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Severity-Dependent Neuroaxonal Damage Assessed by Serum Neurofilaments in ICANS Patients Undergoing CD19-Targeted CAR T-Cell Therapy

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ABSTRACT

Background and Objectives: Immune effector cell-associated neurotoxicity syndrome (ICANS) is a potential complication following Chimeric Antigen Receptor (CAR) T-cell infusion. Biomarkers to aid in early diagnosis and severity assessment are lacking. We aim to describe and compare serum neurofilament light chain (sNfL) dynamics in non-Hodgkin lymphoma (NHL) patients undergoing anti-CD19 CAR T-cell therapy, based on ICANS presence and severity.

Methods: This is a case-control study nested within a cohort of NHL patients treated with anti-CD19 CAR T-cells at a tertiary care center. From this cohort, we selected those who developed ICANS and had available blood samples. These patients were compared to matched NHL patients without ICANS from the same cohort. sNfL concentrations were measured immediately pre-infusion and on days 7 and 14 post-infusion, with z-scores calculated against a normative database. Mixed linear and ROC analysis assessed sNfL dynamics by ICANS presence and severity.

Results: Of 159 patients treated, 54 (34%) developed ICANS. We included 32 patients with ICANS and 22 matched controls. Baseline sNfL concentrations were similarly elevated in both ICANS and non-ICANS patients. However, on day 7, patients with moderate-severe ICANS (grade ≥ 2) had higher sNfL levels (median z-score 2.33) than those with mild or no ICANS (median z-score 1.72; $p=0.022$). The optimal cutoff to discriminate moderate-severe ICANS from other patients based on sNfL was a z-score of 2.14 on day 7 ($p=0.004$).

Discussion: Moderate-severe ICANS is associated with elevated sNfL levels by day 7 post-infusion, indicating early neuroaxonal damage and underscoring sNfL as a valuable biomarker for assessing ICANS severity.

1 | Introduction

Chimeric Antigen Receptor (CAR) T-cells have revolutionized the treatment landscape of patients with relapsed or refractory hematological diseases [1]. They are engineered by

reprogramming a patient's own T cells with a CAR construct targeting specific antigens, which exerts anti-tumor effects without major histocompatibility complex restriction [2]. Currently, commercial CAR T-cell products available for lymphoma are CD19-targeted [3–9].

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Despite this paradigm shift, two potential life-threatening complications can occur after the CAR T-cell infusion [10]. Cytokine release syndrome (CRS) is a systemic inflammatory response resulting from the overactivation of host bystander effector cells, especially macrophages, triggered by CAR T-cells, which takes place in 42–100% of infused patients [11, 12]. On the other hand, immune effector cell-associated neurotoxicity syndrome (ICANS) is a closely related syndrome consisting of the manifestation of neurologic symptoms due to blood–brain barrier dysfunction [11, 13] with a reported incidence ranging from 21 to 64% with CD19-targeted CAR T-cells [3, 5, 14]. It typically manifests as a fluctuating encephalopathic syndrome, usually accompanied by aphasia or tremor [15]. The diagnostic workup is often normal or exhibits non-specific abnormalities such as general or focal abnormalities in electroencephalographic studies and, rarely, patchy multifocal T2-lesions, multifocal leptomeningeal enhancement, or cerebral edema on the brain magnetic resonance imaging (MRI) [16–18]. Depending on the clinical severity and the type of abnormalities in the diagnostic work-up, a score from 1 to 5 is assigned, according to the international consensus grading [19].

Since the diagnosis of ICANS is based on clinical findings, quantitative biomarkers contributing to ICANS diagnosis or establishing its severity are warranted. Utilizing serum neurofilament light chain (sNfL) as a surrogate marker may offer valuable insights into the extent of neuronal damage associated with ICANS [16, 20]. Neuroaxonal damage has been implicated in ICANS [21–24], though findings on neurofilament light chain (NfL) level increases remain contradictory [20]. In pediatric patients treated with anti-CD19 CAR T-cells for acute lymphoblastic leukemia, elevated cerebrospinal fluid (CSF) NfL levels were observed in those developing ICANS [21]. In contrast, adult patients with high-grade lymphoma showed higher baseline sNfL levels, even before lymphodepleting chemotherapy [25], in those who developed ICANS [21–25], suggesting that preexisting neuroaxonal vulnerability may play a greater role than the acute insult itself [20]. Nonetheless, the dynamic concentration of sNfL after anti-CD19 CAR T-cell treatment has not been thoroughly explored.

In this context, our goal was to describe and compare the dynamic concentration of serum sNfL after anti-CD19 CAR T-cell therapy in patients with non-Hodgkin lymphoma, based on the presence of ICANS and its severity.

2 | Methodology

2.1 | Study Design and Participants

From a cohort comprising all adult patients who received anti-CD19 CAR T-cell therapy at Vall d'Hebron University Hospital for hematological malignancies from July 2018 until May 2022, we selected patients with a diagnosis of non-Hodgkin lymphoma (NHL) who developed ICANS with stored serum samples available, and a control group of NHL patients who received CAR T-cells but did not develop ICANS from the same cohort [13], matched for age and sex through frequency matching. Exclusion criteria encompassed patients with prior central nervous system

(CNS) disease involvement and ICANS onset beyond 14 days after CAR T-cell infusion.

