixed upper limb nerve injuries and a clear focus on objective sensory outcome measures in hand sensory nerve injury with only 2 studies assessing disability, using tools that are not validated for use in hand sensory nerve injury.

Time points for assessment were highly variable and appeared to follow arbitrary end points. In distal sensory injuries or mixed upper limb nerve injuries where the regenerative distance to the end organ is less than 300 mm, follow-up of 12 mo is logical given regenerative rates of approximately 1 mm/day in humans (Pfister *et al.*, 2011; Grinsell and Keating, 2014). In brachial plexus injuries where the distance to the end organ can be 800 mm or more (Pfister *et al.*, 2011), follow-up of 2 to 3 yr may be more appropriate. Standardizing time points for measurement will ensure comparable phases of post-treatment recovery, which is particularly important for PNI at different levels of injury.

Our primary aim was to describe and classify outcome measures used in clinical PNI research. The 56 individual outcome measures were grouped into 10 core domains based on their implied definition and using a “best-fit” approach. There is no consensus on how to categorize outcome domains (Boers *et al.*, 2014; Smith *et al.*, 2015; Dodd *et al.*, 2018) and where domain taxonomies have been published for medical research (Dodd *et al.*, 2018) it was unclear how PNI outcome measures would fit into these domains. Previous reviews have suggested domains such as sensory function, motor function, pain and discomfort, PROMs, and

neurophysiological outcome measurements (Wang, Sunitha and Chung, 2013; Rayner *et al.*, 2020). We utilized these domains within our taxonomy but added new domains given the large number of diverse PNI outcome measures identified.

Within this domain structure, each outcome measure is likely to be of differing importance to the critical stakeholders (patients/clinicians/researchers/healthcare funders); however, per-haps the most significant determinant of their relevance will be the anatomical level of PNI. Therefore, allocating outcome measures within each domain that are appropriate to anatomical level seems a pragmatic next step. Historically, there has been a focus on domains such as sensory and motor function as a barometer of success of repair and regeneration. While biological outcomes are clearly important, PNI and its complex interplay with the brain, highlighted eloquently in Lundborg''s “The Hand and the Brain,” (Lundborg, 2014) cannot so easily be assessed using just biological outcome measures. Domains such as psychology and well-being, disability, and quality of life need to be explored further and included in future assessment of PNI patients. In addition, neurotrophic measures utilizing the latest imaging technology are likely to provide even greater insight into the interplay between PNI and the central nervous system.

Collating and describing these measures into domains allows us to begin the process of stratifying this wide selection of outcome measures into a core outcome set (COS). The Core Outcome Measures in Effectiveness Trials initiative began in 2010 to develop COS in clinical trials (Williamson *et al.*, 2017). They have published a set of 11 standards required for the development of COS (Kirkham *et al.*, 2017). This systematic review has met the first 4 of these standards that look to specify the scope of the COS in PNI surgery. The remaining standards require definition of the stakeholders involved (standards 5-7) and subsequently development of the consensus process (standards 8-11) utilizing the scope of the COS between stakeholders (Kirkham *et al.*, 2017).

Limitations

Limitations of this study include the search for articles that were solely retrieved from established databases with no additional manual searches conducted; in addition, articles not published in English were excluded. International publications were included, however. The results of this systematic review reveal important trends in

outcome measure use in PNI research that could be used to help inform the development of a core outcome set.

Conclusion

We have described and categorized outcome measure use in clinical upper limb PNI research identifying a lack of consensus among researchers over which outcome measures to use for a particular PNI. Common time points for the use of sensory and motor outcome assessment have been established, and we have demonstrated a lack of validated measures of psychology and well-being, disability, and quality of life after PNI of the upper limb.

We now need to develop a COS of validated outcome measures for PNI research that are inclusive of patient-reported measurements of psychology and well-being, disability, and quality of life. Objective and sensitive measures of PNI that are inclusive of all stakeholders (patients, clinicians, and researchers) will allow us to collect effective data that are comparable between PNI centers both nationally and internationally. In addition, it would allow researchers and policymakers to develop more accurate guidelines for the management of PNI patients, which in turn would standardize care and improve outcomes.

CHAPTER 5 Optical coherence tomography in digital nerve injury (ODEN Study)

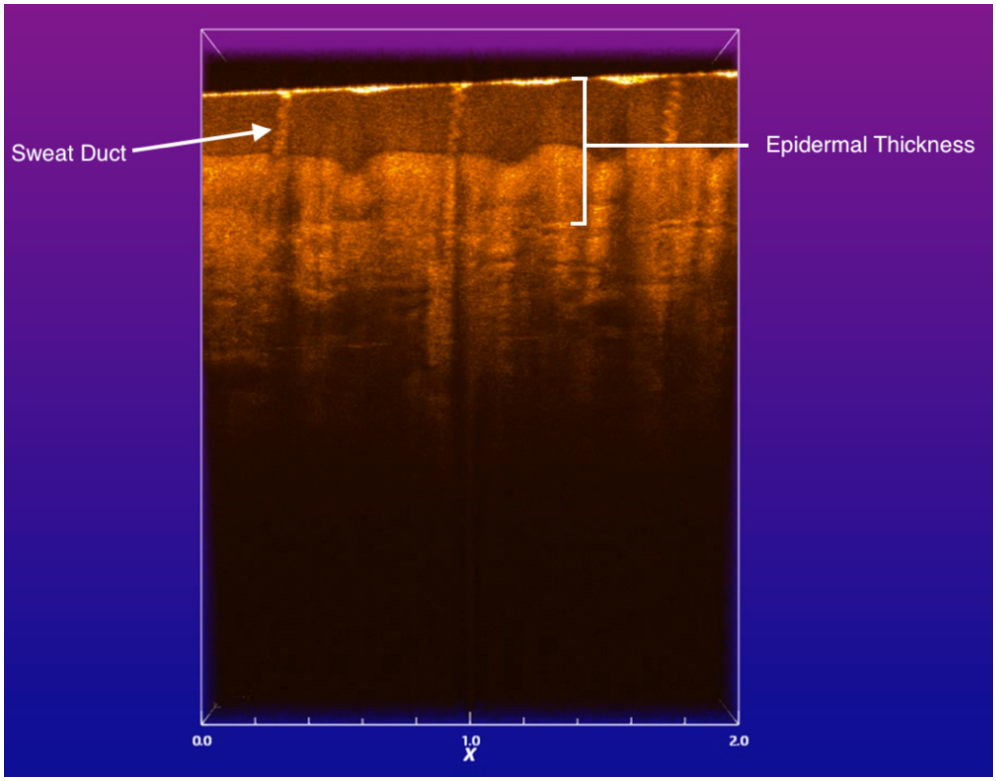
5.1 Introduction:

PNIs are common and significantly impact on patients' lives with reduced sensation, pain and cold intolerance frequently leading to an inability to work or return to normal activities after injury. Microsurgical repair techniques have developed over recent decades yet there remains clinical uncertainty as to which operations will result in the best outcomes; and the development of novel therapies has been hindered by the inability to detect incremental improvements in nerve regeneration across heterogeneous patient groups and injury patterns. We now need non-invasive, reproducible, objective methodologies to evaluate the success of these novel treatments and to optimise their delivery at the right time and to the right patient.

Clinical management of PNI has not advanced significantly over recent decades despite greater scientific insight into the fundamental biology of peripheral nerve regeneration. This is due in part, to the lack of sensitive and objective outcome measures to compare novel treatments both in the lab and in clinical trials. Optical coherence tomography (OCT) is a light-based imaging technique analogous to ultrasound that is rapid, non-invasive and can measure epidermal thickness and sweat duct density. Our preliminary data suggests that these measures bear a correlation to the degree of nerve regeneration into skin and further investigation is required.

An initial proof of concept study performed by our group using OCT has demonstrated its effectiveness at analysing epidermal thickness and sweat gland density in nerve injured patients (Figure 5.1).

Figure 5.1: Two-Dimensional Optical Coherence Tomography Image of volar hand skin.



A sweat duct (white arrow) and the epidermal layer are highlighted on this two-dimensional image of uninjured volar finger pulp skin (the X axis at the bottom demonstrates a scale in millimetres).

Our aims were two-fold. Firstly, we sought to determine the mean epidermal thickness and sweat duct density of volar digital skin in a healthy, non-injured population and investigate whether differences exist between genders, hand dominance, age or ethnic background. Secondly we investigated the predictive capacity of skin changes (epidermal thickness and SDD) as measured by OCT to monitor nerve regeneration as compared to current clinical outcome measures.

5.2 Methods:

A non-randomised longitudinal study of patients with injured sensory nerves in the hand who underwent surgical repair with primary neurorrhaphy and were followed-up for 6 months. Patients were seen as out-patients and scanned at regular intervals following the standard course of care (1-week post-operative visit served as baseline) with further research visits at 4-/8-/12-weeks and 6-months. This study was purely observational and no change to standardised treatment was made.

At each visit, OCT scans were performed of the injured digits at three points along the hemi-pulp and the same hemi-pulp of the contralateral control finger (Figure 5.2). In addition, clinical sensory outcome measures were used to assess the degree of sensory nerve regeneration and patient-reported outcome measures (DASH and iHand) completed to assess the impact on the patient's life.

Healthy non-injured members of staff and students at Manchester University NHS Foundation Trust and the University of Manchester were recruited to generate the normative data.

The trial was conducted in accordance with the principles of GCP. The UK Health Research Authority (HRA) provided ethical permissions for the clinical investigation through the London-Fulham Research Ethics Committee (REC reference: 19/LO/0691).

Outcome

Primary:

- Changes in SDD and epidermal thickness as measured by OCT.

Secondary:

- Sensory outcome measures – 2PD, WEST monofilament and locognosia.
- Patient-Reported Outcome Measurement (PROMs) – Disabilities of the Arm, Shoulder and Hand (DASH) and Impact of Hand Nerve Disorders (I-HaND) questionnaires.

Eligibility Criteria

Non-injured cohort: Healthy non-injured adults willing to consent to have their finger skin measured by OCT.

Injured cohort: Adult patients between 18–- 80 years old with a sensory nerve injury to the hand who had undergone primary neurorrhaphy and had the capacity to consent for the study.

For the non-injured cohort, we included any adult over 18 years old with the capacity to consent and no previous upper limb nerve injury. For the injured cohort we included adult patients (18 – 80 years old) with a sensory PNI of the hand, who had direct, epineurial surgical repair within 1 week after injury. These patients were required to have the capacity to consent onto the study.

OCT imaging

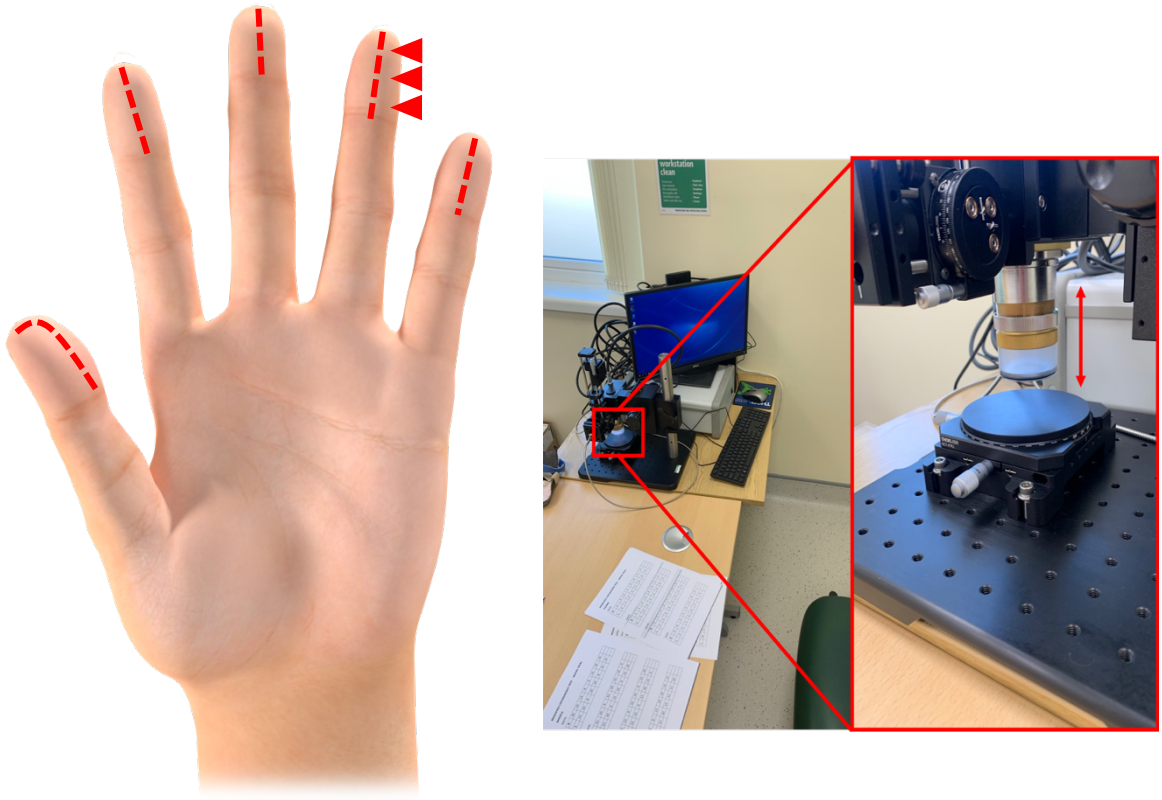
A Thorlabs Telesto® 220 PSC2 1300nm OCT system was used with a user-customizable scanner allowing adjustment of the reference arm length to achieve optimal focal distance of the sample, in this case a finger placed on the sample plate. A Thorlabs® OCT-LK4 scan lens kit was added in order to increase the working distance of the imaging equipment to allow for easier placement of participants' hands on the sample plate. This allowed participants to rest their arms at 90 degrees on a table with their hands supine which was the most comfortable position for scanning. A Thorlabs® OCT-IMM4 air sample spacer was added in order to lightly compress the fingers between sample plate and lens which eradicated movement during scanning allowing for improved image acquisition (Figure 5.2).

The injured finger pulp was divided into halves longitudinally (Figure 5.2), representing the anatomically dominant areas supplied by each digital nerve (Bas and Kleinert, 1999). We then tested the hemi-pulp supplied by the injured digital nerve and compared with the corresponding uninjured hemi-pulp on the contralateral finger. We performed two-dimensional scans to obtain depth measurements and three-dimensional scans to perform direct counts of sweat ducts in a defined area (1x1mm). Each set of scans took less than 30 seconds and was repeated at three separate points along the hemi-pulp, equidistant from each other. These were marked out using a non-permanent marking pen and ruler. When the finger was placed underneath the scanner, the camera within the scan lens allowed accurate identification of scan location. All images were taken and then calculations performed. A mean epidermal thickness (mm) was calculated using three separate depth measurements, taken at random, across each two-dimensional image using the line measure tool available within the software. SDD (number of sweat ducts/mm^2) was then calculated for each three-dimensional image by manually

counting the number of sweat ducts in each 1x1mm recording and a mean of the three hemi-pulp recordings was then calculated.

Figure 5.2: OCT hand imaging (a) an image of the hand and finger pulps divided into hemi-pulp (dashed red lines) areas for testing and examples of OCT imaging points (red arrow heads); (b) OCT hardware setup with zoomed-in demonstration of lens and finger mount where participants' digits were placed volar-side up.

(a) (b)



Three two-dimensional images and three three-dimensional recordings (link) were recorded at each optical coherence tomography (OCT) imaging point. The hemi-pulp representing the dermatome of the injured digital nerve was analysed (experimental) along with the contralateral hemi-pulp of the opposite hand (control).

Clinical Testing

Sensory Testing; Three tests of sensory function were used: (i) the Weinstein Enhanced Sensory Test (WEST) to assess single-point pressure threshold and the integrity of the afferent fibre populations; (ii) the static two-point discrimination test (s2PD) to assess sensory discrimination; and (iii) locognosia testing to assess functional sensibility of the injured nerves.

All tests were performed in a quiet room with the patient sitting opposite the examiner with arms rested on a pillow and elbows at ~45-90 degrees of flexion and supination. This position was chosen as the most comfortable position for most patients to sit through the sensory testing without movement of the hands. The hands were then placed through a JAMAR sensory testing shield (Performance Health, IL, USA) so as to blind the patient to the testing instrument during the examination. Before the start of each test, and as needed, patients were reminded to keep their hands rested into the pillow in order to prevent lifting of the fingers towards the testing instrument.

The WEST monofilament tool (Fabrication Enterprises Inc., NY, USA) developed by Sidney Weinstein (Weinstein, 1993) was used for pressure threshold testing. This tool includes five standard filaments which apply a standard calibrated force of 0.07g, 0.2g, 2.0g, 4.0g and 200g. For the purposes of this study a corresponding integer from 5 (0.07g) to 1 (200g) represented the incremental monofilaments with 0 representing an inability to detect any force. A random monofilament test was employed whereby each filament was applied in random order to the test sites with the lowest detectable stimulus recorded in terms of the corresponding integer.

The s2PD test was performed incrementally using a blunt-tipped discriminator (Baseline DISCRIM-A-GON, Fabrication Enterprises Inc., NY, USA) as described by Mackinnon and Dellon (Mackinnon and Dellon, 1985) starting with the smallest width and just sufficient pressure for the subject to detect the stimulus. Each pin set distance was performed 10 times and a score of 7 positive responses out of 10 was deemed a positive response.

The locognosia test was adapted from Jerosch-Herold et al. JBJS 2006 (Jerosch-Herold, 2006) for use in digital nerve injury patients. A diagram of the hand with a superimposed grid of zones, numbered is presented to the patient. The patient is asked to identify the zone where a suprathreshold stimulus has been perceived. The stimulus is delivered using a WEST monofilament (Fabrication Enterprises Inc., NY, USA), which upon contact with the skin bends, providing a repeatable peak force of 200g.

Patient-Reported Outcome Measures (PROMs)

Two patient-reported outcome measures were utilised. DASH, which is a 30-item (scored 1-5) self-reported questionnaire reported as a percentage whereby the sum of responses is divided by number of responses minus 1 and multiplied by 25 (Institute for Work and Health, 2006). And the I-HaND scale V2 which is a 32-item (scored 1-5) self-reported questionnaire, reported as a percentage as described for the DASH score (Ashwood, Jerosch-Herold and Shepstone, 2018).

Statistics

Data are presented as mean (SD), median [interquartiles] and count (%). Effect sizes are presented as difference with 95% confidence interval (CI) and within-subject correlation (95% CI). Gaussian normality was assessed using Kolmogorov-Smirnov omnibus tests and normality plots. 95% reference intervals were estimated using two-sided robust analysis with 3000 bootstrap samples. General linear and mixed-effects models (GLMM) were used to estimate effects over time in patients. Mixed-effects linear regression models were used to estimate regression coefficients for age with both optical coherence tomographic measures. Within-subject correlations were estimated for both optical measures and PROMs to estimate the relative usefulness of the optical measures. Correlation coefficients are presented as positive to show the strength of association, with the sign shown for each measure as appropriate. Tukey-Kramer tests were used to adjust *p* values and 95% CIs for multiple comparisons. Significance was defined at $p<0.05$ (two-sided). Analyses were conducted using Number Cruncher Statistical Systems 2020 (NCSS), NCSS Inc., Kaysville, UT; Stata 17.0, Stata Corp, College Station, TX and GraphPad Prism 7.04, La Jolla, CA.

5.3 Results:

Normative sample participants

A total of 53 healthy participants had bilateral OCT scans of all digits. Ages ranged from 18 – 63 with a median age of 32. There were 21 males and 32 females included. 49 participants were right-handed with only 4 left-handed participants. There were 41 white, 7 Asian and 5 Afro-Caribbean participants. There were no participants with previous upper limb nerve injuries. The mean ambient temperature at time of measurement was 21.04 (+/- 0.34) °C.

Digits

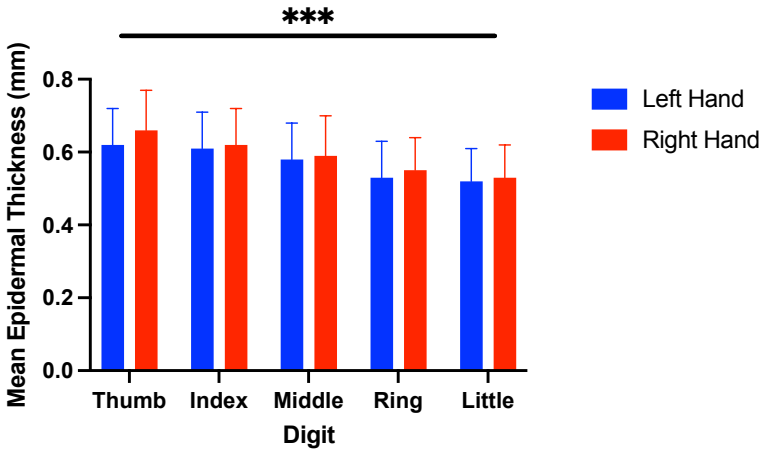
There was a significant progressive reduction (p<0.0001) in epidermal thickness from thumb to little finger. There were significant differences (p<0.0001) in SDD between digits (Figure 5.3). The thumb had significantly (p<0.030) lower SDD compared to middle, ring and little fingers (Figure 5.3).

