Quantifying regeneration in patients following peripheral nerve injury.

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Abstract:

Healthy nerve function provides humans with the control of movement, sensation (such as pain, touch and temperature) and the quality of skin, hair and nails. Injury to this complex system creates a deficit in function which is slow to recover and rarely, if ever, returns to what patients consider to be normal.

Despite promising preclinical experiments in animals, a significant limitation in the translation of emerging therapies is the lack of effective measures with which to quantify nerve regeneration in patients and to relate this to clinical recovery.

In animal models, tissue can be obtained interventionally following treatment to quantify muscle mass and structure and the number of axons in nerve. This would incur a significant functional deficit if undertaken in humans, and furthermore, quantification of such biological features does not necessarily reflect patient experience of functional recovery. This article presents a combined commentary of current practice from a specialist clinical unit and research team in regard to laboratory and clinic quantification of nerve regeneration. We highlight how electrophysiological diagnostic methods (which are used with significant recognised limitations in assessment of clinical medicine) can potentially be used with more validity to interpret and assess the processes of neural regeneration in the clinical context. Thus throwing light on the factors at play in translating lab advances into the clinic.

Keywords:

Peripheral nerve injury, axon regeneration, functional recovery, patient reported outcome measures, neurophysiology, MUNE, sensibility, clinical assessment.

Introduction:

Peripheral nerve injuries (PNI) can have profound effects on an individual's quality of life (QoL) and often result in life-long loss or disturbance of function [1, 2]. Ideally any incised or transected nerves should be treated urgently with a primary repair under no tension. For any repair with evident tension or significant gap between the stumps, autografts can be inlaid for repair. Complex injuries benefit from bespoke combined, and often multimodal, approaches to treatment of the lesion(s). For example, combining neurolysis, graft, distal nerve and/or tendon transfers. As successful as these techniques are we must recognise they only provide a benefit when deployed in those with the most severe injuries.

We have no methods to improve the outcome for the vast majority of those with a nerve injury. In those, whose injuries have been deemed not severe enough to warrant surgical treatment supportive physiotherapeutic care is typically favoured in place of a surgical intervention. Following a severe injury restoration of normal function is infrequently achievable and the overall prognosis following injury remains difficult to determine. One aspect that hinders the development and testing of improved treatments is the lack of assessment tools to evaluate the impact of any intervention in patients. This is in contrast to the plethora of options available for assessing outcomes in animal models; which underpin the understanding of nerve repair at a cellular and molecular level. This provides the evidence base for emerging treatments. An appreciation of the methods used to measure nerve recovery in animals is important for clinicians wishing to interpret preclinical literature. An appreciation of the additional challenges faced when assessing recovery in patients is important for all researchers in the field.

Methods to measure nerve recovery in animals

Various different animal models are used routinely in PNI research and while there is no particular consensus, it is clear that rodents (and in particular rats) are used most commonly in models of nerve injury and surgical repair [3]. As with humans, functional outcomes such as

motor, sensory and sympathetic recovery can be measured following PNI in animals. The key differences being that (1) experimental injury, repair and monitoring are reproducible and carefully controlled to address specific scientific hypotheses and (2) tissue can be harvested and analysed at the end point of the experiment. Injury models normally involve damaging a nerve in one forelimb or hindlimb then distributing animals into test and control groups to investigate outcomes from treatments.

Following initial neural degeneration in both animals and humans, recovery from PNI can be separated into three main phases: the regeneration of axons, reinnervation of targets and recovery of function. Each phase depends upon the preceding stage for a successful outcome. Experiments are designed with these different phases in mind [4]. The regeneration of axons involves the emergence of sprouts and their growth across the injury site. The axons that successfully regenerate will reinnervate the target organ, establishing connections which will in turn restore function such as motor control, sympathetic function and sensory discrimination.

Each evaluation method for PNI recovery has its strengths and weaknesses. Assessments selected based upon the type of data required to test a specific hypothesis [5, 6]. The technical difficulty, cost and time requirements to conduct the assessments are of course also a consideration [5]. Table 1 lists a selection of commonly used outcome assessments for each stage of regeneration.

