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Longitudinal profiles of routine serum biomarkers and pain from the nadir through recovery in Guillain–Barré syndrome

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Article

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Longitudinal profiles of routine serum biomarkers and pain from the nadir through recovery in Guillain–Barré syndrome

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Abstract

Background: Pain is a frequent and disabling manifestation of Guillain–Barré syndrome (GBS), particularly during the acute nadir phase, yet its biological correlates remain poorly understood. Identifying accessible serum biomarkers associated with disease recovery and pain-related outcomes may improve patient monitoring and generate insights into associated mechanisms.

Methods: A longitudinal analysis of serum biochemical parameters was performed in patients with GBS evaluated at three recovery stages: nadir phase (GBS-T1), onset of recovery (GBS-T2), and late recovery (GBS-T3). Healthy donors and patients with traumatic spinal cord injury (SCI) were included as reference cohorts. Non-parametric statistical analyses were used to identify biomarkers showing recovery-associated changes. Pain intensity, pain interference, and neuropathic pain symptoms were assessed using the Brief Pain Inventory (BPI) and Neuropathic Pain Symptom Inventory (NPSI). Exploratory associations between pain-related outcomes and selected serum biomarkers were assessed using Spearman's rank correlation.

Results: Several inflammatory and metabolic biomarkers exhibited stage-dependent changes throughout recovery. C-reactive protein (CRP) and interleukin-6 (IL-6) were highest during GBS-T1 and progressively decreased during recovery, whereas albumin, bilirubin, creatinine, and creatine kinase increased over time. Pain intensity, pain interference, and neuropathic pain symptoms were greatest during GBS-T1 and progressively improved during recovery. At GBS-T1, serum creatinine showed a moderate positive association with pain intensity but did not reach statistical significance (Spearman's $\rho = 0.534$, exact two-tailed $p = 0.051$; $n = 14$). No significant associations were observed between pain outcomes and C-reactive protein, albumin, bilirubin, or creatine kinase.

Conclusions: Recovery from the nadir phase of GBS is accompanied by coordinated inflammatory, metabolic, and homeostatic adaptations reflected in circulating serum biomarkers. CRP and IL-6 reflected inflammatory activity, whereas albumin and bilirubin were consistent with restoration of systemic metabolic homeostasis. Serum creatinine showed a moderate but non-significant positive correlation with pain intensity during GBS-T1. This unconfirmed exploratory signal should not be interpreted as evidence that serum creatinine is a biomarker of pain in GBS. Overall, these findings characterize recovery-associated biochemical patterns that warrant further evaluation in larger prospective longitudinal cohorts.

Keywords: Guillain–Barré syndrome; pain; biomarkers; creatinine; C-reactive protein; albumin; bilirubin; recovery

Background

The Guillain–Barré syndrome (GBS) is an acute immune-mediated neuropathy characterized by rapidly progressive weakness and variable sensory symptoms. In addition to motor impairment, pain is a highly prevalent and clinically relevant feature, particularly during the acute nadir phase, where it can significantly impact patient well-being and recovery. Despite its clinical importance, the biological mechanisms underlying pain in GBS remain poorly understood, and reliable biomarkers that could assist in its assessment and management are currently lacking.

Pain as a major clinical burden in GBS

Pain is one of the most common and clinically relevant non-motor manifestations of Guillain–Barré syndrome (GBS). While the disease has traditionally been characterized by rapidly progressing muscle weakness and areflexia, pain can appear even before the onset of motor deficits, persist during the acute phase, and even continue for months or years after the onset of the disease. Several longitudinal studies have shown that between 55% and 89% of patients experience some type of pain during the course of GBS, including radicular pain, musculoskeletal pain, and symptoms consistent with neuropathic pain [1–4].

In addition to its high prevalence, pain is a major determinant of disability and quality of life. It has been associated with sleep disorders, functional limitations, anxiety, depression, and an increased need for analgesic treatment, even in patients who achieve satisfactory motor recovery. Prospective studies have shown that the evolution of pain does not always follow the same trajectory as the recovery of muscle strength, suggesting that both processes may be mediated, at least partially, by different pathophysiological mechanisms [1–4].

Despite its clinical relevance, pain remains an underdiagnosed and undertreated symptom in Guillain–Barré syndrome. Clinical attention tends to focus on motor deficits and respiratory complications, while systematic pain assessment remains limited, especially in critically ill patients or those admitted to intensive care units [2,3]. Therefore, readily available biological

measures that complement, rather than replace, patient-reported outcomes could improve pain characterization during recovery.

Lack of reliable serum biomarkers associated with pain

Multiple biomarkers have been proposed as being associated with the severity, prognosis, and immune activity of Guillain–Barré syndrome. These include inflammatory cytokines, complement proteins, antiganglioside autoantibodies, cerebrospinal fluid proteins, and various markers of neuronal damage and systemic inflammation [5–8]. However, most of these biomarkers have been studied as indicators of disease activity or functional recovery, while their relationship to pain has received less attention.

From a clinical perspective, there is growing interest in identifying readily available serum biomarkers that can complement subjective pain assessment scales. While instruments such as the Brief Pain Inventory (BPI) or the Neuropathic Pain Symptom Inventory (NPSI) provide a standardized assessment of the pain experience, both rely on individual patient perception and do not offer insights into the underlying biological mechanisms [9,10]. Identifying circulating biomarkers associated with pain could contribute to better clinical stratification of patients, facilitate longitudinal monitoring of recovery, and provide new therapeutic targets.

Evidence directly linking routine biochemical parameters to patient-reported pain in GBS remains sparse and heterogeneous. The few studies that have explored the relationship between routine biochemical parameters and pain in Guillain–Barré syndrome have shown inconsistent results and have generally focused exclusively on the acute phase of the illness, leaving uncertainty about whether biomarker–pain relationships persist or change during recovery. Therefore, there remains a significant need to identify accessible and reproducible serum biomarkers that reflect the biological progression of pain during clinical recovery.

Rationale for studying inflammatory and metabolic parameters

The immune response characteristic of Guillain–Barré syndrome triggers profound inflammatory and metabolic changes that evolve throughout the course of the disease. Several studies have demonstrated elevated levels of inflammatory biomarkers such as C-reactive protein (CRP) and interleukin-6 (IL-6), whose concentrations typically correlate with inflammatory activity and clinical severity during the acute phase [7,11].

At the same time, metabolic and homeostatic biomarkers such as albumin, bilirubin, creatinine, and creatine kinase may also reflect biologically relevant processes during recovery. Albumin serves as a comprehensive marker of inflammatory and nutritional status [12]; bilirubin has recognized antioxidant and cytoprotective properties [13]; creatinine reflects not only renal function but also muscle mass and energy metabolism; while creatine kinase can provide information on neuromuscular activity and muscle damage associated with immobilization and functional recovery[14].

Most published studies have evaluated these biomarkers cross-sectionally or by focusing on their prognostic value [8,11–14]. Few studies have analyzed their longitudinal evolution from the most critical phase (nadir phase) to clinical recovery, or explored their potential association with pain intensity and neuropathic pain symptoms. This lack of information limits our understanding of the biological mechanisms involved in GBS recovery and underscores the need for longitudinal studies that integrate serum biomarkers and patient-reported clinical outcomes.