2.2 | Patient Evaluation and Follow-Up

We collected demographic characteristics, including age, sex, body mass index (BMI), underlying hematological disease and staging, number of previous therapies, need for bridging therapy, type of CAR T-cell, baseline neurological examination, and functional status. Moreover, blood samples for sNfL concentration were collected the day before CAR T-cell administration (after lymphodepleting therapy consisting on fludarabine and cyclophosphamide), on day 7, and 14 after infusion.

All patients were evaluated at baseline by a neurologist and hematologist, prior to the start of lymphodepleting therapy. Patients presenting signs or symptoms of ICANS after infusion were re-evaluated by neurology and graded according to the American Society for Transplantation and Cellular Therapy (ASTCT) Consensus Guidelines [19]. ICANS was classified as moderate–severe when the grading scale was 2 or higher (ICANS grade ≥ 2). Following the development of ICANS, various diagnostic tests were performed (CT, blood tests, brain MRI, CSF study, and electroencephalogram) as per clinical practice. Results were collected along with details of ICANS treatment, duration, symptoms, and follow-up. Functional outcomes were assessed using the modified Rankin Scale (mRS) at the final follow-up [26]. Functional independence was defined as mRS 0–1.

2.3 | Biomarkers Measures

Blood was collected by standard venipuncture and allowed to clot spontaneously for 30 min. Serum was obtained by centrifugation and stored frozen at -80°C until used. We analyzed sNfL levels in previously frozen serum samples at baseline (day before treatment, prior to CAR T-cell infusion), on day 7, and 14 after CAR T-cell infusion. sNfL levels were determined using a commercially available single-molecule array kit (Quanterix, cat#103186) run on the fully automated ultrasensitive Simoa HD-1 Analyzer (Quanterix). Samples were run in duplicate in accordance with manufacturers' instructions, with appropriate standards and internal controls assay (SIMOA). z-scores were calculated by comparing the absolute sNfL concentrations to a normative database of healthy controls, adjusting for age and BMI [27].

2.4 | Statistical Analysis

We calculated the required sample size to detect differences in mean NFLs concentration depending on the presence of ICANS. We utilized a type I error rate (α) of 0.05 and a power ($1-\beta$) of 0.8. To determine the sample size, we utilized the means and standard deviations of sNfL in patients with ICANS and those without ICANS from previous studies [24].

Descriptive analysis of the entire study sample and categorized by the presence or absence of ICANS was performed. Central tendency measures (mean or medians) and dispersion measures

(standard deviations, interquartile ranges, range intervals or ranges) were utilized for quantitative variables, while counts and frequencies were employed for qualitative variables. Differences between groups were assessed using the T student and Chi-square tests for quantitative and qualitative variables, respectively. Non-parametric tests, such as the Mann–Whitney U test, were used when normal distribution could not be assumed, instead of the Student's t-test. We also conducted logistic regression to investigate variables posing a higher risk for ICANS. In our multivariable model, we included all variables with a significance level greater than 0.1 from the univariate analysis.

Furthermore, we performed a mixed linear analysis, incorporating repeated measurements of sNfL and comparing concentration dynamics across different time points among the various groups. For the analysis, outcomes were grouped based on the presence of any grade of ICANS or the presence of moderate–severe ICANS (grade 0–1 vs. grade ≥ 2), as previously reported [25].

We also examined the concentration of sNfL z-scores at each specific timepoint and evaluated their association with the presence of ICANS, the severity of ICANS (grade 0–1 vs. grade ≥ 2), the relapse of ICANS, ICANS symptoms, and functional outcome (defining a poor functional status as mRS ≥ 2) through logistic regression.

We then used Receiver Operating Characteristic (ROC) to explore whether there was a z-score sNfL cutoff point that would allow us to correctly classify the presence of ICANS and ICANS

grade ≥ 2 . Subsequently, we analyzed the association between sNfL above the cutoff and the presence of the outcome.

2.5 | Ethical Issues

This study was approved by the Ethics Committee of Vall d'Hebron Research Institute (PR[AG]404/2020). An informed consent was obtained from all patients.

3 | Results

From a cohort of 159 patients treated with anti-CD19 CAR T-cell therapy for high-grade B-cell lymphoma between July 2018 and May 2022, 54 patients (34%) developed ICANS. Of these, 32 had stored serum samples available and were included in the ICANS group for analysis. Following the sample size calculation, a minimum of 22 patients in each group was recommended. Therefore, 22 age- and sex-matched patients without ICANS, who also had stored serum samples available, were selected from the same cohort to serve as controls (Figure 1).