When assessing motor recovery most tests are simple and non-invasive which can be monitored repeatedly throughout the recovery period. Walking track/SFI analysis provides a functional index score as a measure of motor recovery [7]. The static sciatic index (SSI) is a more basic assessment which involves the analysis of foot muscle function by measuring the toe spread in static stance. This is often limited by a difficulty in achieving a clear consistent print from the whole paw to allow the precise measurements of the toe spread. This can be overcome by using video analysis of gait which allows the assessment of SFI from the recording and provides

additional information about stance, stride length, ankle angle and swing phase duration [4]. With both of these assessments a comparison to premorbid function and to the uninjured contralateral limb allows the degree of recovery to be assessed [8]. Other common tests include whisker motion for facial nerve injuries, muscle tension, and grasping transduced by strain gauges which can be used in forelimb and hind limb injuries [4]. A novel method of grasp assessment using video recording has more recently been established, which allows the grasp abilities of both the injured and contralateral paw to be assessed at intervals over the recovery period. The technique is less time-consuming than the traditional grasping methodologies involving analysis by observation and reduces the stress put upon the animals [9].

Sensory recovery outcome measures typically evaluate the sensitivity of animals in response to a controllable and quantifiable level of stimulation [7]. Such tests include responses to localised hot and cold temperatures, von Frey filaments, nociceptive electric stimulation and incremental electrical stimulation. However, assessment of sensation independent of motor assessment in animals is challenging as most sensory tests are predicated on a motor response as an indication that a stimulus has been detected [5].

Multiple non-invasive or minimally-invasive functional tests can be performed at intervals throughout the study in animals. Such studies in comparison to post mortem assessments reduce the number of animals necessary for a study, provide longitudinal measures of change and reduce the effect of inter-individual variability. While there are many parallels, at least in theory, between functional outcome measures that can be used in both animals and humans, the key divergence is that highly invasive studies can be conducted in animals at the end-point of the study under terminal anesthesia or post mortem. Such tests to determine axonal regeneration include immunohistochemical and histological analysis of harvested tissue, and retrograde/anterograde tracing [4]. Histology is used routinely to assess the number of axons present in a repaired nerve and to distinguish nerve fibre populations (e.g. sensory, motor, and

sympathetic) [6, 10]. It also provides information about the non-neuronal cells such as Schwann cells and macrophages, and the recovery of neural architecture. Other parameters can be quantified from harvested nerve tissue using electron microscopy and other high-power imaging techniques including the number of myelinated or unmyelinated axons, myelinated axon diameter and area, myelin thickness, g-ratio, n-ratio and blood vessel profiles [11, 12].

Furthermore, muscle tissue distal to the injured nerve can be harvested and the weight determined as another useful outcome measure that is indicative of the degree of innervation [13]. The test is easy to perform and the muscle weight from the nerve-injured side is compared with the contralateral uninjured side.

Another commonly utilised outcome measure in animal studies is neurophysiology involving the stimulation of the nerve with electrical pulses, delivered by electrodes placed proximal to the injury and recording the compound muscle action potential (CMAP) from the corresponding target muscle. This is performed under anesthesia either through exposure of the relevant nerve and muscles and direct placement of electrodes, or less invasively by using percutaneous needle electrodes. CMAP is a useful indicator of nerve regeneration as the amplitude is related to the number of functionally reinnervated muscle nerve fibres, so CMAPs can be used to quantify motor nerve regeneration. It is important to note that; although CMAPs may recover to their pre-operative value this does not necessarily mean that the number of axons has returned to normal [7]. Regenerating axons branch and sprout forming many distal sprouts form one injured axon and these sprouts can form neuromuscular junctions with a greater number of muscle fibres than in uninjured animals [4]. As CMAP amplitude can overestimate nerve regeneration, motor unit number estimations (MUNE) can potentially be used as an alternative to quantify the number of motor axons supplying the muscles under test. Another useful electrophysiological technique is to record compound nerve action potentials

(CNAPs) between two positions on the nerve, providing details about conduction through specific nerve segments [7].