Study hypothesis and objectives

We hypothesized that routinely measured serum biochemical parameters would exhibit distinct recovery stage-associated profiles in patients with GBS and that selected parameters might show stage-specific relationships with pain-related outcomes. The primary objective of this study was to characterize changes in serum biochemical parameters across three clinically defined stages of GBS recovery—the nadir phase, the onset of functional recovery, and later

recovery or hospital discharge—and to compare these profiles with those observed in healthy participants and patients with traumatic spinal cord injury. The secondary objective was to explore the associations between selected serum biomarkers and pain intensity, pain interference, and neuropathic pain symptoms at each GBS recovery stage.

Methods

Study population and cohort definition

This retrospective, observational, longitudinal study analysed serial serum biochemical parameters and pain-related scores in patients with GBS during hospitalization and recovery. Patients with traumatic SCI were included as a neurological reference cohort exposed to hospitalization-associated conditions and pain, and healthy donors served as a non-neurological reference cohort. The study was approved by the Clinical Research Ethics Committee of the Hospitals of Toledo, Castilla-La Mancha, Spain (approval no. 17). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Serum samples were obtained from the previously characterized Toledo Guillain–Barré Syndrome (TOSGB) Biobank Collection [15]. Samples corresponding to predefined GBS recovery stages—GBS-T1 (nadir phase), GBS-T2 (onset of recovery), and GBS-T3 (later recovery/discharge)—were selected together with samples from SCI controls and healthy donors. Blood was collected by qualified nursing personnel at the National Hospital for Paraplegics and the Regional Blood Transfusion Centre in Toledo, Spain.

The study included 61 patients with GBS (45 men and 16 women; median age, 56.0 years [IQR, 42.5–65.5]), 13 SCI controls (11 men and 2 women; median age, 52.0 years [IQR, 42.5–62.5]), and 30 healthy controls (20 men and 10 women; median age, 48.5 years [IQR, 36.5–56.3]). No formal a priori sample size calculation was performed because this retrospective study used an

availability-based cohort derived from the TOSGB Biobank Collection. The sample size was determined by the number of participants for whom suitable stored serum samples and the corresponding clinical data were available and met the prespecified eligibility criteria[15]. Analyte-specific sample sizes varied because of missing laboratory values and prespecified exclusions.

The overall study design and allocation of patient cohorts are summarized in Figure 1.

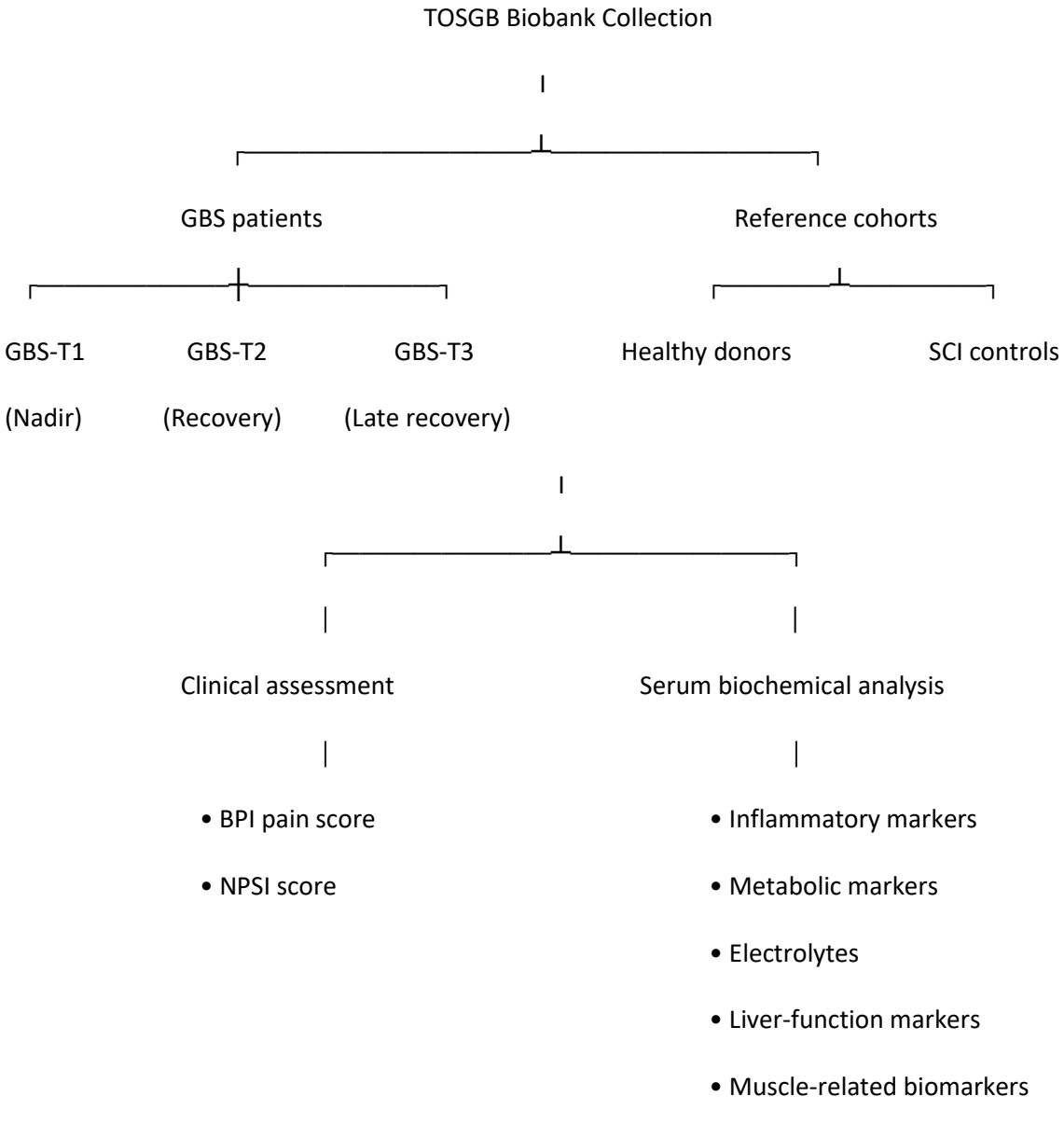


Figure 1. Study design and cohort allocation from the Toledo Guillain–Barré Syndrome (TOSGB) Biobank Collection.

Clinical assessment and pain measurement

Clinical and functional evaluation of patients with GBS was performed during different recovery stages defined as GBS-T1 (peak disease severity/nadir phase), GBS-T2 (onset of functional recovery), and GBS-T3 (later recovery/discharge stage). Pain assessment was performed using the Brief Pain Inventory (BPI), including pain intensity and pain interference domains, together with the Neuropathic Pain Symptom Inventory (NPSI) to evaluate neuropathic pain-associated symptoms[9,10]. At each clinical stage, the BPI and NPSI were administered within seven days after the first serum collection. Pain-related outcomes were compared across GBS recovery stages and with those of SCI controls. Biomarker–pain correlation analyses used only the first serum sample obtained at each stage and its temporally matched pain assessment. Additional serum samples collected within the same stage were excluded because they were not accompanied by repeat BPI or NPSI assessments.

