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Temporal development of peripheral neuroinflammation in whiplash-associated disorder grade II and its role in chronicity

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Abstract

Whiplash injuries cause considerable pain and disability. Most individuals are diagnosed with whiplash-associated disorder grade II (WADII), which is defined by the absence of frank nerve injury. However, studies indicate possible peripheral neuroinflammation in some individuals with WADII that may contribute to symptoms. The temporal changes of peripheral neuroinflammation in WADII remain unclear. This study aimed to investigate the course of peripheral neuroinflammation from acute to chronic stages and assess whether neuroinflammation in the acute stage predicts recovery 6 months postinjury. Sixty-two WADII participants, who were examined within 4 weeks of a whiplash injury, returned for a follow-up appointment at 6 months. Thirty-two percent (n = 20) of participants considered themselves to be all better at 6 months based on a global recovery question. Magnetic resonance imaging T2-weighted signal ratio of the C5 to C8 roots of the brachial plexus, associated dorsal root ganglia, and median nerve, were similar at both time points. Signs of heightened nerve mechanosensitivity reduced significantly at 6 months, as did mechanical and thermal hyperalgesia in the upper limb. Inflammatory mediator serum levels were unaltered at 6 months, except for tumour necrosis factor-α, which was reduced. Multivariable regression analysis indicated that heightened nerve mechanosensitivity (reduced elbow range of motion) in the acute stage was weakly prognostic for neuropathic pain classification at 6 months. Although many participants recovered at 6 months, the data show that peripheral neuroinflammation may persist in some individuals. These findings highlight the complexity of WADII and the contribution of neuroinflammation in both acute and chronic stages.

Keywords: Whiplash, Whiplash-associated disorder grade II, Peripheral neuroinflammation, Neuroinflammation, Magnetic resonance imaging, Heightened nerve mechanosensitivity

1. Introduction

Whiplash injuries often cause considerable pain and disability,[42] and as such, present a significant healthcare challenge. Most individuals are diagnosed with whiplash-associated disorder grade II (WADII), which is characterised by neck pain and musculoskeletal symptoms, such as impaired movement and tenderness, but without frank neurological signs (ie, decreased or absent deep tendon reflexes, weakness, and sensory deficits).[33]

Although frank neurological signs are not a feature of WADII, studies, including our own, have shown that many individuals exhibit subtle signs of peripheral nerve involvement.[15,16,27] For example, in acute WADII, hyperalgesia and hypoaesthesia have been reported in the upper limb dermatomes, as well as heightened nerve mechanosensitivity, which is assessed using clinical tests that increase the tensile load in upper limb nerves or palpation over nerve trunks. Approximately two-thirds of individuals with acute WADII are considered to have neuropathic pain,[16] which may also indicate peripheral nerve involvement.

Despite the lack of discernible structural nerve pathology on bedside examination, our findings suggest that some individuals

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with acute WADII may have peripheral neuroinflammation. Magnetic resonance imaging (MRI) studies have shown increased T2-weighted signal in the brachial plexus in acute WADII,[27] which is an indicator of intraneural oedema associated with inflammation.[3,34] In addition, the presence of heightened nerve mechanosensitivity and hyperalgesia may also indicate peripheral neuroinflammation, as supported by findings from preclinical models.[10,11,18,31]

The long-term prognosis for individuals with WADII remains poor, with reports of approximately 50% experiencing pain and disability 5 years after injury.[6] Some individuals continue to experience persistent neuropathic symptoms, as well as heightened nerve mechanosensitivity.[9,38,39] However, the temporal changes of peripheral neuroinflammation in the development of chronic WADII has not been examined in detail. In a small cohort of individuals with chronic WADII, increased T2-weighted signal intensity of the brachial plexus and median nerve at the wrist was identified on MRI, indicating likely neuroinflammation.[19]

Few prognostic factors have been identified to predict poor recovery in WADII. Examples include increased initial neck-related disability, age, post-traumatic hyperarousal, and cold hyperalgesia.[5,12,21,40,45] We have also identified the presence of acute hypoesthesia in the index finger as a prognostic indicator, which further links neuropathology with long-term outcomes in WADII.[16]

In this study, we have determined the course of peripheral neuroinflammation from the acute to chronic stages of WADII injuries. Specifically, we have focussed on T2-weighted MRI signal in the brachial plexus and median nerve as a measure of peripheral neuroinflammation, as well as surrogate measures of neuroinflammation, including nerve mechanosensitivity, sensory hyperalgesia (gain of sensation), and raised serum cytokine levels. We have serially examined a subgroup of participants from our larger WADII cohort,[27] within 1 month of injury and at 6 months. In addition, we have determined whether the presence of peripheral neuroinflammation in the acute stage can predict participant recovery status at 6 months.

2. Methods

2.1. Protocol registration

This article reports the temporal and prognostic findings of a 2-centre (Brighton and Oxford, United Kingdom) longitudinal cohort study prospectively registered at ClinicalTrials.gov (reference number NCT04940923; protocol version V2 25/9/20). The full study protocol was published in 2022,[28] and baseline findings were published in 2025 (acute WADII).[27] Temporal changes in dermatomal sensations and quantitative sensory testing (QST) parameters were also published in 2025.[16]

2.2. Ethical approval

Ethical approval was received from London-Brighton and Sussex Research Ethics Committee (20/PR/0625) and South Central-Oxford C Ethics Committee (18/SC/0263). All participants provided informed written consent before participating.

2.3. Participants

Participants aged 18 to 60 years were recruited within 4 weeks after a whiplash injury from the Emergency Departments of NHS Trusts in the Southeast of England. Details of the recruitment strategy can be found in Ridehalgh et al.[27] Those participants (n = 122) who met the Quebec Task Force WADII classification[33] were invited to complete a series of questionnaires (see Section 2.4) and attend an initial baseline appointment for a clinical assessment (see Section 2.5). Six months after the date of their whiplash injury, all participants were invited to complete the same questionnaires, as well as a global recovery questionnaire (GRQ).[7] Furthermore, participants were sequentially invited to attend a follow-up appointment for a repeat clinical assessment. In accordance with the study protocol, the number of participants required to attend the follow-up appointment was 50, and therefore, not all participants were invited back for a follow-up appointment. As the researchers collected information from the participants during the initial appointment, they were unable to remain blinded to the participants' status. However, all analyses of clinical and MRI data were performed blinded.

Exclusion criteria included pregnancy, recent history of cervical/arm pain lasting >3 months, previous diagnosis of a peripheral neuropathy, history of systemic illness or autoimmune disease, nonmedically controlled hypertension and ongoing steroid treatment, and a history of previous whiplash injury or whiplash-related symptoms within the past 12 months. Participants were also excluded if they were not eligible to undergo MRI.

2.4. Questionnaires

Participants were asked to complete the following questionnaires: (1) Neck Disability Index (NDI), which comprises 10 questions, scored from 0 (no disability) to 5 (complete disability; maximum score = 50)[43,44]; (2) Pain Catastrophising Scale (PCS), which comprises 13 questions, scored from 0 (not at all) to 4 (all the time; maximum score = 52).[41] A score of ≥30 indicates significant pain-related worry; (3) Depression, Anxiety and Stress Subscale (DASS), which is a 42-item questionnaire that assesses emotional states of depression, anxiety, and stress (maximum score = 126).[23] Scores of <6 indicate mild symptoms, 7 to 12 suggest moderate symptoms, and ≥13 indicate severe symptoms; and (4) Post-traumatic Stress Disorder Inventory-8 (PTSD-8), which consists of 8 items focused on how much symptoms have troubled the individual since the traumatic event, scored from 0 (not at all) to 3 (most of the time). It is further divided into 3 subscales: intrusion (4 items), avoidance, and hypervigilance (2 items each).[20]

At follow-up, the GRQ was also completed, which is a single question that asks, "How do you feel you are recovering from your injury?" It has 6 response options from "all better" to "getting much worse." Both the NDI and the GRQ were used as measures of recovery.

2.5. Clinical assessment

The clinical assessments are described in detail in Reference 27. All clinical assessments were performed by C.R. and J.F., who are experienced physiotherapists with more than 10 years of clinical experience and postgraduate qualifications, and S.K., who is a clinical research coordinator with 5 years of experience in clinical research and data collection. S.K. received comprehensive training in all clinical assessments before conducting evaluations with research participants, ensuring standardized and reliable data collection across all assessors. Demographic information (age, sex, height, and weight) was collected at the baseline appointment. Whiplash-related upper quadrant pain levels were recorded using a visual analogue scale (VAS; 0-100 mm).

A neurological assessment of the upper limbs was performed using Neurotips and cotton wool to assess sensation over the C5 to T1 dermatomes, isometric manual muscle testing to assess muscle strength, and a reflex hammer to assess deep tendon reflexes.

Tests for heightened nerve mechanosensitivity of the brachial plexus, median and ulnar nerves were performed bilaterally. The upper limb neurodynamic test 1 (ULNT1; median nerve bias) and 3 (ULNT3; ulnar nerve bias) were both performed as previously described.[27] A positive test was recorded when there was at least partial reproduction of the participant's symptoms and these changed with structural differentiation.[24] A digital inclinometer (Trend, DLB Swansea, United Kingdom) was also used to record the degree of elbow extension at the point of symptom onset for ULNT1. Pressure mechanosensitivity over the median nerve at the wrist and ulnar nerve in the cubital tunnel (pressure pain threshold [PPT]) was tested using an algometer (tip size = 1 cm^2; Wagner, Greenwich, CT), and digital pressure was applied over the brachial plexus in the supraclavicular area. Three PPT measurements were taken at each site, and mean values were obtained. Brachial plexus mechanosensitivity was graded according to symptom response: 0 = no pain or discomfort, 1 = local discomfort, 2 = local painful response, and 3 = referred pain/symptoms. Scores of 2 and 3 were considered a positive painful response to digital palpation.

A composite score for heightened nerve mechanosensitivity of 0 (negative) or 1 (positive) was calculated based on a "positive test" from at least one of 3 tests: ULNT1, ULNT3, and brachial plexus pain on digital palpation.

2.6. Quantitative sensory testing

Quantitative sensory testing was carried out over the ventral aspect of the proximal phalanx of the index finger on the most symptomatic side using the German Network for Neuropathic Pain protocol.[29] Cold detection, warm detection, cold and heat pain thresholds (CDT, WDT, CPT, HPT) were measured using a thermotester (Somedic, Norra Mellby, Sweden). Thermal sensory limen was calculated from 3 repeats of alternating CDT and WDT. Thermal stimuli were ramped at a rate of 1°C/second, starting at 32°C.

The mechanical detection threshold (MDT) was determined using von Frey filaments (Optihair 2 MRC systems, Heidelberg, Germany). Starting at 16 mN, 5 ascending and descending stimuli were applied. Mechanical pain threshold (MPT) was measured using pinprick stimulators within a range of 8 to 512 mN (MRC Systems). Mechanical pain sensitivity (MPS) was evaluated by applying pinprick stimulators randomly to the testing site. Dynamic mechanical allodynia (DMA) was assessed using 3 light touch stimuli (cotton wool, a cotton bud, and a standardized soft brush; MRC Systems). For both MPS and DMA, which were tested together, participants were asked to rate the pain intensity on a scale from 0 to 100. The protocol included a total of 14 pinprick and 6 nonnoxious stimuli.[25] The geometric mean values were calculated for MDT, MPT, MPS, and DMA.