3.1 | Clinical Characteristics and Risk Factors for ICANS

At baseline, there were no differences regarding age (mean 55.7 years, SD 11.6) and gender (22 out of 54 (40.7%) were

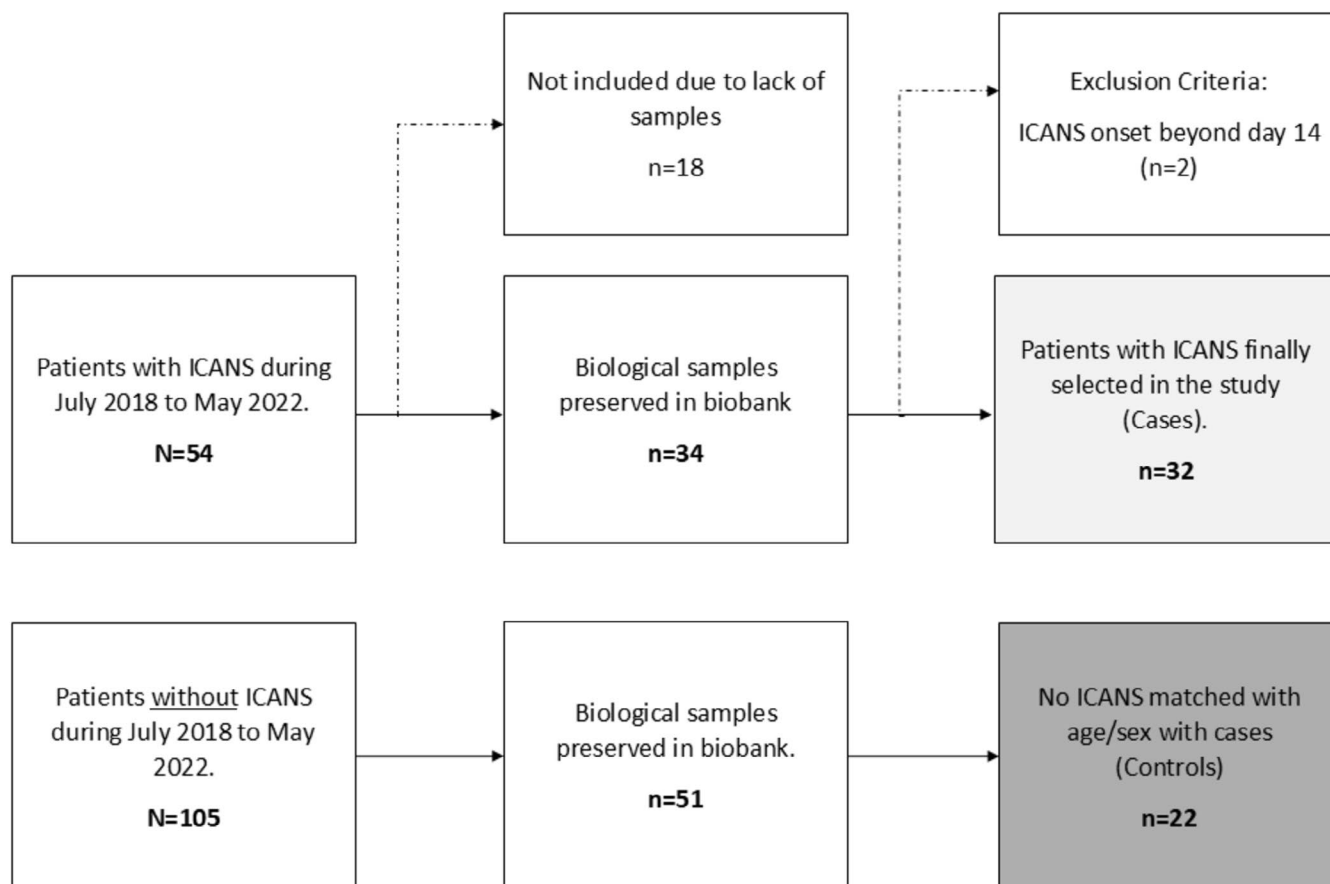


FIGURE 1 | Flowchart of patients with high-grade B-cell lymphoma treated with anti-CD19 CAR T-cell therapy included in the sNfL study.

women) depending on the presence of ICANS. In terms of previous treatments, 41 out of 54 (75.9%) received bridging chemotherapeutic treatment before CAR-T therapy. The use of bridging therapy prior to CAR-T treatment was not associated with either the occurrence ($p=0.851$) or the

severity of ICANS ($p=0.540$). Other variables are shown in Table 1.

Median time of onset for ICANS was 6 days (IQR 3) following the administration of CAR T-cells (Table S1). All patients

TABLE 1 | Baseline characteristics of ICANS (cases), no ICANS (controls), and all Subjects, with corresponding p -values for comparisons.