It is important to select the most suitable outcome measures that provide data to answer specific experimental questions. In an animal study it is common to take a multimodal approach and combine electrophysiology and behavioural tests with histological and morphometric analysis in order to build up a comprehensive assessment of nerve recovery from axonal regeneration to the recovery of target organ function.

Current methods to measure nerve recovery in humans

Clinical assessment is ultimately an assessment of function and not of nerve recovery in isolation. The human experience of outcome following nerve injury is a process which has not been correlated directly with the recovery of nerve tissue. The modalities of human nerve function that are measured in clinical medicine, are not similar to or even analogous to those assessed in experimental conditions in laboratory research. Assessment of nerve regeneration with animal and cellular models is achievable in the lab, however, not easily seen, monitored or measured in humans. In essence scientists and clinicians are looking at two very different faces of the same problem. This is the challenge of all translational research, but it is particularly challenging in nerve injury medicine.

The World Health Organisation (WHO) developed the International Classification of Functioning, Disability and Health (ICF) as a conceptual framework for measuring health and disability at both an individual and population level. This framework recognises that a disease or injury has multimodal effects. In this model an individual's structural impairments should be considered alongside their limitations in activities which will affect their ability to participate in everyday life. In addition, the framework recognises that personal factors such as

psychological well-being and environmental factors such as the ability to obtain effective treatments will affect their current state and future outcome.

Dy *et al* (2016) conducted a systematic review of outcome reporting for brachial plexus injury. Quantitative measures of outcome (for example, active range of movement and muscle strength testing) were almost exclusively used to determine the effectiveness of interventions. However, this empirical approach does not consider the wider impact of loss of nerve function. Patients often report that recovery following nerve injury is painful, slow and incomplete [14]. Furthermore, qualitative studies of lived experience following brachial plexus nerve injuries highlight the importance of psychosocial factors within the process of recovery. Body image, employment and the hope/expectations of recovery are all key issues that can be overlooked in studies reliant on quantification of isolated physiological responses [14-16].

Assessment of outcomes from nerve injury.

The hope of regenerative medicine is to improve the rate and quality of recovery following PNI which may then provide a good base for suitable clinical recovery. Clinical assessment is ultimately an assessment of function and not of nerve regeneration in isolation. However, one assessment undertaken clinically which is a pure assessment of neuronal regeneration unhindered by the subsequent impact of end-organ reinnervation is the Tinel Sign [17]. First described more than a hundred years ago this clinical test assesses the progression of axonal regeneration by tapping on the skin along the course of the nerve [18]. This is the only method in use which can monitor the recovery of a degenerative nerve injury prior to any tissue renervation.

It must be kept in mind that there is not necessarily a linear relationship between good quality neuronal recovery and the ability to carry out functional tasks. Healthy nerve function in humans creates and gives quality and enjoyment to our experiences of movement, touch, pain and interaction with the world. Therefore clinical assessments should be holistic and represent

all of these areas. Clinician reported measures of sensibility, motor function and electrodiagnostics should be combined with patient reported measures of quality of life, function and pain. Some of the recommended and commonly used measures within clinical practice will be discussed (Table 2).

Sensibility

Sensibility is described as the ability to receive and perceive sensation. There are four levels of sensibility: detection, discrimination, quantification and identification. These include all characteristics of touch, stereognosis and proprioception. The neuroanatomy and physiology of touch via the skin are well characterised with differing kinds of cutaneous mechanoreceptors [19]. This information is projected to the sensory cortex. The complex events within the central nervous system (CNS) which interpret these signals mean that there is no straightforward relationship between stimulus and experience, thus no one specific clinical test exists to evaluate this.

The ability of the patient to sense cutaneous pressure and static touch is commonly evaluated using Semmes-Weinstein monofilament test and the Weinstein Enhanced Sensory Test (WEST). These tests have been shown to have good responsiveness to monitor change over time [20]. Spatial discrimination tests constitute the next level of sensibility. Two-point discrimination is the most commonly used test and is incorporated into the Medical Research Council (MRC) score of sensory recovery. When used over time this, system provides an account of recovery on an 8-point scale. However, the responsiveness of the 2PD test has been shown to be poor and its clinical utility therefore questioned [20]. The pressure specified sensory device (PSSD) was developed to standardise pressure detection testing [21]. This tool has been applied in both research and clinical settings [21].