For calculation of the wind-up ratio (WUR), a 256-mN pinprick stimulus was applied to the test area and a pain rating determined (out of 100). The stimulus was applied at 1 Hz for 10 repeats, and an average pain rating determined for the repeats. The WUR was determined from a ratio of the average pain rating for the repeated stimuli to the rating for the single stimulus.

Vibration detection threshold (VDT) was assessed using a Rydel–Seiffer tuning fork, which was placed over the palmar aspect of the metacarpophalangeal joint of the index finger. Pressure pain threshold was determined using an algometer (Wagner) applied over the thenar eminence. Participants were asked to indicate the first onset of pain.

For CDT, WDT, CPT, HPT, WUR, VDT, and PPT, the mean of 3 repeats was determined.

2.7. Magnetic resonance imaging

2.7.1. Magnetic resonance imaging acquisition

The same 3-T scanner (Siemens Prisma; Siemens Medical Solutions, Erlangen, Germany) with a dedicated 64-channel head/neck coil was used to acquire images of the roots of the brachial plexus and dorsal root ganglia (DRG) at both research sites. Coronal images were obtained of participants lying supine in the scanner using a 2D multislice T1-weighted (echo time [TE] = 8.7 ms; repetition time [TR] = 500 ms; voxel size = 0.9 × 0.9 mm; slice thickness = 3 mm; field of view [FoV] = 330 mm) and a T2-weighted short tau inversion recovery (STIR) 3D SPACE sequence (TE = 166 ms; TR = 3500 ms; inversion time [TI] = 230 ms; voxel size = 0.8 × 0.8 × 0.8 mm; FoV = 205 mm). In addition, T1-weighted images were acquired for anatomical reference. The most symptomatic side of the wrist was imaged using a flex coil positioned at the level of the carpal tunnel, with participants placed in a supine superman position with the wrist positioned centrally in the scanner. Axial images were acquired using a T2-weighted STIR sequence (TE = 21 ms; TR = 6030 ms; TI = 220 ms; voxel size = 0.4 × 0.4; slice thickness = 2 mm; interslice spacing = 0.2 mm; FoV = 100 mm).

2.7.2. Magnetic resonance imaging analysis

Coded image files were stored in 16-bit Digital Imaging and Communications in Medicine (DICOM) format and converted to NIfTI format for the analysis by a blinded investigator. T2-weighted signal intensity ratios from the STIR sequence were determined for the roots of the brachial plexus, associated DRG, and the median nerve at the wrist using code developed in MATLAB (MathWorks, Natick, MA) as described previously.[27]

Briefly, coronal image slices were extracted for the C5 to C8 roots. From a single maximum intensity projection image, a mask, drawn freehand around the roots, was applied to all extracted slices. Within the region of the mask, the roots were segmented for each slice using thresholding and an active contours model ("snakes"). The segmented regions were overlaid and overlapping voxels averaged. From the resulting segmented image of the brachial plexus, regions of interest were drawn around each root and the median grayscale values were determined. For the C5 to C8 DRG, a similar methodology was applied. Dorsal root ganglia were identified from individual coronal image slices and segmented using thresholding and active contour modelling within bounding boxes that were placed around each DRG. Median grayscale values were obtained.

The C5 to T1 vertebral bodies were used to normalise the median T2-weighted signal intensities of both the roots of the brachial plexus and the DRG, which was necessary to account for variation in the positioning of individuals and between study sites. For normalisation, the median grayscale values of the C5 to T1 vertebral bodies were determined from regions of interest (10 × 10 voxels) positioned in the centre of each vertebral body on 6 coronal slices. After subtraction of a constant from the roots of the brachial plexus and DRG median T2-weighted signal intensities (equivalent to the y-intercept for a best fit line when the roots of the brachial plexus and DRG median T2-weighted signal intensities [y-axis] are plotted against their respective median vertebral body T2 values),[27] T2-weighted signal intensity ratios were determined.

For the median nerve at the wrist, axial T2-weighted image slices were examined at the level of the distal radio-ulnar joint, as well as the proximal and distal carpal rows. A freehand mask was drawn around the median nerve on 3 adjacent slices at each of these locations. The nerve was segmented using thresholding

Table 1

Demographic data.

	Acute (n = 62)	6-mo follow-up (n = 62)
Age, y (mean, SD)	35.7 (12.3)	
Sex (n = females) (%)	33 (53%)	
Height, cm (median, IQR)	170.0 (15.0)	
Weight, kg (median, IQR)	75 (21.0)	
Time since injury, days (mean, SD)	23.7 (7.7)	
Right symptomatic side	59.7%	
Proportion bilateral symptoms	48.4%	
Symptom visual analogue scale (median, IQR)	34.0 (36.0)	16.0 (30.5) ($P < 0.0001$)
Neck disability index (median, IQR)	15.5 (10.0)	7.5 (11.0) ($P < 0.0001$)
painDETECT (median, IQR)	10.0 (8.0)	7.0 (9.0) ($P < 0.0001$)
Positive Tinel's sign %	30.6%	22%
Positive Phalen's test %	31.1%	11.9%
Positive ULNT1 %	39.7%	13.6%
Positive ULNT3 %	22.0%	6.8%
Pain Catastrophising Scale (median, IQR)	12.0 (19.0)	9.0 (14.0) ($P = 0.0009$)
DASS score (median, IQR)		
Depression	7.0 (10.25)	4.0 (12.0)
Anxiety	8.0 (7.25)	5.0 (8.5) ($P = 0.0006$)
Stress	15.0 (15.25)	10.0 (13.0) ($P = 0.0019$)
PTSD-8 (median, IQR)		
Intrusion	10.0 (5.0)	9.0 (5.5) ($P = 0.0127$)
Avoidance	4.0 (3.0)	4.0 (4.0)
Hypervigilance	5.0 (3.0)	4.0 (3.75) ($P = 0.0027$)

DASS, depression, anxiety and stress subscale; PTSD-8, post-traumatic stress disorder inventory-8; ULNT, upper limb neurodynamic test.

and an active contours model. From the segmented slices, median grayscale values, nerve area, and aspect ratio were measured. A circular control region of interest (radius = 10 voxels) was placed at the centre of a neighbouring bone (radius at the distal radio-ulnar joint, lunate in the proximal carpal row, and capitate in the distal carpal row) on each slice, and the median grayscale value for each region was recorded. After subtraction of a constant from the median nerve T2-weighted signal intensities (equivalent to the y-intercept of a best fit line when the nerve T2-weighted signal intensities [y-axis] are plotted against their respective underlying bone T2-weighted intensity values), T2-weighted signal intensity ratios were determined.

2.8. Venipuncture and analysis of serum inflammatory cytokines

To obtain serum, whole blood collected from the antecubital fossa using Vacutainer tubes (BD Vacutainer SST II advanced tubes) was centrifuged at 3000 rpm for 10 minutes at 4°C and stored at −80°C in cryotubes until processing.

Serum inflammatory mediators (interferon-γ [IFN-γ]; interleukins [IL]-1β, 2, 4, 6, 8, 10, 12, and 13; and tumour necrosis factor-α [TNF-α]) were measured using multiplex electrochemiluminescence (V-Plex Proinflammatory Panel 1 [human] kit; Meso Scale Discovery, Rockville, MD). All assays were conducted following the manufacturer's guidelines. Samples were diluted 1:2 and tested in duplicate. Readings were taken using a Meso QuickPlex SQ (Meso Scale Discovery), with concentrations determined from the standard curves generated from each plate. Serum samples from acute and follow-up WADII participants were randomly assigned to wells on the plates to minimize potential batch effects. Five of the inflammatory mediators (IL-1β, IL-2, IL-4, IL-12, and IL-13) were below detectable levels and were therefore not included in the analysis.

2.9. Neuropathic pain special interest group classification of neuropathic pain

Neuropathic pain was identified using the hierarchical grading system of the IASP Neuropathic Pain Special Interest Group (NeuPSIG) as unlikely, possible, or probable.[14] To meet the requirement of "possible neuropathic pain," participants' symptoms must fall within a neuroanatomically plausible distribution, and they must have a medical history suggestive of a "neurological lesion" (eg, neuropathic pain descriptors such as burning, shooting, or tingling). Probable neuropathic pain requires the aforementioned criteria *and* reduced sensory signs or allodynia in the same neuroanatomically plausible distribution. In this study, we used the participants' description of symptoms and altered sensory examination findings in the main pain area. For definite neuropathic pain, a diagnostic test to identify a lesion or disease of the somatosensory nervous system is necessary. Because the MRI scans were not clinically evaluated, a classification of definite neuropathic pain could not be reached.

2.10. Data analysis

Details of the sample size calculation for this study have been previously described.[28] Each participant was given a unique study-specific code to ensure blinding, and the data were stored

on a secure web platform (REDCap, Vanderbilt University, Nashville, TN). All MRI and blood marker analyses were blinded to participant group (acute phase and 6 months). Data were exported to GraphPad Prism (10.03) or R for analysis. Normality of the data was checked using the Kolmogorov–Smirnov test, with subsequent use of parametric or nonparametric methods as appropriate.

Quantitative sensory testing data were log transformed (except for CPT, HPT, and VDT) to achieve normality and were expressed as z-scores using the data of age matched healthy participants (at least n = 7 controls per decade).[1] A gain of function is represented as a positive z-score, whereas negative z-scores indicate a loss of function.[29] For MPS, a constant of 0.1 was added to avoid losing zero-rated values. A ceiling effect was noted for the 20-year age group in VDT z-scores because there was little variability, preventing z-score calculation. As a result, VDT z-scores for individuals in the 20- and 30-year age groups were calculated using a combined mean and SD, incorporating both decades into a single calculation. Z-scores for other age groups were calculated separately for each decade. Participants were considered to have a gain in sensation if the z-score was above the upper 95% confidence limit of our published healthy control group.[27]

Comparisons of mean values (QST parameters) and medians (PCS, DASS, PTSD-8, pain scale, NDI, painDETECT, T2 signal ratios, nerve area, aspect ratio, elbow range of motion [ROM] during ULNT1, carpal tunnel and cubital tunnel PPT, and inflammatory mediator levels) between the acute and follow-up groups were performed using paired *t*-tests and Wilcoxon tests, respectively. For the median nerve, mean T2-weighted signal ratios at each level were analysed using 2-way analysis of variance (with location and group as the independent factors) followed by Tukey's post hoc tests. For each parameter, a cutoff of 0.2 SDs of the absolute difference between time points was used to determine the proportion of participants who had a positive or negative change at 6 months. A cutoff of 0.2 SDs was used because less than 0.2 SD was considered to be a trivial effect size. A cutoff was not calculated for the QST data because of the skewed distribution of the differences. Fisher exact tests were used to assess the association between categorical variables. Spearman rank correlations were used to examine the relationship between measures of pain and recovery (VAS, NDI, GRQ, and painDETECT) and T2-weighted signal intensity ratios, as well as other surrogate measures of neuroinflammation. Correlation (r) values of ≥ 0.4 were considered indicative of a moderate correlation.[32] The correlation analyses were exploratory and therefore not corrected for multiple comparisons. Follow-up data were also separated into recovered and unrecovered groups based on GRQ, with scores of 1 and 2 ("all better" and "There has been quite a bit of improvement") considered recovered. Bonferroni corrections were applied to adjust for multiple testing in the analyses of T2-weighted signal ratios for the brachial plexus and dorsal root ganglia, and the blood serum inflammatory mediator analyses (corrected α provided for each result). Quantitative sensory testing data were not adjusted for multiple comparisons, as applying such corrections for the 11 measures, many of which are mechanistically unrelated, could lead to an underestimation of any true effect.[30]

The prognostic role of peripheral neuroinflammation in WADII was examined using multivariable linear regression analysis, with NDI as the measure of recovery, and multivariable logistic regression, with GRQ as the clinical endpoint. The a priori selected acute measures of neuroinflammation included in the models were as follows: (1) heightened nerve mechanosensitivity (elbow ROM and composite score); (2) QST (CPT, MPS, and PPT); (3) maximum T2-weighted signal ratio (roots of the brachial plexus and DRG); and (4) at least one elevated cytokine. Cold pain threshold and MPS were chosen because both thermal hyperalgesia and mechanical allodynia are features of the neuritis model,[31] and CPT is associated with chronic disability after whiplash.[12,37] Pain pressure threshold was also chosen because deep C-fibre neurons are preferentially affected in the neuritis model.[4,10] Three covariates were added to the models: age, gender, and baseline NDI, which have been found to be associated with recovery.[45] A secondary exploratory analysis was also performed using the NeuPSIG grading system for neuropathic pain and the VAS as outcome measures. The NeuPSIG grading system was dichotomised for this exploratory analysis (unlikely = 0; possible or probable = 1). The prognostic modelling was undertaken on data from the main whiplash cohort, which included individuals who completed the NDI and GRQ at 6 months but who did not return as part of the subgroup.