Variable	Total ($n=54$)	ICANS ($n=32$)	No ICANS ($n=22$)	Odds ratio (95% CI)
Age, years—mean (SD)	55.7 (11.6)	54.9 (11.9)	56.7 (11.4)	1.7 (−4.8–8.2)*
Sex (female)— n (%)	22 (40.7)	12 (37.5)	10 (45.5)	1.0 (0.9–1.1)
Body Mass Index, Kg/m ² —mean (SD)	26.0 (5.9)	25.7 (5.3)	26.3 (6.7)	1.0 (0.9–1.1)
Abnormal baseline neurological examination— n (%)	22 (40.7)	12 (37.5)	10 (45.5)	0.1 (0.1–1.3)
Length-dependent Polyneuropathy	20 (37.0)	11 (34.4)	9 (40.9)	
Non-long-dependent Polyneuropathy	1 (1.9)	1 (3.1)	0 (0)	
Visual Disturbance	2 (3.7)	1 (3.1)	1 (4.5)	
Action Tremor	8 (14.8)	5 (15.6)	3 (13.6)	
mRS > 1— n (%)	4/56 (7.1)	4/32 (12.5)	0 (0.0)	1.4 (0.6–3.2)
Hematological disease				
Diffuse large B-cell lymphoma— n (%)	44 (81.5)	23 (71.9)	21 (95.5)	0.4 (0.1–1.5)
Mantle-cell lymphoma—(%)	3 (5.6)	3 (9.4)	0 (0)	—
Other High-grade B-cell lymphoma— n (%)	7 (11.1)	6 (18.8)	1 (4.5)	—
Stage III or IV Ann-Arbor— n (%)	37 (68.5)	23 (71.9)	14 (63.6)	1.5 (0.5–4.7)
Hematopoietic stem cell transplantation— n (%)	15 (27.8)	7 (21.9)	8 (36.7)	0.5 (0.2–1.6)
Days lymphodepletion before CART—mean (SD)	6.3 (2.1)	6.6 (3.2)	5.9 (1.2)	1.2 (0.7–1.5)
Bridging Therapy— n (%)	41 (75.9)	24 (75.0)	17 (77.3)	1.2 (0.4–4.1)
CAR T-cell				
Axicabtagene ciloleucel	28 (51.9)	22 (68.8)	6 (27.3)	6.6 (2.0–21.7)
Tisagenlecleucel	16 (29.6)	5 (15.6)	11 (50.0)	0.2 (0.1–0.6)
Lisocabtagene marleucel	6 (11.1)	2 (6.3)	4 (18.2)	0.3 (0.0–1.4)
Clinical Trial	4 (7.4)	3 (9.4)	1 (4.6)	2.4 (0.2–24.4)
CRS— n (%)	44 (81.5)	29 (90.6)	15 (68.2)	4.8 (1.1–20.8)
CRS Grade 2 or more— n (%)	18 (33.3)	14 (43.8)	4 (18.2)	3.0 (1.0–10.0)
Time to CRS, days—median (IQR)	1 (3)	1 (2)	1 (1.5)	1.0 (0.9–1.1)
High ⁺ Ferritin pre CART— n (%)	34 (63.0)	12 (52.2)	13 (59.1)	0.4 (0.1–1.6)
High ⁺ LDH pre CART— n (%)	25 (61.0)	19 (59.4)	15 (68.2)	0.9 (0.3–2.6)
High ⁺ CRP pre CART— n (%)	40 (74.1)	23 (71.9)	17 (77.3)	0.8 (0.2–2.7)
Platelet count—mean (SD)	171.1 (101.0)	167.7 (108.0)	175.5 (92.9)	1.1 (0.9–1.3)
EASIX [^] —median (IQR)	4.1 (15.0)	3.2 (16.9)	4.3 (11.2)	1.0 (1.0–1.0)

*Mean differences in quantitative values.

⁺High denotes elevated above the bounds of normal limits for the general population.

[^]Endothelial Activation and Stress Index (EASIX) includes the formula lactate dehydrogenase (U/L)×creatinine (mg/dL) / thrombocytes (10⁹ cells per L).

fulfilled the diagnostic criteria for ICANS, which requires the presence of encephalopathy, regardless of severity [19]. In addition, 24 patients (75.0%) had an action tremor, 16 patients (50%) had language impairment, five patients (16.5%) had a decreased level of consciousness, and only one patient (3.1%) had signs of pyramidal tract damage. Brain MRI was conducted in 20 patients (62.5%), revealing abnormalities in 7 patients (35.0%), five out of seven (71.4%) with probable inflammatory hyperintense T2-lesions (four patients with subcortical lesions, two with cortical lesions, and one with a single lesion in the vermis). Based on ICANS severity, 17 patients (53.1%) were identified as having mild ICANS (grade 1), and 15 patients (46.9%) as having moderate-severe ICANS (grade ≥ 2). Most patients required treatment (25, 78.1%), all with corticosteroids as first-line therapy; anakinra was added in 3 patients (9.4%). An ICANS recurrence occurred in seven patients (21.9%) during steroid tapering, after a median time of 8 days after initial ICANS onset. Additional variables collected during ICANS are detailed in Table S1.

In terms of the risk of developing ICANS, only the administration of axicabtagene ciloleucel (axi-cel) (OR 6.6; 95% CI 2.0–21.7) and the occurrence of CRS (OR 4.8; 95% CI 1.1–20.8) were significantly associated with an increased risk of ICANS (Table 1). However, following a multivariable logistic regression analysis, including the former variables, only the use of axi-cel retained statistical significance (OR 5.8, 95% CI 1.7–19.6). The influence of CRS as a risk factor for ICANS was higher in patients receiving other constructs, in comparison with the influence it had for axi-cel recipients (OR of 5.7 [95% CI 0.6–55.6] in patients treated with axi-cel vs. 2.0 [95% CI 0.1–22.7] in those not treated with it).

3.2 | Serum Neurofilament Light Chain (sNfL) Measurements and Associations

Baseline concentrations of sNfL were significantly elevated in both groups of patients with and without ICANS (ICANS: median z -score 2.23, range interval—2.0–3.4, $p=0.025$; No-ICANS: median z -score 2.14, range interval 0.3–3.4, $p=0.032$, respectively), without differences between them ($p=0.717$). The elevated baseline concentrations of sNfL were associated with the bridging chemotherapeutic treatment that the majority of patients (41 out of 54; 75.9%) received before CAR-T therapy. Patients who underwent bridging therapy had a significantly higher median z -score (2.29) compared to those who did not (median z -score: 1.22; $p<0.001$).