Effect of Side: Left vs Right Hand

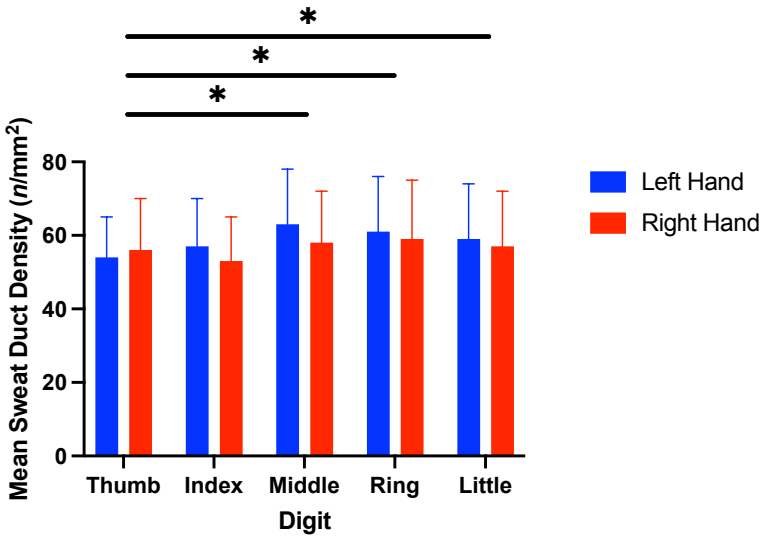
The effect of side on digits was not significant for epidermal thickness (p=0.11) but was (p=0.039) for SDD (Figure 5.3). The overall mean left-hand epidermal thickness was 0.57 (+/- 0.10) mm and right-hand was 0.59 (+/- 0.11) mm, difference -0.014 (95%CI -0.031, 0.003) mm; p=0.11. The mean left-hand SDD was 59.0 (+/- 14.3)/mm^2 and right-hand was 56.78 (+/- 14.23)/mm^2, difference 2.13 (95%CI 0.11, 4.16) mm^2; p=0.039.

Figure 5.3: Mean epidermal thickness and sweat duct density values for each digit

(a)



(b)



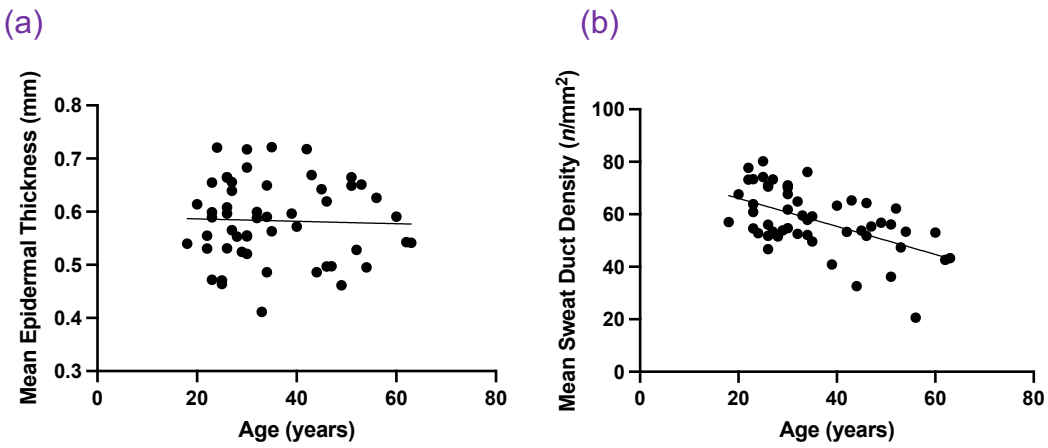
Bar chart (a) demonstrates the mean (+/- S.D.) epidermal thickness of each digit comparing left (blue) vs right (red) hands with a significant reduction in epidermal thickness ($p<0.0001$) from thumb to little finger. Bar chart (b) demonstrates the mean (+/- S.D) SDD of each digit comparing left (blue) vs right (red) hands where the thumb had significantly ($p<0.030$) lower SDD compared to middle, ring and little fingers.

Analysis of hand dominance showed no significant effect (p=0.10) for epidermal thickness. SDD was significantly lower in the dominant hand by 2.70 (95%CI 0.73, 4.67)/mm^2; p=0.0082.

Age

Taking the mean of all digits of both hands, there was no significant change (p=0.79) in epidermal thickness with age. However, there was a significant decrease (p<0.0001) in SDD with age at -0.53 (95%CI -0.75, -0.31)/mm^2/year (Figure 5.4).

Figure 5.4: Mean (combined) epidermal thickness and sweat duct density values by age of participant



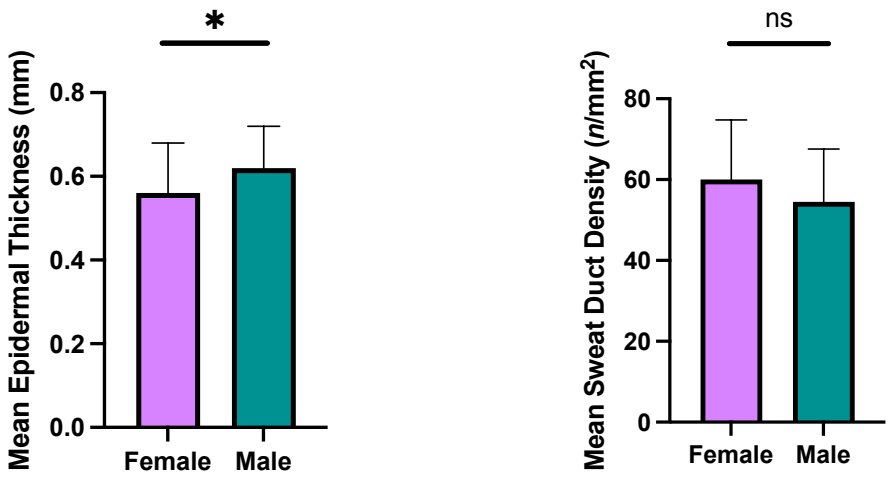
Scatter plot (a) demonstrates mean epidermal thickness values based on age of patient with a simple linear regression line showing no significant trend; (b) demonstrates mean sweat duct density values by age of patient with a simple linear regression line showing a decreasing trend in SDD with age.

Sex

Taking the mean of all digits on both hands, mean female epidermal thickness 0.56 (+/- 0.12) mm was significantly less (p=0.011) than male epidermal thickness 0.62 (+/- 0.10) mm; difference -0.05 (95%CI -0.09, -0.01) mm. There was no significant sex difference (p=0.093) between mean female SDD 60.05 (+/- 14.7)/mm^2 and male SDD 54.50 (+/- 13.1)/mm^2; difference 5.56 (95%CI -0.97, 12.09)/mm^2 (Figure 5.5).

Figure 5.5: Mean (combined) epidermal thickness and sweat duct density values by gender of participant

(a) (b)



(a) bar chart of significant difference between mean epidermal thickness of female and male participants; (b) bar chart of mean SDD of female and male participants

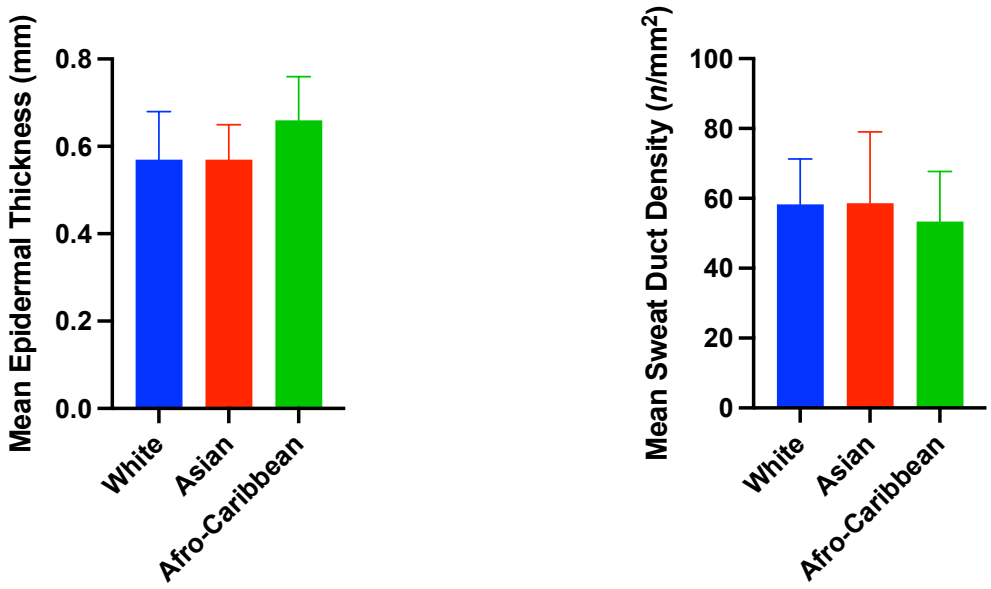
Ethnic Background

White participants had a mean epidermal thickness of 0.57 (+/- 0.11) mm, which was similar to Asian participants who had a mean of 0.57 (+/- 0.08) mm. Afro-Caribbean participants had a higher, but not significantly different epidermal thickness of 0.66 (+/- 0.10) mm compared to white (p=0.052) or Asian (p=0.087) participants (Figure 5.6).

White participants had a mean SDD of 58.3 (+/- 13.0)/mm^2 which was similar to Asian participants 58.6 (+/- 20.4)/mm^2. Afro-Caribbean participants had a mean of 53.4 (+/- 14.3)/mm^2 which was lower but not significantly different to white (p=0.66) or Asian (p=0.74) participants (Figure 5.6).

Figure 5.6: Mean (combined) epidermal thickness and sweat duct density values by ethnicity of participant

(a) (b)



(a) bar chart demonstrating significant difference between mean epidermal thickness of Afro-Caribbean vs white and Asian participants; (b) bar chart of mean SDD values of white, Asian and Afro-Caribbean participants

Reference intervals

The 95% reference intervals for all digits combined for epidermal thickness of volar digital hand skin were calculated to be 0.367 – 0.790 mm (Table 5.1). For SDD of volar digital hand skin, the values were calculated to be 29.773 – 86.236/mm^2 (Table 5.2).

Reference intervals of each digit were calculated in addition to combined values:

Table 5.1: Epidermal thickness (mm) reference intervals

Digit	2.5% lower reference limit (mm)	97.5% upper reference limit (mm)
Thumb	0.418	0.840
Index Finger	0.420	0.814
Middle Finger	0.377	0.788
Ring Finger	0.356	0.726
Little Finger	0.339	0.712
Combined	**0.367**	**0.790**

Table 5.2: Sweat duct density (number/mm^2) reference intervals

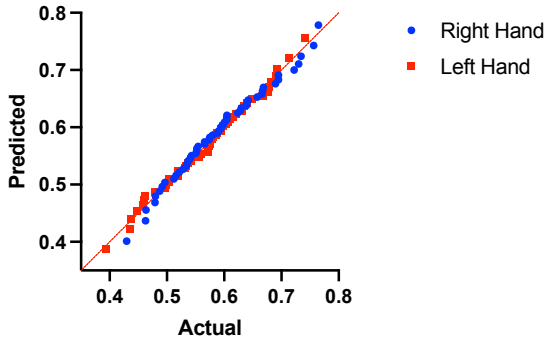
Digit	2.5% lower reference limit (number/mm^2)	97.5% upper reference limit (number/mm^2)
Thumb	28.527	80.739
Index Finger	30.510	80.379
Middle Finger	31.396	90.211
Ring Finger	29.725	91.543
Little Finger	29.188	88.717
Combined	**29.773**	**86.236**

Normality of epidermal thickness and sweat duct density values

The normality of epidermal thickness and SDD values was assessed within our sample population (Figure 5.7) which demonstrated that both measurements were normally distributed.

Figure 5.7: A normality distribution analysis of (a) epidermal thickness and (b) sweat duct density values.

(a)



Anderson-Darling test		
A2*	0.2606	0.1753
P value	0.6962	0.9198
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

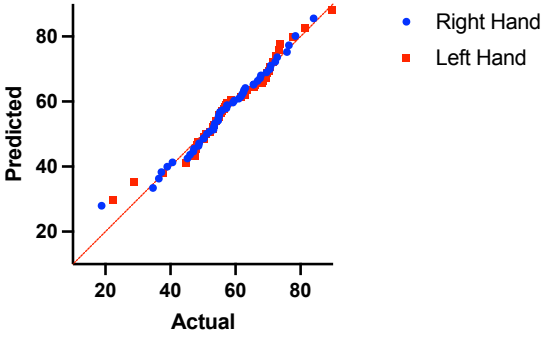
Agostino & Pearson test		
K2	1.391	0.3796
P value	0.4988	0.8271
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

Shapiro-Wilk test		
W	0.9817	0.9899
P value	0.5898	0.9335
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

Kolmogorov-Smirnov test		
KS distance	0.08579	0.08638
P value	>0.1000	>0.1000
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

Number of values	53	53

(b)



Anderson-Darling test		
A2*	0.2777	0.5098
P value	0.6384	0.1891
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

Agostino & Pearson test		
K2	2.919	2.929
P value	0.2323	0.2312
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

Shapiro-Wilk test		
W	0.9829	0.9727
P value	0.6428	0.2637
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

Kolmogorov-Smirnov test		
KS distance	0.06939	0.08300
P value	>0.1000	>0.1000
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

Number of values	53	53

Digital nerve injury participants

A total of 27 patients with digital nerve injuries consented to take part in the study between 6th November, 2019 and 26th February, 2021 (Table 5.3). Ages ranged from 20 – 74 with 18 males and 9 females. There were no participants with previous upper limb nerve injuries. A total of 18 patients completed study follow-up.

Table 5.3: Demographics of digital nerve injury group

Study I.D.	Age	Gender	Occupation	Injured nerve	Injury Method	PMH	PSH	SH
8	28	M	Dentist	RIF RDN	circular saw	nil	nil	nil
9	74	M	retired joiner	LIF RDN	tin can	nil	nil	smoker
10	34	F	Gas engineer	LIF RDN	knife lac	nil	nil	smoker
11	30	F	full-time mother	RIF RDN+UDN	glass lac	nil	nil	smoker
12	29	M	corporate researcher	LIF RDN	knife lac	nil	nil	smoker
13	35	M	joiner	LMF RDN+UDN	circular saw	nil	nil	smoker
14	30	M	bar manager	LIF RDN	circular saw	nil	nil	smoker
15	24	F	building manager	LMF RDN+UDN	metal fence	Raynaud's syndrome	nil	smoker
16	22	M	student	RRF UDN	glass lac	nil	nil	smoker
17	37	M	joiner	RRF RDN	glass lac	nil	nil	smoker
18	51	M	admin officer civil service	RLF RDN	glass lac	nil	nil	nil
19	45	F	ward clerk	LIF UDN	knife lac	nil	nil	nil
20	29	F	property manager	LIF RDN	knife lac	nil	nil	nil
21	63	M	milkman	LMF RDN	glass lac	nil	nil	nil

22	33	M	unemployed	Lt.Th.UDN	metal sink	nil	nil	nil
23	33	F	bar manager	LLF RDN	slate step FOOSH	nil	nil	nil
24	44	M	machine technician	RLF UDN	glass lac	nil	nil	nil
25	58	M	site services manager	LIF RDN	chainsaw	nil	nil	nil
26	20	F	student	RMF RDN	knife lac	nil	nil	nil
27	33	M	cladder	LRF UDN	knife lac	nil	fracture LRF, left ^{5t}h MC fracture fixation	smoker
28	23	M	unemployed	RLF UDN	glass lac	nil	previous bilateral wrist fractures	smoker
29	52	M	control supervisor	LMF UDN	circular saw	T2DM, HTN, high cholesterol	gastric bypass 2010, kidney biopsy 2001	smoker
30	50	M	general engineer	LLF UDN	knife lac	glaucoma	nil	nil
31	65	F	retired	LMF RDN	knife lac	nil	nil	nil
32	52	F	teacher	RIF RDN	glass lac	nil	right wrist fracture fixation 2017	nil
33	67	M	joiner	Lt.Th. RDN	circular saw	nil	nil	nil
34	61	M	retired white goods engineer	RIF RDN	metal tin	nil	left wrist fusion 2019	nil

*LIF = left index finger; RIF = right index finger; LMF = left middle finger; RMF = right middle finger; RRF = right ring finger; LRF = left ring finger; LLF = left little finger; RLF = right little finger; Lt. Th. = Left thumb. RDN = radial digital nerve; UDN = ulnar digital nerve.

Epidermal thickness was significantly reduced over the 24-weeks (p<0.0001) on the injured compared to corresponding uninjured hemi-digit by -0.09 (95%CI -0.11, -0.07) mm. Epidermal thickness showed no significant change (p=0.47) in the injured hemi-digit vs control (0.50 vs 0.53 mm) at 1-week post repair, with difference of 0.04

(95%CI -0.02, 0.11) mm. However, by 4 weeks a significant thinning (p<0.0001) of the epidermis had occurred (0.37 vs 0.53 mm, difference -0.15 (95%CI -0.22, -0.08) mm) which returned towards the control value by 24 weeks (0.50 vs 0.56 mm, difference -0.05 (95%CI -0.13, 0.02); p=0.43) when no significant difference was found between injured and control digits (Figure 5.8 (a)).

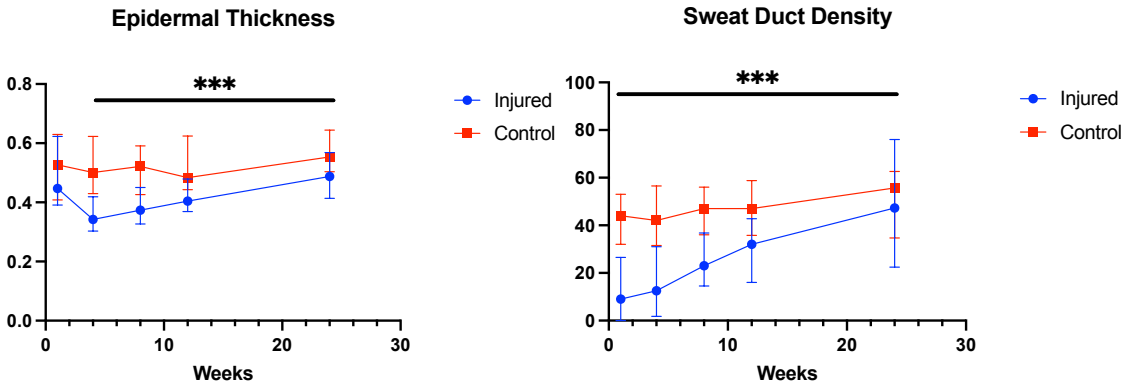
SDD was significantly reduced over the 24-weeks (p<0.0001) on the injured compared to corresponding uninjured hemi-digit by -17.4 (95%CI -20.7, -14.2)/ mm^2. In contrast to epidermal thickness, sweat duct density was significantly reduced (p<0.0001) in the injured hemi-digit compared to the control side by the first week after repair (15 vs 43/mm^2, difference -27.0 (95%CI -37.7, -16.3)/mm^2. This then returned towards the control value by 24-weeks (48 vs 47/mm^2, difference -0.1 (95%CI -13.2, 13.0)/mm^2; p=0.99) when no significant difference was found between injured and control digits (Figure 5.8 (a)).

S2PD (p<0.0001), WEST (p<0.0082) and locognosia (p<0.0001) scores all demonstrated expected significant improvements from baseline to 24-weeks after repair (Figure 5.8 (b)). However, there was still a significant difference between injured and control s2PD values by 24 weeks of 2.44 (95%CI 0.61,4.28); p=0.001) mm which was not the case for WEST measurements (mean difference of 0.44 (95%CI -0.25,1.14; p=0.57).

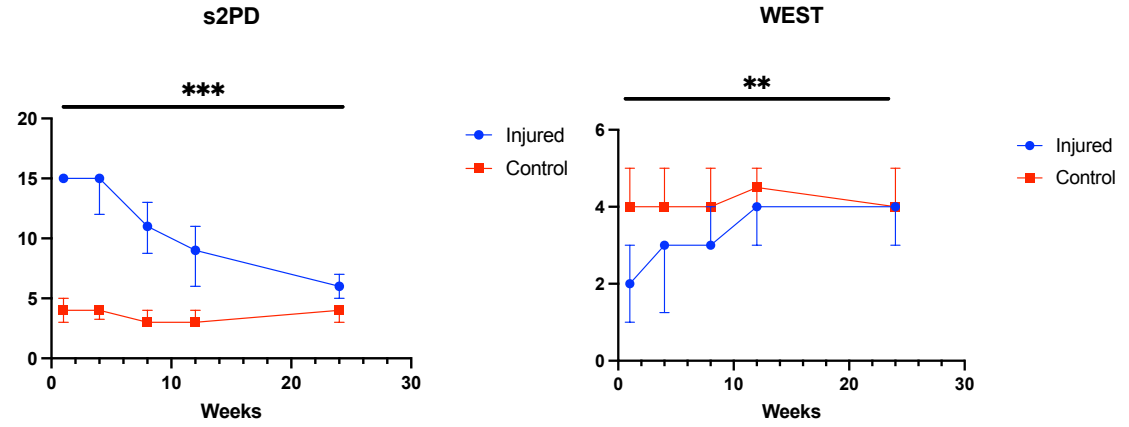
DASH and iHand scores significantly decreased (p<0.0001) over time as the nerves regenerated. Scores from both measures at 24-weeks after repair achieved DASH = 14.2 (+/-22) and iHand = 21.1 (+/-23). The reductions in disability with DASH and iHand at 24 weeks were 40.7 (95%CI 29.1, 52.3) and 35.0 (95%CI 24.6, 45.4) respectively (Figure 5.8 (c)).