3. Results

3.1. Demographics

Sixty-two participants returned for a follow-up appointment at 6 months (see Supplementary Table 1, http://links.lww.com/PAIN/C391 for summary of acute findings) and are included in these analyses. This represents 124% of the originally targeted follow-up sample size (n = 50). The demographics for this subset of participants is shown in **Table 1** (see Supplementary Table 2, http://links.lww.com/PAIN/C391 for a comparison of demographics between this subgroup and those from the main cohort who did not return for follow-up).

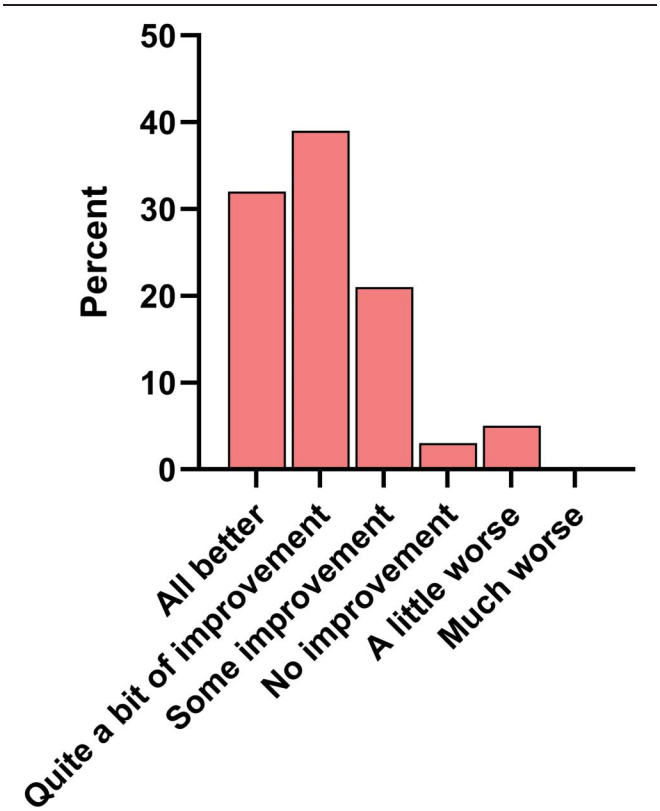

Figure 1. Global recovery question for those individuals who returned for a follow-up appointment at 6 months (n = 62).

At 6 months, there was an observed reduction in pain-related worrying on the pain catastrophising scale compared with the acute phase ($P = 0.0009$). Moderate symptoms of stress ($P = 0.0019$) and mild symptoms of anxiety ($P = 0.0006$) on the DASS at the acute phase were also reduced at 6 months. In addition, the high levels of post-traumatic stress that were reported acutely were reduced for both intrusion and hypervigilance at 6 months ($P = 0.0127$ and 0.0027, respectively).

3.2. Pain, disability, and recovery

The GRQ indicated that only 32.3% of the individuals who returned for a follow-up appointment considered themselves to be all better at 6 months (**Fig. 1**). By contrast, 8.1% of participants considered themselves to have not improved or had got worse. The median rating on the whiplash-related upper quadrant pain VAS was reduced at 6 months ($P < 0.0001$; **Table 1**; **Fig. 2A**). There was also an improvement in neck disability at 6 months ($P < 0.0001$; **Table 1**; **Fig. 2B**). The median painDETECT score was similarly reduced at 6 months ($P < 0.0001$; **Table 1**; **Fig. 2C**). At 6 months, only 10% (n = 6) of participants had a score greater than 13, indicating possible neuropathic pain compared with 35.6% (n = 21) in the acute phase. For all 3 measures, the frequency distributions were bimodal in the acute phase, which were less pronounced at 6 months (**Figs. 2D–F**). All 3 measures show a notable right skew at this later time point. For the individual painDETECT items, there was a reduction in 5 of the 7 items at 6 months (**Fig. 2G**). More than 73.7% of individuals were considered to have a decrease in pain or neck disability

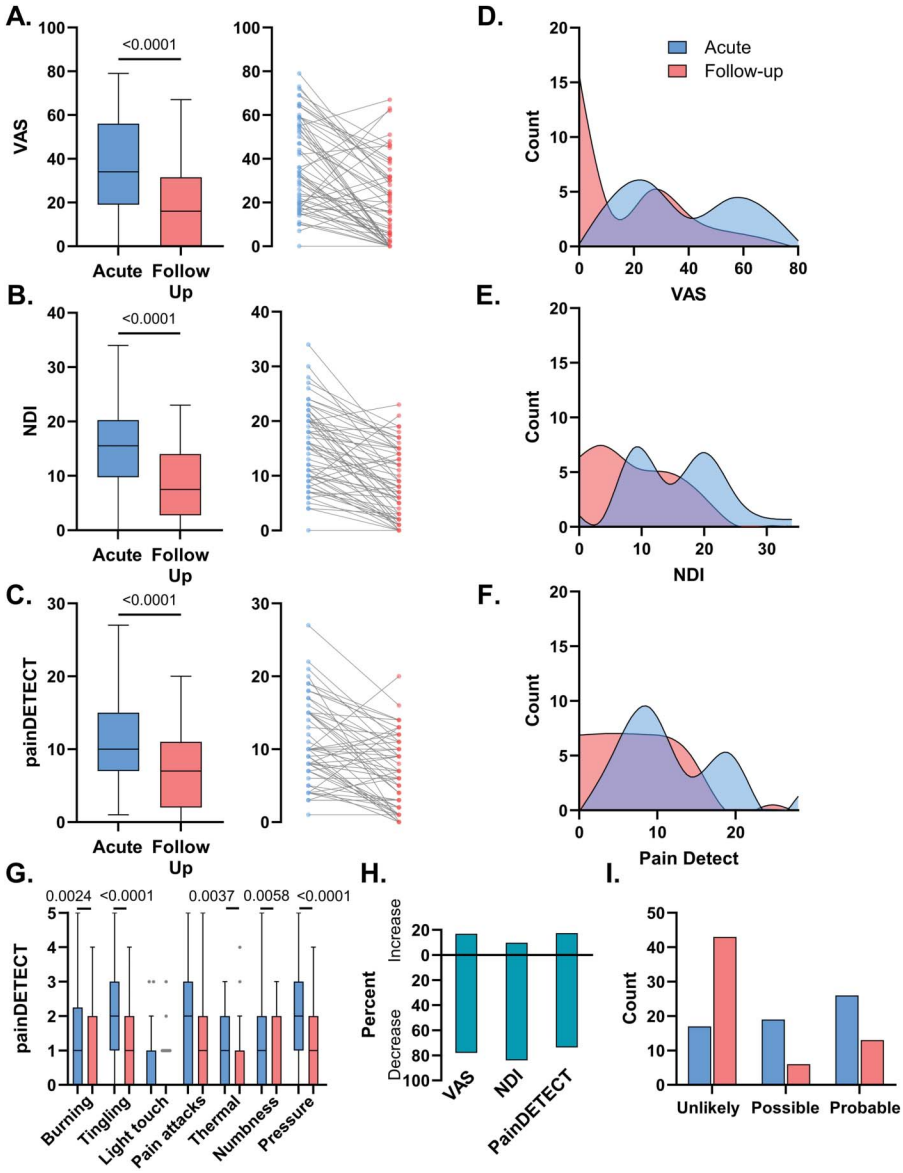

Figure 2. Temporal changes in pain and neck disability. (A and D) Whiplash-related upper quadrant pain visual analogue scale (VAS; 0-100), (B and E) neck disability index (NDI), and (C and F) PainDETECT scores within 1 month after injury (acute) and at 6-month follow-up. Box plots (A–C; median/IQR) and frequency distribution curves (D–F) are displayed. Individual paired scores are also shown. (G) Box plots (median/IQR) for individual painDETECT items. (H) Proportion of participants with a change (ie, >0.2 SDs of the absolute difference between time points) in pain and neck disability at 6-month follow-up compared with 1 month after injury. (I) Number of individuals with unlikely, possible, or probable neuropathic pain at 1 month and at follow-up based on the NeuPSIG grading system. Blue, acute. Red, follow-up. n = 59 to 62. Corrected α for painDETECT items in G = 0.007. NeuPSIG, neuropathic pain special interest group.

at 6 months compared with <17.5% where there was an increase (**Fig. 2H**). The NeuPSIG grading system for neuropathic pain indicated that 30.6% of participants had possible or probable neuropathic pain at 6 months compared with 72.6% of participants in the acute phase ($P < 0.0001$; **Fig. 2I**).

3.3. Brachial plexus magnetic resonance imaging

Example images of the brachial plexus in the acute phase and at follow-up are shown in **Figure 3A**. At 6 months, there were no observed changes in T2-weighted signal ratio of the C5 to C8 roots of the brachial plexus in participants compared with the acute phase for both the most symptomatic and less symptomatic sides (**Figs. 3B–E**). The proportion of individuals with increased T2 signal at 6 months was similar to the proportion with decreased signal for each root of the brachial plexus (**Fig. 3F**).

3.4. Dorsal root ganglia magnetic resonance imaging

Example images of the C5 to C8 dorsal root ganglia in the acute phase and at follow-up are shown in **Figure 3A**. There were no observed changes in T2-weighted signal ratio of the C5 to C8 DRG in participants at 6 months compared with the acute phase (**Figs. 4A–D**). The proportion of individuals with increased T2 signal at 6 months was similar to the proportion with decreased signal for each DRG (**Fig. 4E**).