Patients with ICANS grade 3 or 4 had higher baseline NfL levels (median z -score 2.56, range interval 1.6–3.4) compared to those with less severe ICANS (Grade 1 or 2) (median z -score 2.04, range interval—2.0–3.4), although the difference was not statistically significant ($p=0.138$). Overall, sNfL levels remained elevated, with a mean z -score of 1.50 compared to normalized data, in both groups until day 14 after CAR T-cell infusion, with a tendency to decrease at day 7 compared to pre-treatment. sNfL concentrations at various time points following CAR T-cell infusion are presented in Table 2.

When assessing patients who developed any grade of ICANS, we did not find a significant difference in sNfL dynamics compared

to patients who did not develop ICANS. In contrast, when assessing sNfL dynamics based on ICANS severity, a significantly higher increase of sNfL concentration at day 7 (the closest time point to the median onset of ICANS) was observed in patients presenting moderate-severe ICANS (grade ≥ 2) (median z -score 2.33, range interval—0.0–5.0) compared to patients without ICANS or with mild ICANS (grade 0–1) (median z -score 1.72, range interval—0.6–3.4) in (grade 0–1; $p=0.022$) (Figure 2). No differences in sNfL dynamics were observed based on the presence of CRS (Figure 2). To mitigate confounding bias, the mixed analysis confirmed no significant differences in sNfL dynamics based on the presence of CRS or the type of CAR T-cell therapy.

We determined the optimal cut-off value for sNfL z -score at different timepoints to classify patients depending on the presence of moderate-severe ICANS (grade ≥ 2) through ROC analysis (Figure 3). The best cut-off to classify patients with moderate-severe ICANS was 2.14 at day 7, significantly associated with ICANS grade ≥ 2 ($p=0.004$). This cut-off showed 0.73 sensitivity (95% CI 0.45–0.92) and 0.71 specificity (95% CI 0.53–0.84), 0.51 positive predictive value (PPV) (95% CI 0.37–0.65) and 0.86 negative predictive value (NPV) (95% CI 0.72–0.94) considering ICANS prevalence, with an AUC of 0.68 (accuracy 71.2%). Considering only those patients whose samples were collected prior to ICANS onset (15 out of 32 patients with ICANS), the same cut-off at day 7 (sNfL z -score 2.144) was significantly associated with moderate-severe ICANS ($p=0.01$), showing 0.83 sensitivity (95% CI 0.36–1.00) and 0.72 specificity (0.53–0.87), 0.56 PPV (95% CI 0.40–0.72) and 0.91 NPV (95% CI 0.63–0.98) considering ICANS prevalence with an AUC of 0.67 (accuracy 75.7%).

We investigated the concentration of sNfL and functional outcomes, assessed by the mRS, with a focus on functional independence (mRS 0–1) at the last follow-up visit. At baseline, 28 patients in the ICANS group (87.5%) and 22 patients (100%) in the non-ICANS group had an mRS of 0 or 1 (OR 1.4 (0.6–3.2)). Functional independence was achieved in 17 patients with ICANS (53.3%) after a median time of follow-up of 8.52 months (IQR 8.55). There was no significant association between functional outcome at the last follow-up visit and sNfL concentration at baseline (OR 1.0; 95% CI 0.5–1.9), day 7 (OR 1.2; 95% CI 0.7–2.3), or day 14 (OR 1.1; 95% CI 0.6–2.1). Furthermore, sNfL concentration at baseline, day 7, or day 14 did not predict recurrence of ICANS ($p=0.295$, $p=0.149$, and $p=0.346$, respectively).

4 | Discussion

In this study, we found that although baseline sNfL levels could not discriminate between patients who would develop ICANS and those who would not, a significant increase in sNfL concentration was observed on day 7 in patients with moderate-severe ICANS (grade ≥ 2). This finding suggests that, besides the pre-existing axonal damage in these patients with refractory high-grade B-cell lymphoma, acute neuroaxonal injury occurs after CAR T-cell infusion and around the time of ICANS onset.

Regarding risk factors for ICANS, patients who received axi-cel and/or developed any grade CRS had a higher risk of ICANS, as previously described [11, 16]. Other variables with a reported

TABLE 2 | sNfL concentration at baseline (day 0, prior to CAR T-cell infusion), day 7, and 14 after infusion in the full patient population, and according to the development of ICANS.