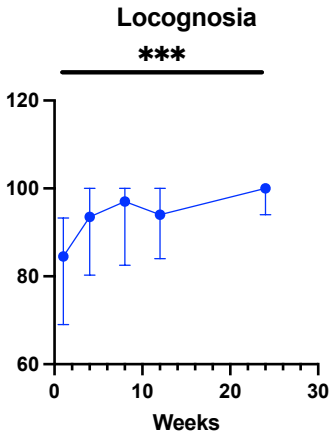
Figure 5.8: OCT measures, clinical outcomes and PROMs for digital nerve injury cohort.

(a)

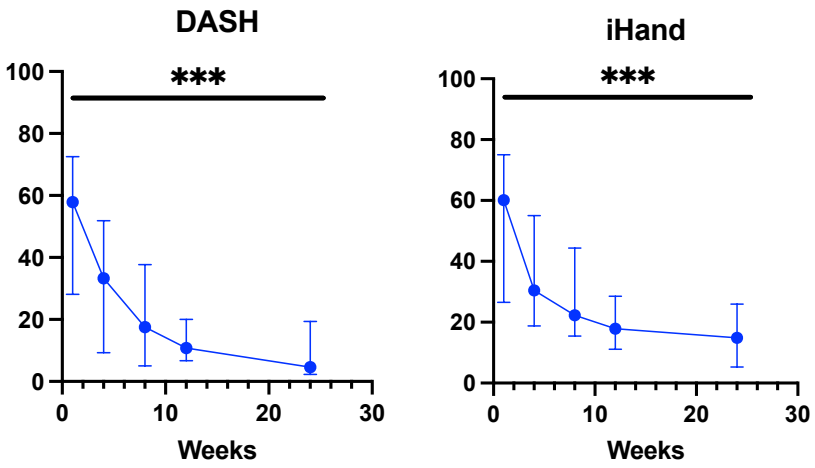


(b)





(c)



(a) OCT-measured volar digital epidermal thickness (mm) of control vs injured hemi-digits and volar sweat duct density (number/mm^2) of control vs injured hemi-digits (medians +/- IQR.); (b) static two-point discrimination (s2PD), Weinstein-Enhanced Sensory Testing (WEST) and locognosia scores of control vs injured hemi-digits (medians +/- IQR); (c) Patient-reported outcome measures (DASH and iHand) (medians +/- IQR).

Correlation of optical coherence tomographic measures with patient reported outcome measures.

Within-subject correlations were estimated for the OCT measures (epidermal thickness and sweat duct density) and clinical outcome measures (s2PD, WEST, locognosia) with PROMs (DASH and i-HAND), across the 24-week period after injury and surgical repair (Table 5.4).

Table 5.4 Within subject correlations (95% Cis) and *p* values between OCT and clinical outcome measures compared to DASH and iHand PROMS (**weeks 1 to 24**)

Measure (*r* sign)	**DASH**	**iHAND**
Epidermal Thickness (-)	0.13 (-0.09, 0.34), p=0.24	0.12 (-0.10, 0.33), p=0.28
Sweat Duct Density (-)	0.49 (0.30, 0.64), p<0.0001	0.54 (0.37, 0.68), p<0.0001
Difference (-)	**0.47 (0.28, 0.63), p<0.0001**	**0.53 (0.35, 0.67), p<0.0001**
S2PD (+)	0.52 (0.34, 0.66), P<0.0001	0.53 (0.35, 0.67), P<0.0001
WEST (-)	0.56 (0.39, 0.70), P<0.0001	0.57 (0.40, 0.70), P<0.0001
Locognosia (-)	0.59 (0.42, 0.72), P<0.0001	0.47 (0.28, 0.63), P<0.0001

SDD was more significantly correlated (p<0.0001) with both DASH and i-HAND (Table 5.4) and is therefore a more significant predictor of nerve recovery, compared to epidermal thickness.

SDD compared well with clinical outcome measures in terms of correlation to PROMS and had better correlation than s2PD and locognosia to iHand which is more peripheral nerve specific (Table 5.4).

As a sensitivity analysis, within-subject correlations were also estimated from week 4 to remove the slow thinning of the epidermis noted at week 1 (Table 5.5).

Table 5.5: Within subject correlations (95% Cis) and *p* values between OCT and clinical outcome measures compared to DASH and iHand PROMS (**weeks 4 to 24**)

Measure (*r* sign)	DASH	iHAND
Epidermal Thickness (-)	0.38 (0.13, 0.58), P=0.003	0.37 (0.13, 0.57), P=0.003
Sweat Duct Density (-)	0.49 (0.26, 0.67), P<0.0001	0.53 (0.31, 0.69), P<0.0001
Difference (-)	**0.30 (0.05, 0.52), p=0.022**	**0.37 (0.13, 0.58), p=0.0038**
S2PD (+)	0.47 (0.24, 0.65), P=0.0002	0.50 (0.28, 0.67), P<0.0001
WEST (-)	0.44 (0.20, 0.63), P=0.0005	0.53 (0.31, 0.69), P<0.0001
Locognosia (-)	0.56 (0.34, 0.72), P<0.0001	0.38 (0.13, 0.59), P=0.0032

Although there were considerable improvements in the correlations with epidermal thickness, SDD still significantly out-performs epidermal thickness in trending with both PROMs (Table 5.5).

SDD also out-performs s2PD and WEST compared to DASH and outperforms s2PD and locognosia compared to iHand. It had identical correlation to WEST when compared against iHand.

5.4 Discussion:

We have investigated volar hand epidermal thickness and SDD in both health and disease in order to test development of an early, objective and quantifiable measure of sensory nerve regeneration. In our normative sample of 53 healthy participants we investigated the epidermal thickness (mm) and SDD (n/mm^2) of all digits of bilateral hands. We found that the volar hand epidermis became progressively thinner from the thumb to little finger and the thumb has significantly lower sweat duct density compared to ulnar-sided digits. Our sample population was derived from a professional cohort who may often use precise prehension grips regularly as part of their work. Prehension grips predominantly utilise the thumb and radially sided-digits which could lead to a progressive hypertrophy of volar skin in this region through repetitive use (Kinoshita, Kawai and Ikuta, 1995). Whether this leads to a

reduction in identifiable sweat ducts is uncertain but may explain our findings of reduced SDD in more radial-sided digits. Manual workers, for example, who use more power grips on a daily basis, could have thicker epidermis in ulnar-sided digits with converse reduction in sweat duct density from ulnar to radial sided digits. Further work is needed to explore occupation differences.

We found no significant differences between hands in terms of epidermal thickness, however there was a significant reduction of SDD in the right hand and also when hand dominance was taken into account. However only 4 of the 53 participants were left-handed and therefore may not be representative of all left-handed individuals. We found that SDD significantly reduced with age which may be due to reduced sympathetic control of skin with increasing age (Greaney, Alexander and Kenney, 2015) that prevents sweat duct dilatation and thus sweating. Male epidermal thickness was significantly thicker than female epidermal thickness (mean of 0.62 vs 0.56 mm) which was expected given known sex differences in skin thickness (Rahrovan *et al.*, 2018), however there was no sex difference for SDD. There was no significant difference between ethnic groups in terms of epidermal thickness or SDD. The normative sample study was limited by occupation amongst participants, however a broader sample selection was not possible due to coronavirus restrictions at the time.

From our sample population we estimated reference intervals for volar hand epidermal thickness and SDD in order to evaluate change in disease states such as sensory nerve injury to the hand. This may also be useful for future diagnostic studies, where uncertainty remains as to whether a digital nerve is injured or not.

After sensory nerve injury in the hand, we found that the epidermis in the corresponding area of cutaneous innervation significantly thinned by 4 weeks after surgery compared to the contralateral uninjured side (0.34 vs 0.50 mm (p<0.0001)). This was below the lower reference range for epidermal thickness defined in our normal population (0.367 mm). This returned towards the control value by 24-weeks (0.50 vs 0.56 mm (p=0.43)). We found a similar but more pronounced change in SDD which was significantly reduced after only 1 week after surgery (15 vs 43/mm^2 (p<0.0001)), returning towards the control value by 24-weeks (48 vs 47/mm^2(p=0.99)). SDD values remained below the lower reference value (29.77 sweat ducts/mm^2) from our normal population until 12 weeks after surgery.

Notably s2PD still demonstrated a significant difference between injured and control digits at 24 weeks after repair whereas OCT measures and WEST did not. This may indicate that s2PD, the most commonly utilised outcome measure of sensory nerve regeneration in the hand (Chapter 4), may lack the sensitivity to detect when sensory nerve regeneration is complete.

Both epidermal thickness and SDD correlated well with PROMs, however SDD was significantly better correlated to PROMs over the study period. SDD had better correlation to the peripheral nerve specific PROM (iHand) than both s2PD and locognosia, with equivalent correlation to WEST monofilament testing from 4-24 weeks after surgery.

OCT assessment of epidermal thickness and SDD can provide an objective method of quantifying peripheral nerve regeneration that is simple to perform, non-invasive and immediately clinically available. In addition, SDD measurements may offer greater sensitivity than other clinical measures (e.g. s2PD) of complete regeneration. The expertise required to use the equipment is minimal, and with simple instructions, can be performed easily by clinicians or researchers. However, at this stage in the development of this outcome measure the image processing time would likely deter clinicians and would therefore be of greatest utility to researchers looking to gain more accurate and objective measures of peripheral nerve regeneration when trialling novel treatments for PNI. In time, and with appropriate software development, it would be possible to automate the epidermal thickness and SDD measurement thereby reducing the image processing time. Reducing the size of the hardware needed would also be important in order to allow for greater flexibility in use at the point of care, storage and functionality. Similarly, to community opticians who use OCT to analyse retinal changes, the ultimate goal would be to develop a tool that patients could simply place their finger on and be provided with an immediate readout of their epidermal thickness and sweat duct density across the digital pulp. Not only could this provide an important tool for outcome measurement, but it could also be a useful diagnostic device for suspected sensory/mixed nerve injury. This may be of even more clinical urgency by providing a way to determine if a digital nerve is transected or not thereby reducing the surgical burden of digital nerve exploration.

Objective measures of nerve regeneration are clearly needed that can provide quantification of the microbiological changes that occur in the regenerating nerve in order to demonstrate effectiveness of novel treatments. Quantification of epidermal thickness and sweat duct density changes may provide a more detailed and accurate measurement of sensory nerve regeneration than current clinical outcome measures. In addition, these measurements may be better at identifying when nerve regeneration is complete compared to current clinical measures.

A third of patients did not return for their final research visit despite transport and time costs covered by study funding. It is difficult to determine the exact cause of this high dropout at the 24-week timepoint especially given the ongoing lockdowns associated with the COVID-19 pandemic. It appears that regeneration would be complete by 24-weeks in our patient cohort therefore it may be that the perceived increased risk of contracting COVID-19 by visiting a hospital site outweighed participants' desires to continue with the study knowing that their nerve had fully healed.

We have demonstrated the feasibility of OCT as a novel outcome measure for peripheral nerve regeneration and can use the data we have obtained here to perform sensitivity analyses in order to inform sample size calculations for future studies. Future multicentre, prospective studies that are appropriately powered are needed to ascertain whether the OCT biomarkers (epidermal thickness and sweat duct density) defined in this paper can provide a more accurate assessment of sensory nerve regeneration than current clinical outcome measures. In addition, we will look to investigate whether they are better able to diagnose hand sensory nerve injury than current clinical measures.

CHAPTER 6 Novel outcome measures in peripheral nerve injury (OPEN Study)

6.1 Introduction:

The ubiquitous availability of ultrasound, its relative inexpensiveness and its excellent spatial resolution has led to it becoming the initial imaging of choice for investigating peripheral nervous system disorders of the upper limb, especially in entrapment syndromes and suspected tumours (Ohana *et al.*, 2014). In addition, the advent of high frequency ultrasound probes (>15mHz) and improved software development over the past 30 years (Fornage, 1988)(Stuart, Koh and Breidahl, 2004) has led to the ability to identify much greater neural architectural detail. It is now possible to differentiate between fascicular structures within peripheral nerves at the level of the wrist in vivo (Suk, Walker and Cartwright, 2013) which demonstrates a spatial resolution down to 0.38mm (Brill and Tyler, 2017). This high frequency, 3D ultrasound has already been used to assess median nerves in the forearm (Pelz *et al.*, 2017) and a recent case report has demonstrated its potential for assessing nerve regeneration through synthetic, polymer nerve conduits at the level of the wrist (Billakota *et al.*, 2018). Novel imaging techniques such as this are needed to objectively assess and quantify the microbiological changes that occur at the site of PNI and repair in order to provide researchers and clinicians with much more detailed information on the regenerative process.

Combining high-frequency, three-dimensional, tomographic ultrasound (Hf,3D,tUS) hardware and advanced artificial intelligence imaging software from PIUR Imaging Austria now gives us the ability to interrogate morphometric, volumetric and intra-neural grey-scale changes. Intra-neural grey-scale, or echogenicity of the internal structure of a peripheral nerve, is influenced by the degree of axonal density (Powles *et al.*, 2018) with injured nerves displaying a hypoechoic appearance (Mayans, Cartwright and Walker, 2012) which then becomes more isoechoic as the nerve regenerates (Hudson *et al.*, 1996). These ultrasound-based biomarkers may provide a novel way to quantify early (less than 6 months) peripheral nerve regeneration.

To do this, we sought to determine what volumetric and intra-neural grey-scale changes, as measured by Hf,3D,tUS, occur during nerve regeneration and how they correspond to currently used clinical outcome measures and patient-reported outcome measures. Specifically, we aimed to qualitatively described the ultrasound-based morphometric changes and quantified volume and intraneural grey-scale

changes in regenerating nerve segments (proximal stump, repair site and distal stump) compared to contralateral, uninjured control nerves. In addition to sensory and motor assessment and DASH and iHand patient-reported outcome measurement (PROMs).

6.2 Methods:

We conducted an open, non-randomised, prospective, longitudinal study of patients with transection of median and/or ulnar nerves who had undergone surgical repair with primary neurorrhaphy within 5 days of injury. Participants were scanned at regular intervals during the study coinciding with their visits to the out-patient centre for clinical assessment in the standard course of care (2- and 6-week visits; 3, 6 and 12 month visits). The study was purely observational and no change to standardised treatment was made.

The trial was conducted in accordance with the principles of GCP. The UK Health Research Authority (HRA) provided ethical permissions for the clinical investigation through the East Midlands – Nottingham 1 Research Ethics Committee (REC reference: 18/EM/0426).

At baseline and at each subsequent patient visits, Hf,3D,tUS was performed. At baseline, contralateral, uninjured arm nerves were also scanned to obtain a control measurement. Sensory (s2PD, WEST monofilament and locognosia testing) and motor (manual muscle testing (MRC Grading) and grip strength (Jamar Dynamometer)) clinical outcome measures assessed the degree of nerve regeneration and PROM forms (DASH and i-HAND Questionnaires) were completed to assess the impact on the patient's life.

Participants were identified by the direct care team prior to surgery and recruited either prior to surgery or at the time of surgery, in the department of plastic surgery at Wythenshawe Hospital, Manchester University NHS Foundation Trust.

Patients underwent Hf,3D,tUS in the Vascular Imaging Department which is directly adjacent to the plastic surgery outpatient department. Following this, patients were invited to the clinical research facility (CRF) at Wythenshawe Hospital to undergo clinical peripheral nerve testing and completion of PROMs.

We included adults aged 18-80 years old with an ulnar &/median nerve injury of the arm who had direct, epineurial repair within 5 days of injury and had capacity to consent.

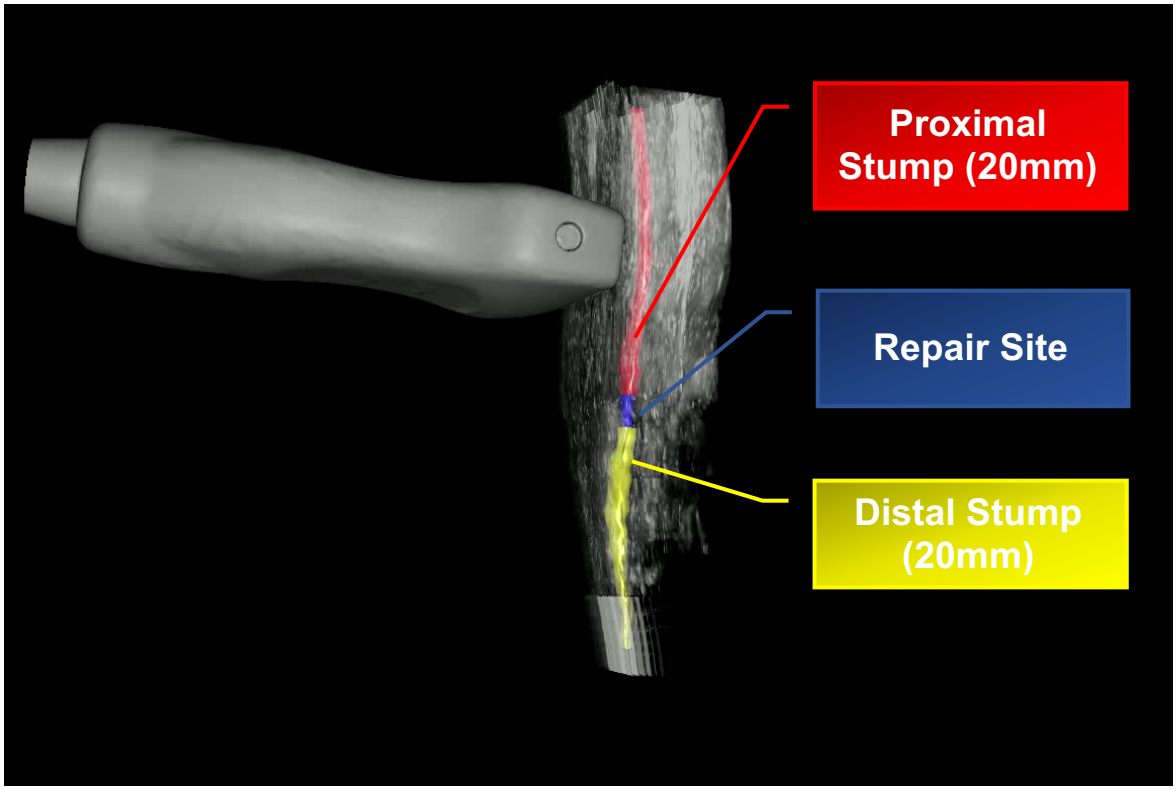
Hf,3D,tUS

Participants underwent Hf,3D,tUS in the Department of Vascular Imaging, where a research ultrasonographer from Independent Vascular Services, trained in limb-based imaging, undertook the scans. Transverse tomographic 3D ultrasound scans were produced using a L20 MHz transducer, a Resona 7 high-resolution ultrasound scanner (Mindray, Shenzhen, China) and the PIUR tUS system (PIUR Imaging, Vienna, Austria). The L20 transducer had electromagnetic sensors attached and tUS images were captured from the Resona via video capture. Individual 2-dimensional ultrasound frames were extracted from the Resona 7 machine and compiled together using the PIUR tUS system to create a 3-dimensional image dataset which can be analysed cross-sectionally similarly to computed tomography and MRI. All scans were performed in B-Mode with standardised settings of dynamic range = 110, persistence = 2 and a starting gain of 48 that produced a frame rate of at least 62. Sound speed compensation was individual to each patient. Time gain compensation was set diagonally and not adjusted. Minimal gain adjustments were made on each participant to optimise the images. The 20mHz probe frequency was chosen as an optimal balance between neural architectural detail and technical difficulty obtaining scan images.

Patients were positioned in the most comfortable position depending on their injury pattern and their arms were placed in an extended position at the elbow and supine hand and wrist position. The original injury site was marked then further marks were made at 5 cm proximal and distal to this wound overlying the position of the relevant nerve. Scans were performed from a proximal to distal direction in one continuous smooth motion incorporating a similar length of nerve in each scan and at each visit producing a fused series of tUS scans (Figure 6.1). During the initial baseline scans, where wounds were not fully healed, aseptic non-touch procedures were employed, whereby the wounds were exposed from overlying dressings, a sterile transparent primary dressing was applied (Tegaderm™, 3M™ Minnesota, USA) and a sterile

probe cover applied to allow application of ultrasound gel and probe contact to patients' arms without contaminating the wound. Fresh dressings were then applied after the scan. At baseline, contralateral uninjured nerves, and injured nerves, were scanned in order to provide an internal control grey-scale value and volume measurement for future injured nerve scans.

Figure 6.1: Illustration of Hf,3D,tUS scans.



Example high-frequency, three-dimensional, tomographic ultrasound scan (Hf,3D,tUS) of the proximal stump, repair site and distal stump peripheral nerve-structures that were visualised during scanning.