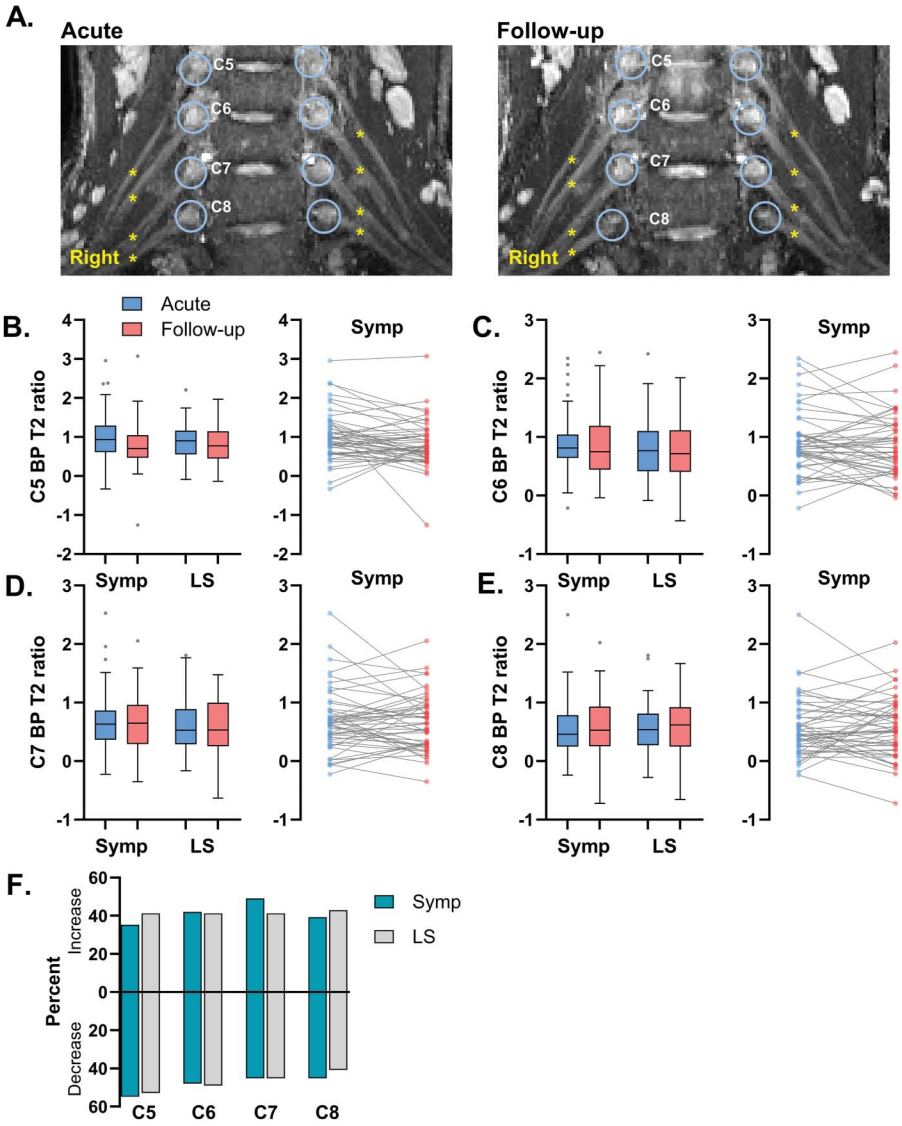

Figure 3. Temporal changes in T2-weighted signal ratios for the C5 to C8 roots of the brachial plexus (BP). (A) Example coronal T2-weighted maximum intensity MR images of the brachial plexus at 1-month (acute) and 6-month follow-up in the same person with WADII. Yellow asterisks indicate the respective roots of the brachial plexus. Note the subtle increase in MRI signal intensity of the roots of the brachial plexus at follow-up in this example. The C5 to C8 dorsal root ganglia (DRG) are circled and their cervical level labelled. (B–E) Box plots (median/IQR) for the (B) C5, (C) C6, (D) C7, and (E) C8 roots of the brachial plexus at 1-month (acute) and 6-month follow-up. Individual paired T2 ratios are shown. (F) Proportion of participants with a change (ie, >0.2 SDs of the absolute difference between time points) in MRI T2-weighted signal ratio in the C5 to C8 roots of the brachial plexus at 6-month follow-up compared with 1 month after injury. Blue, acute. Red, follow-up. LS, less symptomatic side; Symp, most symptomatic side; WADII, whiplash-associated disorder grade II. n = 51.

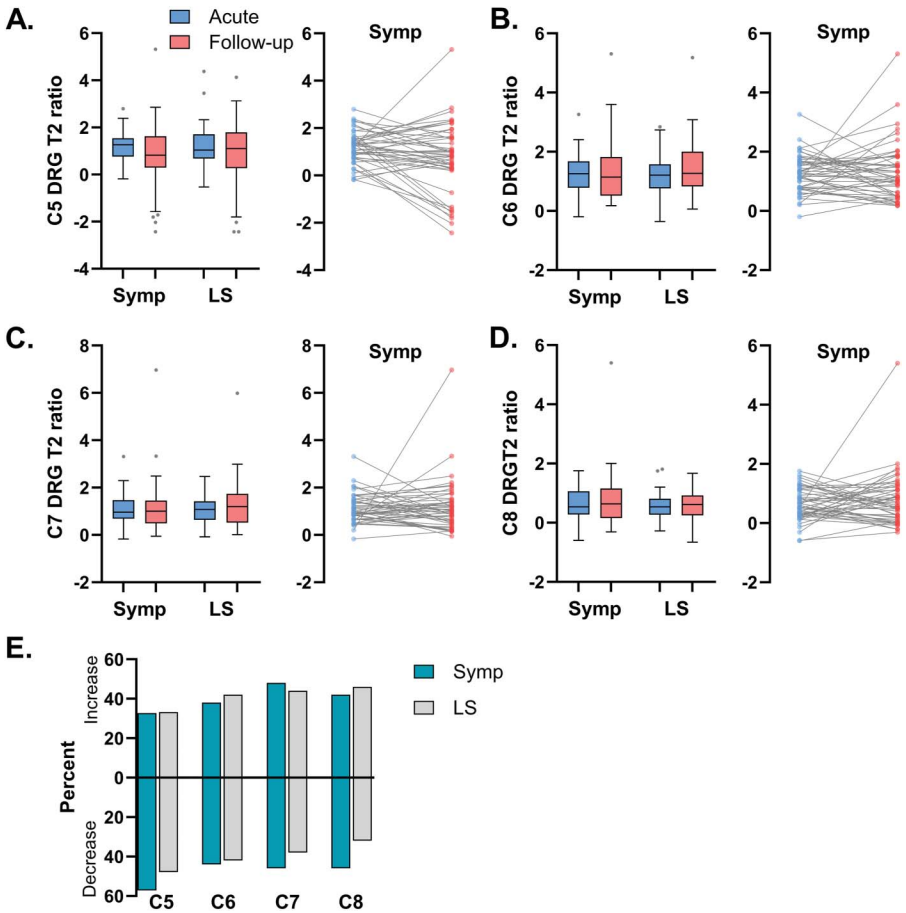

Figure 4. Temporal changes in T2-signal ratios for the C5 to C8 dorsal root ganglia (DRG). (A–D) Box plots (median/IQR) for the (A) C5, (B) C6, (C) C7, and (D) C8 DRG of the brachial plexus at 1-month (acute) and 6-month follow-up. Individual paired T2 ratios are shown. (E) Proportion of participants with a change (ie, > 0.2 SDs of the absolute difference between time points) in MRI T2 signal in the C5 to C8 DRG at 6-month follow-up compared with 1 month after injury. Blue, acute. Red, follow-up. LS, less symptomatic side; Symp, most symptomatic side. n = 50.

3.5. Median nerve magnetic resonance imaging

Example MR images of the median nerve at each site are shown in **Figure 5A**. There was no observed interaction between median nerve T2-weighted signal ratio at each site (distal radioulnar joint, proximal carpal row, or distal carpal row) and time (baseline and follow-up; $P = 0.65$) or main effect for time ($P = 0.091$; **Figs. 5B and C**). However, there was a main effect for location ($P = 0.0033$), with a significant decrease in T2-weighed signal ratio at the proximal carpal row compared with the distal radioulnar joint ($P = 0.0070$) and distal carpal row ($P = 0.0121$). There was a trend for a higher proportion of individuals to have a decrease in T2-weighted signal ratio at 6 months at each site, compared with an increase (**Fig. 5D**).

There was a significant decrease in median nerve area at the distal carpal row (R3) at follow-up compared with the acute phase, but not at the distal radioulnar joint or proximal carpal row (**Fig. 5E**). There were no significant differences in median nerve aspect ratio between time points (**Fig. 5F**).

3.6. Heightened nerve mechanosensitivity

Signs of heightened nerve mechanosensitivity were reduced at 6 months compared with the acute phase on the symptomatic side, which was indicated by an increase in elbow extension range of motion during the ULNT1 (6-month median = 80°, IQR = 26.58; acute median = 66.95°, IQR = 37.2, $P = 0.0009$; **Fig. 6A**) and increases in both PPT over the median nerve at the carpal tunnel (6-month median = 7.8 kg/cm^2, IQR = 3.55; acute median = 5.57 kg/cm^2, IQR = 4.88, $P < 0.0001$; **Fig. 6B**), and over the ulnar nerve at the cubital tunnel at 6 months (median = 4.45, IQR = 2.82; acute median = 3.53, IQR = 3.10, $P = 0.0013$; **Fig. 6C**). No temporal changes were apparent on the less symptomatic side. Changes in the frequency distribution curves for the symptomatic side were seen at 6 months compared with the acute phase (**Figs. 6D–F**). More than 55% of individuals had an increased elbow range of motion or PPT over the median or ulnar nerves at 6 months, indicating a reduction in nerve mechanosensitivity compared with <28.6% who had a decrease in these measures (**Fig. 6G**). At 6 months, fewer individuals reported pain in response to palpation over the cords of the brachial plexus compared with the acute phase on the symptomatic side ($P = 0.022$) but not on the less symptomatic side ($P = 0.38$; **Fig. 6H**).

3.7. Somatosensory hyperalgesia (gain of sensation)

The proportion of WADII participants with altered sensation in the C5 to T1 dermatomes and QST parameters on the most symptomatic side in the acute phase and 6 months are summarised in Supplementary Figure 1, http://links.lww.com/PAIN/C391.