Variable	Total (n = 54)	Patients with ICANS (n = 32)	Patients without ICANS (n = 22)	p*	Patients ICANS Grade 0–1 (n = 39)	Patients ICANS Grade 2–4 (n = 15)	p*
NfLs at baseline (pg/mL)—median (range)	25.31 (7.7–135.8)	24.17 (8.3–105.2)	26.11 (7.7–135.8)	0.717	21.31 (7.7–135.8)	31.65 (8.3–105.2)	0.349
NfLs at baseline (z-scores)—median (range)	2.14 (–2.0–3.4)	2.23 (–2.0–3.4)	2.14 (0.3–3.4)		2.10 (–0.6–3.4)	2.35 (–2.0–3.4)	
NfLs at day 7 (pg/mL)—median (range)	20.40 (3.9–253.5)	21.30 (9.0–253.5)	19.34 (3.9–110.3)	0.632	17.91 (3.9–110.3)	38.97 (9.0–253.5)	0.045
NfLs at day 7 (z-scores)—median (range)	1.83 (–2.5–3.6)	2.04 (–1.7–3.6)	1.62 (–2.5–3.4)		1.73 (–2.5–3.4)	2.33 (–1.7–3.6)	
NfLs at day 14 (pg/mL)—median (range)	23.07 (9.0–281.8)	23.12 (9.2–281.8)	23.07 (9.0–122.2)	0.808	20.02 (9.0–122.2)	30.70 (9.9–281.8)	0.361
NfLs at day 14 (z-scores)—median (range)	2.14 (–1.5–3.8)	2.12 (–1.5–3.8)	2.14 (–0.5–3.4)		1.98 (–0.5–3.4)	2.33 (–1.5–3.8)	

Abbreviation: NfLs, Serum Neurofilaments light chain.
*P-values are calculated by comparing z-scores and using the Mann–Whitney U test.

association with ICANS [16], such as tumor burden [13], lactate dehydrogenase [28], ferritin [16], C reactive protein [16], platelet count [28], severe CRS [16], or a pre-existing neurologic condition [13] did not harbor a significant impact on the risk of ICANS in our study. This could have been related to the fact that our study was not adequately powered nor designed to evaluate risk factors for ICANS; however, identifying the increased risk of axi-cel and prior CRS development suggests that our cohort is representative of a patient population receiving anti-CD19 CAR T-cell therapy.

Focusing on the patients developing ICANS, the median time to onset was 6 days, consistent with previous studies [15, 29]. Noteworthy, the proportion of patients with ICANS grade 1 with the standard therapeutic protocol was higher than other reports, potentially reflecting the acquired expertise of treating hematologists and neurologists to identify very early signs of ICANS and early treatment in certain high-risk patients. This different distribution in ICANS grade/severity rates could also explain the absence of associations with other previously published risk factors, beyond CRS and axi-cel use [16, 29].

For the sNfL analysis, we took into account baseline levels, concentration at different evaluated time points, and the dynamics

throughout the study period. We used normalized z-score values for sNfL analysis to enable comparisons between patients, accounting for factors such as age and body mass, both of which can influence sNfL concentrations [27]. Prior to CAR T-cell infusion, both ICANS and non-ICANS exhibited a significantly higher concentration of sNfL z-scores in comparison to the reference population. Although there was a tendency for higher baseline sNfL z-scores in patients who will develop a more severe ICANS, the baseline sNfL concentrations were similarly elevated in both ICANS and non-ICANS. These results differ from previous studies, which reported significantly higher baseline sNfL concentrations in patients who developed ICANS compared to those who did not [24]. There is evidence suggesting a relationship between elevated baseline sNfL concentrations and prior chemotherapy [1, 30] and we found in this cohort an association between the administration of bridging therapy prior to CAR-T treatment and significantly higher baseline sNfL levels. The limited sample size and different timing from chemotherapy to CAR-T [25] may have masked the ability of baseline sNfL concentrations to predict the risk and severity of ICANS.

When examining the dynamics of sNfL concentration based on ICANS severity, we identified a significant difference at day 7 after infusion between patients with moderate–severe ICANS