Serum biochemical analysis

Serum isolation and storage procedures from the TOSGB Biobank Collection were previously described by our group [15]. Briefly, peripheral venous blood samples were collected in sterile gel-barrier tubes and centrifuged to obtain the serum. Serum aliquots were coded and stored at −80°C in the TOSGB Biobank Collection until biochemical analysis. For the present study, serum biochemical analyses were performed in samples corresponding to different GBS recovery stages (T1, T2, and T3), together with SCI and healthy donor reference cohorts. Samples from the same GBS patient were analysed simultaneously whenever possible to minimize inter-assay variability. Biochemical analyses included markers associated with inflammatory status, metabolic regulation, liver function, electrolyte balance, and muscle damage. Parameters analysed included creatinine, uric acid, sodium, potassium, chloride, calcium, phosphate,

magnesium, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total bilirubin, C-reactive protein (CRP), lipase, cholinesterase, creatine kinase (CK), and interleukin-6 (IL-6). Biochemical determinations were performed as previously described [15]. Fourteen biochemical parameters were measured using spectrophotometric techniques on a cobas c 702 analyser (Roche, Barcelona, Spain). Sodium, potassium, and chloride concentrations were determined by indirect potentiometry using the ion-selective electrode module of the cobas c 702 analyser. Serum IL-6 concentrations were quantified by electrochemiluminescence immunoassay using a cobas e 801 analyser (Roche). The specific detection method used for each biochemical parameter is detailed in Table 1 of reference 15.

Statistical analysis

Serum biochemical markers were assessed across three recovery stages in patients with Guillain–Barré syndrome: T1 (nadir phase, peak disease severity), T2 (onset of recovery), and T3 (later recovery/discharge). Healthy donors were sampled once, whereas patients with traumatic spinal cord injury (SCI) were sampled once during hospitalization. At T2, 53 serum samples were available from 45 patients because seven patients underwent additional within-stage blood collections. For each biochemical parameter, all valid T2 measurements from the same patient were averaged arithmetically before group-level analyses, yielding a maximum of 45 patient-level T2 values; analyte-specific sample size varied because of missing laboratory values and prespecified exclusions. Since most variables did not meet normality assumptions, non-parametric analyses were performed. Global differences among groups were evaluated using the Kruskal–Wallis test. For each biochemical parameter, Dunn's multiple-comparisons test with adjustment for multiple testing was used to evaluate four prespecified pairwise comparisons. GBS-T2 was selected as the reference group for biomarker comparisons because it represents the onset of functional improvement following peak disease severity. Adjusted *p* values are

reported, statistical significance was established at $p < 0.05$, and data are shown as median and interquartile range (IQR).

Differences in BPI pain intensity, BPI pain interference, and NPSI scores among GBS stages and SCI controls were assessed using the Kruskal–Wallis test followed, when appropriate, by Dunn's multiple-comparisons post hoc test, using GBS-T1 as the reference group. Associations between selected biomarkers and pain-related outcomes were evaluated separately within each study group using Spearman's rank correlation coefficient (ρ). Correlation analyses used only the first serum sample obtained at each stage and the corresponding pain assessment completed within the subsequent seven days. Exact two-tailed p values were calculated. These correlation analyses were exploratory and were not adjusted for multiple testing; effect estimates, p values, and numbers of paired observations are therefore reported transparently. All tests were two-sided, and statistical significance was defined as $p < 0.05$. Analyses and graphical representations were performed using GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, CA, USA).

Results

Circulating biochemical markers across GBS recovery stages

To characterize systemic biochemical alterations associated with GBS recovery, serum biomarkers were analysed across three clinically defined recovery stages (GBS-T1, GBS-T2, and GBS-T3) and compared with healthy donors and SCI controls (Table 1).

Table 1. Circulating biochemical markers across Guillain–Barré syndrome recovery stages and control cohorts

Serum biomarker	GBS-T1 (n = 46)	GBS-T2 (n = 45)	GBS-T3 (n = 33)	Healthy (n = 30)	SCI (n = 13)
Creatinine (mg/dL)	0.36 (0.29-0.48)	0.46 (0.32-0.62)	0.60 (0.46-0.79)	0.92 (0.77-1.00)	0.67 (0.53-0.79)
Uric acid (mg/dL)	3.80 (2.97-4.72)	5.10 (3.70-6.10)	5.35 (4.25-6.47)	5.4 (4.52-6.00)	6.30 (3.95-7.25)
Sodium (mmol/L)	140.0 (136.5-141.0)	141.0 (139.0-143.0)	141.0 (140.0-142.0)	142.0 (141.0-143.0)	140.0 (139.5-142.0)
Potassium (mmol/L)	4.10 (3.80-4.25)	4.20 (3.90-4.60)	4.20 (3.90-4.40)	4.55 (4.20-4.72)	4.20 (3.80-4.50)
Chloride (mmol/L)	103.0 (101.0-106.0)	102.00 (99.12-104.00)	102.0 (100.0-104.0)	103.0 (101.0-105.0)	103.0 (100.0-105.0)
Calcium (mg/dL)	8.85 (8.60-9.42)	9.40 (9.20-9.70)	9.40 (9.3-9.60)	9.45 (9.30-9.70)	9.35 (8.95-9.47)
Phosphate (mg/dL)	3.80 (3.20-4.40)	4.20 (3.60-4.55)	4.00 (3.70-4.20)	3.10 (2.72-3.62)	3.95 (3.72-4.37)
Magnesium (mg/dL)	1.88 (1.82-1.95)	1.95 (1.85-2.11)	1.96 (1.89-2.03)	2.01 (1.93-2.14)	2.08 (1.89-2.19)
Albumin (g/dL)	3.40 (3.00-3.80)	4.00 (3.60-4.20)	4.10 (4.00-4.40)	4.65 (4.40-4.82)	4.20 (3.89-4.30)
AST (U/L)	13.50 (6.75-22.25)	11.0 (8.0-16.0)	10.00 (7.00-14.75)	16.50 (13.00-19.25)	16.00 (13.25-18.75)
ALT (U/L)	11.00 (5.25-19.50)	7.0 (4.5-11.0)	4.50 (4.50-8.75)	10.50 (8.00-15.25)	7.50 (4.87-11.50)
GGT (U/L)	67.50 (33.75-124.8)	27.0 (15.5-48.0)	16.00 (11.00-31.00)	21.00 (13.75-28.50)	15.00 (12.00-21.50)
Total bilirubin (mg/dL)	0.16 (0.14-0.24)	0.26 (0.17-0.34)	0.31 (0.23-0.41)	0.51 (0.35-0.66)	0.43 (0.30-0.52)
C-reactive protein (mg/L)	8.00 (4.05-19.33)	4.65 (1.20-14.82)	1.40 (0.75-2.95)	0.95 (0.50-1.82)	4.90 (2.60-17.85)
Lipase (U/L)	30.50 (15.75-48.00)	30.5 (19.0-44.8)	28.00 (19.00-38.00)	39.50 (33.00-49.00)	27.50 (24.25-41.75)
Cholinesterase (U/L)	5316 (4190-7231)	6855 (5560-8595)	7038 (6173-8594)	8269 (6676-9321)	6986 (5484-9200)
Creatine kinase (U/L)	17.0 (6.5-36.5)	28.5 (15.2-59.8)	45.0 (20.0-78.0)	98.0 (75.0-135.0)	75.0 (34.5-250.5)
IL-6 (pg/mL)	11.65 (6.14-24.00)	4.93 (2.55-12.35)	3.03 (1.80-5.17)	2.81 (1.45-4.38)	4.09 (3.09-20.67)

Data are presented as median (25th–75th percentile) for each study group. Sample sizes varied slightly across biomarkers because of missing laboratory values and prespecified exclusions. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; IL-6, interleukin-6. Detailed statistical results, including Kruskal–Wallis and Dunn's multiple-comparisons post hoc tests, are provided in Supplementary Table S1

Several circulating biomarkers showed dynamic changes throughout GBS recovery. Early-stage GBS patients (GBS-T1) exhibited reduced serum creatinine, albumin, calcium, and uric acid levels compared with later recovery stages and healthy donors. In contrast, inflammatory-associated markers, including C-reactive protein (CRP), gamma-glutamyl transferase (GGT), and interleukin-6 (IL-6), tended to be elevated during the nadir phase and progressively decreased during recovery.