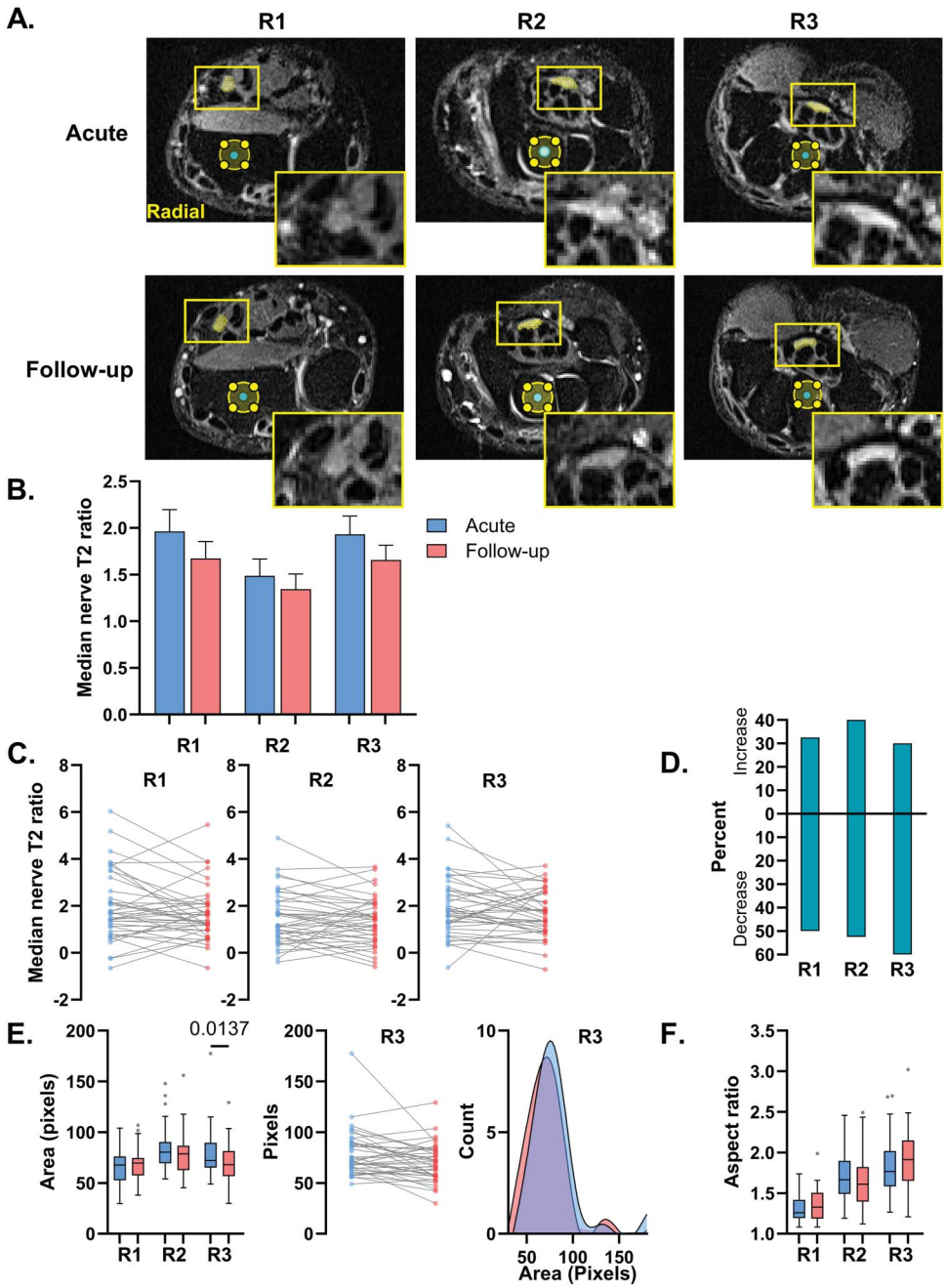

Figure 5. Temporal changes in median nerve T2-weighted signal ratios, area, and aspect ratio. (A) Example axial T2-weighted MRI slices of the median nerve (segmented nerve highlighted in yellow) at the distal radioulnar joint (R1), proximal (R2), and distal carpal row (R3). The yellow circle indicates the control region of interest in the neighbouring bone. Inserts show an enlarged image of the median nerve at each location. (B) Mean median nerve T2-weighted signal intensity ratios at each region at 1-month (acute) and 6-month follow-up. (C) Individual paired T2 ratios at each region. (D) Proportion of participants with a change (ie, >0.2 SDs of the absolute difference between time points) in MRI T2 signal in each region at 6-month follow-up compared with 1 month after injury. (E) Box plots (median/IQR) of median nerve area at 1- and 6-month follow-up at each region. Individual paired areas and frequency distribution curves are shown for R3. (F) Box plots (median/IQR) of median nerve aspect ratios for each region. Higher values indicate a flatter nerve profile. Error bars = SEM. Blue, acute. Red, follow-up. n = 40. Corrected $\alpha = 0.017$ for nerve areas.

A total of 16.1% (n = 10) of WADII participants had signs of pinprick hyperalgesia in one or more upper limb dermatomes in the acute phase, which was recovered in 60% (n = 6) of participants at 6 months, whereas 8.1% (n = 5) of participants developed pinprick hyperalgesia at 6 months, which was not present in the acute phase. In addition, 4.8% (n = 3) of WADII participants had signs of mechanical (light touch) allodynia in one or more upper limb dermatomes in the acute phase, which was not present at 6 months (**Fig. 7A**).

Figure 7B illustrates the temporal changes in WADII participants with a gain in sensation on QST either during the acute phase or at 6 months. A total of 18.0% (n = 11) showed signs of thermal hyperalgesia (gain on CPT or HPT testing) in the acute stage, which was recovered in 72.7% (n = 8) at 6 months,

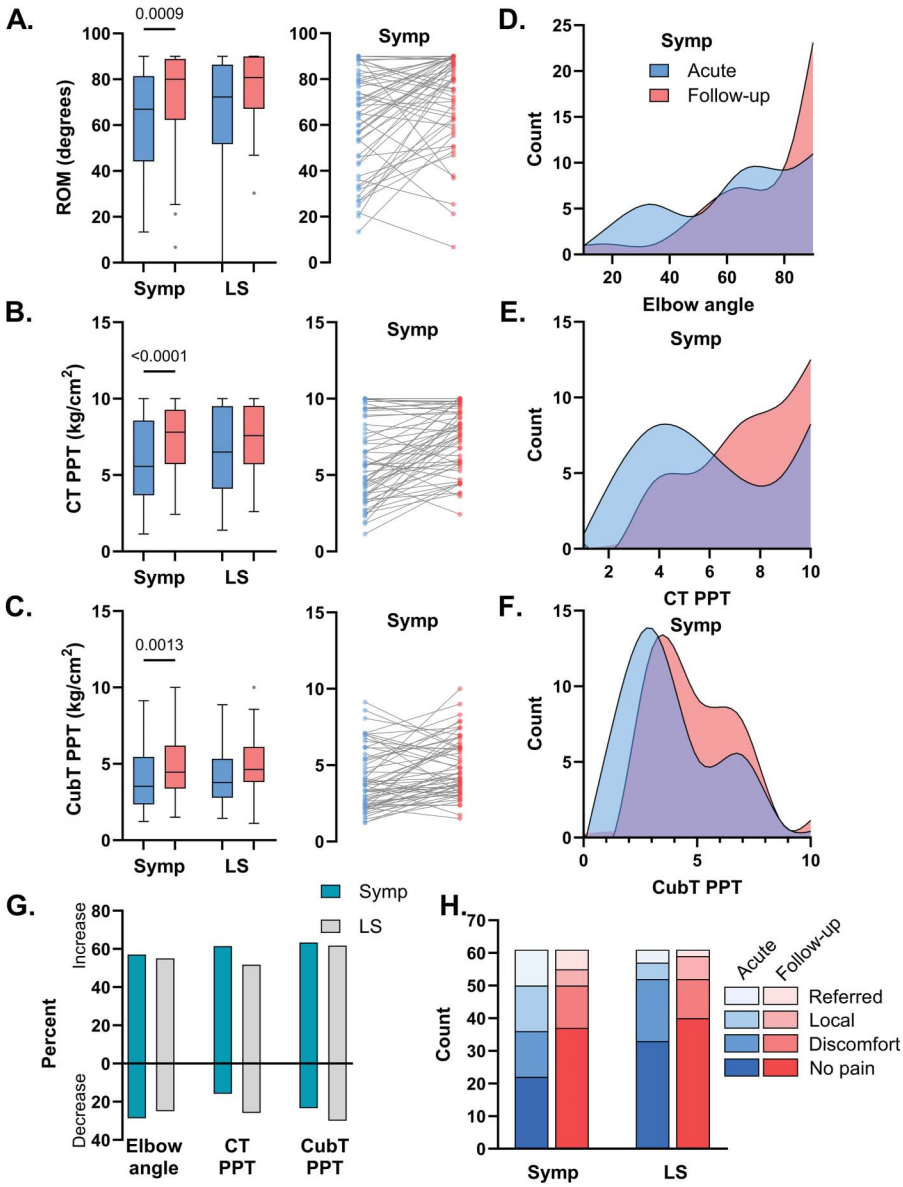

Figure 6. Temporal changes in heightened nerve mechanosensitivity. (A and D) Elbow extension range of motion (ROM) during the ULNT1 (median nerve bias), pressure pain thresholds over the (B and E) median nerve at the carpal tunnel (CT PPT) and (C and F) ulnar nerve at the cubital tunnel (CubT PPT) within 1 month after injury (acute) and at 6-month follow-up. Box plots (A–C; median/IQR) and frequency distribution curves (D–F) are displayed. Individual paired scores are also shown. (G) Proportion of participants with a change (ie, >0.2 SDs of the absolute difference between time points) in elbow angle, CT PPT, or CubT PPT at 6-month follow-up compared with 1 month after injury. (H) Digital palpation of the brachial plexus. The number of participants with no pain, discomfort, local and referred pain is shown at 1 month and at follow-up. LS, less symptomatic side; Symp, most symptomatic side. Blue, acute. Red, follow-up. n = 57 to 62. Corrected α = 0.017.

whereas 14.8% (n = 9) of participants developed thermal hyperalgesia at 6 months, which was not present in the acute phase. A total of 11.5% (n = 7) of WADII participants showed signs of mechanical hyperalgesia (gain on MPT) in the acute stage, which was recovered in all participants at 6 months, whereas only 1.6% (n = 1) of participants developed mechanical hyperalgesia at 6 months, which was not present in the acute phase. A similar proportion of individuals showed signs of mechanical pain sensitivity in the acute phase (13.1%; n =8), who all recovered at 6 months. Only 3.3% (n = 2) participants developed mechanical pain sensitivity at 6 months, which was not present in the acute phase. Furthermore, 14.8% (n = 9) WADII participants showed signs of deep mechanical hyperalgesia (gain on PPT) in the acute stage, which was recovered in all participants at 6 months, whereas 3.3% (n = 2) of participants developed a gain of PPT at 6 months, which was not present in the acute phase.

3.8. Serum cytokines

IFN-γ, IL-6, IL-8, and IL-10 serum levels were similar at 6 months compared with the acute phase. However, there was a reduction in TNF-α levels at 6 months (median = 1.78 pg/mL, IQR = 0.58; acute median = 2.05 pg/mL, IQR = 0.78, P = 0.0027; **Figs. 8A–F**). A total of 67.3% of individuals had a decrease in TNF-α levels at 6 months compared with only 26.9% where there was an increase (**Fig. 8G**), which is also reflected in the left shift of the frequency distribution curve at 6 months (**Fig. 8F**).

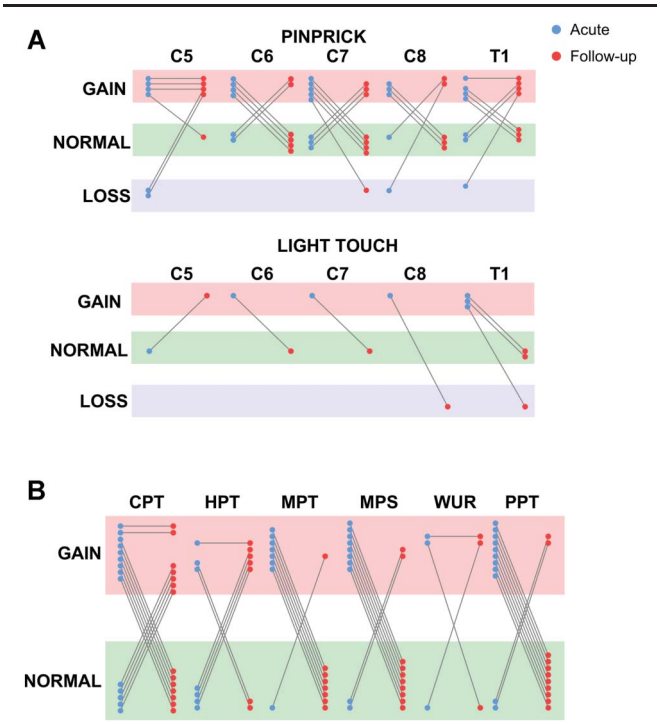

Figure 7. Temporal changes of gain of sensation. (A) Individual paired data for those WADII participants with a gain in sensation to pinprick (mechanical hyperalgesia) or light touch (mechanical allodynia) in one or more upper limb dermatomes (C5-T1) at 1 month after injury (acute) or at 6-month follow-up. Participants who had normal dermatomal sensation at both time points are not shown. (B) Individual paired data for those participants with a gain in sensation on QST at 1 month after injury or at 6-month follow-up. Only those QST parameters where WADII participants had a gain of sensation are shown. CPT, cold pain threshold; HPT, heat pain threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PPT, pressure pain threshold; QST, quantitative sensory testing; WUR, wind-up ratio. Blue, acute. Red, follow-up.