Stereognosis is the ability of the hand to recognise objects in the absence of any visual clues. It is assessed using tactile gnosis tests which are arguably more clinically relevant than the

simplistic assessments of touch which are described above. The Shape Texture Identification (STI) test is an assessment tool which has had extensive research regarding its validity, reliability and responsiveness. This tool is recommended particularly for use when assessing recovery of the median and ulnar nerves [20].

Sensory recovery can also be informed through the use of Quantitative Sensory Testing (QST). QST is a multimodal method of assessment including temperature and pain as well as sympathetic function [22].

Pain

PNI can lead to both nociceptive and neuropathic pain [23]. Reports of the incidence of pain with injuries to the brachial plexus are high with neuropathic pain being recorded in 50% of all patients [24]. The symptom of pain is most commonly measured in clinical practice by rating scales of severity. These assess verbally (VRS) or visually (VAS) a numerical scale from 0 – 10 or analogue scales anchored with words ranging from "no pain" to "worse pain imaginable" [25]. The PNI score is a simplified version of this [26].

Pain is described as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (as defined by the International society of pain – IASP); [27]. This description demonstrates that pain is multifactorial and severity alone is not enough to encapsulate the whole pain experience. Tools such as the Neuropathic Pain Inventory (NPI) and McGill pain questionnaire encourage patients to record descriptors of quality, severity and temporal characteristics of the pain as well as traditional numerical assessment.

Motor function

Muscle function (as controlled by nerve) provides active range of movement (AROM) of a joint. Combined together many muscle groups coordinate movement across joints. The movement of many joints together creates function. Functional assessment of the patient should therefore reflect all of these elements. Measuring a joint's AROM and its change over time gives an indication of recovery after paralysis. The strength of contraction is measured most commonly through peak volitional force (PVF). Manual muscle testing (MMT) has been the mainstay of PVF assessment. MMT has been shown to discriminate reliably between muscle groups innervated by injured and uninjured nerves [28]. The MRC method of grading outcomes is universally deployed but has deficiencies as an assessment tool [29]. In research the use of a device to measure force (dynamometry) is now increasingly common [30]. The understanding of the lived experience of muscle recovery has been explored. The importance of fatigue, coordination of motor function and pain are all reported as being significant contributors to a patient's perception of outcome [14].

With this in mind, it is also important to consider how the whole limb is integrated into functional activities. Specific functional tests such as the Sollerman Hand function test, Action Research Arm Test (ARAT) and Adult Assisting Hand Assessment (AHA) have all been widely used to assess upper limb function in a hemiplegic population. They await validity testing for peripheral nerve injuries but show good potential clinical utility.

It can be seen that in spite of the wide range of tests that exist, there is little consensus on what clinical tests should be used in PNI outcome studies. Combination or cluster testing in other disease conditions is advocated to assist with assessments [31]. The Rosen score is a well recognised and well validated protocol for documentation of hand function after nerve injury; uniting clinical tests of sensation, motor and function with patient reported measures [32].

Patient Reported Outcome Measures

The collection of patient perceptions of recovery and satisfaction is endorsed through the use of patient reported outcome measures (PROMs) [33]. The psychological aspects of resilience and coping have been shown to be important predictors of clinical outcome in many injuries [34, 35]. It is likely to also be proved so in nerve injury.

Many self-reported measures for upper limb function exist. The Disability of the Arm, Shoulder and Hand (DASH) has been used extensively to measure functional outcomes following nerve injuries to the upper limb [36]. However, it has not yet gained validation or wide spread acceptance for use with complex nerve injuries, such as brachial plexus injuries [36]. The Brachial plexus Assessment Tool (BrAT) is a new measure which has shown good construct validity, reproducibility and responsiveness with this cohort of patients [37].

Quality of Life (QoL) has been shown to be severely reduced following PNI [38, 39]. The SF36 is the most frequently used QoL tool within nerve injury literature [36]. However, a validated nerve specific measure that rigorously encompasses multiple patient-centered outcomes has yet to be developed.