Biomarkers associated with muscle metabolism and tissue injury also demonstrated recovery-associated changes. Creatine kinase (CK) and total bilirubin levels increased progressively from GBS-T1 to GBS-T3, approaching values observed in healthy donor cohorts. Electrolyte-associated parameters, including sodium, potassium, phosphate, and magnesium, showed moderate variations across recovery stages but remained within relatively conserved ranges.

Interestingly, several biomarkers in GBS-T3 patients approached values observed in SCI controls and healthy donors, suggesting partial normalization of systemic biochemical homeostasis during recovery progression. Overall, these findings support the existence of stage-dependent biochemical dynamics during GBS recovery and identify circulating biomarkers potentially associated with inflammatory status, metabolic adaptation, and neurological recovery.

Recovery-associated biochemical trajectories in GBS

Based on the magnitude of the global Kruskal–Wallis H statistics, biological relevance, and consistency of recovery-associated patterns, selected circulating biomarkers were visualized in Figure 2. C-reactive protein (CRP) concentrations were markedly elevated during the nadir phase (GBS-T1) and progressively decreased throughout recovery, suggesting attenuation of systemic inflammatory activity. In contrast, albumin levels increased steadily from the acute phase toward later recovery stages, approaching values observed in healthy donors and indicating restoration of systemic homeostasis.

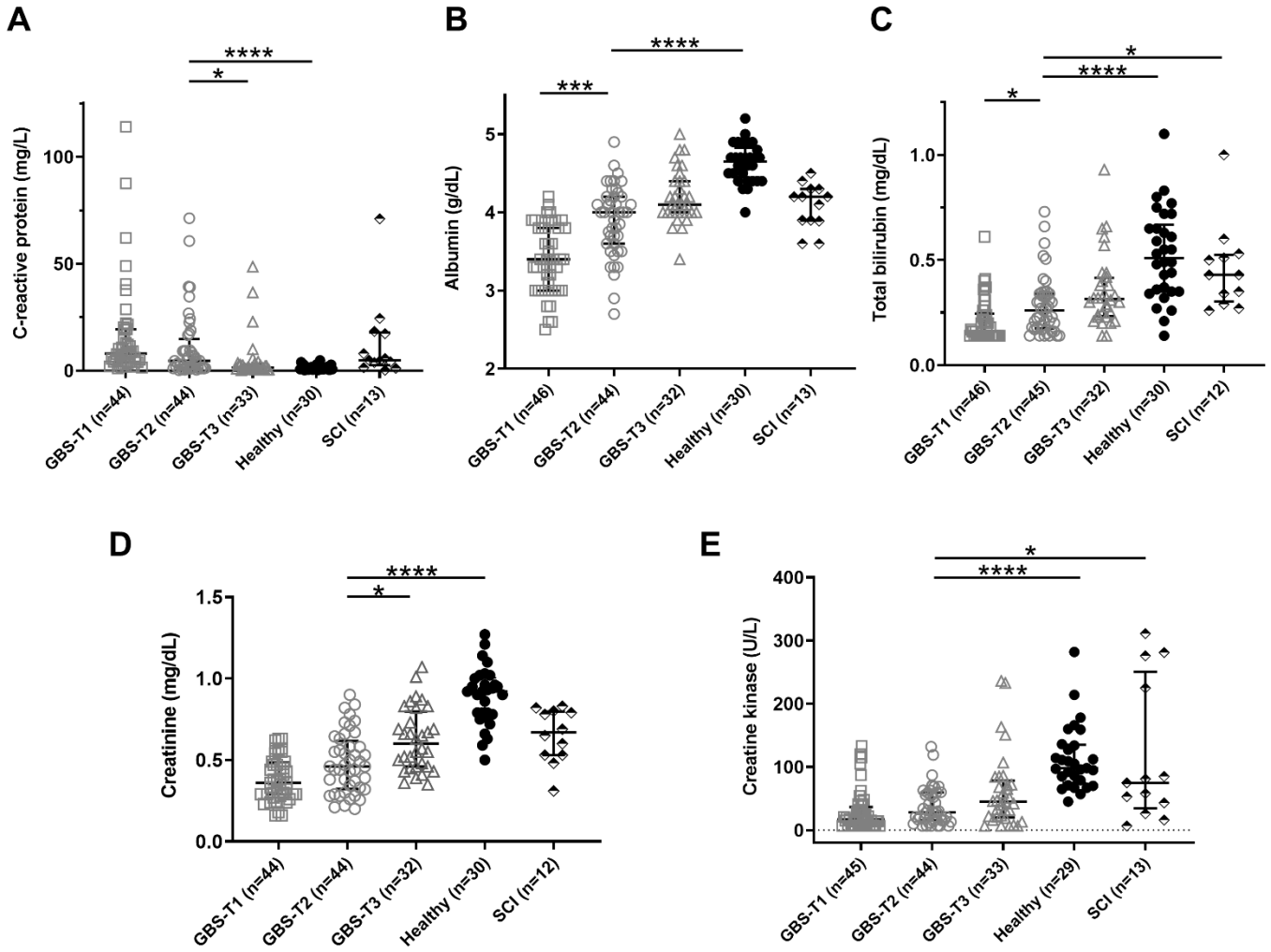


Figure 2. Recovery-associated trajectories of selected circulating biochemical biomarkers across Guillain–Barré syndrome stages and reference cohorts. Serum concentrations of C-reactive protein (A), albumin (B), total bilirubin (C), creatinine (D), and creatine kinase (E) were evaluated in Guillain–Barré syndrome (GBS) patients at different recovery stages (GBS-T1, GBS-T2, and GBS-T3), healthy donors, and spinal cord injury (SCI) controls. Data are presented as median with interquartile range (IQR) and individual data points. Statistical comparisons among groups were performed using the Kruskal–Wallis test followed by Dunn's multiple-comparisons *post hoc* test. These biomarkers were selected based on large global Kruskal–Wallis H statistics and consistent recovery-associated patterns. Significance levels are indicated as: **p* < 0.05; *** *p* < 0.001; **** *p* < 0.0001.

Total bilirubin and creatinine also exhibited recovery-related changes. Total bilirubin gradually increased from GBS-T1 to GBS-T3, reaching levels closer to those observed in healthy donors. Similarly, creatinine concentrations increased during recovery, although values remained lower than those observed in healthy donors and SCI controls. In contrast, creatine kinase displayed substantial interindividual variability and was consistently higher in healthy donor and SCI reference groups than in GBS patients during the acute disease stage. Collectively, these findings reveal coordinated inflammatory and metabolic adaptations accompanying functional recovery following GBS.