3.9. Correlations between measures of recovery and neuroinflammation

At 6 months, there were moderate negative correlations between painDETECT scores and MPT ($r = -0.41$; $P = 0.0014$), VAS and MDT ($r = -0.40$; $P = 0.0019$), and NDI and elbow extension range of motion during the ULNT1 ($r = -0.42$; $P = 0.0008$). There were no other notable correlations between pain scale, NDI, painDETECT or GRQ scores and our measures of peripheral neuroinflammation at 6 months (Supplementary Table 3, http://links.lww.com/PAIN/C391).

A secondary analysis was performed whereby the follow-up data were separated into recovered and unrecovered based on the GRQ. There were no observed differences in measures of neuroinflammation between recovered and unrecovered groups (Supplementary Fig. 2, http://links.lww.com/PAIN/C391).

3.10. Prognostic factors for recovery

The GRQ (perceived recovery) and follow-up NDI questionnaire (neck disability) were sent to all 122 participants who took part in the acute phase of the study and were used as measures of recovery for the regression analysis. The GRQ was completed by 91 participants (74.6%) and the NDI by 90 participants (73.8%), which included the 62 participants who returned for a follow-up appointment (**Figs. 9A and B**). Follow-up NDI and GRQ were comparable between participants who did (GRQ = 2 [2]; NDI = 7.5 [11.0]) and who did not attend the follow-up appointment (GRQ = 2 [1], $P = 0.19$; NDI = 7 [8.5], $P = 0.82$; median [IQR]). Scores ≥3 on the GRQ were considered unrecovered. There was a large positive correlation between GRQ and follow-up NDI scores ($r = 0.65$, $P < 0.0001$; **Fig. 9C**).

Multivariable linear regression analysis indicated that the a priori selected acute measures of neuroinflammation were not prognostic for neck-related disability at 6 months (**Table 2**). Multivariable logistic regression analysis also indicated that the same acute measures of neuroinflammation in WADII were not prognostic for perceived recovery at 6 months (**Table 2**).

A secondary regression analysis showed that elbow range of motion during the ULNT1 was prognostic for both certainty of neuropathic pain, based on the NeuPSIG grading system ($P = 0.01$), and whiplash-related upper quadrant pain VAS rating at 6 months ($P = 0.03$; **Table 3**).

4. Discussion

The aims of this study were to examine the temporal development of measures of neuroinflammation in individuals with WADII, and to determine whether such measures predict recovery at 6 months. We focussed on a subgroup from our larger whiplash cohort who were examined within 1 month after injury and at 6 months. In our larger whiplash cohort, we showed evidence of neuroinflammation in the acute phase based on MRI T2-weighted signal ratio of the brachial plexus, heightened nerve mechanosensitivity, somatosensory hyperalgesia, and serologic inflammatory mediators.[27] In the subgroup who returned after 6 months, the MRI T2-weighted signal ratio remained unaltered, whereas heightened nerve mechanosensitivity and somatosensory hyperalgesia improved in many individuals. Except for TNF-α, serum inflammatory mediator levels remained similar at 6 months. Although there were signs of recovery at 6 months, our findings suggest that peripheral neuroinflammation may persist in a subgroup. Furthermore, peripheral neuroinflammation in the acute stage may in part predict recovery at 6 months.

4.1. Magnetic resonance imaging signs of peripheral neuroinflammation persist in a subgroup

Given our previous findings of heightened T2-weighted signal ratios of the C5 to C6 roots of the brachial plexus and C5 to C8 DRGs in acute WADII compared with healthy participants,[27] the unaltered T2-weighted signal ratios over time suggests ongoing neuroinflammation in some individuals. However, our data likely reflect the complexity in the underlying pathology. It is established that WADII encompasses a heterogeneous group with different patterns of recovery. Therefore, varying temporal changes in T2-weighted signal ratio, as well as the lack of an observed difference between time points, may reflect this heterogeneity.

Because median nerve T2-weighted signal ratios were not increased compared with healthy controls in the acute study, the lack of observed differences in T2-weighted ratios at 6 months suggests that distal neuroinflammation is not a prominent feature of WADII. This finding contrasts with our earlier study that showed the likely presence of median nerve neuroinflammation in a small cohort of chronic whiplash patients.[19] However, in that study, the median time since injury was 18 months. In addition, many of our present cohort were recovered, whereas participants in our previous study only included those with persistent pain.

4.2. Reduced nerve mechanosensitivity indicates decreased neuroinflammation

Evidence from animal models suggests that heightened nerve mechanosensitivity is driven by peripheral neuroinflammation.[4,10,18] In this cohort, heightened nerve mechanosensitivity was a prominent feature during the acute stage, supporting a potential role for neuroinflammation. The observed reduction in nerve mechanosensitivity at 6 months on the most symptomatic side is consistent with a resolution of neuroinflammation for many individuals. Although we previously reported a lack of correlation between brachial plexus T2-weighted signal intensity and heightened mechanosensitivity in the acute stage,[27] this discrepancy is likely attributed to the heterogeneity of WADII and limitations in the sensitivity of neuroinflammation assessments.

There was a similar recovery of hyperalgesia in many individuals at 6 months. Because hyperalgesia is also associated with peripheral neuroinflammation,[26,31] this recovery may be a consequence of reduced neuroinflammation. However, there was also a subset of individuals who developed mostly thermal hyperalgesia at 6 months. This later development of hyperalgesia might indicate the onset of a more chronic phenotype. Similarly, there was a small proportion of individuals who showed an increase in heightened nerve mechanosensitivity at 6 months, also reflecting a more chronic phenotype.

4.3. Systemic inflammation persists at 6 months

From the acute data, raised levels of IFN-γ, IL-6, and IL-8 were consistent with systemic inflammation.[27] The lack of change at 6 months for these mediators suggests that inflammation may persist in some individuals. Similar studies have also demonstrated systemic inflammation after acute[35] and chronic whiplash injury.[13] From the data, it is not possible to ascertain whether the inflammation is related to the presence of neuroinflammation or other soft tissue injury. Reduced systemic TNF-α levels at 6 months suggest that the inflammatory milieu has changed compared with the acute stage. Because TNF-α plays a significant role in neuropathic pain mechanisms,[22] decreasing levels might reflect a reduction in neuropathic pain symptoms at 6 months. However, there was no correlation between TNF-α levels and painDETECT scores. Previously, elevated TNF-α concentrations have been identified in a subset of WADII participants who were recovered or had mild neck-related disability both acutely and at

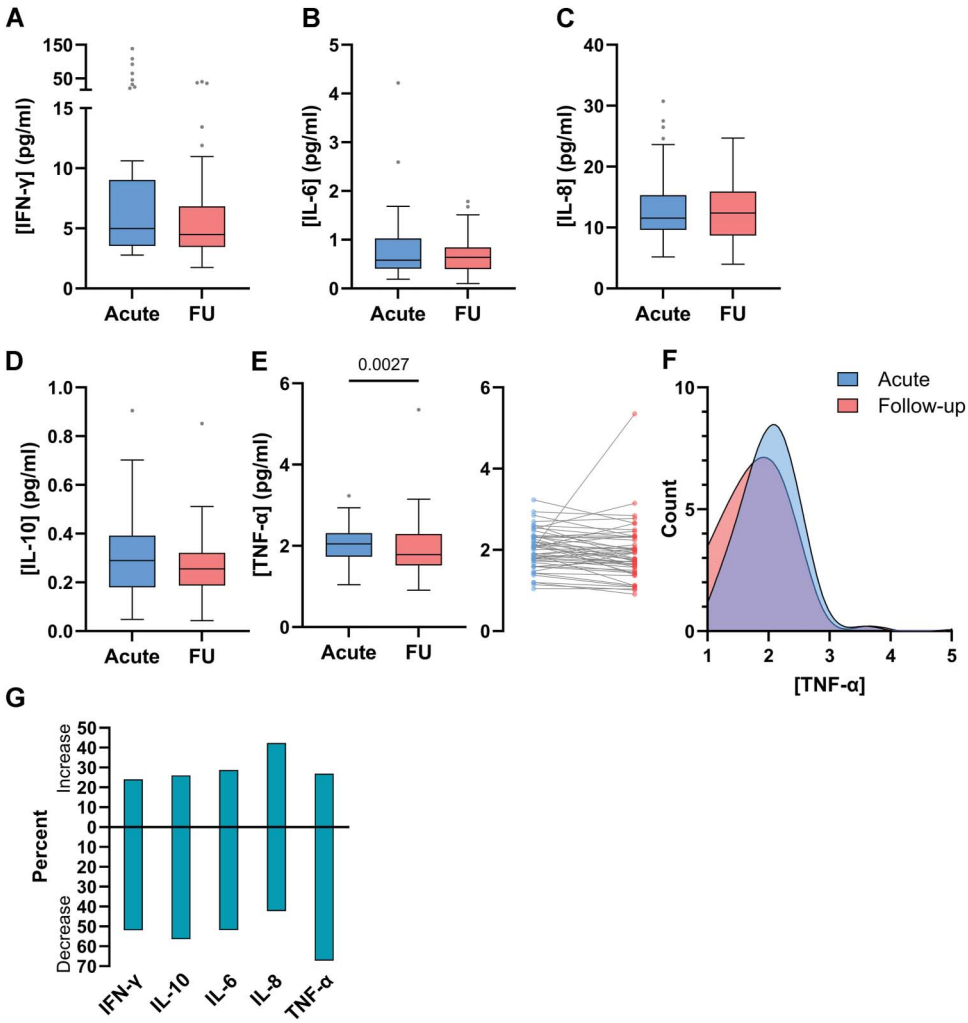

Figure 8. Temporal changes in serum cytokine levels. (A–E) Box plots (median/IQR) showing serum levels of (A) IFN-γ, (B) IL-6, (C) IL-8, (D) IL-10, and (E) TNF-α at 1-month (acute) and 6-month follow-up (FU). Individual paired scores are also shown for TNF-α. (F) Frequency distribution curve for TNF-α. (G) Proportion of participants with a change (ie, >0.2 SDs of the absolute difference between time points) in each cytokine at 6-month follow-up compared with 1 month after injury. Blue, acute. Red, follow-up. n = 46 to 52. Corrected α = 0.01. IFN, interferon; IL, interleukin; TNF-α, tumour necrosis factor-α.

follow-up, although this was observed in a smaller cohort with a shorter (3-month) follow-up.[35]

4.4. Whiplash-associated disorder grade II recovery levels remain high

Previous reports have indicated that approximately 50% of individuals develop chronic symptoms after a whiplash injury.[6,8,21,36] In this study, there was a trend for recovery based on whiplash-related upper quadrant pain (VAS), NDI, and painDETECT scores in three-quarters of participants. This was further supported by the reduction in pain catastrophising, DASS, and PTSD-8 scores. Although high levels of recovery may reflect an unknown characteristic of the participants who returned for a follow-up appointment, there were no clear baseline differences that might infer a different outcome at 6 months between those who returned and those who did not (Supplementary Table 2, http://links.lww.com/PAIN/C391). Furthermore, because NDI and GRQ were comparable between participants who did and did not attend the follow-up assessment, and participants were sequentially invited to return, it is unlikely that attrition bias influenced the returning group composition. Despite the high rate of improvement, less than one-third of individuals considered themselves fully recovered on the GRQ. Furthermore, a similar proportion of individuals continued to exhibit symptoms of neuropathic pain at 6 months, consistent with a persistent minor nerve injury, including peripheral neuroinflammation.