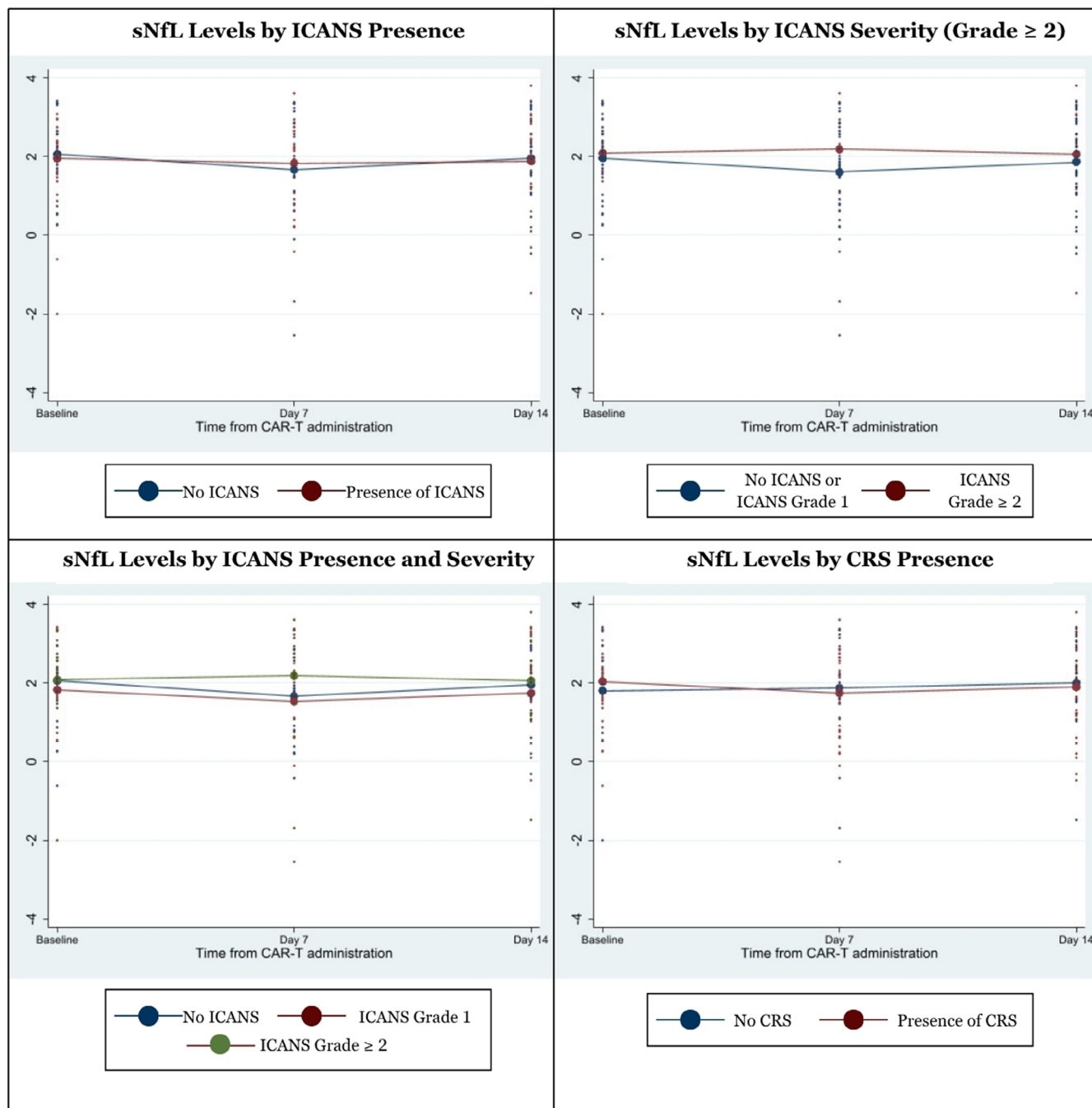


FIGURE 2 | sNfL Dynamics Over Time by the presence of ICANS (A) and ICANS Severity (grade ≥ 2) (B) After CAR T-Cell Therapy (B) using mixed linear regression analysis, including mean sNfL (line) and all patients individually (dots). In (A), sNfL show no significant increment at any timepoint ($p=0.119$ at day 7, 0.430 at day 14) compared to baseline levels. In (B), a significant increase is observed at day 7 ($p=0.022$) compared to baseline level, while no significance is found at day 14 ($p=0.362$). Additionally, panel (C) presents sNfL concentrations based on ICANS severity, with patients separated into those with no ICANS, ICANS grade 1, and higher-grade ICANS. (D) The presence of CRS does not influence the observed curve dynamics in patients with ICANS ($p=0.062$ at day 7, 0.285 at day 14).

and those with grade 1 or non-ICANS. Notably, day 7 represents the median time from CAR T-cell administration to ICANS onset, making it a key time point for analysis. Other studies have evaluated sNfL concentration over time, yielding contradictory results as they did not find an increase in sNfL levels in the acute phase [22]. However, these studies only classified groups based on the presence of ICANS, and it is possible that an increase in sNfL levels occurs primarily in more severe cases. In this

context, a pediatric study found that the concentration of NfL in the CSF increased during ICANS, suggesting that only those patients with significant symptoms who required a lumbar puncture will increase NfL [21]. Adding biological plausibility to our findings, sNfL concentrations have been observed to be elevated during the acute phase in patients with delirium, a condition with a pathophysiology similar to ICANS [13], compared to those without delirium [31]. Our study, unveiling previously

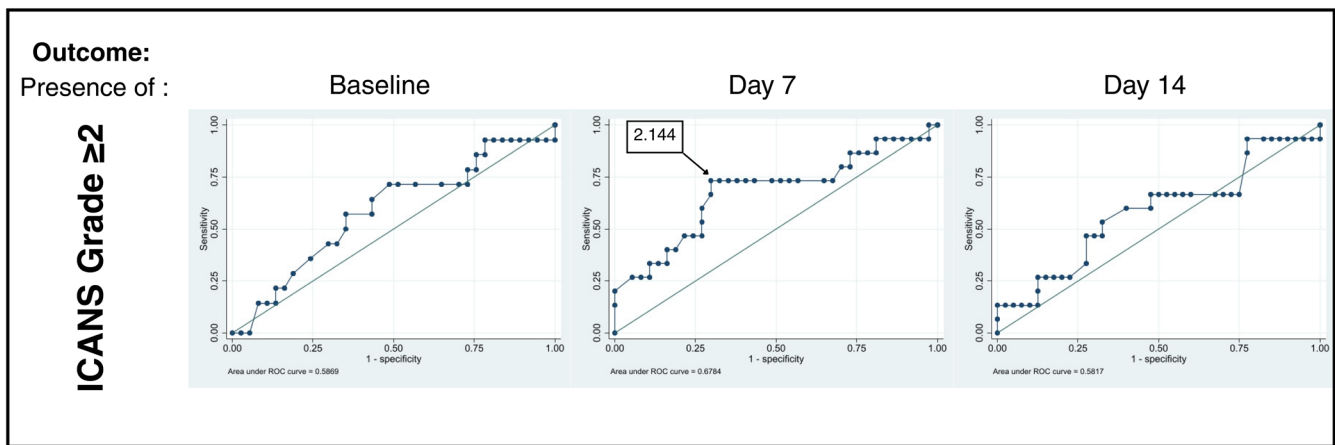


FIGURE 3 | Different ROC curves using sNfL at each timepoint as predictors of moderate–severe ICANS (grade ≥ 2 according to the American Society for Transplantation and Cellular Therapy (ASTCT) Consensus Guidelines). ROC, Receiver Operating Characteristic (ROC) study; sNfL, Serum Neurofilaments Light chain.