Image analysis was performed using PIUR imaging (Vienna, Austria) 3D, tomographic ultrasound system (tUS) software (version 2.1 #15) (Downey, Fenster and Williams, 2000). A plastic surgeon trained in ultrasound image analysis performed the analysis of the regenerating nerve images after all images were obtained. Analysis was undertaken by the first author following training by a Professor of Musculoskeletal Imaging. Fused tUS scans were used to measure segmental nerve volume using a standard technique to measure digital artery volumes (Hughes *et al.*, 2021) and were presented in centimetres-cubed (cm^3). Peripheral nerve analysis software developed by PIUR imaging (Vienna, Austria) was used to measure the intraneural three-dimensional-grey scale median (3D-

GSM) value in each segment. The 3D-GSM is calculated, taking all voxels (volume and pixel) inside the segmentation into account and ranges from 0 (black) to 255 (white) (Casella *et al.*, 2015). A standardised distance of 20mm from repair site was used for proximal and distal stump analysis, where possible, with a variable length of repair site reported based on its clear morphological appearance during scanning. The overlying cutaneous scar served as a reference point to locate the repair site. Results were presented as a percentage of the contralateral control 3D-GSM. Contralateral, un-injured control nerves scanned at the baseline visit, at the same anatomical location as the injured nerve, were segmentally (10mm) analysed to establish the volume and 3D-GSM variability along their length. A mean control volume (cm^3) and 3D-GSM was subsequently calculated and results from the injured nerve were presented as a percentage of the control values.

Clinical Testing

Sensory Testing; Three sensory tests were utilised to measure sensibility outcomes including: the Weinstein Enhanced Sensory Test (WEST) to assess single-point pressure threshold and the integrity of the afferent fibre populations, the static two-point discrimination test (s2PD) to assess sensory discrimination and locognosia testing to assess functional sensibility of the injured nerves. Testing was performed by a plastic surgeon competent in using the described sensory testing techniques.

All tests were performed in a quiet room with the patient sat opposite the examiner with the arms rested on a pillow with elbows at ~45-90 degrees of flexion and predominant (not complete) supination of the wrists. This position was chosen as the most comfortable position for most patients to sit through the sensory testing without movement of the hands. The hands were then placed through a JAMAR sensory testing shield (Performance Health, IL, USA) so as to blind the patient to the testing instrument during the examination. Before the start of each test, and as needed, patients were reminded to keep their hands rested into the pillow in order to prevent lifting of the fingers towards the testing instrument.

The WEST monofilament tool (Fabrication Enterprises Inc., NY, USA) developed by Sidney Weinstein (Weinstein, 1993) was used for pressure threshold testing. This tool includes five standard filaments which apply a standard calibrated force of 0.07g, 0.2g, 2.0g, 4.0g and 200g. For the purposes of this study a corresponding

integer from 5 (0.07g) to 1 (200g) represented the incremental monofilaments with 0 representing an inability to detect any force. A random monofilament test was employed whereby each filament was applied in random order to the test sites with the lowest detectable stimulus recorded in terms of the corresponding integer.

The s2PD test was performed incrementally using a blunt-tipped discriminator (Baseline DISCRIM-A-GON, Fabrication Enterprises Inc., NY, USA) as described by Mackinnon and Dellon (Mackinnon and Dellon, 1985) starting with the smallest width and just sufficient pressure for the subject to detect the stimulus. Each pin set distance was performed 10 times and a score of 7 positive responses out of 10 was deemed a positive response.

The locognosia test was performed as described by Jerosch-Herold et al. JBJS 2006 (Jerosch-Herold, 2006). A diagram of the hand with a superimposed grid of zones, numbered is presented to the patient. The patient is asked to identify the zone where a suprathreshold stimulus has been perceived. The stimulus is delivered using a WEST monofilament (Fabrication Enterprises Inc., NY, USA), which upon contact with the skin bends, providing a repeatable peak force of 200g.

Motor Testing; two motor tests were employed, with the patient in the same position as described for sensory testing and with their hands through the same JAMAR sensory testing shield (Performance Health, IL, USA) so as to blind the patient to the examination. Manual muscle testing utilising the Medical Research Council scale (Riddoch *et al.*, 1976) was performed as described except with the examiner utilising the same tendon/muscle group to oppose that being tested in the patient. Isometric grip force was measured using a JAMAR Smart Hand Dynamometer (Performance Health, IL, USA) with a mean of 3 trials in the second position using the uninjured followed by the injured side. Scores of both the manual muscle testing and isometric grip force were recorded as per the Rosen Score (Roseén and Lundborg, 2000).

A modified version of the Rosen Scoring system (Roseén and Lundborg, 2000) was utilised in order to generate a combined sensory and motor score. The STI test and Sollerman test were removed and replaced with locognosia testing as previously described above with the injured score represented as a quotient of the contralateral control score with a maximum score of 5 achievable if the injured scoring matched

the control scoring. No change was made to the Semmes-Weinstein monofilament, s2PD or motor score reporting whilst cold intolerance and hyperaesthesia testing were excluded.

Patient-Reported Outcome Measures (PROMs)

Two patient-reported outcome measures were utilised. The Disabilities of the Arm, Shoulder and Hand (DASH) measure, which is a 30-item (scored 1-5) self-reported questionnaire reported as a percentage whereby the sum of responses is divided by number of responses minus 1 and multiplied by 25 (Institute for Work and Health, 2006). And the Impact of Hand Nerve Disorders (I-HaND) scale V2 which is a 32-item (scored 1-5) self-reported questionnaire, reported as a percentage as described for the DASH score (Ashwood, Jerosch-Herold and Shepstone, 2018).

6.3 Results:

A total of 5 participants were recruited to the trial between 3rd September, 2019 and 6th March, 2020 with a mixture of arm and forearm based median and ulnar nerve injuries that were directly repaired within 1 week of injury (Table 6.1). One patient was lost to follow-up with 4 patients continuing with longitudinal follow-up however the timing of their follow-up appointments was significantly impacted by the COVID-19 pandemic as described at the start of this thesis.

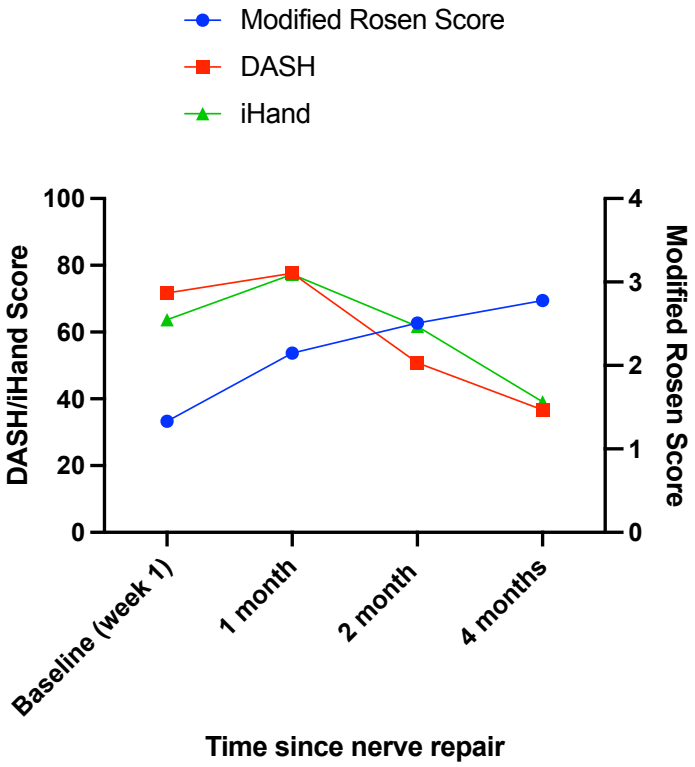
Table 6.1: Demographics of OPEN participants

Study I.D.	Age	Gender	Race	Hand Dominance	Occupation	Injured Nerve	Mechanism of Injury	Significant PMH	Smoker
001	35	F	Bangladeshi	Right	Bank Clerk	Left median nerve	knife lac	N	N
002	33	F	White - British	Right	Care Worker	distal right ulnar nerve	glass lac	N	N
003	37	F	Asian/Asian Brit - Indian	Right	Mental Health Worker	Proximal right median nerve	glass lac	Hypothyroidism	N
004	27	F	White - British	Right	Cleaner	distal left median nerve (DSH)	Razor blade (DSH)	Bipolar disorder	N
005	48	M	Asian/Asian Brit - any other asian b/g	Right	Takeaway Owner	Right distal median and ulnar nerve	Circular saw injury	N	N

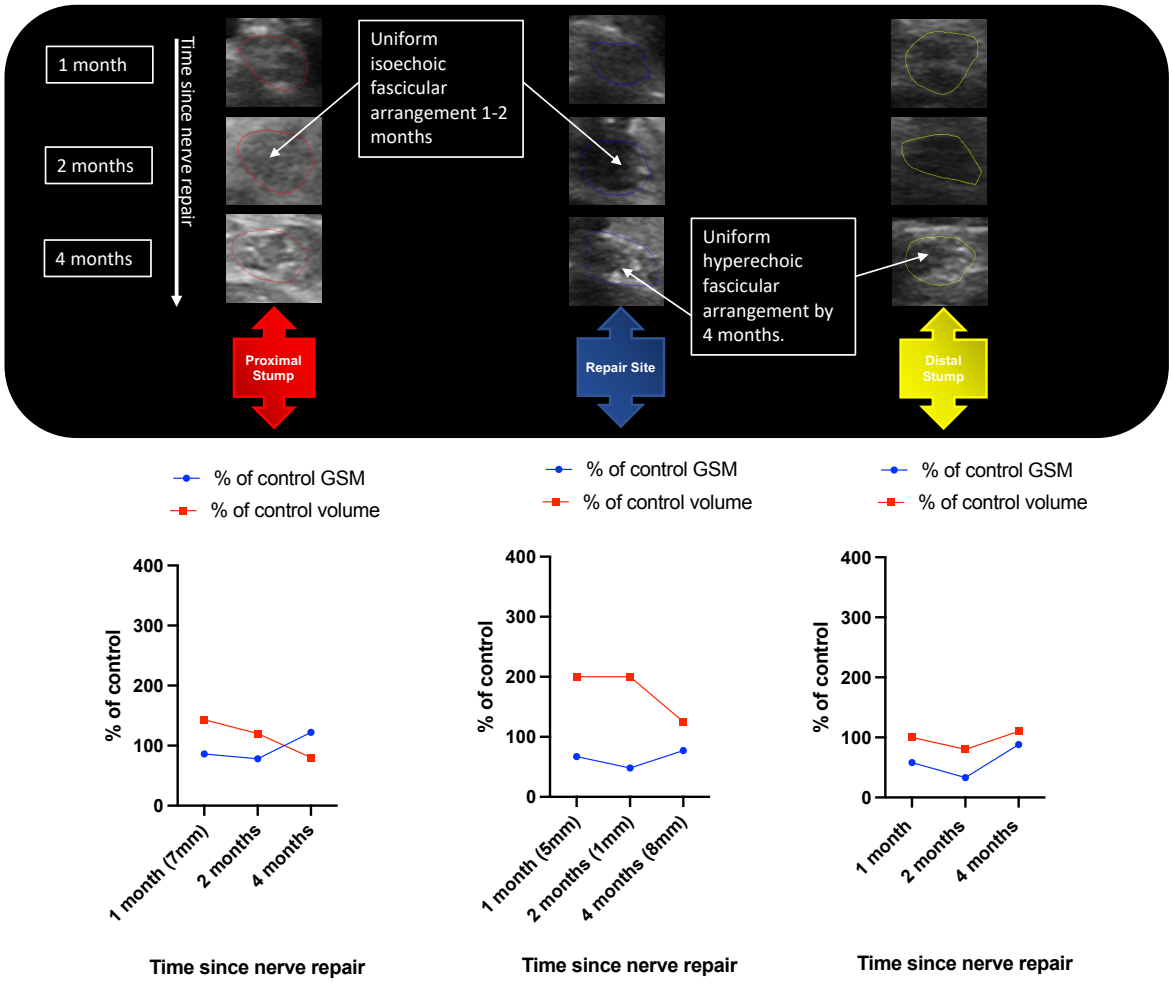
Individual Participant Follow-up Data

OPEN 002

Figure 6.2: Participant 002 clinical, patient reported and Hf,3D,tUS outcome data. (a)



(b)



(a) clinical and patient-reported outcome measures: Disability of Arm Shoulder and Hand (DASH) and Impact of Hand Nerve Disorders (iHand) scores (left-hand y-axis) and Modified Rosen Scores (right-hand y-axis); (b) ulnar nerve Hf,3D,tUS imaging with volumetric and 3D-GSM analysis of participant 002's injured ulnar nerve at proximal, repair site and distal sections of the nerve and at their corresponding timepoints with the graphical representation of their (%) percentage of control grey-scale median and volumetric (cm^3) values over these nerve segments.

Participant 002 (33-year old, female, care worker) sustained a glass laceration to the right wrist between the proximal and distal wrist creases on 21/07/2019 after accidentally hitting her right hand on a glass on the side of her bed. She had a repair of her ulnar artery and nerve in her right wrist on 21/07/2019 under general anaesthetic with tourniquet control.

Figure 6.2 (a) above shows a decrease in PROM scores over the study period of DASH 77.6 to 36.7 and iHand 77.3 to 39.1. Modified Rosen Scores demonstrated a

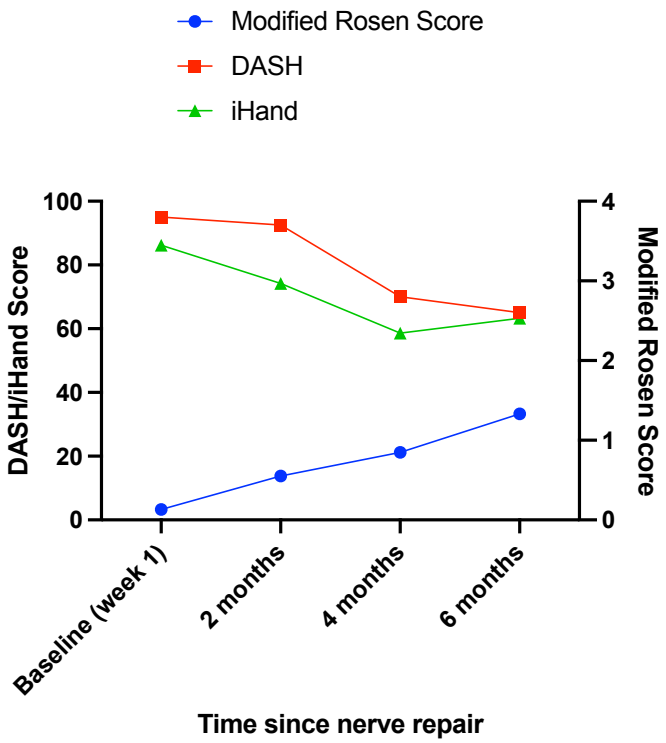
limited improvement from 2.15 at 1 month (from a baseline value of 1.33) to 2.78 at 4 months.

In Figure 6.2 (b) the transverse Hf,3D,tUS morphology demonstrates a uniform isoechoic fascicular pattern extending from proximal to distal stump. This pattern becomes more hyperechoic by 4 months after repair.

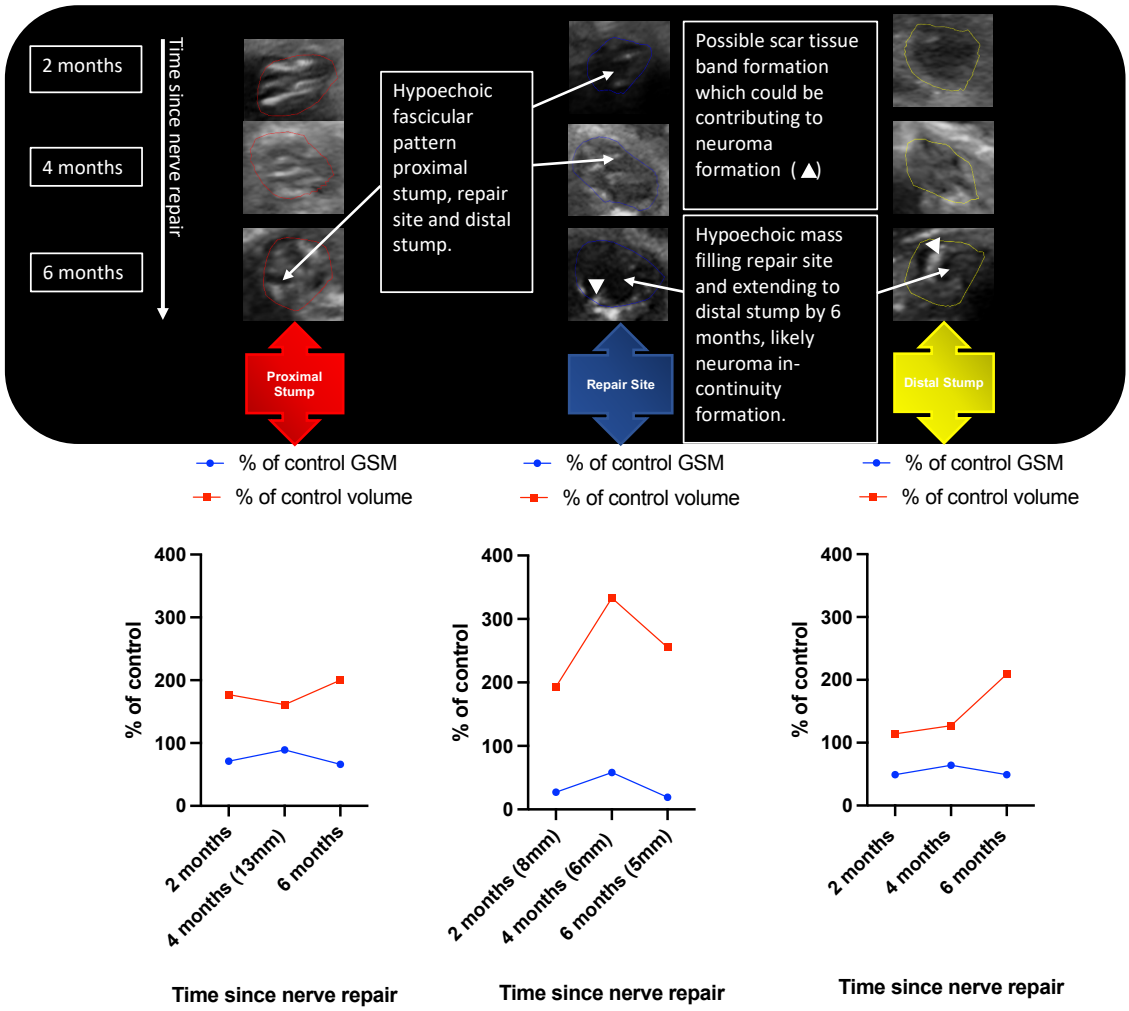
Proximal stump 3D-GSM values remain close to 100% of contralateral control nerve values over the time period (86%, 78%, 122%) whilst both repair site and distal stump values initially decreased compared to control at 2 months (48% and 33% respectively) and then increased to 77% and 88% of control values by 4 months as expected with axonal regeneration. The repair site volume remained at 200% of the contralateral control until the 4-month visit when it returned to 125% of control. Both proximal and distal nerve stumps remained at similar volumes to control at all assessment time points.

OPEN 003

Figure 6.3: Participant 003 clinical, patient reported and Hf,3D,tUS outcome data. (a)



(b)



(a) clinical and patient-reported outcome measures: Disability of Arm Shoulder and Hand (DASH) and Impact of Hand Nerve Disorders (iHand) scores (left-hand y-axis) and Modified Rosen Scores (right-hand y-axis); (b) cross-sectional tUS images of participant 003's injured median nerve at proximal, repair site and distal sections of the nerve and at their corresponding timepoints with the graphical representation of their (%) percentage of control grey-scale median and volumetric (cm^3) values over these nerve segments.

Participant 003 (37-year old, female, mental health worker) suffered a glass laceration to her right arm just proximal to the antecubital fossa on 22/07/2019. She completely transected her right median, radial and lateral antebrachial cutaneous nerves, her brachial artery and her biceps muscle.

She was transferred to a major trauma centre by ambulance where she underwent exploration of her right arm wound and brachial artery interposition bypass grafting

(using long saphenous vein from right thigh) with prolene suture tagging of the transected nerves and forearm flexor compartment fasciotomy.

Four days later, on 26/07/2019, she was transferred to a nearby hospital with specialist plastic surgery services and had her nerve injuries directly repaired using epineurial interrupted suturing under general anaesthetic with tourniquet control.

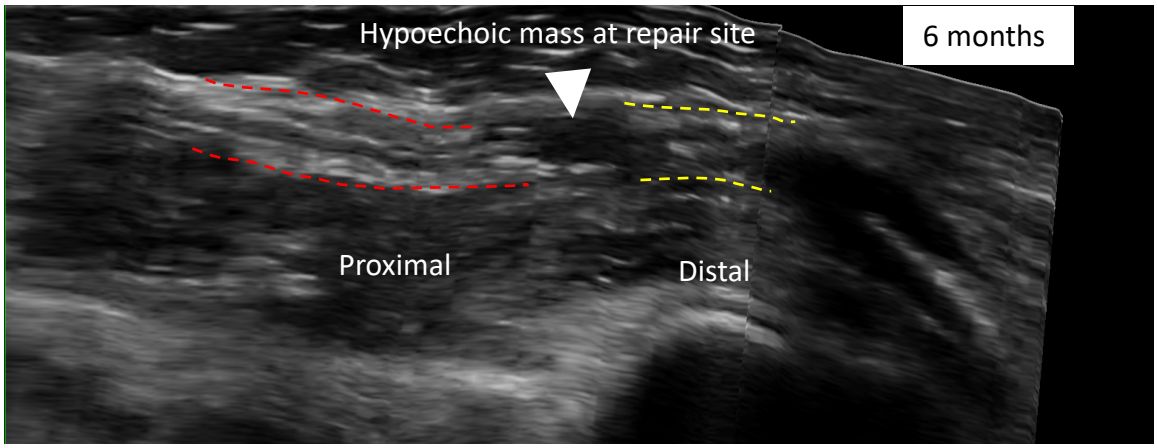
Her PROM scores showed minimal change over the study period: DASH 92.5 to 65 and iHand 74.2 to 63.3. Modified Rosen Scores demonstrated little improvement from 0.53 at 2 months (from a baseline/post-op value of 0.13) to 2.78 at 4 months (Figure 6.3 (a)).