Neurophysiology

The clinical tools, described above, are all infused with subjectivity from either the patient or clinician; as they reflect the *outcome* of renervation. In contrast, laboratory measurements objectively assess the *process* of nerve regeneration. Much like the laboratory setting, the application of clinical neurophysiology can provide measurements of nerve regeneration. These data can be used to characterise differing types of nerve injury and to monitor recovery or progression. Despite this apparent objectivity, there is a subjective aspect of the interpretation; there is no absolute meaning to the numerical data in relation to either nerve physiology or disease [40].

In surgical practice neurophysiology can be applied pre, intr- and post-operatively to determine both localise and characterise the degree of damage to the nerve. The main aspects of neurophysiology applied are Nerve Conduction Studies (NCS) and Electromyography (EMG). NCS assess the speed and amplitude of a stimulated current passed along a nerve. Somatosensory Evoked Potentials (SSEP's) can be studied inter-operatively to assist the nerve surgeon in determining the anatomical site of the nerve lesion [40, 41]. For example, the presence of an SSEP when stimulating a nerve root distal to the foramen and recording from the upper cervical spine demonstrates a neural connection between the two; supporting continuity of the nerve root. The absence of an SSEP in this situation would provide information supportive of an avulsion [40].

Electromyography (EMG) is an assessment of the function of the nerve and muscle interaction. Motor Unit Number Estimation (MUNE) is not a commonly used clinical tool but is a quantitative assessment that has been used to characterise the progression of a number of different progressive denervating neurological pathologies [42, 43].

A number of different methods have emerged to assess MUNE based on the phenomenon that it is possible to observe an increase in amplitude of response within a muscle with an increasing proximal nerve stimulus [44-46]. If the intensity is incrementally increased, it is possible to stimulate single motor units. Therefore, through this process, an estimate of the total number of MU's innervating a muscle can be obtained. MUNE is minimally invasive with high reproducibility [42]. This characteristic has allowed it to be employed as a quantitative primary outcome measure. Clinical trials have been undertaken with MUNE as a primary outcome to assess the efficacy of therapeutics in the treatment of denervating conditions [42]. Applying this test to regenerating motor nerves may be the first step towards obtaining a quantitative primary motor outcome for the heterogeneous PNI demographic.

Imaging

Imaging modalities are widely deployed by clinicians to support clinical examination findings and to inform diagnosis in CNS pathologies. However, imaging techniques have provided less utility in the assessment of peripheral nerve disorders [41]. MRI often demonstrates diffuse regional tissue changes of oedema and haemorrhage at the level of injury with clear intraneural signal changes being difficult to detect. On T2-weighted scans the injured nerve may return a hyperintense signal within 48 hours following injury. These signals have been observed to return towards normal levels following regeneration [47]. These changes correlate with and often precede changes on needle EMG examinations [47].

Tractography and Diffusion tensor imaging (DTI) techniques present graphical representation of neural anatomy [48]. However the 'tracts' of compressed, injured or degenerative nerves following trauma are difficult to differential from those of functioning anatomy. Thus whilst this technique has potential to offer valid assessment of surrogate markers of neural regeneration, the interpretation and application of the images to clinical care is still awaited [48].

Ultrasound (US) imaging has have shown benefit in the acute phase of nerve injuries. Its role in identification of level of injury and entrapment is evident; but still not widely applied [49]. High resolution US has been reported to a sensitive method for distinguishing between axonotmesis and neurotmesis lesions pre-operatively [50, 51]. However, the technique is highly operator dependent and imaging deeper nerves such as the Sciatic nerve is often challenging.

Summary:

The clinical assessment of degree of injury at the time of initial assessment and following recovery from PNI remains a challenge for therapists and surgeons. Clinicians and patients require a variety of tools specific to different areas of assessment (for all modalities of nerve function) and for a variety of aims (diagnosis, assessment of recovery, outcome). There is a

key challenge to bridge the void between the assessments used to measure nerve regeneration in laboratory experiments and the clinical outcome measures that will make a meaningful difference to patients. Overcoming this will involve the identification of methods which provide parallel or translatable measures between animal models and patient studies.