Although IL-6 showed significant global group differences, post hoc comparisons were less consistent after correction for multiple testing; therefore, IL-6 findings were interpreted as supportive rather than primary. Moreover, marked intraindividual fluctuations in IL-6 levels were observed during clinical follow-up, suggesting considerable temporal variability in IL-6 expression. Complete inferential statistical analyses for all evaluated biomarkers, including Kruskal–Wallis and Dunn's multiple-comparisons post hoc tests, are provided in **Supplementary Table S1**.

Pain-related outcomes across GBS recovery stages

Pain-related outcomes were evaluated using the Brief Pain Inventory (BPI) and the Neuropathic Pain Symptom Inventory (NPSI) across Guillain–Barré syndrome (GBS) recovery stages and spinal cord injury (SCI) controls (Figure 3, Supplementary Table S2). Patients assessed during the nadir phase (GBS-T1) exhibited the highest pain burden across all evaluated domains.

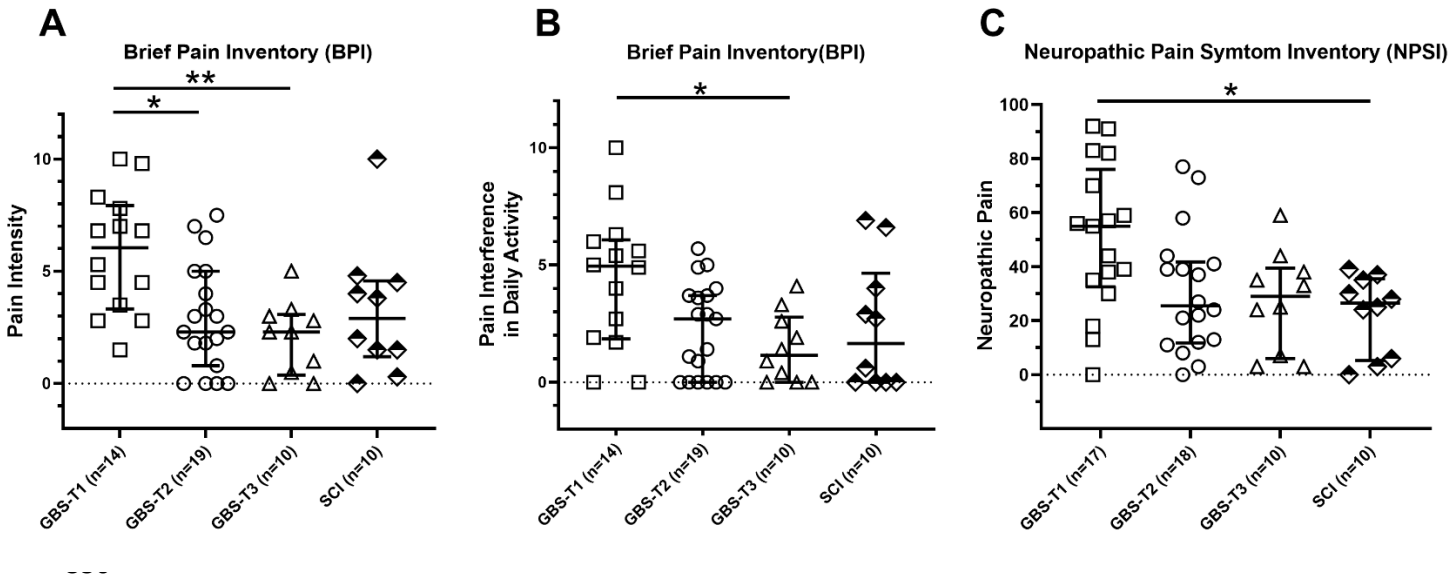


Figure 3. Pain-related outcomes across Guillain–Barré syndrome recovery stages and spinal cord injury controls. (A) Pain intensity and (B) pain interference with daily activities were assessed using the Brief Pain Inventory (BPI), whereas (C) neuropathic pain symptoms were evaluated using the Neuropathic Pain Symptom Inventory (NPSI). Data are presented as median with interquartile range (IQR) and individual data points. Statistical comparisons between groups were performed using the Kruskal–Wallis test followed by Dunn's multiple-comparisons *post hoc* test. GBS-T1 represents patients at the nadir phase with poorest functional status, GBS-T2 patients at the onset of recovery, and GBS-T3 patients at later recovery stages. SCI subjects were included as neurological controls. Asterisks indicate statistically significant differences relative to the GBS-T1 group (*$p < 0.05$; **$p < 0.01$).

Pain intensity progressively decreased during recovery, with significantly lower scores observed in both GBS-T2 and GBS-T3 compared with GBS-T1. Pain interference with daily activities was significantly reduced at GBS-T3 relative to the nadir phase, indicating a slower improvement in pain-related functional impact.

Neuropathic pain symptoms, assessed using the NPSI, were descriptively lower at GBS-T2 and GBS-T3; however, neither comparison with GBS-T1 reached statistical significance. SCI controls showed significantly lower NPSI scores than patients at GBS-T1. SCI controls displayed pain-related scores within the range observed during GBS recovery stages. Overall, these findings indicate that both pain intensity and neuropathic pain burden are greatest during the severe phase of GBS and tend to improve during recovery, supporting the association between clinical recovery and progressive attenuation of pain-related symptoms.

Correlations between pain intensity and circulating biomarkers

To explore potential biological correlates of pain, correlation analyses were performed between pain intensity scores and selected serum biomarkers across GBS recovery stages and SCI controls (**Figure 4**). At GBS-T1, serum creatinine showed a moderate positive association with pain intensity, although this association did not reach statistical significance (Spearman's ρ = 0.534, 95% CI -0.012 to 0.835, exact two-tailed *p* = 0.051). This association was not observed during subsequent recovery stages (GBS-T2 and GBS-T3) or in SCI controls.