The transverse Hf,3D,tUS morphology images in Figure 6.3 (b) demonstrate a hypoechoic fascicular pattern developing at the repair site by 2 months. By 6 months this pattern has extended from proximal to distal stump with evidence of a hypoechoic mass filling the repair site and extending into the distal stump along with what appears to be a band of scar tissue within the repair site and distal stump (white arrow heads).

The images in Figure 6.3 (b) also show no 3D-GSM changes between 2 – 6 months after repair. Proximal stump 3D-GSM values were 71% of control at 2 months and 66% of control by 6 months, with repair site values of 27% and 19% and distal stump values of 49% and 49% respectively. Proximal stump volume was 177% of control volume at 2 months and whilst decreasing slightly at 4 months (161%) it remained enlarged at 6 months (200% of control). Repair site volume increased from 193% of control at 2 months to 333% of control at 4 months and by 6 months was still 255% of control volume. Distal stump volume stayed relatively static with a value of 114% of control at 2 months, 127% of control at 4 months and then jumped in volume to 209% of control by 6 months.

Figure 6.4 below further characterises the hypoechoic mass at the repair site at 6 months with incomplete fascicles from proximal to distal stump. The epineurium around the repair site appears intact and therefore represents a neuroma-in-continuity.

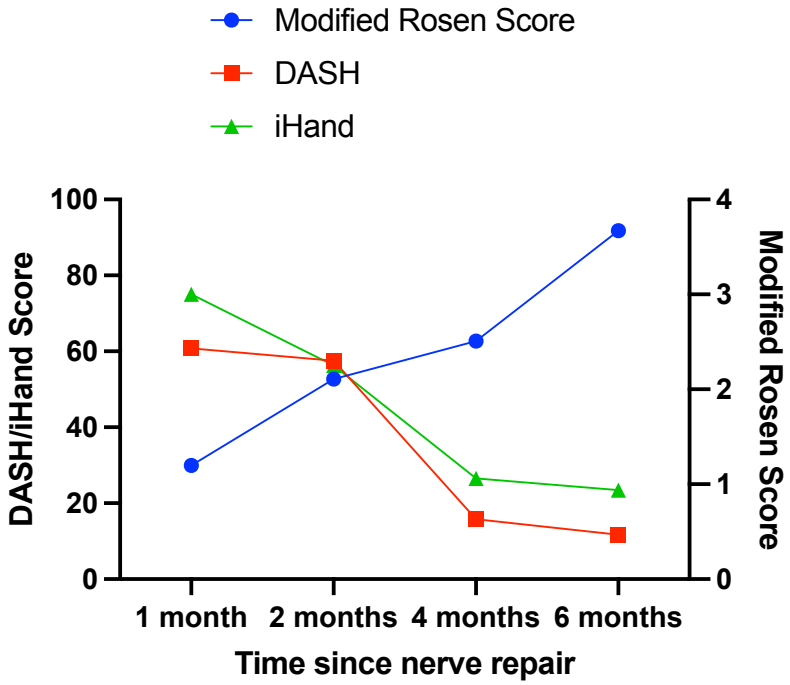
Figure 6.4: Sagittal-view of participant 003's injured median nerve at 6 months.



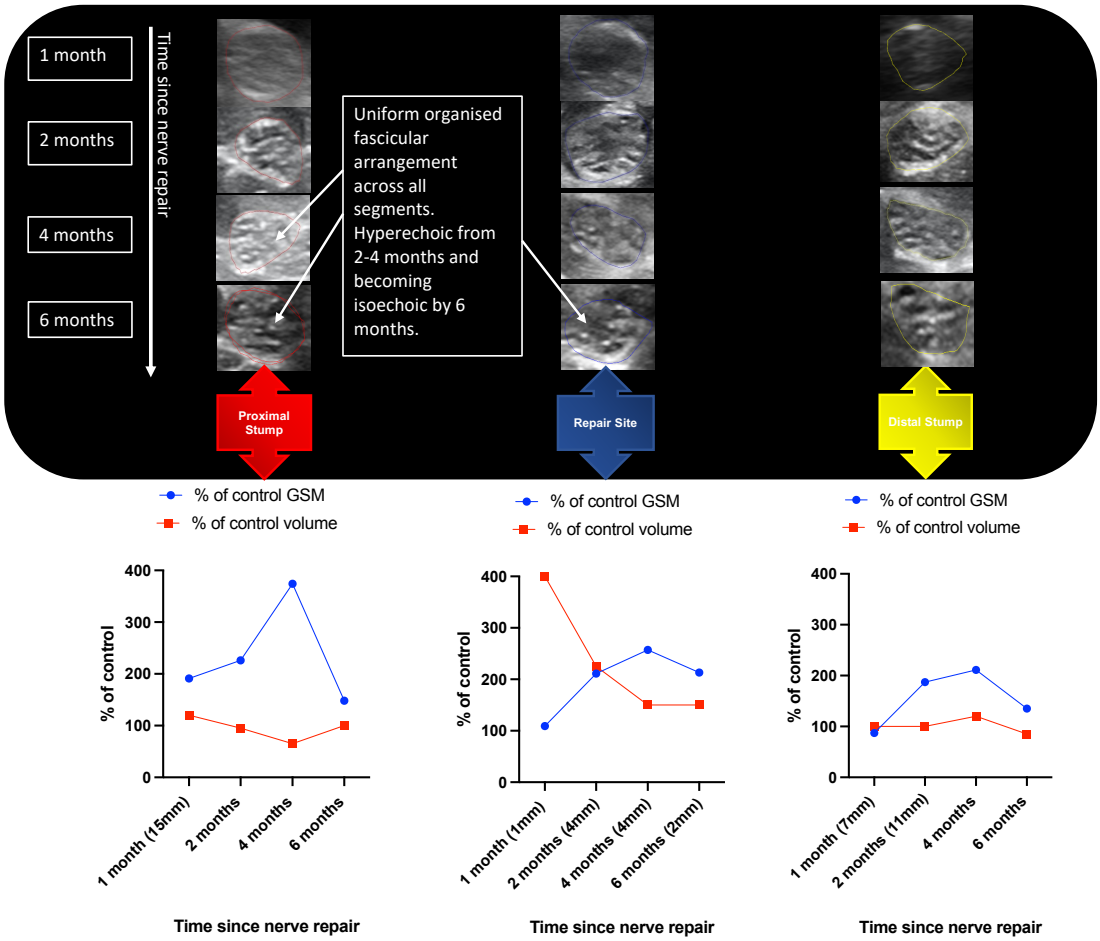
There is a hypoechoic mass at the repair site (white arrowhead) and incomplete fascicles from proximal (red-dash lines) to distal stumps (yellow-dash lines).

OPEN 004

Figure 6.5: Participant 004 clinical, patient reported and Hf,3D,tUS outcome data (a)



(b)



(a) clinical and patient-reported outcome measures: Disability of Arm Shoulder and Hand (DASH) and Impact of Hand Nerve Disorders (iHand) scores (left-hand y-axis) and Modified Rosen Scores (right-hand y-axis); (b) cross-sectional tUS images of participant 004's injured median nerve at proximal, repair site and distal sections of the nerve and at their corresponding timepoints with the graphical representation of their (%) percentage of control grey-scale median and volumetric (cm^3) values over these nerve segments.

Participant 004 deliberately cut her left wrist using a razor blade on the 05/08/2019. She underwent surgery the following morning (06/08/2019) to explore the left wrist wound and repair her flexor carpi radialis tendon, median nerve and ulnar artery under general anaesthetic with tourniquet control.

Both DASH 60.83 to 11.67 and iHand 75 to 23.44 greatly improved over the 6-month observation period and the Modified Rosen Scores were also much improved from a value of 1.2 at 1 month to 3.67 by 6 months (Figure 6.5 (a)).

The transverse Hf,3D,tUS morphology in Figure 6.5 (b) demonstrates a uniform hyperechoic fascicular pattern extending from proximal to distal stump from 2-4 months after repair. This becomes more isoechoic by 6 months.

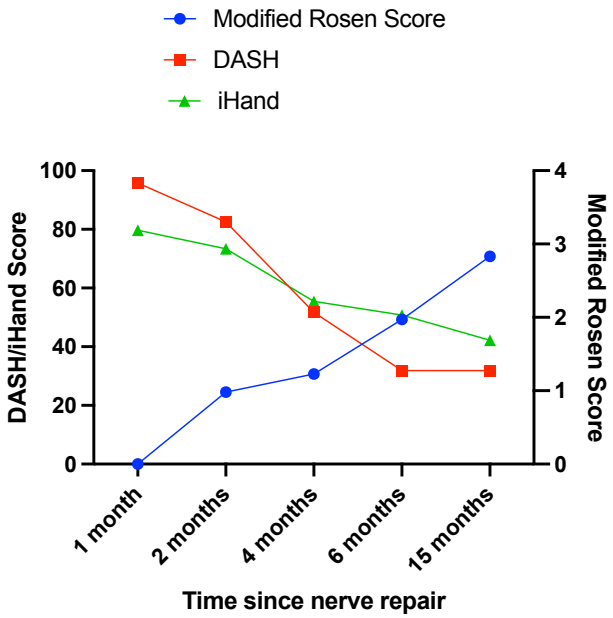
There is incremental 3D-GSM changes from 1-4 months in all segments. Proximal stump 3D-GSM values increased from 191% of control at 1 month to 374% of control by 4 months. Repair site 3D-GSM values demonstrated a similar increase from 109% of control at 1 month to 257% of control by 4 months. Distal stump 3D-GSM values increased from 87% of control at 1 month to 211% of control by 4 months. 3D-GSM value of all segments then returned towards control by 6 months (Figure 6.5 (b)).

By 1 month, the repair site volume had jumped to 400% of control with proximal and distal stumps remaining close to control volumes. Repair site volume gradually decreased to a value of 150% of control by 6 months whilst proximal and distal stump volumes remained largely unchanged throughout, staying at values close to the contralateral control volume (Figure 6.5 (b)).

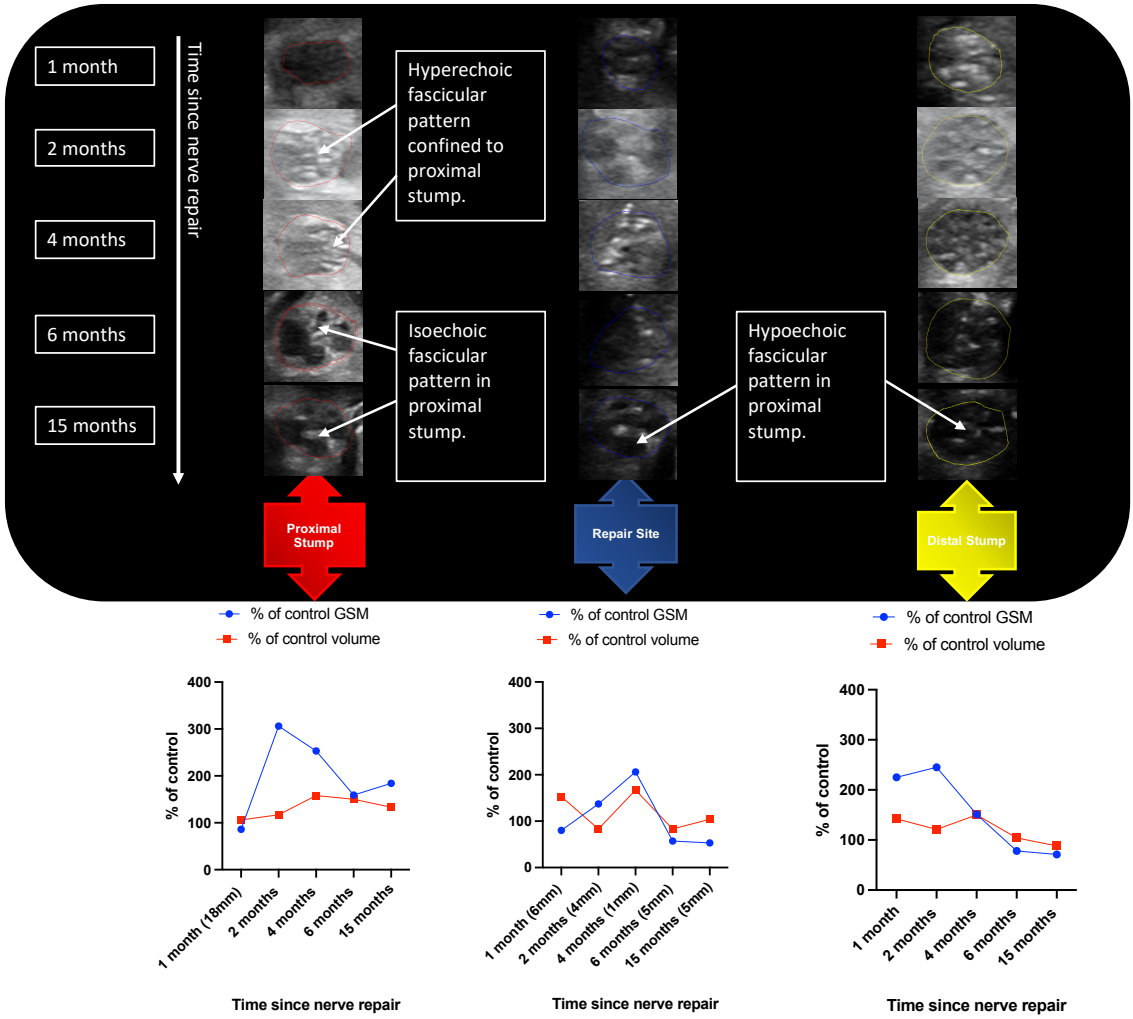
OPEN 005

Figure 6.6: Participant 005 clinical, patient reported and Hf,3D,tUS outcome data (median nerve).

(a)



(b)



(a) clinical and patient-reported outcome measures: Disability of Arm Shoulder and Hand (DASH) and Impact of Hand Nerve Disorders (iHand) scores (left-hand y-axis) and Modified Rosen Scores (right-hand y-axis); (b) cross-sectional tUS images of participant 005's injured median nerve at proximal, repair site and distal sections of the nerve and at their corresponding timepoints with the graphical representation of their (%) percentage of control grey-scale median and volumetric (cm^3) values over these nerve segments.

Participant 005 suffered a right wrist laceration from an angle grinder on 26/08/2019. The following day he had the wound explored and median and ulnar nerves, ulnar artery and all tendons (FPL, FCR, FCU, PL and FDS and FDP to all digits) repaired under general anaesthetic with tourniquet control.

Both DASH 95.83 to 31.9 and iHand 79.69 to 42.19 improved over the 15-month observation period and the Modified Rosen Scores improved from a baseline value of 0 at 1 month to 2.83 by 15 months (Figure 6.6 (a)).

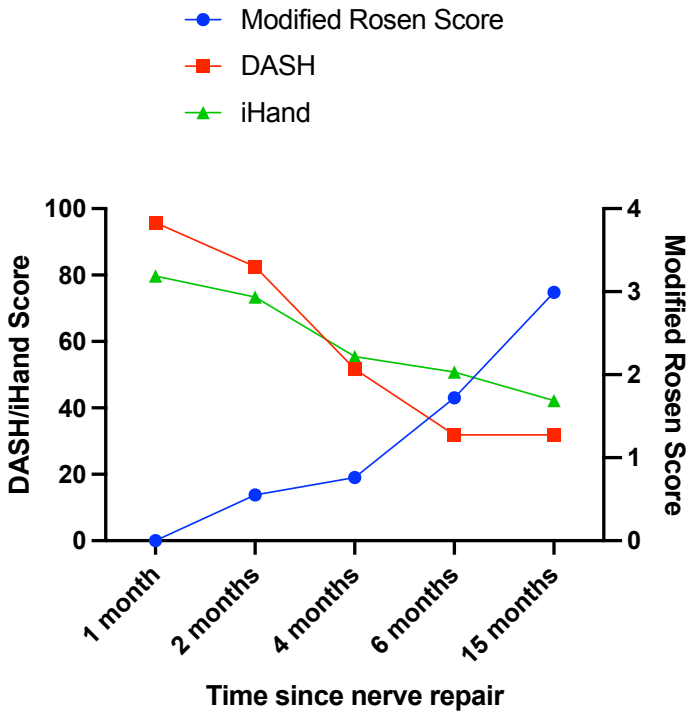
The transverse Hf,3D,tUS morphology in Figure 6.6 (b) demonstrates a hyperechoic fascicular pattern in the proximal stump at 2-and 4-month timepoints which became isoechoic by 6 months. However, the repair site and distal stump remained comparatively isoechoic up until 6 months before developing a hypoechoic pattern.

The images in Figure 6.6 (b) also demonstrate large increases in 3D-GSM values from 1 to 2 months after repair in all segments (proximal 86% to 306%, repair site 80% to 137% and distal 224% to 245%). These values began a downward trend from 4 months through to 15 months (proximal 253% to 184%, repair site 206% to 53% and distal 151% to 71%).

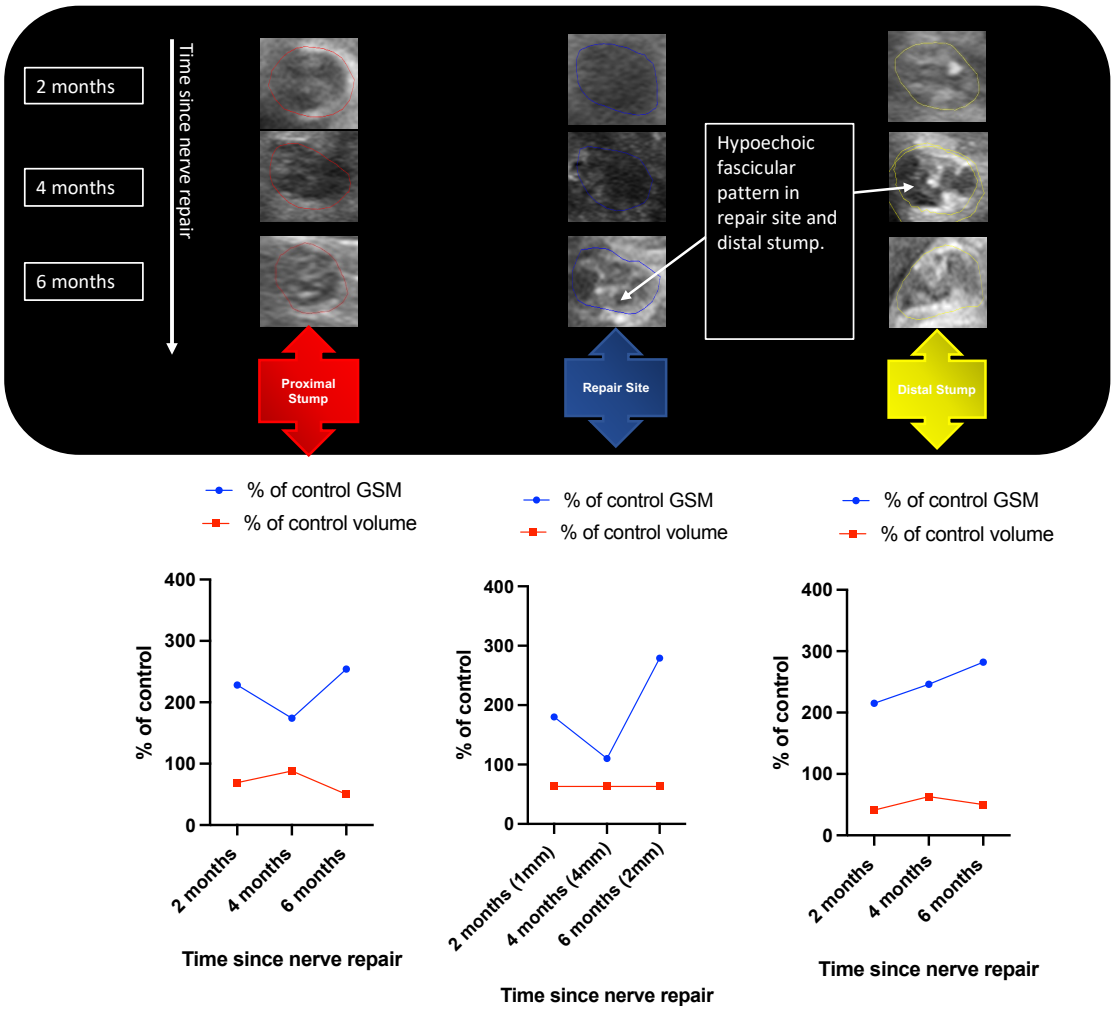
Volumes of all segments increased from 1 - 4 months after repair (proximal 106% to 158%, repair site 153% to 167% and distal 142% to 150%) followed by a decrease through to 15 months after repair (proximal 158% to 113%, repair site 167% to 104% and distal 150% to 88%) (Figure 6.6 (b)).