The relationship between the cellular and tissue changes in nerve regeneration and the human experience of recovery from nerve injury is complex and currently unquantifiable. Understanding this link is likely to be a key step for future translational research in nerve injury medicine.

Conflict of interest: None

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Table 1: Common functional outcome tests used following PNI in animal models. Adapted from [4].

OUTCOME MEASURES in laboratory animal models	
Axon regeneration	
Pinch Test	Measure of muscle contraction following pinching an exposed nerve with fine forceps.
Nerve conduction	Electrically stimulating a nerve and recording at a set distance to obtain a compound nerve action potential (CNAP) (*in situ* or *in vivo*).
Tracing methods	Retrograde tracing of neurons with dye from a distal point back to the neuronal cell body.
Target reinnervation	
Pinprick test	Light pricking of skin with blunt needle.
Foot flick test	An electrical stimulus is applied to the paw and the withdrawal response measured.
Evans blue extravasation test	Evans blue solution is injected intravenously and the nerve electrically stimulated leading to an accumulation of Evans blue in the target skin.
Toe spread	The toe spread is measured when the rodent is gently lifted by the tail.
Motor nerve conductions tests	The CMAP is measured from the target muscle following electrical stimulation of the proximal stump of the nerve and MUNE (Estimation of the motor unit number by electrically stimulating the nerve using an incremental stimulation technique).
Muscle tension	Muscle contractile force is transduced following electrical stimulation of the nerve.
Muscle weight	The muscle distal to the injured nerve is harvested and weighed and compared to the contralateral uninjured side.
Functional recovery	

Von Frey	The animal is placed on a grid and plantar skin stimulated using filaments with increasing force until it responds by paw withdrawal.
Hot and cold response	The animal is placed into a box and heat or cold is applied until paw withdrawal.
Sweat gland function	Silastic impression method is used to quantify the number of secreting sweat glands.
Grasping test	The animal grasp a grip strength apparatus which is a bar connected to strain gauges.
Whisker motion function	Animals are video-recorded for 3-5 min and analysed for angle, amplitude of retraction, protraction and whisking frequency etc.
Rotarod test	The animals are placed onto a rotating rod and recorded until they can no longer maintain balance on the rod and fall off.
Walking track/ Sciatic Functional Index (SFI)	The animal walks along a narrow corridor and the placement of the paw is recorded. Parameters are then measured to calculate SFI.
Static Sciatic Index (SSI)	Toe spread is measured from a static photographic image of the animal in stance.
Gait/ kinematics	The recording from a walking track is used to analyse gait parameters such as stride length, stance and swing phase duration, and ankle angle.
Neurophysiology	Electromyography (EMG) is used to record activity from the muscles. Monosynaptic H waves and polysynaptic withdrawal reflexes are measured following electrically stimulation Motor and Sensory Evoked Potentials (MEPs and SEPs) can be recorded by stimulating the brain or the peripheral nerve to evaluate the whole neural pathway.

Table 2: A list of commonly deployed clinical tools for the assessment of PNI. (See text for further information on these assessments).

OUTCOME MEASURES in clinical practice	
Sensibility	Semmes-Weinstein monofilament (SWM) Weinstein enhanced sensory test (WEST) Medical Research Council (MRC) scale Stereognosis; Shape tecture identification (STI) Quantitative Sensory testing (QST) Pressure specified sensory device (PSSD)
Pain/Discomfort	Numerical Rating Scale (NRS) PNI score Visual Analog Scale (VAS) McGill Pain Questionnaire Neuropathic Pain Index (NPI)
Motor function	Active range of movement (AROM) Peak volitional force (PVF) : MRC strength testing/ Dynamometry Tender Muscle Sign Specific functional Tests (Sollerman, Adult AHA, ARAT)
Combined Clinician reported outcome (CRO) and Patient Reported Outcome (PRO)	Rosen Score
Patient Reported Outcome (PRO) measures	DASH BrAT SF36 QoL Psychosocial
Neurophysiology	Nerve conduction studies (NCS) Electromyography (EMG)