unreported findings, holds important potential implications. If confirmed, these results suggest that moderate–severe ICANS might be linked to neuroaxonal injury [20], suggesting a potential impact on neurological disability and/or long-term sequelae post-CART, especially if the damage persists or occurs in a vulnerable patient population [20]. We did not find associations with functional neurological outcomes (mRS) at 9 months, but it is known that this scale relies too much on motor dysfunction and is not sensitive enough to capture cognitive impairment. Even though most patients will recover with corticosteroids [32], a global decline in perceived cognition has been reported in CAR T-cell recipients within the first year after experiencing ICANS [33]. In that case, the identification of this injury in moderate or severe ICANS could leverage a window of opportunity for a prompt immunosuppressive intervention to mitigate neurological harm. Notably, these findings align with current guidelines, recommending rapid treatment initiation in patients with ICANS grade ≥ 2 , while advocating for a more conservative management approach in ICANS grade 1 [34].

Evidence indicates that prophylactic corticosteroids within the first 3 days post-infusion, along with early corticosteroid or tocilizumab intervention for mild CRS or ICANS, reduce severe events while preserving CAR T-cell efficacy [11, 35]. In order to select patients who might benefit from these preventive strategies, tools such as the Multivariable Predictive Score of Neurotoxicity [29] have been developed to identify patients at higher risk of ICANS before receiving the CAR T-cells, with modest accuracy. Measuring sNfL concentration could serve as a valuable predictor for ICANS grade ≥ 2 after CAR T-cell administration. ROC analysis identified an sNfL cutoff of 2.14, also measured prior to ICANS onset, as predictive of moderate–severe ICANS specifically. This threshold was significantly associated with the likelihood of moderate–severe ICANS in patients, even before clinical signs of ICANS appeared. However, considering the prevalence of ICANS, these results are more informative for predicting the absence of moderate–severe ICANS rather than the occurrence of moderate–severe ICANS, as evidenced by the higher NPV compared to the PPV. Despite its conceptual interest, the feasibility of using sNfLs for this purpose remains

limited due to the lack of routine sNfL testing in clinical practice at many centers and the unavailability of immediate results after testing. Further studies with larger sample sizes and earlier time points are necessary to confirm and enhance the area under the curve of the ROC analysis.

Several limitations of the study warrant acknowledgment. Firstly, as an observational study following a case–control design, most neurological variables were collected retrospectively; however, the main outcomes of the study (presence and grade of ICANS) were collected prospectively by trained hematologists and neurologists with ample experience in CAR T-cell therapy. Secondly, the sample size was determined to detect differences in sNfL concentration between patients with and without ICANS, potentially limiting the statistical power for analyses involving other outcomes, such as the grade of ICANS or mRS at last follow-up. However, the analysis of ICANS prevalence, features, and associated factors is in line with the available literature, suggesting that our cohort is representative of this patient population.

In conclusion, our study highlights that patients who develop ICANS grade 2 or higher present a significantly higher peak concentration on day 7 after CAR T-cell infusion, even before presenting symptoms, as opposed to patients with grade 1 or non-ICANS, indicating potential neuroaxonal injury in moderate–severe ICANS. These sNfL dynamics could potentially serve as a real-time biomarker, reflecting the biological impact of CAR T-cell therapy and guiding early treatment interventions.

Author Contributions

Andreu Vilaseca: conceptualization, investigation, writing – original draft, methodology, visualization, writing – review and editing, formal analysis, data curation. **Helena Ariño:** conceptualization, writing – review and editing, methodology, supervision. **Gloria Iacoboni:** data curation, conceptualization, investigation, writing – review and editing. **Samantha Feijóo:** writing – review and editing, data curation. **Ana Zabalza:** writing – review and editing, conceptualization, methodology. **Nicolás Fissolo:** data curation, formal analysis, software,

methodology, validation, writing – review and editing. **María Jesús Arévalo:** conceptualization, investigation, writing – review and editing. **Cecilia Carpio:** conceptualization, writing – review and editing, data curation. **Mario Sánchez:** conceptualization, writing – review and editing, data curation. **Mar Tintoré:** conceptualization, writing – review and editing, funding acquisition, methodology, investigation. **Manuel Comabella:** conceptualization, investigation, funding acquisition, writing – review and editing, methodology, resources. **Xavier Montalbán:** funding acquisition, writing – review and editing, supervision, resources, project administration. **Pere Barba:** funding acquisition, conceptualization, writing – review and editing, supervision. **Àngela Vidal-Jordana:** conceptualization, investigation, writing – review and editing, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** ene70305-sup-0001-Supinfo.docx.