Figure 6.7: Participant 005 clinical, patient reported and Hf,3D,tUS outcome data (ulnar nerve).

(a)



(b)



(a) clinical and patient-reported outcome measures: Disability of Arm Shoulder and Hand (DASH) and Impact of Hand Nerve Disorders (iHand) scores (left-hand y-axis) and Modified Rosen Scores (right-hand y-axis); (b) cross-sectional tUS images of participant 005's injured ulnar nerve at proximal, repair site and distal sections of the nerve and at their corresponding timepoints with the graphical representation of their (%) percentage of control grey-scale median and volumetric (cm^3) values over these nerve segments.

DASH 82.5 to 31.9 and iHand 73.38 to 50.83 improved between 2 and 6 months after ulnar nerve repair, whilst the Modified Rosen Scores improved from a value of 0.55 at 2 months to 1.72 by 6 months (going on to a score of 2.99 by 15 months) (Figure 6.7 (a)).

The transverse Hf,3D,tUS morphology in Figure 6.7 (b) demonstrates a uniform isoechoic fascicular pattern extending from proximal to distal stump at 2 months after injury. However, from 4 months after injury the repair site and distal stump have developed areas of hypoechoic fascicular patterning.

The images in Figure 6.7 (b) also show decreases in 3D-GSM values from 2 to 4 months after repair in proximal stump (228% to 174%) and repair sites (180% to 110%), whereas there was an increase in 3D-GSM in the distal stump (215% to 246%). From 4 to 6 months after repair, all segments demonstrated an increase in 3D-GSM values (proximal 174% to 254%, repair site 110% to 279% and distal 246% to 282%).

There was little change in volumes of all segments throughout the observation period.

6.4 Discussion:

There are limited primary studies utilising ultrasound in the assessment of peripheral nerve regeneration in humans. Those studies that exist often use lower frequency probes to identify pathology when clinically indicated (Peer and Bodner, 2006; Wijntjes, Borchert and van Alfen, 2021). In order to explore peripheral nerve regeneration in vivo, investigators have used longitudinal MR images and correlated these with other outcome measures (Bendszus *et al.*, 2004). Clinically, MR neurography provides accurate levels of signal intensity to determine location of a lesion and detail regarding surrounding structures but it lacks meaningful intraneural detail. There is a significant trade-off between resolution and field of view which inhibits accurate intra-neural visualisation of long nerves (Rangavajla *et al.*, 2014). The next generation of MR imaging with functional MR neurography uses techniques such as Diffusion Tensor Imaging which analyses water diffusion to quantify nerve health. Injured nerves demonstrate disorganised water movement compared to healthy nerves with more organised movement (Noguerol *et al.*, 2019). These image acquisition techniques may improve spatial resolution, providing greater intraneural detail to monitor nerve regeneration however they require a prolonged image-acquisition time (+/- stronger magnets), which may preclude clinical use (Lehmann *et al.*, 2010). In addition, it is not possible to scan the full length of a nerve in the limb during the image acquisition process (Rangavajla *et al.*, 2014).

Ultrasound, in comparison to MRI, is more readily available, costs less and provides excellent spatial resolution of nerves in the arm (Suk, Walker and Cartwright, 2013; Brill and Tyler, 2017). This allows for detailed morphological images to be produced,

providing superior intra-neural detail which has the potential to be refined and further interrogated by clinicians and researchers. In comparison to MRI, scan image acquisition takes seconds even when scanning the entire length of upper limb nerves from axilla to hand. Ultrasound is therefore regarded as the initial imaging modality of choice in imaging upper limb peripheral nerves.

In this pilot study, we have used high-frequency probes (20mHz) and 3D, tomographic software to obtain detailed neuronal architectural images of the early stages of peripheral nerve regeneration in human participants undergoing standard surgical repair (direct neurorrhaphy). We analysed the ultrasound-based morphometry and used novel image analysis software (PIUR imaging (Vienna, Austria)) to quantify intraneural grey-scale (echogenicity) and volumetric changes within key segments of the nerve during regeneration. Specifically, the proximal stump, the repair site and the distal stump. These areas were chosen and standardised between participants where possible to allow for direct comparison during regeneration. In addition, we scanned contralateral control nerves at baseline to demonstrate uniformity of grey scale along the length of the nerve. We expected regeneration to be complete prior to 6 months however we looked to follow participants for longer time periods to explore whether any further changes occurred.

Participant 004 demonstrated excellent clinical outcomes (Modified Rosen Score = 3.67) and PROMs (DASH = 11.67 and iHand = 23.44) by 6 months after repair. Her nerve morphometry demonstrated a uniform hyperechoic fascicular pattern from proximal to distal stump through earlier timepoints, returning to a more isoechoic fascicular pattern by 6 months. Notably, there was minimal volume changes occurring in the proximal and distal stumps compared to the contralateral nerve. At the repair site, although the volume was initially 4x the contralateral control, this returned close to control levels by 4 months. The 3D-GSM increased in all areas after repair, the greatest increases occurring in the proximal stump with repair site and distal stump changes increasing sequentially as the nerve regenerated. Between 4 and 6 months after injury the 3D-GSM values began returning towards the control values as nerve regeneration was expected to be complete and as clinical outcome measures plateaued.

Conversely, participant 003, achieved poor clinical outcomes (Modified Rosen Score = 1.33) and PROMs (DASH = 65 and iHand = 63.28) by 6 months after repair. The morphometry demonstrated a disorganised fascicular pattern across all segments and by 6 months it was clear that a neuroma-in-continuity was developing (confirmed by sagittal views). The volume of all segments was more than double the volume of the control by 6 months, with the repair site segment demonstrating the highest volume differences. The 3D-GSM values remained significantly lower than the control throughout the follow-up period in all segments.

Participants 002 and 005 demonstrated variations of patterns seen in participant 004. Participant 002 demonstrated a much slower, gradual improvement in clinical and PROMs, reaching reasonably good outcomes (Modified Rosen Score = 2.78, DASH = 36.7 and iHand = 39.1) by 4 months after repair. She had similar volumetric changes as participant 004, however morphometry demonstrated a more isoechoic fascicular pattern at 1-and 2-month visits, with a hyperechoic fascicular pattern developing by 4 months. The 3D-GSM levels only began increasing at the 4-month timepoint in all segments, again with the proximal stump demonstrating the largest increase.

Participant 005 also demonstrated a slower pattern of recovery reaching a (Median Nerve) Modified Rosen Score of 2.83 and DASH score of 31.9 and iHand score of 42.19 by 15 months after repair. He developed a hyperechoic fascicular pattern in the proximal stump at 2-and 4-month timepoints which became isoechoic by 6 months. However, the repair site and distal stump remained comparatively isoechoic up until 6 months before developing a hypoechoic pattern. Volume levels remained grossly unchanged compared to control levels throughout, except at the 4-month timepoint at the repair site segment. This appeared to coincide with the decrease in 3D-GSM levels in the same segment although it is not clear if these changes are linked. Participant 005's ulnar nerve changes followed a more typical pattern with volumetric changes in all segments remaining low throughout and 3D-GSM levels showing an overall increasing trend by 6 months, despite dips at 4 months in the proximal stump and repair site segments. A modified Rosen Score of 1.72 was recorded by 6 months for the ulnar nerve which improved to 2.99 by 15 months despite a lack of Hf, 3D, USS data at this timepoint due to poor image acquisition. The only other difficult image acquisitions occurred at the first post-operative visit in participant 003 and 005 (ulnar nerve) at 1 month after repair.

These findings can be explained by the clinical situation. In participant 004 who had the best clinical and Hf,3D,tUS outcomes, her nerve was explored and repaired in less than 24 hours after injury. However, in participant 003 who had the worst clinical and Hf,3D,tUS outcomes, she had an initial operation at a major trauma centre with suture “tagging” of the nerve ends followed by a further definitive procedure nearly 4 days later to debride and repair the severed nerve ends. The repeated operation and delay to definitive repair likely increased the risk of scar formation at the repair site which ultimately contributed to her developing a neuroma-in-continuity.

We have identified trends in the Hf,3D,tUS morphology, 3D grey-scale median (echogenicity) and volume measurements that appear to correlate well with clinical and PROMs. Firstly, morphometric analysis allows for identification of abnormalities in regeneration and localisation of causative factors, such as participant 003 who developed a neuroma-in-continuity due to intraneural scarring. These abnormalities appear to correlate strongly with volumetric analysis (participant 003) whereby areas of abnormal growth, or neuromas-in-continuity, lead to intraneural swelling. Volumetric changes at the repair site also help to confirm whether post-operative swelling has improved (participant 004).

The 3D-GSM allows us to quantify the degree of echogenicity of intraneural tissues for the first time. Detailed echogenic analysis of these specific three-dimensional injured nerve segments has never previously been described and may allow quantification of nerve regeneration. In similar studies of peripheral nerve regeneration in animal models and using magnetic resonance imaging, hyperintense signals were noted distal to the injury and regressed in a proximal to distal direction as concomitant electrophysiology and histology showed evidence of regeneration (Bendszus *et al.*, 2004). We have demonstrated an equal but opposite change in the echogenicity that occurs during regeneration with an increase from a proximal to distal direction across the repair site before decreasing back to levels of the contralateral control uninjured nerves once regeneration is complete. This may be explained by the fact that collagen and myelin are more hyperechoic than collagen alone (Byra *et al.*, 2019) thus the laying down of new myelin as the nerve regenerates presents a more hyperechoic signal than the empty collagen “shell” of a nerve that has undergone Wallerian degeneration.

These ultrasound-based biomarkers of peripheral nerve regeneration may provide nerve surgeons and researchers with a novel, early and objective measure of peripheral nerve regeneration in humans that is immediately clinically available. Clinically important changes can be detected in the first 2 - 4 months after repair which could help guide whether the nerve is going to regenerate and produce a good clinical outcome or not. The tomographic, three-dimensional imaging software allows for detailed interrogation of the neuronal structural changes in three planes (cross-section/transverse, sagittal and coronal) even when the technician performing the scans has limited experience in peripheral nerve imaging. The equipment, in relation to a standard ultrasound machine, is a laptop and a probe attachment approximately the size of a printer cartridge. The cost is less than MRI and the intuitive image analysis can easily be performed by clinicians with minimal prior ultrasound training. The lead author who analysed the images required two clinical half days and subsequent reading in order to develop the knowledge and skills required.

This pilot study was limited in participant numbers and timing and number of follow-up visits due to the coronavirus pandemic leading to a pause in face-to-face clinical research. However, it provides a clear structure and basis to inform a future prospective, multicentre study, adequately powered, in order to demonstrate the relationship between these novel ultrasound biomarkers and clinical and patient-reported outcome measures of peripheral nerve regeneration. Addition of intravenous ultrasound-contrast may further help to explore the neovascularisation processes during regeneration. This combined with grey-scale analysis on Hf,3D,tUS may help to highlight areas for targeted therapies or interventions that could improve the regenerative process to ensure outcomes are optimised beyond simple repair strategies.

CHAPTER 7 Summary, Discussion & Future Work

7.1: Summary

7.2: Discussion of results from:

Chapter 2: The national epidemiology of peripheral nerve injury (2005-2019)
Chapter 3: Translation of a novel treatment for peripheral nerve gap injuries: The UMANC Trial
Chapter 4: Systematic review of outcome measures used in clinical peripheral nerve research
Chapter 5: Optical coherence tomography in digital Nerve Injury (ODEN Study)
Chapter 6: Novel Outcome Measures in Peripheral Nerve Injury (OPEN Study)

7.3: Future Direction

7.1 Summary

Professor Göran Lundborg highlighted the need for greater understanding of the neurobiology of nerve injury and regeneration at the turn of the century (Lundborg, 2000). Over the past 20 years, much of this neurobiology has been explored in laboratories around the world but this has not translated into improved clinical treatments for PNI. There is a translational barrier that prevents laboratory advancements reaching patients which is in part due to the lack of understanding of the clinical landscape and how we, as peripheral nerve surgeons, approach the assessment of our patients. We lack basic knowledge of who is most commonly affected by these injuries, their cause and how they are being treated. Through the interrogation of national epidemiological data of PNI I have been able to identify the who, what and why of upper limb PNI in addition to exploring the current management of these patients.

Novel treatments that show great promise in pre-clinical models may not demonstrate the expected treatment effect in clinical trials due to a lack of objective, detailed outcome measures that can demonstrate the early microbiological effects of nerve regeneration. Performing a Phase I clinical trial of a novel treatment for PNI has demonstrated the need for these improved outcome measures.

A plethora of outcome measures exist, and I have now demonstrated what these are, and which are the most commonly used through systematic review of clinical outcome measures of peripheral nerve regeneration. The most commonly used were often developed over half-a-century ago and new outcome measures utilising the latest technology are clearly needed. These need to be able to objectively explore and quantify the early microbiological changes after nerve repair in order to determine the effectiveness of treatments, current or new, and improve patient care. I have achieved this through assessment of end-organ skin changes during sensory nerve regeneration utilising real-time *in-vivo* histology with OCT. Identifiable change in biomarkers such as epidermal thickness and sweat duct density provide a detailed, objective insight into the early stages of peripheral nerve regeneration. At the repair site of mixed nerve injuries, I utilised Hf,3D,US in order to explore regrowth and complications of regeneration, which has demonstrated detectable

greyscale and volumetric changes which may provide a novel biomarker of regeneration.

7.2: Discussion of results

The national epidemiology of peripheral nerve injury (2005 - 2019)

Incidence of PNI in England is high compared to other European countries. Men are over twice as likely to sustain a PNI as women and distal sensory nerves of the upper limb represent the most commonly injured nerves (>75% of all PNIs). These types of injury are now more commonly being managed as an outpatient whereas more proximal injuries often require prolonged inpatient stays that have been increasing over the past 15 years. Knife lacerations as a cause of PNI have shown a significantly increasing trend and further work is needed to determine the exact cause and whether prevention strategies can be implemented to reduce these injuries. More proximal injuries are commonly caused by falls or road-traffic accidents which are likely to result in significant trauma. Ensuring these types of injury are effectively managed in major trauma centres is of utmost importance for rehabilitation and functional outcomes. Plastic surgeons are increasingly managing the majority of nerve injuries and certainly the highest proportion of upper limb nerve injuries. The most common operation remains neurosynthesis with techniques first described over a century ago. Nerve grafting is becoming more common for forearm and distally based nerve injuries of the upper limb and therefore development of alternatives to nerve grafts are needed to reduce donor site morbidity. The next chapter explores the translation of a novel alternative to nerve grafting from the lab to the clinic.

Translation of a novel treatment for peripheral nerve gap injuries: The UMANC Trial

Polynerve appears safe to use in humans and has proved to be more effective at peripheral nerve regeneration than other clinically available, synthetic, hollow tube conduits. However, randomised clinical trials are needed to compare it to autograft and Neurolac® in order to compare its effectiveness at regeneration. In addition, phase II/III trials are needed to explore its safety profile in a larger more diverse population. Through the process of translating Polynerve from lab to clinic through a Phase I clinical trial, I have identified a lack of objective and quantifiable measures

of peripheral nerve regeneration. In some patients, the ability of currently available clinical outcome measures to detect change was very limited. Some measures were more effective than others and some measures showed little to no change throughout the study. In order to develop more robust and objective clinical outcome measures it is important to determine what outcome measures are currently in clinical use, which should be used and when.

Systematic review of outcome measures used in clinical peripheral nerve research

Through systematic interrogation of currently used clinical outcome measures I have found that the most commonly used, such as the MRC scale for assessment of motor function, are subjective methods often developed over 50 years ago. In addition, there is no guidance on to which outcome measures to use and when. These commonly used clinical outcome measures lack the ability to provide detailed information of the early stages of nerve regeneration. Nerve surgeons and researchers need objective, detailed information of the early stages of nerve regeneration to be able to intervene when regeneration is failing or when developing novel treatments.

Optical coherence tomography in digital nerve injury (ODEN Study)

I have used optical coherence tomography to measure epidermal thickness and sweat duct density in an uninjured population to identify if any variability exists between hands, age, sex or ethnicity. In addition, I have produced estimated reference intervals of these values in order to compare their values in disease states. I have explored the epidermal thickness and sweat duct density changes after sensory nerve injuries in the hand which has demonstrated that the epidermis of volar digital skin thins after nerve injury returning to normal as the nerve regenerates. In addition, sweat-duct density significantly reduces immediately after digital nerve injury and only returns to normal by 6 months after repair. Quantification of these biomarkers of nerve regeneration is relatively simple, immediate and completely non-invasive and SDD correlation to PROMs appears better than current clinical outcome measures. OCT measurement of epidermal thickness and SDD may provide a more objective and detailed quantification of the early stages of peripheral nerve regeneration that is immediately clinically available. In addition, it

may provide a useful diagnostic tool in the future in order to reduce the burden of digital nerve exploration +/- repair.

Novel outcome measures in peripheral nerve injury (OPEN Study)

Hf,3D,US provides non-invasive visualisation of fascicular level structures within nerves of the arm. In cross-sectional views, grey-scale (linear echogenicity) and volumetric changes can be quantified, and longitudinal assessments made as the nerve heals. These measurements correlate well with clinical outcome measures during nerve regeneration. In addition, causes of impaired regeneration can be identified in multi-planar views. Hf,3D,US may provide a non-invasive, objective immediately available outcome measure of the early (first 4 months) microbiological changes of peripheral nerve regeneration.

7.3: Future direction

Prospective audit registries of peripheral nerve injury are needed which have recently been developed by the BSSH with their UK Hand Registry (BSSH, 2020), in order to provide a more detailed understanding of the mechanism of these injuries and how they are managed.

A randomised, multicentre trial is needed to compare Polynerve to autograft and Neurolac® in order to determine whether it is more effective than Neurolac® at nerve regeneration and whether either can replace the surgical gold standard of autograft repair of nerve gap injuries.

As part of this process, a core outcome set of current outcome measures is needed in order to standardise outcome reporting in clinical peripheral nerve injury research. This will require definition of the stakeholders involved and subsequently development of a consensus process. In addition to current clinical outcome measures, the novel, objective, early measures of regeneration developed in this thesis are needed immediately for research purposes and in the future, for clinical practice. Larger, multi-centre studies will be required, that are adequately powered in order to assess whether these novel outcome measures are able to more effectively detect change after peripheral nerve injury compared with current clinical measures. In addition, a prospective pilot study is planned to explore whether OCT

measured epidermal thickness and SDD can be used as a diagnostic tool in assessing digital nerve-injured patients. This may allow us to forgo the need for exploration of some injuries where uncertainty remains as to whether the nerve has been injured or not.

Appendix 1: Supplementary tables from chapter 2

Supplementary Table 1: ICD-10 Codes for peripheral nerve injury

ICD-10 code	Description of injury level
S04.0–04.9	cranial nerves
S14.3, S14.4, S14.6	neck level (*including brachial plexus S14.3)
S24.3, S24.5, S24.6	thorax level
S34.6–34.8	abdomen, lower back and pelvis level
S44.0–44.9	shoulder and upper arm level
S54.0–54.9	forearm level
S64.0–64.9	wrist and hand level
S74.0–74.9	hip and thigh level
S84.0–84.9	lower leg level
S94.0–94.9	ankle and foot level
T11.3 T11.6	unspecified level, upper limb
T13.3 T13.6	unspecified level, lower limb
T14.4	nerves of unspecified body region

Supplementary Table 2: OPCS-4 Codes for peripheral nerve surgery

OPCS-4 Code	Description of procedure
A62.1	Primary microsurgical graft to peripheral nerve
A62.3	Microsurgical graft to peripheral nerve NEC
A62.4	Primary microsurgical repair of peripheral nerve NEC
A62.7	Microsurgical repair of multiple peripheral nerves NEC
A63.1	Primary graft to peripheral nerve NEC
A64.2	Primary repair of peripheral nerve NEC
A68.4	Primary neurolysis of peripheral nerve NEC
A73.4	Exploration of peripheral nerve
A73.6	Transfer and reimplantation of peripheral nerve NEC

Appendix 2: Polynerve: Instructions for Use

Indications for use

The Polynerve conduit is indicated for the repair of peripheral nerve gap up to 2cm in patients where nerve continuity has been damaged.

Contraindications

Polynerve conduits must not be used in contaminated wounds.

Warnings

Ensure wounds are clean before use.

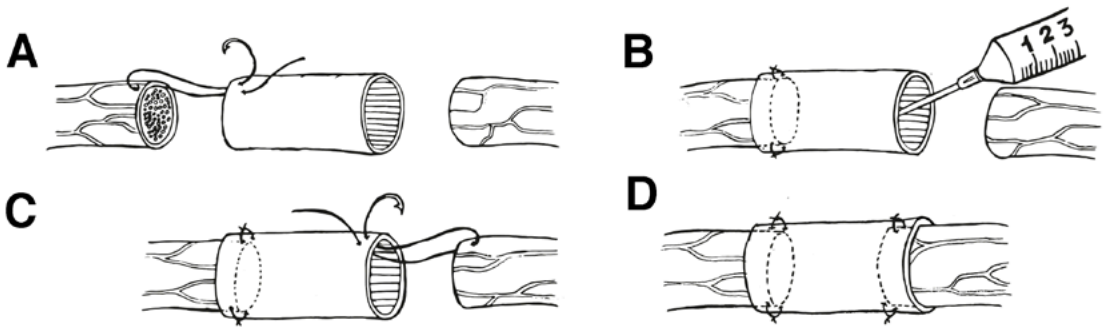
Precautions

• Return unused conduits to pharmacy once the outer package has been opened.
• The use of 9-0 non-resorbable sutures is recommended.
• Avoid crushing, kinking or damage to the conduit using surgical instruments and while handling.
• Prevent any compression or tension of the nerve stumps during insertion into the conduit.