4.5. Measures of recovery do not correlate with neuroinflammation

The lack of correlation between recovery measures at 6 months (ie, whiplash-related upper quadrant pain, NDI and GRQ) and measures of neuroinflammation highlights the complexity of WADII and the presence of multiple phenotypes. This complexity is reflected in the absence of observed differences between recovered and unrecovered groups when comparing neuroinflammatory measures, although the small number of unrecovered participants may be partly responsible. Furthermore, recovery cannot be attributed to a single measure of neuroinflammation but is instead multifactorial. Each of the neuroinflammatory measures we examined pertain to distinct aspects of the pain mechanism. Moreover, our measures of recovery are not specifically designed to evaluate nerve pathology. The observed negative correlation between painDETECT and mechanical pain threshold suggests that hypoaesthesia may be linked to alterations in nociceptor function.

4.6. Heightened nerve mechanosensitivity in the acute stage predicts recovery

Although the presence of neuroinflammation was not prognostic for NDI or GRQ, our secondary exploratory analysis showed that elbow range of motion during the ULNT1 was a weak prognostic

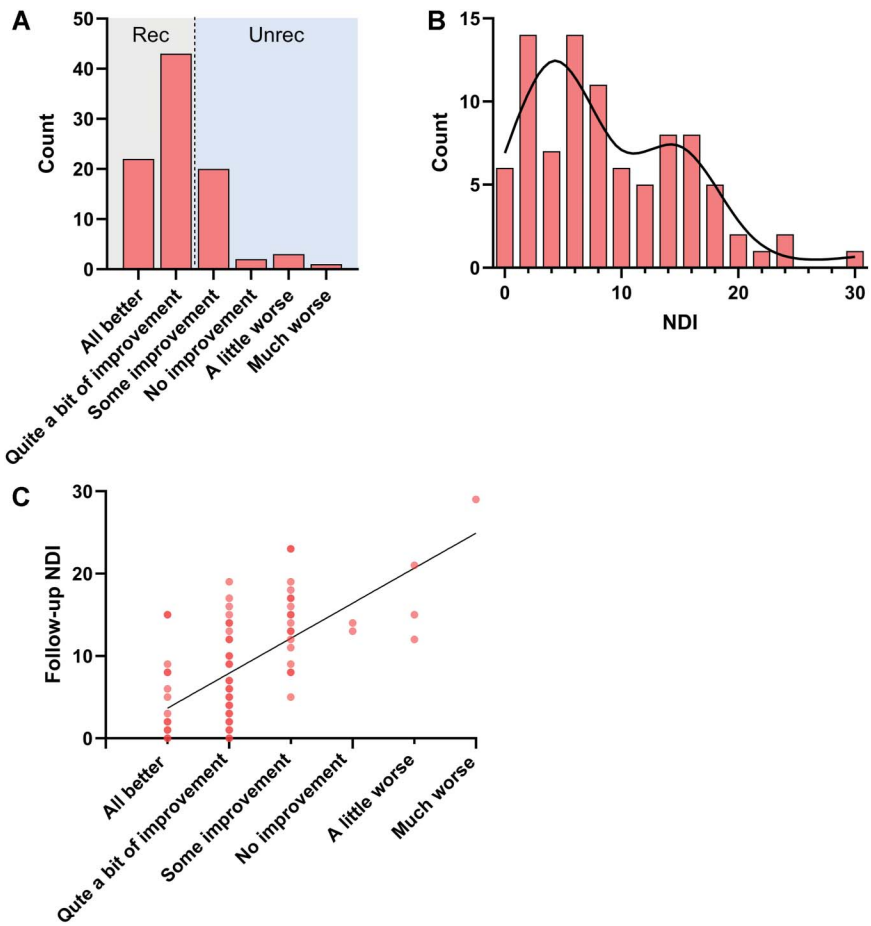

Figure 9. Measures of recovery for the main WADII cohort. (A) Histogram of global recovery questionnaire (GRQ) scores, with vertical line separating recovered (Rec) from unrecovered (Unrec). (B) Histogram of neck disability index (NDI) scores with overlaid frequency distribution curve. (C) Correlation between GRQ and NDI scores. $r = 0.67$. n = 91 (GRQ) and 90 (NDI). WADII, whiplash-associated disorder grade II.

Table 2

Results of multivariable regression analyses for neck-related disability and perceived recovery.

Neck-related disability (NDI)					
Variable	**No. of participants**	**Estimated coefficient**	**Standard error**	***P***	**Overall model adjusted r^2 value**
Heightened nerve mechanosensitivity					
Elbow ROM	64	−0.05	0.03	0.12	0.38
Composite score	67	−0.38	1.36	0.78	0.38
QST					
CPT	79	−0.73	0.47	0.12	0.38
MPS	79	−0.38	0.59	0.52	0.36
PPT	82	−0.36	0.35	0.32	0.37
MRI					
Max T2 BP	66	−1.44	2.67	0.21	0.39
Max T2 DRG	68	0.51	0.95	0.59	0.37
Cytokines					
Elevated cytokines	60	0.88	3.10	0.52	0.37
Perceived recovery (GRQ)					
Variable	**No. of participants**	**Estimated coefficient (log-odds)**	**Standard error**	***P***	**Odds ratio (95% CI)**
Heightened nerve mechanosensitivity					
Elbow ROM	64	−0.01	0.01	0.57	0.99 (0.97-1.02)
Composite score	67	0.13	0.61	0.83	1.14 (0.34-3.84)
QST					
CPT	80	−0.24	0.23	0.30	0.79 (0.48-1.21)
MPS	79	0.18	0.26	0.49	1.20 (0.71-2.00)
PPT	82	−0.01	0.17	0.96	0.99 (0.70-1.38)
MRI					
Max T2 BP	66	−0.20	0.56	0.72	0.82 (0.25-2.31)
Max T2 DRG	68	0.62	0.44	0.16	1.86 (0.81-4.83)
Cytokines					
Elevated cytokines	60	0.50	0.91	0.59	1.64 (0.31-12.91)

Each regression model included follow-up WADII Neck Disability Index (NDI) scores (linear regression) or global recovery questionnaire (GRQ) scores (logistic regression) as the dependent (outcome) variable, with acute phase measures of heightened nerve mechanosensitivity (elbow range of motion [ROM], and composite score, based on either a positive upper limb neurodynamic test-1 or 3, or painful response to digital palpation over the brachial plexus), quantitative sensory testing (QST) (cold pain threshold [CPT], mechanical pain sensitivity [MPS] and pain pressure threshold [PPT]), maximum (Max) T2-weighted signal ratio (roots of the brachial plexus [BP] and dorsal root ganglia [DRG]), and elevated cytokines (at least one elevated cytokine) as the independent variables, controlling for age, sex, and initial neck-related disability.
WADII, whiplash-associated disorder grade II.

indicator for neuropathic pain and whiplash-related upper quadrant pain. Although its prognostic value is limited, indicated by only 4% odds of experiencing a worse outcome in neuropathic pain and accounting for just 16% of the variability in pain, this finding suggests that heightened nerve mechanosensitivity during the acute stage may be one factor that leads to poor outcomes. Future prognostic studies are required to confirm this finding.

A potential explanation for why our other measures of neuroinflammation did not predict recovery may be related to the specificity of these measures. It is difficult to measure specific neuroinflammatory components or events in humans. The measures used in this study indicate the general presence of neuroinflammation or systemic inflammation. Although inflammation may persist in some people at 6 months, its presence in the acute stage is not specific to the development of chronic symptoms. Instead, it suggests that inflammation could contribute to chronicity if it has not resolved.

Although several studies have identified prognostic markers, such as increased initial neck-related disability, age, and post-traumatic hyperarousal, that are associated with poor outcome,[5,21,45] very few studies have identified prognostic markers that are specific to an underlying pathophysiological mechanism. We previously found that hypoaesthesia tested at the index finger was a modest prognostic indicator for neck and arm pain, accounting for 13% of the observed variance.[16] In terms of neuroinflammatory markers, there is some evidence for cold hyperalgesia as a potential prognostic marker for chronicity in WADII[17] and other neuropathic pain conditions.[2] Interestingly, in the acute stage in our cohort, cold hyperalgesia was not significantly different between healthy controls and WADII participants,[27] and we could not replicate its prognostic role. However, in previous studies, cold hyperalgesia was tested over the cervical spine rather than the index finger.[38]

5. Conclusions

This longitudinal study demonstrates significant self-reported functional and symptomatic improvement 6 months after a whiplash injury. However, some individuals continue to experience persistent symptoms, with continuing signs of peripheral neuroinflammation. Although no clear correlation was found between recovery and neuroinflammatory measures, the ongoing presence of neuroinflammation in some individuals infers its potential role in chronicity. The variability in the temporal changes of the neuroinflammatory measures underscores the complexity of WADII and the presence of multiple phenotypes. Although the predictive value of specific measures of peripheral neuroinflammation for recovery is limited, our findings highlight the potential broad role of inflammation in both acute and chronic symptoms in WADII.

Table 3

Results of multivariable regression analyses for neuropathic pain grading and whiplash-related upper quadrant pain.

Neuropathic pain grading					
Variable	**No. of participants**	**Estimated coefficient**	**Standard error**	***P***	**Odds ratio (95% CI)**
Heightened nerve mechanosensitivity					
Elbow ROM	51	−0.04	0.02	0.01	0.96 (0.93-0.99)
Composite score	53	0.33	0.70	0.64	1.34 (0.35-5.70)
QST					
CPT	59	0.06	0.25	0.80	1.06 (0.65-1.73)
MPS	59	−0.33	0.35	0.35	0.72 (0.34-1.40)
PPT	59	−0.04	0.19	0.83	0.96 (0.64-1.40)
MRI					
Max T2 BP	53	0.52	0.68	0.44	1.69 (0.45-6.81)
Max T2 DRG	53	−0.08	0.45	0.86	0.92 (0.35-2.25)
Cytokines					
Elevated cytokines	50	−0.20	0.97	0.84	0.82 (0.13-6.81)
Whiplash-related upper quadrant pain					
Variable	**No. of participants**	**Estimated coefficient (log-odds)**	**Standard error**	***P***	**Overall model adjusted r^2 value**
Heightened nerve mechanosensitivity					
Elbow ROM	52	−0.25	0.11	0.03	0.16
Composite score	51	0.70	5.73	0.90	0.07
QST					
CPT	59	−1.13	2.00	0.57	0.08
MPS	59	−4.58	2.70	0.10	0.12
PPT	59	0.25	1.47	0.86	0.07
MRI					
Max T2 BP	54	4.45	5.51	0.42	0.09
Max T2 DRG	54	3.50	3.74	0.35	0.09
Cytokines					
Elevated cytokines	51	−0.39	7.78	0.96	0.10

Each regression model included the NeuPSIG grading system for neuropathic pain (logistic regression) or the whiplash-related upper quadrant pain visual analogue scale (VAS) (linear regression) as the dependent (outcome) variable, with acute phase measures of heightened nerve mechanosensitivity (elbow range of motion [ROM] and composite score), quantitative sensory testing (QST) (cold pain threshold [CPT], mechanical pain sensitivity [MPS] and pain pressure threshold [PPT]), maximum T2 signal ratio (roots of the brachial plexus [BP] and dorsal root ganglia [DRG]), and elevated cytokines as the independent variables, controlling for age, sex, and initial neck-related disability.