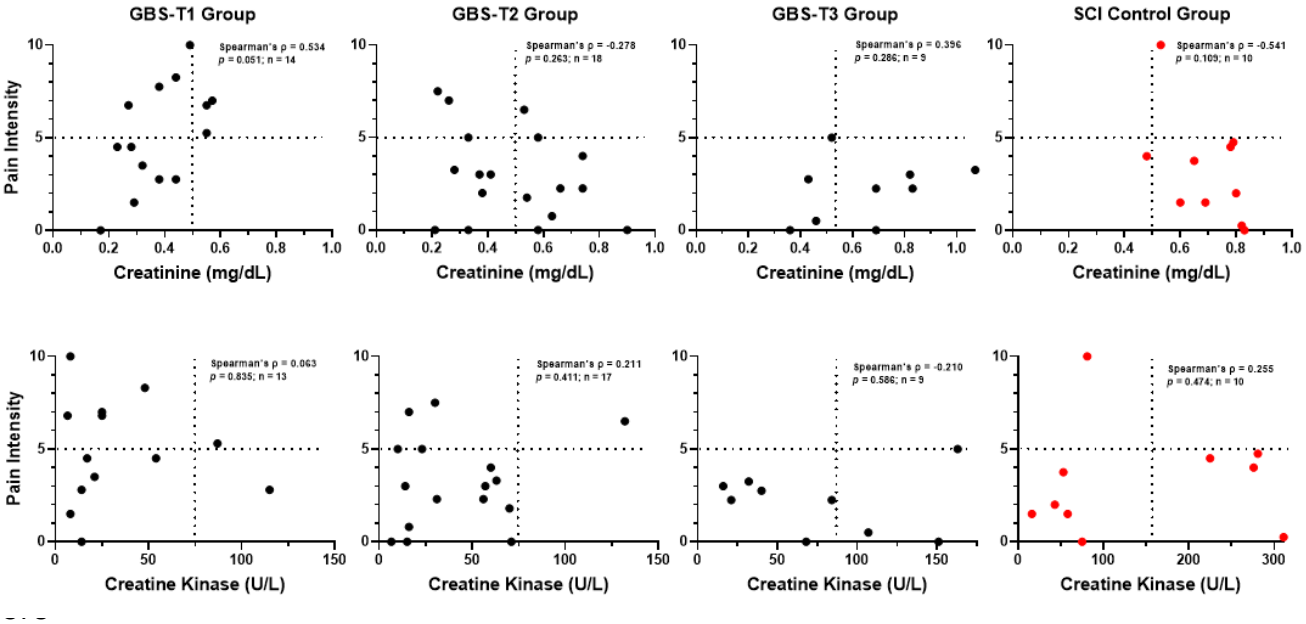


Figure 4. Associations of pain intensity with serum creatinine and creatine kinase across study groups. Upper panels show the associations between Brief Pain Inventory (BPI) pain intensity scores and serum creatinine concentrations (mg/dL), whereas lower panels show the associations between pain intensity and serum creatine kinase activity (U/L). Analyses were performed separately for GBS-T1, GBS-T2, GBS-T3, and spinal cord injury (SCI) control groups. GBS-T1 corresponds to peak disease severity/the nadir phase, GBS-T2 to the onset of functional recovery, and GBS-T3 to later recovery/the discharge stage. Each dot represents an individual participant with paired biomarker and pain measurements; black dots represent patients with GBS and red dots represent SCI controls. Associations were evaluated using Spearman's rank correlation analysis. Spearman's correlation coefficients (ρ), exact two-tailed p values, and the number of paired observations (n) are shown within each panel. Dotted vertical and horizontal lines indicate the median biomarker concentration and median pain intensity score, respectively. GBS, Guillain–Barré syndrome; SCI, spinal cord injury.

Serum creatine kinase levels were not significantly correlated with pain intensity in any study group. Thus, the moderate creatinine–pain correlation estimate at GBS-T1 was a stage-specific exploratory signal rather than evidence of a consistent association across recovery stages. Given

the exploratory nature of these analyses and the known influence of factors such as sex, age, and muscle mass on circulating creatinine concentrations, these findings should be interpreted with caution and warrant validation in larger independent cohorts.

Correlations between neuropathic pain severity and circulating biomarkers

Associations between serum creatinine or creatine kinase levels and neuropathic pain severity, assessed using the Neuropathic Pain Symptom Inventory (NPSI), were evaluated across GBS recovery stages and SCI controls (Supplementary Figure S1). No significant correlations were identified between NPSI scores and either serum creatinine or creatine kinase levels in any GBS subgroup (all $p > 0.05$). Correlation coefficients were generally weak and inconsistent across recovery stages.

In contrast, a significant inverse correlation between serum creatinine levels and neuropathic pain severity was observed in the SCI control group (Spearman's $\rho = -0.806$, two-tailed $p = 0.007$; $n = 10$), indicating that lower creatinine concentrations were associated with higher NPSI scores in this cohort. No significant association was detected between NPSI scores and creatine kinase levels in SCI controls. In GBS, the moderate but nonsignificant positive correlation observed between serum creatinine and overall pain intensity at T1 was not reproduced for neuropathic pain-specific symptoms. This pattern tentatively suggests that any relationship between serum creatinine and pain during the nadir phase of GBS may be more closely related to overall pain intensity than to neuropathic pain symptomatology; however, the exploratory nature and limited sample size of these analyses preclude firm conclusions.

We additionally explored potential associations between pain-related outcomes and circulating inflammatory and antioxidant-related biomarkers. No consistent correlations were identified between albumin, total bilirubin, or C-reactive protein levels and either pain intensity (BPI) or neuropathic pain severity (NPSI) across GBS recovery stages (Supplementary Figures S2–S3).

Discussion

Main findings

The present study provides a longitudinal characterization of circulating biochemical markers and pain-related outcomes during recovery from the nadir phase of GBS. Three principal findings emerged. First, several inflammatory and metabolic biomarkers exhibited stage-dependent changes throughout recovery. Second, pain intensity, pain interference, and neuropathic pain symptoms improved following the nadir phase. Third, serum creatinine showed a moderate positive correlation with pain intensity at GBS-T1, but the estimate did not reach statistical significance and was not reproduced at later stages; it should therefore be regarded as an exploratory signal.

Clinical relevance of inflammatory and metabolic biomarkers in GBS recovery

C-reactive protein (CRP) showed one of the largest global differences among the study groups, with higher median concentrations during the nadir phase and lower concentrations during subsequent recovery. This observation is consistent with previous studies reporting increased CRP levels in patients with Guillain–Barré syndrome and supporting its association with disease activity and clinical severity [11]. Elevated CRP concentrations have been linked to higher disability scores, cranio-bulbar involvement, need for mechanical ventilation, and more severe electrophysiological abnormalities, suggesting that systemic inflammatory activation contributes to disease burden [16]. More recently, composite inflammatory indices incorporating CRP, such as the CRP-to-albumin ratio, have also been proposed as clinically relevant biomarkers associated with disease severity and prognosis in GBS [17]. Although CRP is a non-specific acute-phase reactant and cannot be considered a disease-specific marker, accumulating evidence from neurological autoimmune disorders supports its value as an accessible biomarker reflecting systemic inflammatory activity and disease burden [18]. In agreement with these reports, the marked elevation of CRP observed during the nadir phase in

our cohort likely reflects the heightened inflammatory state associated with peak disease severity and its subsequent attenuation during functional recovery.

IL-6 also displayed significant overall differences across study groups, although post hoc comparisons were less consistent and substantial interindividual variability was observed. As a central mediator of the acute-phase response and a major inducer of hepatic C-reactive protein synthesis, IL-6 is closely linked to the inflammatory cascade underlying autoimmune and neuroinflammatory disorders[7]. Previous studies have reported increased IL-6 concentrations during the acute phase of inflammatory neuropathies, including Guillain–Barré syndrome and COVID-19-associated neuropathic syndromes, with levels tending to decline during clinical recovery [19]. Elevated IL-6 levels have also been associated with greater disease severity and enhanced systemic inflammatory responses in patients presenting inflammatory polyneuropathies [20]. In our cohort, however, the marked temporal fluctuations and limited consistency of post hoc comparisons suggest that IL-6 may be more sensitive to dynamic inflammatory changes than CRP, reducing its utility as a stable stage-specific biomarker. Consequently, our findings support a complementary rather than primary role for IL-6 in monitoring inflammatory activity during recovery from Guillain–Barré syndrome.

Overall, the CRP and IL-6 patterns were consistent with greater systemic inflammatory activity during severe disease and its attenuation during recovery, although the substantial variability and non-specificity of these markers limit their interpretation at the individual-patient level. Concurrent changes in albumin, bilirubin, CK, and creatinine may reflect broader shifts in nutritional, hepatic, redox, and neuromuscular status accompanying recovery.