The injured nerve is exposed and mobilised following standard surgical procedure. The nerve size is assessed in millimetres (mm) and a conduit of suitable diameter is selected in order to insert the nerve stumps into the conduit. The nerve gap is also measured and where this is less than 2 cm the Polynerve conduit can be reduced to a suitable length by cutting with a sharp blade. The nerve stumps should be inserted into the conduit by 1-2 mm at each end. Care should be taken that no tension occurs and the nerve is handled gently with no crushing.

The Polynerve conduit is sutured using 9-0 non-resorbable sutures and non-traumatic technique. Insert the suture through the wall of the conduit from outside to inside, at least 1 mm from the edge of the conduit. Then suture transversely and superficially through the epineurium of one nerve stump, reversing the suture and passing it through the wall of the conduit from inside to the outside of it (A). Gently pull the end of the nerve stump into the conduit for at least 1-2 mm, avoiding tension and crushing of the nerve ends, and tie a secure knot in the suture. Gently flush the conduit lumen with sterile saline (B), then repeat the procedure for the other nerve stump (C) to conclude the repair (D).

For the repair of extremities associated with a joint, immobilization is recommended for 1 week after repair.



Appendix 3: Supplementary data from chapter 4

Supplementary Table 1: Sensory outcome reporting

Outcome Measure Domains	Outcome Measures	No. of studies reporting outcome measure	Instrument	Metric	Specific Time points
Sensory Objective	Cutaneous Pressure Threshold	25	25	23	8
	Static Tactile Discrimination (2PD)	30	30	28	13
	Moving Tactile Discrimination (2PD)	16	16	16	5
	Vibration Detection Threshold	7	7	7	2
	Thermal Detection Threshold	10	9	9	5
	Stereogenesis	10	10	8	3
	Mechanical detection threshold	4	4	4	2
	Ninhydrin sweat test	1	1	1	0
Sensory Subjective	MRC Sensory Scale	16	–	16	2
	Discrimination between sharp and dull stimuli	1	1	1	0

Sensory

Sensory Objective

Eight specific outcomes measures were classified under the sensory outcome domain. These were further subdivided into objective (9 outcome measures, used a total of 94 times) and subjective measures (2 outcome measures, used a total of

17 times) with a clear tendency towards the use of objective measures. The two most commonly assessed sensory objective outcomes were tactile discrimination and cutaneous pressure threshold.

Tactile discrimination, or the ability to discriminate between two points using touch alone, was utilised in a total of 31 studies. Of these 31 studies, it was assessed using static two-point discrimination (2-PD) in 30 studies (Chow *et al.*, 1980; Mailander *et al.*, 1989; Nunley *et al.*, 1989; Novak and Mackinnon, 1993; Tadjalli *et al.*, 1995b, 1995a; Aszmann, Muse and Dellon, 1996; Lundborg *et al.*, 1997; Battiston *et al.*, 2000; Weber *et al.*, 2000; Cheng, 2000; Cheng *et al.*, 2001; Sungpet, Suphachatwong and Kawinwonggowit, 2003; Bertleff, Meek and Nicolai, 2005; Bjorkman, Rosen and Lundborg, 2005; Rosén, Björkman and Lundborg, 2006; Taylor *et al.*, 2010; MahmoudAliloo *et al.*, 2011; Rinker and Liau, 2011; Martins *et al.*, 2013; He *et al.*, 2015; Wong *et al.*, 2015; H. J. Klein *et al.*, 2016; Hsu *et al.*, 2016; Goswami *et al.*, 2016; Ahmad, Mir and Khan, 2017; Krarup *et al.*, 2017; Rinker *et al.*, 2017; Foroni *et al.*, 2017; Saeki *et al.*, 2018) and/or moving 2-PD in 16 studies (Walton *et al.*, 1989; Mailander *et al.*, 1989; Novak and Mackinnon, 1993; Tadjalli *et al.*, 1995b, 1995a; Aszmann, Muse and Dellon, 1996; Lundborg *et al.*, 1997; Battiston *et al.*, 2000; Weber *et al.*, 2000; Cheng, 2000; Cheng *et al.*, 2001; Meek, Coert and Wong, 2003; Bertleff, Meek and Nicolai, 2005; Rinker and Liau, 2011; Hsu *et al.*, 2016; Foroni *et al.*, 2017). It was uniformly measured in millimetres but was occasionally (Tadjalli *et al.*, 1995a, 1995b; Wong *et al.*, 2015) additionally stratified using the Mackinnon/Dellon modified Highet Classification (Mackinnon and Dellon, 1988) which groups scores according to the Highet Classification but with additional s2PD and m2PD measurements. Others stratified the raw measurements using the Rosen Score (Roseén and Lundborg, 2000), Dellon's Highet Scale (Mackinnon and Dellon, 1988) or the American Society for Surgery of the Hand classification (as described by Moberg)(Moberg, 1960). The time points for assessment varied widely between studies. If specified, in digital/hand sensory nerve injury studies the range of follow up times varied from 3 weeks up to 87 months after surgery with the most common time points for assessment at 6 and/or 12 months (8 studies). In mixed upper limb nerve injuries, the range of recorded follow up times was between 1 month and 8 years after injury, with the most common period for follow up being between 12 – 24 months after injury (6 studies). After brachial plexus injury tactile discrimination was most commonly assessed between 2 – 4 years after surgery (2 studies).

Cutaneous pressure threshold assessed using Semmes-Weinstein Monofilaments (Weinstein, 1993) or von Frey filaments (Moberg, 1960) was utilised in a total of 25 studies (Nunley *et al.*, 1989; Walton *et al.*, 1989; Tadjalli *et al.*, 1995a, 1995b; Aszmann, Muse and Dellon, 1996; Lundborg *et al.*, 1997; Cheng, 2000; Cheng *et al.*, 2001; Meek, Coert and Wong, 2003; Attal *et al.*, 2004; Bjorkman, Rosen and Lundborg, 2005; Rosén, Björkman and Lundborg, 2006; Taylor, Anastakis and Davis, 2009; MahmoudAliloo *et al.*, 2011; Martins *et al.*, 2013; Chen *et al.*, 2013; Wong *et al.*, 2015; He *et al.*, 2015; Hsu *et al.*, 2016; H. J. Klein *et al.*, 2016; Krarup *et al.*, 2017; Rinker *et al.*, 2017; Foroni *et al.*, 2017; Saeki *et al.*, 2018; Bertelli *et al.*, 2018) all of which specified the instrument used. The specific metric used for reporting was force (in g or g/mm^2) in 8 studies or score (1-5) based on individual monofilaments where each monofilament is given a score instead of its actual force (Weinstein, 1993) in 7 studies. Colour of the monofilament was used in 3 studies, whilst the remaining studies used: the fibre size or a visual analogue scale. The time points for assessment in digital/hand sensory nerve injury studies varied but were most commonly found between 6 – 24 months after surgery. In mixed upper limb nerve injuries, the range of recorded follow up times was between 1 month and 10 years after injury although most studies made assessments between 6 – 24 months. After brachial plexus surgery time points for assessment ranged between 1 – 5 years.

The vibration detection threshold was assessed in 7 studies (Lundborg *et al.*, 1997; Taylor, Anastakis and Davis, 2009; Taylor *et al.*, 2010; Gierthmuhlen *et al.*, 2012; Goswami *et al.*, 2016; Vollert *et al.*, 2016; Foroni *et al.*, 2017) using either a tuning fork (256Hz) or a bio-thesiometer. A bio-thesiometer is a handheld mains operated rubber tactor (antenna) which vibrates at 100 Hz displaying a linear scale showing the applied voltage where an increase in voltage increases the amplitude of vibration (Bloom *et al.*, 1984). All studies described a specific metric in reporting outcomes using binary terms of perceptible (+) or not (-) (Foroni et al. (Foroni *et al.*, 2017)) or the specific threshold limit voltage (Taylor et al. (Taylor, Anastakis and Davis, 2009)). Lundborg et al. (Lundborg *et al.*, 1997) used their own previously described sensibility index (Lundborg *et al.*, 1992). Six studies assessing mixed upper limb nerve injuries assessed outcomes at a range of time periods from 3 weeks up to 8 years after surgery but where specified these were assessed at 3, 6 and 12 months (Lundborg *et al.*, 1997) and 3 weeks and 1 year after surgery (Goswami *et al.*, 2016).

One brachial plexus study assessed outcomes at a mean of 41 months (range 36-52 months) (Foroni *et al.*, 2017).

The thermal detection threshold was utilised in 10 studies (Attal *et al.*, 2004; Gottrup *et al.*, 2006; Witting *et al.*, 2006; Gordh *et al.*, 2008; Taylor *et al.*, 2010; Gierthmuhlen *et al.*, 2012; Wong *et al.*, 2015; Goswami *et al.*, 2016; Vollert *et al.*, 2016; Foroni *et al.*, 2017). Wong et al. (Wong *et al.*, 2015) assessed the cold detection threshold using just noticeable differences (Yarnitsky, 1997) (which is the minimal difference required to detect between two stimuli of close intensity) on a monthly basis between 1-6 months after digital nerve injury. Foroni et al. (Foroni *et al.*, 2017) assessed temperature perception in a cohort of pan-plexus injured patients treated with nerve transfers (specifically investigating intercostobrachial nerve (ICBN) as a donor of sensory fibres to the lateral cord contribution to the median nerve (LCMN)) using a steel bar warmed to 50°C and an ice bar recorded as perceptible (+) or not (-). Time points for assessment were between 36-52 months with a mean of 41 months post-operatively. Taylor et al., Goswami et al. and Vollert et al. (Taylor *et al.*, 2010; Goswami *et al.*, 2016; Vollert *et al.*, 2016) used a computer-controlled (Peltier- (TSA-II NeuroSensory Analyzer, Medoc Ltd., Israel), whilst Gottrup et al., Attal et al. and Witting et al. (Attal *et al.*, 2004; Gottrup *et al.*, 2006; Witting *et al.*, 2006) used a thermotester (SOMEDIC,Hörby, Sweden)) device with thermal probe to assess cold and warm detection threshold respectively, using a method of limits, whereby the stimulus intensity increases linearly or exponentially from a neutral temperature until the subject stops it immediately as they detect the specified sensation (Yarnitsky, 1997). Time points for assessment in these studies varied considerably with no commonly used assessment period post-surgery.

Stereogenesis, or the ability to detect three-dimensional objects via touch, was most commonly (5/10 studies) (Rosén, Björkman and Lundborg, 2006; Taylor *et al.*, 2010; MahmoudAliloo *et al.*, 2011; Goswami *et al.*, 2016; Krarup *et al.*, 2017) assessed using the shape-texture identification test described by Rosen and Lundborg (Rosén and Lundborg, 1998). Two studies (Novak and Mackinnon, 1993; Tadjalli *et al.*, 1995a) utilised an object identification test originally described by Dellon (Dellon and Kallman, 1983) and one study (H. J. Klein *et al.*, 2016) utilised the grating orientation test (Van Boven and Johnson, 1994) which consists of gratings of parallel bars and grooves of equal widths on hemispherical plastic domes. Subjects are required to touch the domes and indicate which direction the grooves and bars are before the

stimulus is removed. Bertelli et al. (Bertelli *et al.*, 2018) undertook a locognosia assessment using a 2.0 Semmes Weinstein monofilament and Adson forceps in order to localise touch, whereas Hsu et al. (Hsu *et al.*, 2016) utilised a manual tactile test to assess stereogenesis and barogenesis. The Rosen Score was the most commonly used metric for reporting results (3/10 studies), whereas those studies using Dellon's object identification test reported time and correct number/percentage of objects identified. Time points for assessment in digital sensory nerve injury studies were at a minimum of 12 months after surgery up to 87 months, whereas in mixed nerve injury studies assessment timepoints ranged from 1 month after surgery up to 8 years. The most common time points for assessment were between 18 months and 3 years after surgery.

The mechanical detection threshold was assessed in 4 studies (Bertleff, Meek and Nicolai, 2005; Gierthmuhlen *et al.*, 2012; Goswami *et al.*, 2016; Vollert *et al.*, 2016) using either von Frey hairs with rounded tips (to avoid nociceptor activation) between 0.25 and 512 mN or a pressure-specifying sensory device (Bertleff, Meek and Nicolai, 2005). Goswami et al. (Goswami *et al.*, 2016) specified time points for assessments at 2-3 weeks after surgery and after one year post-mixed upper limb nerve injury when using the mechanical detection threshold, whereas Bertleff et al. (Bertleff, Meek and Nicolai, 2005) assessed hand sensory nerve injury patients repaired using a nerve conduit at 3, 6 and 12 months.

Walton et al. (Walton and Finseth, 1977) was the only author to use the Ninhydrin sweat test to assess sudomotor nerve regeneration after nerve grafting for mixed upper limb nerve injury. A positive or negative result was recorded, and it was utilised between 10-31 months after surgery.

Sensory Subjective

Subjective sensory outcome measures were utilised far less than objective measures. The original Highet (Highet and Holmes, 1943) classification (S0-4) of sensory recovery, devised during the second world war, was used in 5 studies (Amillo *et al.*, 1993; Kalomiri and Soucacos, 1994; Meek, Coert and Robinson, 2005; Roganovic and Pavlicevic, 2006; Bai *et al.*, 2015) and often termed the MRC sensory scale, despite being a misnomer (Brushart, 2011). The modification of the original Highet classification by Mackinnon-Dellon (1988) (Mackinnon and Dellon,

1988) which includes further 2-PD criteria was used more often (Sakellarides, 1962; Walton and Finseth, 1977; Amillo *et al.*, 1993; Daoutis *et al.*, 1994; Taha and Taha, 1998; Cheng *et al.*, 2001; Lin *et al.*, 2007; Vaughn *et al.*, 2016; Rinker *et al.*, 2017; Wang *et al.*, 2017). Other modifications in the 20th Century include the Millesi score (Brushart, 2011) which combines ratings of joint motion in the hand, 2-PD and a pick-up test in addition to strength measurements which was utilised by one group (Samardzic and Rasulic, 1997). Where specified Cheng et al. (Cheng *et al.*, 2001) used the modified Highet classification in hand sensory nerve injured patients at 3 weeks and 6 months post op. Bai et al. (Bai *et al.*, 2015) used the original Highet classification in mixed upper limb nerve injuries at 3, 6 and 12 months post-operatively.

Supplementary Table 2: Motor Outcome Reporting

Outcome Measure Domains	Outcome Measures	No. of studies reporting outcome measure ment	Instrument	Metric	Specific Time points
Motor objective	Dynamometry (Grip/Pinch Strength)	9	9	9	1
Motor Subjective	Manual Muscle Testing (BMRC Scale)	30	30	30	4
	Manual Muscle Testing (Louisiana State University (LSU) Scale)	2	2	2	0
	Clinical range of motion	3	3	3	0
	Functional assessment	3	3	3	1

Motor Objective

In contrast to sensory outcome measures, grip and/or pinch strength measured using dynamometry was the only commonly utilised objective measure (Bosnjak *et al.*, 1992; Williams, 1996; Nunley, Saies and Sandow, 1997; Sungpet, Suphachatwong and Kawinwonggowit, 2003; Schreuders *et al.*, 2004; Cheing and Luk, 2005; Taylor *et al.*, 2010; Martins *et al.*, 2013; Hu *et al.*, 2018). It was uniformly well described whilst the specific reporting metric of force in Newtons or kilograms of force (kgf) was used throughout. Williams et al. (Williams, 1996) and Taylor et al. (Taylor *et al.*, 2010) reported percentage of normal grip strength as compared with the contralateral uninjured side in mixed upper limb nerve injured patients, whereas Cheing et al. (Cheing and Luk, 2005) reported grip, key pinch and oppositional pinch strengths compared to similar measurements in an uninjured control group. Only Taylor et al. (Taylor *et al.*, 2010) described specific assessment time points for assessment at 3, 6 and 12 months after surgery, the remaining studies described

ranges. In mixed nerve injuries, time points between 1 year and 18 years were used. In three brachial plexus studies a range of 12 – 42 months was used.

Motor Subjective

Subjective measures of motor function include Manual Muscle Testing using the British Medical Research Council (MRC) scale for individual muscles which was used in 30 studies (Sakellarides, 1962; Walton and Finseth, 1977; Boonstra *et al.*, 1987; Mailander *et al.*, 1989; Young, Hudson and Richards, 1990; Bosnjak *et al.*, 1992; Amillo *et al.*, 1993; Kalomiri and Soucacos, 1994; Daoutis *et al.*, 1994; Williams, 1996; Lundborg *et al.*, 1997; Nunley, Saies and Sandow, 1997; Samardzic and Rasulic, 1997; Battiston *et al.*, 2000; Jaquet *et al.*, 2001; Becker *et al.*, 2002; Sungpet, Suphachatwong and Kawinwonggowit, 2003; Bertelli and Ghizoni, 2003; Schreuders *et al.*, 2004; Meek, Coert and Robinson, 2005; Daneyemez *et al.*, 2005; Roganovic and Pavlicevic, 2006; Lin *et al.*, 2007; Bai *et al.*, 2015; Ko *et al.*, 2016; Vaughn *et al.*, 2016; Ahmad, Mir and Khan, 2017; Frueh *et al.*, 2017; Wang *et al.*, 2017; Hu *et al.*, 2018); the original Highet's staging of motor recovery (Highet and Holmes, 1943) from which the MRC scale was based, was utilised in four studies (Mailander *et al.*, 1989; Nunley, Saies and Sandow, 1997; Samardzic and Rasulic, 1997; Becker *et al.*, 2002). It was uniformly well described with the same specific measurement used throughout. Time points for assessment were rarely specified, where stated in brachial plexus injuries, the MRC scale was used anywhere between 20-73 months post-operatively. In mixed upper limb nerve injury studies, it was used anywhere between 3 months – 18 years after injury. The Louisiana State University (LSU) scale developed by Kline and Hudson (Kim *et al.*, 2007), modified the MRC scale, helping to differentiate patients in grade 4 and create a specification for each nerve and was used in 2 studies (Sulaiman *et al.*, 2009; Ferreira, Martins and Siqueira, 2017). It was well described, however neither study clarified time points for its use.

Clinical assessment of range of motion was assessed in three studies. Chu et al. (Chu *et al.*, 2016), Hu et al. (Hu *et al.*, 2018) and Baltzer et al. (Ko *et al.*, 2016) assessed range of movement at the shoulder measured in degrees. These were respectively assessed post-surgery at 1-7years, when recovered and between 12-40 months.

Three studies made functional assessments using a variety of scores. Bai et al. (Bai *et al.*, 2015) used the Mayo Elbow Score to assess elbow function at 3, 6 and 12 months after high median, ulnar and radial nerve injuries. Zhou et al. (J.-M. Zhou *et al.*, 2012) used the Upper Extremity Functional Evaluation of the Chinese Hand Surgery Academy to assess functional recovery in mixed upper limb nerve injury patients which was performed 4 weeks post-surgery. Baltzer et al. (Baltzer *et al.*, 2016) assessed ulnar intrinsic function compared to an uninjured control group at 1 year post-op in patients undergoing end-to-side anterior interosseous nerve to ulnar motor nerve transfer.

Supplementary Table 3: Sensorimotor Function Outcome Reporting

Outcome Measures	No. of studies reporting outcome measurement	Instrument	Metric	Specific Time points
Moberg's Pick-up Test	1	1	1	0
Minnesota Rate of Manipulation Test	1	1	1	0
Southampton Hand Assessment Procedure (SHAP)	1	1	1	0
Grooved Pegboard	1	1	0	0

Four separate sensorimotor tests were employed to assess sensorimotor function. Tadjalli et al. used Moberg's Pick-up Test (Tadjalli *et al.*, 1995a), whilst Nunley et al. (Nunley, Saies and Sandow, 1997) used the Minnesota Rate of Manipulation Test and Taylor et al. (Taylor, Anastakis and Davis, 2010) used the Southampton Hand Assessment Procedure (SHAP) and the grooved pegboard test. The Minnesota Rate of Manipulation Test, Moberg's Pick-up Test and the grooved pegboard test were timed tests and thus reported in seconds required to complete the relevant tasks. Contralateral controls were used as the comparator whilst all three studies performed the measures at a considerable time after surgery/injury, Tadjalli et al (Tadjalli *et al.*, 1995a) at a mean of 35 months (16 - 87 months), Nunley et al. (Nunley, Saies and Sandow, 1997) at a mean of 38 months (12 months – 10 years) and Taylor et al. (Taylor, Anastakis and Davis, 2010) at 5 years (+/- 3 years). Taylor et al. (Taylor, Anastakis and Davis, 2010) performed the SHAP test at 5 (+/- 3) years after surgery with a score out of 100 recorded.