NeuPSIG, neuropathic pain special interest group.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Authors' note: Data created during this research are openly available from https://sussex.figshare.com.

Supplemental digital content

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References

[1] Blankenburg M, Boekens H, Hechler T, Maier C, Krumova E, Scherens A, Magerl W, Aksu F, Zernikow B. Reference values for quantitative sensory testing in children and adolescents: developmental and gender differences of somatosensory perception. PAIN 2010;149:76–88.
[2] Boogaard S, Heymans MW, de Vet HC, Peters ML, Loer SA, Zuurmond WW, Perez RS. Predictors of persistent neuropathic pain—a systematic review. Pain Physician 2015;18:433–57.
[3] Boretius S, Gadjanski I, Demmer I, Bahr M, Diem R, Michaelis T, Frahm J. MRI of optic neuritis in a rat model. Neuroimage 2008;41:323–34.
[4] Bove GM, Ransil BJ, Lin HC, Leem JG. Inflammation induces ectopic mechanical sensitivity in axons of nociceptors innervating deep tissues. J Neurophysiol 2003;90:1949–55.

[5] Campbell L, Smith A, McGregor L, Sterling M. Psychological factors and the development of chronic whiplash-associated disorder(s): a systematic review. Clin J Pain 2018;34:755–68.
[6] Carroll LJ, Holm LW, Hogg-Johnson S, Cote P, Cassidy JD, Haldeman S, Nordin M, Hurwitz EL, Carragee EJ, van der Velde G, Peloso PM, Guzman J, Joint Decade-Task Force on Neck P, Its Associated D. Course and prognostic factors for neck pain in whiplash-associated disorders (WAD): results of the bone and joint decade 2000-2010 task force on neck pain and its associated disorders. Eur Spine J 2008;17:S83–92.
[7] Carroll LJ, Jones DC, Ozegovic D, Cassidy JD. How well are you recovering? The association between a simple question about recovery and patient reports of pain intensity and pain disability in whiplash-associated disorders. Disabil Rehabil 2012;34:45–52.
[8] Casey PP, Feyer AM, Cameron ID. Course of recovery for whiplash associated disorders in a compensation setting. Injury 2015;46:2118–29.
[9] Chien A, Eliav E, Sterling M. Hypoaesthesia occurs with sensory hypersensitivity in chronic whiplash–further evidence of a neuropathic condition. Man Ther 2009;14:138–46.
[10] Dilley A, Bove GM. Resolution of inflammation-induced axonal mechanical sensitivity and conduction slowing in C-fiber nociceptors. J Pain 2008;9:185–92.
[11] Dilley A, Lynn B, Pang SJ. Pressure and stretch mechanosensitivity of peripheral nerve fibres following local inflammation of the nerve trunk. PAIN 2005;117:462–72.
[12] Farrell SF, Armfield NR, Kristjansson E, Niere K, Christensen SWM, Sterling M. Trajectories of cold but not mechanical sensitivity correspond with disability trajectories after whiplash injury. PAIN 2025;166:1328–42.
[13] Farrell SF, de Zoete RMJ, Cabot PJ, Sterling M. Systemic inflammatory markers in neck pain: a systematic review with meta-analysis. Eur J Pain 2020;24:1666–86.
[14] Finnerup NB, Haroutounian S, Kamerman P, Baron R, Bennett DLH, Bouhassira D, Cruccu G, Freeman R, Hansson P, Nurmikko T, Raja SN, Rice ASC, Serra J, Smith BH, Treede RD, Jensen TS. Neuropathic pain: an updated grading system for research and clinical practice. PAIN 2016;157:1599–606.
[15] Fundaun J, Kolski M, Baskozos G, Dilley A, Sterling M, Schmid AB. Nerve pathology and neuropathic pain after whiplash injury: a systematic review and meta-analysis. PAIN 2022;163:e789–811.
[16] Fundaun J, Ridehalgh C, Koushesh S, Novak A, Tejos-Bravo M, Bremner S, Baskozos G, Dilley A, Schmid AB. The presence and prognosis of nerve pathology following whiplash injury: a prospective cohort study. Brain 2025;148:3392–3406.
[17] Goldsmith R, Wright C, Bell SF, Rushton A. Cold hyperalgesia as a prognostic factor in whiplash associated disorders: a systematic review. Man Ther 2012;17:402–10.
[18] Goodwin G, Bove GM, Dayment B, Dilley A. Characterizing the mechanical properties of ectopic axonal receptive fields in inflamed nerves and following axonal transport disruption. Neuroscience 2020;429:10–22.
[19] Greening J, Anantharaman K, Young R, Dilley A. Evidence for increased magnetic resonance imaging signal intensity and morphological changes in the brachial plexus and median nerves of patients with chronic arm and neck pain following whiplash injury. J Orthop Sports Phys Ther 2018;48:523–32.
[20] Hansen M, Andersen TE, Armour C, Elklit A, Palic S, Mackrill T. PTSD-8: a short PTSD inventory. Clin Pract Epidemiol Ment Health 2010;6:101–8.
[21] Kamper SJ, Rebbeck TJ, Maher CG, McAuley JH, Sterling M. Course and prognostic factors of whiplash: a systematic review and meta-analysis. PAIN 2008;138:617–29.
[22] Leung L, Cahill CM. TNF-α and neuropathic pain—a review. J Neuroinflammation 2010;7:27.
[23] Lovibond SH, Lovibond PF. Manual for the depression anxiety stress scales. Sydney: Psychology Foundation, 1995.
[24] Nee RJ, Jull GA, Vicenzino B, Coppieters MW. The validity of upper-limb neurodynamic tests for detecting peripheral neuropathic pain. J Orthop Sports Phys Ther 2012;42:413–24.
[25] Pascal MMV, Themistocleous AC, Baron R, Binder A, Bouhassira D, Crombez G, Finnerup NB, Gierthmühlen J, Granovsky Y, Groop L, Hebert HL, Jensen TS, Johnsen K, McCarthy MI, Meng W, Palmer CNA, Rice ASC, Serra J, Solà R, Yarnitsky D, Smith BH, Attal N, Bennett DLH. DOLORisk: study protocol for a multi-centre observational study to understand the risk factors and determinants of neuropathic pain. Wellcome Open Res 2019;3:63.
[26] Pulman KG, Smith M, Mengozzi M, Ghezzi P, Dilley A. The erythropoietin-derived peptide ARA290 reverses mechanical allodynia in the neuritis model. Neuroscience 2013;233:174–83.
[27] Ridehalgh C, Fundaun J, Bremner S, Cercignani M, Koushesh S, Young R, Novak A, Greening J, Schmid AB, Dilley A. Evidence for peripheral neuroinflammation after acute whiplash. PAIN 2025;166:2285–2299.
[28] Ridehalgh C, Fundaun J, Bremner S, Cercignani M, Young R, Trivedy C, Novak A, Greening J, Schmid A, Dilley A. Does peripheral neuroinflammation predict chronicity following whiplash injury? Protocol for a prospective cohort study. BMJ Open 2022;12:e066021.
[29] Rolke R, Baron R, Maier C, Tölle TR, Treede DR, Beyer A, Binder A, Birbaumer N, Birklein F, Bötefür IC, Braune S, Flor H, Huge V, Klug R, Landwehrmeyer GB, Magerl W, Maihöfner C, Rolko C, Schaub C, Scherens A, Sprenger T, Valet M, Wasserka B. Quantitative sensory testing in the German research network on neuropathic pain (DFNS): standardized protocol and reference values. PAIN 2006;123:231–43.
[30] Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology 1990;1:43–6.
[31] Satkeviciute I, Dilley A. Neuritis and vinblastine-induced axonal transport disruption lead to signs of altered dorsal horn excitability. Mol Pain 2018;14:1744806918799581.
[32] Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. Anesth Analg 2018;126:1763–8.
[33] Spitzer WO, Skovron ML, Salmi LR, Cassidy JD, Duranceau J, Suissa S, Zeiss E. Scientific monograph of the Quebec task force on whiplash-associated disorders: redefining "whiplash" and its management. Spine (Phila Pa 1976) 1995;20:1S–73S.
[34] Stanisz GJ, Webb S, Munro CA, Pun T, Midha R. MR properties of excised neural tissue following experimentally induced inflammation. Magn Reson Med 2004;51:473–9.
[35] Sterling M, Elliott JM, Cabot PJ. The course of serum inflammatory biomarkers following whiplash injury and their relationship to sensory and muscle measures: a longitudinal cohort study. PLoS One 2013;8:e77903.
[36] Sterling M, Hendrikz J, Kenardy J. Compensation claim lodgement and health outcome developmental trajectories following whiplash injury: a prospective study. PAIN 2010;150:22–8.
[37] Sterling M, Hendrikz J, Kenardy J. Similar factors predict disability and posttraumatic stress disorder trajectories after whiplash injury. PAIN 2011;152:1272–8.
[38] Sterling M, Jull G, Kenardy J. Physical and psychological factors maintain long-term predictive capacity post-whiplash injury. PAIN 2006;122:102–8.
[39] Sterling M, Jull G, Vicenzino B, Kenardy J. Sensory hypersensitivity occurs soon after whiplash injury and is associated with poor recovery. PAIN 2003;104:509–17.
[40] Sterling M, Jull G, Vicenzino B, Kenardy J, Darnell R. Physical and psychological factors predict outcome following whiplash injury. PAIN 2005;114:141–8.
[41] Sullivan MJL, Bishop SR, Pivik J. The pain catastrophizing scale: development and validation. Psychol Assess 1995;7:524–32.
[42] Tournier C, Hours M, Charnay P, Chossegros L, Tardy H. Five years after the accident, whiplash casualties still have poorer quality of life in the physical domain than other mildly injured casualties: analysis of the ESPARR cohort. BMC Public Health 2016;16:13.
[43] Vernon H. The neck disability index: state-of-the-art, 1991-2008. J Manipulat Physiol Ther 2008;31:491–502.
[44] Vernon H, Mior S. The Neck Disability Index: a study of reliability and validity. J Manipulat Physiol Ther 1991;14:409–415.
[45] Walton DM, Macdermid JC, Giorgianni AA, Mascarenhas JC, West SC, Zammit CA. Risk factors for persistent problems following acute whiplash injury: update of a systematic review and meta-analysis. J Orthopaedic Sports Phys Ther 2013;43:31–43.