Metabolic biomarkers during GBS recovery

Serum albumin levels also exhibited marked stage-dependent changes throughout GBS recovery, with the lowest concentrations observed during the nadir phase followed by progressive normalization during recovery. In addition to its role as a major plasma protein,

albumin is increasingly recognized as an integrative biomarker reflecting the interplay between systemic inflammation, nutritional status, and metabolic homeostasis. Previous studies have demonstrated that reduced serum albumin levels are associated with poorer functional outcomes and diminished response to intravenous immunoglobulin therapy in GBS patients, supporting its prognostic value in clinical practice [12,21] Similarly, albumin-based indices, including the Prognostic Nutritional Index and the CRP-to-albumin ratio, have been associated with disease severity, long-term outcomes, and inflammatory burden in GBS [17,22] Reduced albumin concentrations have also been reported in association with inflammatory hematological markers, further supporting its role as a surrogate indicator of systemic inflammatory activity [23]. In agreement with these observations, the recovery-associated increase in albumin levels observed in our cohort likely reflects the progressive restoration of physiological homeostasis accompanying clinical improvement. Recent evidence linking dynamic alterations in hepatic metabolism with disease outcomes in severe GBS further supports the concept that metabolic recovery represents an important component of the overall recovery process [24]. Given the central role of the liver in maintaining systemic metabolic equilibrium, these findings provide a framework for interpreting additional recovery-associated changes observed in circulating metabolites with antioxidant and cytoprotective functions, including bilirubin.

Bilirubin exhibited a recovery-associated pattern similar to that observed for albumin, with lower concentrations during the nadir phase and progressive normalization during recovery. Traditionally regarded as a marker of hepatic function, bilirubin is increasingly recognized as an endogenous antioxidant and cytoprotective molecule capable of modulating oxidative stress, inflammation, and immune responses. This biological role is particularly relevant in Guillain–Barré syndrome, where inflammatory and oxidative mechanisms contribute to peripheral nerve injury and disease progression. Consistent with our findings, Li et al. reported a significant association between lower serum bilirubin concentrations and greater disease severity in GBS

patients, suggesting that bilirubin depletion may accompany periods of heightened inflammatory burden and neurological dysfunction [13]. The gradual increase in bilirubin levels observed throughout recovery in our cohort may therefore reflect restoration of endogenous antioxidant defenses and attenuation of systemic inflammatory activity. Interestingly, several infectious conditions known to trigger GBS, including hepatitis E, hepatitis A, and dengue infection, have been associated with concomitant alterations in hepatic function and bilirubin metabolism, further highlighting the complex interplay between systemic immune activation, liver-associated metabolic pathways, and peripheral nerve injury. Together, these observations support bilirubin as a biologically plausible biomarker reflecting recovery-associated restoration of redox and metabolic homeostasis in GBS.

Creatine kinase and creatinine

Creatine kinase (CK) was included in our analysis as a surrogate marker of muscle injury and neuromuscular activity. Although GBS is primarily a peripheral nerve disorder rather than a primary myopathy, abnormalities in CK levels have been reported in subsets of GBS patients, particularly those with prominent motor involvement, axonal phenotypes, cramping pain, or prolonged muscle enzyme elevation [14,25,26]. More broadly, CK is widely used as a biomarker of muscle damage in a variety of neuromuscular disorders and may contribute to the differential diagnosis of conditions that clinically mimic GBS, including inflammatory, metabolic, and drug-induced myopathies [27–29]. In our cohort, CK activity increased from the nadir toward later recovery. This pattern may reflect a different temporal aspect of CK biology: profound weakness and immobility during the nadir may be followed by greater muscle activity and neuromuscular re-engagement during rehabilitation. CK was not correlated with pain intensity or neuropathic pain outcomes, suggesting that its recovery-associated change more likely reflects neuromuscular adaptation than a direct contribution to pain severity.

Serum creatinine also exhibited significant stage-dependent changes throughout GBS recovery, with the lowest concentrations observed during the nadir phase followed by a gradual increase during recovery. Although serum creatinine is routinely used as a marker of renal function, it is also influenced by skeletal muscle mass, energy metabolism, physical activity, and overall physiological status. Early observations described altered creatinine levels in patients with severe GBS, suggesting that profound systemic and neuromuscular disturbances accompanying the acute phase may influence creatinine metabolism [30]. The progressive increase in serum creatinine observed in our cohort may therefore reflect recovery-associated restoration of metabolic activity, improved neuromuscular function, and normalization of physiological homeostasis following the nadir phase. Creatinine was the only evaluated biomarker to show a moderate correlation estimate with pain intensity at GBS-T1; however, this association did not meet the prespecified significance threshold ($\rho = 0.534$, $p = 0.051$) and should not be interpreted as a validated pain biomarker.

Pain profiles during recovery

Pain represents one of the most frequent and clinically relevant non-motor manifestations of Guillain–Barré syndrome, contributing substantially to patient disability and impaired quality of life. Beyond the acute phase of the disease, pain may persist throughout recovery and has been recognized as a major determinant of long-term physical and psychosocial well-being in GBS patients [31]. Previous studies have highlighted pain, together with fatigue and emotional distress, as an important component of the overall disease burden, often remaining clinically relevant despite motor improvement [32]. Furthermore, prospective longitudinal studies have demonstrated that patient-reported outcomes continue to evolve during recovery, emphasizing the importance of monitoring symptoms beyond traditional neurological disability measures [33]. In this context, the longitudinal assessment of pain intensity, pain interference, and neuropathic pain symptoms may provide valuable insights into the patient experience of recovery and complement conventional biomarkers of disease progression.

Our findings demonstrate a progressive reduction in pain intensity, pain interference, and neuropathic pain symptoms from the nadir phase to later stages of recovery, supporting the notion that pain is a dynamic clinical manifestation that evolves alongside neurological improvement. To capture both the multidimensional impact of pain and its neuropathic characteristics, pain-related outcomes were assessed using the Brief Pain Inventory (BPI) and the Neuropathic Pain Symptom Inventory (NPSI), two validated instruments widely employed for the evaluation of pain severity, functional interference, and neuropathic symptom profiles [9,10] Previous studies have shown that pain is highly prevalent during the acute phase of Guillain–Barré syndrome and may precede the onset of weakness, persist during disease progression, and remain clinically relevant even after substantial motor recovery [34]. Importantly, persistent pain has been recognized as a major contributor to reduced quality of life and long-term disease burden in GBS survivors, together with fatigue and psychological symptoms [32,33]. Patient-reported experiences further emphasize that pain constitutes a prominent component of the early disease experience and may substantially influence physical and emotional well-being during recovery [35]. Experimental studies have also suggested that inflammatory processes affecting peripheral nerves can directly contribute to pain hypersensitivity, supporting a biological link between immune activation and pain generation during the acute phase of disease [36]. In agreement with these observations, the marked reduction in pain-related outcomes observed in our cohort likely reflects the progressive resolution of inflammatory and neurobiological processes accompanying recovery from the nadir phase of GBS.

Exploratory relationship between serum creatinine and pain during the nadir phase of GBS

Pain is a common and clinically relevant manifestation of Guillain–Barré syndrome, frequently emerging during the severe phase of the disease and contributing substantially to patient disability and reduced quality of life [37]. Given its importance as a patient-centred outcome,

we examined the longitudinal evolution of pain-related measures across recovery stages and explored their relationship with selected serum biochemical markers. Although the association between creatinine and pain intensity at GBS-T1 did not reach statistical significance, its moderate effect size may warrant further investigation in larger cohorts. This exploratory observation should be interpreted cautiously because of the limited sample size and the potential influence of demographic, renal, nutritional, and muscle-related factors.