Supplementary Table 4: Psychology and Wellbeing Outcome Reporting

Outcome Measures	No. of studies reporting outcome measurement	Instrument	Metric	Specific Time points
Hospital Anxiety and Depression Scale (HADS)	2	2	2	0
Post-traumatic Stress Disorder (PTSD) Checklist – civilian version (PCL-C)	1	1	1	0
Neuroticism, Extraversion and Openness - Five Factor Inventory (NEO-FFI)	2	2	2	1
Beck Depression Inventory	1	1	1	0
Sense of Coherence 13-item scale	1	1	1	0

Novak et al. (Novak *et al.*, 2011) and Nashold et al. (Nashold *et al.*, 1982) used a patient-reported outcome measure (PROM) called the Hospital Anxiety and Depression Scale (HADS) to assess psychological distress related to brachial plexus (between 6 months – 15 years post-operatively) or significant mixed upper limb nerve injury (at a mean of 18 months +/- 18 months post-operatively) respectively. Novak et al. (Novak *et al.*, 2011) also used another PROM, the Post-traumatic Stress Disorder (PTSD) Checklist – civilian version (PCL-C), a self-report rating scale for PTSD in the same cohort of patients at similar time points. Goswami et al. (Goswami *et al.*, 2016) and Taylor et al. (Taylor, Anastakis and Davis, 2010) used the Neuroticism, Extraversion and Openness - Five Factor Inventory (NEO-FFI) (a psychological personality inventory providing measures of 5 basic personality factors) to assess the effect of personality on pain and cold pain threshold relationships. Goswami et al assessed patients at 2-3 weeks after surgery and after one-year post-injury, whilst Taylor et al. assessed patients at a mean of 5 years after injury. Ciaramitaro et al. (Ciaramitaro *et al.*, 2010) utilised the Beck

Depression Inventory to assess psychological quality of life at 99 days (range 25 – 150 days) after surgery.

Only one study assessed mental wellbeing, Chemnitz et al. (Chemnitz and Dahlin, 2013) used the Sense of Coherence 13-item scale (Eriksson and Lindström, 2005) which assesses a patients' outlook on life in terms of their ability to see the world as comprehensible, manageable and meaningful. Chemnitz et al used this questionnaire in a long-term (30+ years) follow-up study of patients with forearm/arm (mixed) nerve injuries.

Supplementary Table 5: Disability Outcome Reporting

Outcome Measures	No. of studies reporting outcome measurement	Instrument	Metric	Specific Time points
Disabilities of the Arm, Shoulder and Hand (DASH) PROM	8	8	8	3
Occupational Performance PROM	1	1	1	0
Groningen Activity Restriction Scale PROM	1	1	1	0
The modified Rankin Scale PROM	1	1	1	0
Overall Neuropathy Limitations Scale (OLNS) PROM	1	1	1	0

The Disabilities of the Arm, Shoulder and Hand (DASH) PROM (Institute for Work and Health, 2006) was the most commonly used disability outcome measure with a total of 8 studies using this tool (Novak *et al.*, 2011; Ducic, Fu and Iorio, 2012; Chemnitz and Dahlin, 2013; Bai *et al.*, 2015; Wong *et al.*, 2015; Ko *et al.*, 2016; Ferreira, Martins and Siqueira, 2017; Frueh *et al.*, 2017). Where specified it was used monthly (1-6 months) after hand sensory nerve repair by Wong et al. (Wong *et al.*, 2015); at 3, 6 and 12-months by Bai et al. (Bai *et al.*, 2015) in mixed upper limb nerve injuries and at 1 year after nerve transfer surgery for brachial plexus injured patients by Ferreira et al. (Ferreira, Martins and Siqueira, 2017). Ducic et al. (Ducic, Fu and Iorio, 2012) used the abbreviated version, the quick DASH score after 2 years in patients with mixed upper limb nerve injuries.

Tadjalli et al. (Tadjalli *et al.*, 1995a) used an occupational performance PROM (Townsend, Brintnell and Staisey, 1990) developed by Canadian Occupational Therapists to assess the effect of digital nerve injury and repair on self-care, leisure and productivity. It was used at a mean of 35 months post-operatively (range 16 - 87 months). Meiners et al. (Meiners *et al.*, 2005) used the Groningen Activity Restriction Scale PROM to assess the impact of digital and mixed upper limb nerve

injuries (directly repaired) upon activities of daily living. It was used at a mean of 2 years post-operatively. Ciaramitaro et al. (Ciaramitaro *et al.*, 2010) used the modified Rankin Scale PROM and the Overall Neuropathy Limitations Scale (OLNS) PROM to assess degree of disability, in addition to the DASH score. These were both used at a mean of 99 days (range 25 – 150 days) post-operatively in brachial plexus injured patients.

Supplementary Table 6: Quality of Life Outcome Reporting

Outcome Measures	No. of studies reporting outcome measurement	Instrument	Metric	Specific Time points
The Short Form – 36 PROM	4	4	4	2
The Clinical Global Impressions (CGI) scale	1	1	1	1
The Patient Global Impression of Change (PGIC) scale	3	3	3	3
Visual analogue scale (VAS) to assess the impact of nerve injury on education and leisure activities	1	1	1	0

The Short Form – 36 PROM was the most commonly utilised assessment of quality of life and was used in 4 studies (Gordh *et al.*, 2008; Ciaramitaro *et al.*, 2010; Santana *et al.*, 2016; Colini Baldeschi *et al.*, 2017). Gordh et al. (Gordh *et al.*, 2008) used it in mixed nerve injuries, whereas Colini Baldeschi et al. (Colini Baldeschi *et al.*, 2017), Ciaramitaro et al. (Ciaramitaro *et al.*, 2010) and Santana et al. (Santana *et al.*, 2016) used the form in brachial plexus injured patients. Where specified it was used at 6-months post-operatively.

The Clinical Global Impressions (CGI) scale (Busner and Targum, 2007) comprises three measures: severity of illness, global improvement (both of which are measured through a seven-point scale) and efficacy index. It is intended to be used by a clinician to assess the patient's global functioning prior to and after an intervention. Gordh et al. (Gordh *et al.*, 2008) used the CGI to assess the effect of gabapentin in traumatic nerve injury pain in patients with mixed upper limb nerve injury. The Patient Global Impression of Change (PGIC) scale, which is the patient-reported outcome counterpart to the Clinical Global Impressions (CGI) scale (Busner and Targum, 2007), consists of one item taken from the CGI and adapted to the patient to assess if there has been an improvement or decline in clinical status. It was used in three studies (Gordh *et al.*, 2008; Ciaramitaro *et al.*, 2010; Vollert *et al.*, 2016) two

involving mixed upper limb nerve injuries and one involving brachial plexus nerve injuries and where specified it was used at 1, 3 and 6 months after surgery.

Chemitz et al. (Chemnitz and Dahlin, 2013) used a visual analogue scale (VAS) to assess the impact of the nerve injury on patients' education and leisure activities which was used at a median of 31 years (range 23-40 years) after injury/surgery.

Supplementary Table 7: Pain and Discomfort outcome reporting

Outcome Measure Sub-Domains	Outcome Measures	No. of studies reporting outcome	Instrument	Metric	Specific Time points
Pain Intensity Scales	Visual Analogue Scale (VAS)	12	12	12	10
	Numeric pain rating scale (NRS)	5	5	5	5
Pain Intensity Questionnaires (Patient-Reported)	Short-Form McGill Pain Questionnaire (SF-MPQ)	3	3	3	2
	Mainz Pain Centre Questionnaire	1	1	0	1
Allodynia	Mechanical/ Dynamic Allodynia	9	9	9	7
	Cold allodynia	6	6	6	1
Pain Thresholds	Thermal pain thresholds (heat and/or cold)	8	8	8	3
	Tactile pain threshold	7	7	7	2
	Pressure pain threshold	6	6	6	1
Evoked Pain Intensity	Pinprick-evoked Pain	3	3	3	3
	Wind-up ratio	3	3	3	1
Psychological Impact of Pain	Pain Catastrophizing Scale	3	3	3	1
Neuropathic Pain Questionnaires	Clinician administered DN4 questionnaire	2	2	2	0
	Neuropathic Pain Symptom Inventory (NPSI) (Patient-Reported)	1	1	1	1

The patient-reported Visual Analogue Scale (VAS) was the most common measure used to assess pain after peripheral nerve injury and was used in 12 studies (Gottrup *et al.*, 2006)(Eisenberg, Waisbrod and Gerbershagen, 2004)(Gottrup *et al.*,

2003)(Attal *et al.*, 2004)(Gordh *et al.*, 2008)(Scadding *et al.*, 1982)(Bouhassira *et al.*, 2003)(Colini Baldeschi *et al.*, 2017)(Ciaramitaro *et al.*, 2010)(Santana *et al.*, 2016)(Cheing and Luk, 2005)(Sjolund *et al.*, 2001). It was well described in all studies. Where specified in brachial plexus nerve injury it was assessed at 1, 3 and 6 months after surgery, Ciaramitaro et al. (Ciaramitaro *et al.*, 2010) used most frequently at 3 months after surgery, whereas Santana et al. (Santana *et al.*, 2016) used it up to 18 months after injury.

The patient-reported numeric pain rating scale was used to assess pain in 5 studies (Eisenberg, Waisbrod and Gerbershagen, 2004; Gordh *et al.*, 2008; Nikolajsen *et al.*, 2010; Taylor *et al.*, 2010; Kalliomäki *et al.*, 2013). It was well described in all studies. It is 0 - 10 scale used to measure pain intensity. Specific time points for its use were given in all studies. Gordh et al. (Gordh *et al.*, 2008) used it pre- and post-operatively in mixed nerve injury patients whereas Nikolajsen (Nikolajsen *et al.*, 2010) used it 1 week before neuroma removal surgery and 1, 3 and 6 months after surgery.

Two patient-reported pain intensity questionnaires were used to assess pain intensity. Novak et al. (Novak *et al.*, 2011), Goswami et al. (Goswami *et al.*, 2016) and Witting et al. (Witting *et al.*, 2006) used the Short-Form McGill Pain Questionnaire (SF-MPQ) whereas Eisenberg et al. (Eisenberg, Waisbrod and Gerbershagen, 2004) used the Mainz Pain Centre Questionnaire. The SF-MPQ was well described in all three studies. Novak et al. used the questionnaire in a long term follow up study of brachial plexus patients between 6 months – 15 years after injury, whereas Goswami et al. used it at 2-3 weeks after surgery and after 1-year post injury in mixed nerve injury patients.

Mechanical (or dynamic) allodynia (painful hypersensitivity) was assessed in 9 studies (Lundborg *et al.*, 1997; Sjolund *et al.*, 2001; Bouhassira *et al.*, 2003; Cheing and Luk, 2005; Gottrup *et al.*, 2006; Witting *et al.*, 2006; Kalliomäki *et al.*, 2013; Goswami *et al.*, 2016; Vollert *et al.*, 2016). All studies used a brush as the stimulus with 5 of these studies (Nashold *et al.*, 1982; Novak and Mackinnon, 2000; Gottrup *et al.*, 2006; Gordh *et al.*, 2008; Vollert *et al.*, 2016) measuring the pain response using the VAS and one study (Gierthmuhlen *et al.*, 2012) using the numerical pain rating scale. Time points for assessment were specified in 7 studies – all mixed

upper limb nerve injury studies with completely different time points used in each study.

Cold allodynia was assessed in 6 studies (Lundborg *et al.*, 1997; Gottrup *et al.*, 2006; Taylor *et al.*, 2010; Novak *et al.*, 2011; Chemnitz and Dahlin, 2013; Chen *et al.*, 2013) Chemnitz et al. (Chemnitz and Dahlin, 2013) and Novak et al. (Novak *et al.*, 2011) utilised the patient-reported cold intolerance symptom severity (CISS) questionnaire (IRWIN *et al.*, 1997), which defines the severity of cold induced symptoms, to assess cold allodynia. Lundborg et al. (Lundborg *et al.*, 1997), Chen et al. (Chen *et al.*, 2013) and Taylor et al. (Taylor *et al.*, 2010) assessed cold intolerance using a patient-reported score of 1-4 (minor/moderate/disturbing/hindrance to function) or scale (0 – 10). Where specified assessments were made at 3, 6 and 12 months after surgery after mixed upper limb nerve injury.

Thermal pain thresholds (heat and/or cold) were assessed in 8 studies (Attal *et al.*, 2004; Gottrup *et al.*, 2006; Witting *et al.*, 2006; Nikolajsen *et al.*, 2010; Taylor *et al.*, 2010; Gierthmuhlen *et al.*, 2012; Goswami *et al.*, 2016; Vollert *et al.*, 2016). Gottrup et al. (Gottrup *et al.*, 2006), Nikolajsen et al. (Nikolajsen *et al.*, 2010), Attal et al. (Attal *et al.*, 2004) and Witting et al. (Witting *et al.*, 2006) used a thermotester (SOMEDIC,Hörby, Sweden)) device whereas Taylor et al. (Taylor *et al.*, 2010), Goswami et al. (Goswami *et al.*, 2016) and Vollert et al. (Vollert *et al.*, 2016) used a computer-controlled (Peltier- (TSA-II NeuroSensory Analyzer, Medoc Ltd., Israel) to assess thermal pain threshold. Only Attal et al. (Attal *et al.*, 2004) used a patient-reported visual analogue scale to further quantify the degree of pain felt. Two mixed upper limb nerve injury studies (Nikolajsen *et al.*, 2010; Goswami *et al.*, 2016) specified time points for follow up at before and 3 months after surgery and 2-3 weeks and after 1 year after injury.

Tactile pain threshold was assessed in 7 studies (Sjolund *et al.*, 2001; Gottrup *et al.*, 2006; Witting *et al.*, 2006; Nikolajsen *et al.*, 2010; Gierthmuhlen *et al.*, 2012; Kalliomäki *et al.*, 2013; Vollert *et al.*, 2016). Five studies used von Frey filaments to induce the stimulus, whereas Gierthmuhlen et al. (Gierthmuhlen *et al.*, 2012) and Vollert et al. (Vollert *et al.*, 2016) used pin prick stimuli. Both of these studies used a patient-reported visual analogue scale to measure the effect of the stimulus, whilst Kalliomaki et al. (Kalliomäki *et al.*, 2013) used a numerical rating scale. Only two

studies specified time points for assessment (Sjolund *et al.*, 2001; Nikolajsen *et al.*, 2010) at before and 3 months after surgery and before and after treatment analysing the effect of systemic adenosine infusion to reduce an area of tactile allodynia in neuropathic pain following peripheral nerve injury.

Pressure pain threshold was utilised in 6 studies (Bouhassira *et al.*, 2003; Gottrup *et al.*, 2006; Witting *et al.*, 2006; Nikolajsen *et al.*, 2010; Gierthmuhlen *et al.*, 2012; Vollert *et al.*, 2016). Gottrup et al. (Gottrup *et al.*, 2006), Nikolajsen et al. (Nikolajsen *et al.*, 2010), Bouhassira et al. (Bouhassira *et al.*, 2003) and Witting et al. (Witting *et al.*, 2006) used a pressure algometer to quantify the pressure threshold at which pain was felt, whereas Gierthmuhlen et al. (Gierthmuhlen *et al.*, 2012) and Vollert et al. (Vollert *et al.*, 2016) used a pressure gauge device. Where specified assessments were made before and at 3 months after surgery. However, 2 studies made assessment more than 5 years and up to 10 years after injury.

Pin-prick evoked pain was used in 3 studies (Gottrup *et al.*, 2003, 2006; Nikolajsen *et al.*, 2010). A nylon filament or von Frey hair was used to elicit pain and Gottrup et al. (Gottrup *et al.*, 2003) used a patient-reported visual analogue score to measure the pain response. Nikolajsen (Nikolajsen *et al.*, 2010) used this outcome before and 3 month after neuroma excision, whereas Gottrup et al. (Gottrup *et al.*, 2006) used it to assess the effect of ketamine and lidocaine on mechanical evoked pain after peripheral nerve injury.

The wind-up ratio was used in 3 studies (Witting *et al.*, 2006; Gierthmuhlen *et al.*, 2012; Vollert *et al.*, 2016) and involves a series of repetitive pin-prick stimuli which generates increased pain intensity over time. The pain intensity was assessed using a patient-reported visual analogue score in all studies and was assessed prior to positron emission tomography scanning of the brain in patients with nerve injury pain by Witting et al. (Witting *et al.*, 2006), Vollert et al. (Vollert *et al.*, 2016) and Gierthmuhlen et al. (Gierthmuhlen *et al.*, 2012) used it after 5 years and up to 10 years respectively in patients with neuropathic pain after nerve injury.

The Pain Catastrophizing scale is a patient-reported outcome measure used to assess the psychological impact of pain (Leung, 2012) and was used in 3 studies (Taylor *et al.*, 2010; Novak *et al.*, 2011; Goswami *et al.*, 2016). It was well described in all studies. Only one mixed nerve injury study specified the time points for

assessment (Goswami *et al.*, 2016) at 2-3 weeks after surgery and after 1 year post injury. Novak et al. (Novak *et al.*, 2011) assessed brachial nerve injured patients between 6 months and 15 years after their injuries, whereas Taylor et al. (Taylor *et al.*, 2010) assessed patients at 5 +/- 3 years after injury.

Neuropathic pain was assessed using the clinician administered (DN4) questionnaire by Ciaramitaro et al. (Ciaramitaro *et al.*, 2010) and Satana et al. (Santana *et al.*, 2016) in brachial plexus injured patients and using the Neuropathic Pain Symptom Inventory (NPSI) by Kalliomaki et al. (Kalliomäki *et al.*, 2013) in mixed upper limb nerve injured patients. The clinician administered (DN4) questionnaire (Bouhassira *et al.*, 2005) consists of sensory descriptors and signs related to bedside sensory examination, whereas the NPSI is a patient-reported outcome measure assessing the symptoms of neuropathic pain (Bouhassira *et al.*, 2004). All studies presented the scores of the questionnaires. Where described, time points for assessment were given in ranges. Ciaramitaro et al. (Ciaramitaro *et al.*, 2010) assessed patients at 99 days (range 25-150) and Santana et al. (Santana *et al.*, 2016) at 78 +/- 88 weeks post injury.

Supplementary Table 8: Neurotrophic measure outcome reporting

Outcome Measures	No. of studies reporting outcome	Instrument	Metric	Time points
Electrophysiology	14	14	11	3
Tinel's Test	2	2	2	1
Central Nervous System (CNS) Imaging	3	3	3	1
Magnetic Resonance Neurography	1	1	1	0
End-organ imaging	2	2	2	0
Cognitive capacity testing	1	1	0	0

Fourteen studies utilised electrophysiology as an outcome measure (Zalis *et al.*, 1972; Mackel, 1985; Boonstra *et al.*, 1987; Chu and Chu, 1995; Becker *et al.*, 2002; Sungpet, Suphachatwong and Kawinwonggowit, 2003; Daneyemez *et al.*, 2005; Bilgin *et al.*, 2007; Taylor, Anastakis and Davis, 2009; Theuvenet *et al.*, 2011; J.-M. Zhou *et al.*, 2012; Ko *et al.*, 2016; Frueh *et al.*, 2017; Krarup *et al.*, 2017). In brachial plexus patients time points for assessment ranged from 4 weeks to 73 months after surgery, in mixed upper limb nerve injury patients time points for assessment ranged between 1 month to 8 years after injury. Only one study used electrophysiology in sensory nerve injuries and undertook assessments between 16-68 months after surgery.

Tinel's test was used as an outcome measure in two studies: Lundborg et al. (Lundborg *et al.*, 1997) conventionally assessed the distal most location of Tinel's sign in the hand at 3,6 and 12-months after injury. Whereas Chen et al. graded Tinel's test based on its degree of response to the percussive stimulus ranging from 1 (no tingling) – 4 (severe discomfort caused) (Chen *et al.*, 2013).

Six studies used imaging modalities to assess regeneration. Three of these studies assessed central (cortical) changes during peripheral nerve regeneration. Taylor et al. (Taylor, Anastakis and Davis, 2009) used functional magnetic resonance imaging (MRI) analysis; cortical thickness analysis and diffusion tensor imaging analysis

after a sensory stimulus in brachial plexus injured patients at a mean of 4.8 years post-surgery; whereas Goswami et al. (Goswami *et al.*, 2016) used MRI to perform a cortical thickness analysis between 11-49 days after mixed upper limb nerve injury and Theuvenet et al. (Theuvenet *et al.*, 2011) used MRI to assess magnetoencephalography readings in patients with neuropathic pain at a mean of 5.4 years after mixed upper limb nerve injury. One study used MR neurography to assess regeneration of Oberlin (I) nerve transfers (Frueh *et al.*, 2017) with a mean follow-up of 4 years post-op and two studies used imaging to assess end-organ changes. Bosnjak et al. (Bosnjak *et al.*, 1992) assessed muscle cross-sectional area using MRI at a minimum of 9 years after surgery whilst Boonstra et al. (Boonstra *et al.*, 1987) used computational tomography to assess muscle cross-sectional area (CSA) and muscle density, and also ultrasound scanning to assess muscle CSA.

Only one study used cognitive capacity testing to assess peripheral nerve regeneration. Mahmoud Aliloo et al. (MahmoudAliloo *et al.*, 2011) utilised the Stroop Colour Test and the reaction time and ability to reproduce geometric drawings.

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