Although serum creatinine is traditionally regarded as a marker of renal function, its concentration is also influenced by skeletal muscle metabolism, nutritional status, physical activity, and overall physiological reserve. The biological basis underlying this phase-specific association remains uncertain. One possible explanation is that creatinine levels during the nadir phase may capture acute changes in neuromuscular function, reduced mobility, altered metabolic homeostasis, and systemic physiological stress accompanying severe disease. In this context, serum creatinine may act as an indirect marker of the systemic consequences of acute neurological dysfunction rather than a direct marker of pain mechanisms. Given the limited literature examining creatinine–pain relationships in GBS, these findings should be considered exploratory and warrant confirmation in larger longitudinal cohorts.

Clinical implications and future directions

The findings of this study have several potential clinical implications. First, the identification of distinct biomarker trajectories across recovery stages supports the concept that recovery from GBS involves coordinated changes in inflammatory, metabolic, and homeostatic pathways that extend beyond neurological improvement alone. Because these biomarkers are routinely available, their stage-associated variation provides a basis for future studies evaluating their potential value for monitoring recovery; the present study did not assess their performance for individual patient monitoring. Second, the observed reduction in pain intensity, pain interference, and neuropathic pain symptoms highlights the importance of incorporating

patient-reported outcomes into the longitudinal evaluation of GBS, as these measures capture clinically meaningful aspects of recovery that are not fully reflected by traditional neurological assessments. Finally, the moderate but non-significant creatinine–pain correlation observed at the nadir phase should be regarded as a hypothesis-generating observation and does not establish clinical utility. Future studies involving larger multicentre cohorts and extended follow-up periods are warranted to validate these observations, establish their prognostic significance, and determine whether integrated biomarker panels can improve individualized monitoring and management of patients recovering from GBS.

Limitations

Limitations should be considered when interpreting the findings of this study. First, the relatively modest sample size may have limited statistical power to detect more subtle associations between biochemical markers and clinical outcomes, particularly for biomarkers exhibiting substantial interindividual variability. Second, although the longitudinal design allowed assessment of recovery-associated changes across predefined stages, the observational nature of the study precludes conclusions regarding causal relationships between biomarker dynamics and clinical recovery. Third, because stage-based comparisons were performed using Kruskal–Wallis tests, the analyses did not explicitly account for within-participant correlations across repeated recovery stages. Accordingly, the findings should be interpreted as stage-associated group differences rather than within-patient longitudinal effects. Fourth, several biomarkers analysed in this study are influenced by factors beyond GBS-related pathophysiology, including nutritional status, physical activity, muscle mass, sex, age, and comorbid conditions, which may have contributed to the observed variability. Therefore, the exploratory correlation analyses were not adjusted for sex, age, body composition, or muscle mass, all of which may influence circulating creatinine concentrations. Fifth, pain was assessed using validated patient-reported outcome measures; however, pain perception is inherently subjective and may be influenced by psychological, behavioural, and environmental factors not specifically evaluated in this study.

Sixth, information on analgesic treatment at the time of pain assessment was not systematically available, and its potential influence on the reported pain outcomes could therefore not be evaluated. Finally, the association observed between serum creatinine and pain during the nadir phase should be considered exploratory, given the limited literature addressing this relationship in GBS and the absence of external validation. Consequently, these findings require confirmation in larger multicentre cohorts with extended follow-up and integration of additional clinical, electrophysiological, and molecular measures.

Conclusions

This study demonstrates that recovery from the nadir phase of GBS was accompanied by stage-associated changes in routine serum biochemical parameters and by improvement in pain-related outcomes. CRP and IL-6 decreased across recovery stages, whereas albumin, bilirubin, creatinine, and creatine kinase showed increasing patterns. Most of the selected biomarkers were not significantly correlated with pain-related outcomes. At GBS-T1, serum creatinine showed a moderate but non-significant correlation with pain intensity, which should be regarded as an unconfirmed exploratory signal rather than evidence of a pain biomarker. These findings provide a descriptive biochemical profile of GBS recovery that requires confirmation in larger prospective cohorts using repeated-measures analyses.

List of abbreviations

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BPI: Brief Pain Inventory; CI: Confidence interval; CK: Creatine kinase; CRP: C-reactive protein; GBS: Guillain–Barré syndrome; GBS-T1: GBS at peak disease severity/nadir phase; GBS-T2: GBS at onset of functional recovery; GBS-T3: GBS at later recovery/discharge stage; GGT: Gamma-glutamyl transferase; IL-6: Interleukin-6; IQR: Interquartile range; NPSI: Neuropathic Pain Symptom Inventory; SCI: Spinal cord injury; TOSGB: Toledo Guillain–Barré Syndrome Biobank Collection

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki and the applicable institutional and regional ethical requirements. The study protocol was approved by the Clinical Research Commission of the Hospital Nacional de Parapléjicos (approval No. 2022/02; 20 June 2022) and the Toledo Clinical Research Ethics Committee (approval No. 17; 27 January 2014). The establishment and operation of the Toledo Guillain–Barré Syndrome (TOSGB) Biobank Collection were authorized by the Toledo Research Commission (approval No. 191; 12 February 2018) and the Regional Ministry of Health of the Government of Castilla-La Mancha (Resolution No. 241/2018; file No. 4500038/0004042; 29 November 2018). Written informed consent was obtained from all participants before enrolment and sample collection.

Consent for publication

Not applicable

Availability of data and materials

The biological samples and associated clinical data used in this study were coded (pseudonymized) and handled in accordance with the applicable ethical, confidentiality, and data-protection requirements. The individual-level datasets generated and/or analysed during the current study are not publicly available because they contain protected clinical information. De-identified data may be obtained from the corresponding author upon reasonable request, subject to approval by the relevant institutional bodies and compliance with the applicable ethical and data-protection requirements. Summary data supporting the findings are included in the article and its supplementary information. Access to biological materials held in the TOSGB

Biobank Collection is governed by the collection's established access procedures and corresponding ethical authorizations.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: ED-P and JdlF; Methodology: MZ-L, MSdR, CF-A, RD, and ED-P; Investigation: MZ-L, CF-A, RD, JB-G, JR-G, MIR-C, EV-B, JRT-T, and ED-P; Formal analysis: ED-P; Statistical consultation: LR-R and JM; Visualization: ED-P and MZ-L; Resources: MZ-L, CF-A, AB, MdCM-T, JB-G, JR-G, MIR-C, EV-B, JRT-T, JP-S, CM-A, MM-S and ED-P; Funding acquisition: ED-P; Project administration: ED-P; Supervision: MZ-L, CF-A, and ED-P; Writing – original draft: ED-P; Writing – review and editing: all authors. LR-R and JM contributed to data analysis and interpretation; JdlF, MV and AI contributed to data interpretation and critical revision of the manuscript. All authors read and approved the final manuscript.

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Supplementary information

Additional file 1: Supplementary information (DOCX). Contains Tables S1–S2 and Supplementary Figures S0–S3, including complete statistical comparisons, pain-related outcomes, GBS subtype distribution, and exploratory biomarker–pain correlation analyses.

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