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Research Article

Predictive Value of the Cytokine Kinetic Profiles for the Occurrence of Immune Effector Cell-Associated Neurotoxicity Syndrome

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Abstract

Immune effector cell-associated neurotoxicity syndrome is a major adverse event following CAR-T cell therapy. Peripheral blood cytokine levels are correlated with the severity of ICANS positively, while the relationship between the magnitude of changes and ICANS remains unclear. A retrospective analysis was performed on 120 pediatric relapsed/refractory B-cell acute lymphoblastic leukemia patients treated with CAR-T therapy. Cytokines levels (IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, IL-17) were measured daily for 14 days post-infusion. Neurological symptoms were assessed using the ICE score. 120 pediatric patients receiving CAR-T therapy were enrolled and 31 (25.8%) developed ICANS. CNS infiltration is risk factor for ICANS development ($P < 0.001$). No statistically significant differences were observed between groups for age, gender, chemotherapy outcome, or CAR-T cell dosage. CRS occurred in 91.7% of patients, whose severity demonstrated a significant correlation with ICANS severity ($P < 0.001$). Peak cytokine levels occurred dominantly on days 4 - 6 post-infusion. Within the three days preceding neurotoxicity onset, increased fluctuations of IL-2, IL-4, IL-6, IL-10 and TNF-α, were associated with ICANS occurrence ($P < 0.05$). Elevated GM, mean, and Min of IL-10 ($P < 0.001$), increased SD, Max, and mean of IL-2, IL-4, TNF-α ($P < 0.05$), and Max/Min of IL-6 ($P < 0.05$) were associated with ICANS. ROC curve analysis confirmed the predictive value. Mean IL-10 optimal predictive performance. Significant predictive value was also observed for Max of IL-6, GM of IL-10, mean and GM of TNF-α level. IL-6, IL-10 and TNF-α is possible biomarkers for ICANS prediction, whose monitoring may improve neurotoxicity management.

Keywords: *CAR-T Cell Therapy; ICANS; IL-6; IL-10; TNF-α; Dynamics Prediction*

Abbreviations

R/R ALL: Relapsed/Refractory Acute Lymphoblastic Leukemia; OS: Overall Survival; EFS: Event-Free Survival; CAR-T: Chimeric Antigen Receptor T Cell; CRS: Cytokines Release Syndrome; ICANS: Immune Effector Cell-Associated Neurotoxicity Syndrome; CNS: Central Nervous System; CSF: Cerebrospinal Fluid; ICE: Immune Effector Cell-Associated Encephalopathy; SD: Standard Deviation; CV: Coefficient of Variation; GM: Geometric Mean; MAX: Maximum; MIN: Minimum; HSCT: Hematopoietic Stem Cell Transplantation; ROC: Receiver Operating Characteristic; AUC: Area Under the Curve

Introduction

Acute lymphoblastic leukemia (ALL) is the most prevalent childhood malignancy, accounting for 25% of pediatric cancers, with peak incidence between 2 and 10 years of age [1]. Despite remarkable therapeutic advancements over the past two decades, leading to 5-year overall survival (OS) and event-free survival (EFS) rates of 85 - 90% [2, 3] treatment resistance remains a critical challenge. Approximately 15 - 20% of patients experience bone marrow relapse, and 2 - 5% demonstrate primary refractoriness to conventional chemotherapy [4]. Consequently, relapsed/refractory (R/R) patients exhibit reduced chemosensitivity, achieving complete remission rates of only 30 - 60% following high-dose chemotherapy, although this approach is often limited by severe toxicity.

Chimeric antigen receptor T-cell (CAR-T) therapy has emerged as a novel immunotherapy with strong specificity and improved prognosis, achieving CR rates of 70 - 90%. The FDA has approved six CAR-T regimens for B-cell malignancies such as B-ALL and large B-cell lymphoma [5]. CAR-T therapy utilizes genetically engineered T cells targeting CD19 and CD22 antigens. However, adverse events, particularly CRS and ICANS, remain significant challenges [6]. CAR-T cell rapid expansion induces vascular endothelial damage, with injured endothelial cells subsequently releasing cytokines that trigger systemic inflammatory responses [7].

CRS pathophysiology is characterized by IL-6-driven hyperinflammation, typically manifesting within the initial few days after infusion and follows well-defined cytokines cascades [8, 9]. ICANS frequently develops subsequent to CRS onset [6, 10]. Inflammation biomarkers routinely measured in clinical practice, including maximum daily temperature, C-reactive protein (CRP), and procalcitonin (PCT), have been reported as convenient predictors of ICANS occurrence [7, 11]. These biomarkers demonstrate suboptimal sensitivity or specificity in clinical practice. Animal studies indicate that the pathological mechanism of ICANS primarily involves microglial activation leading to oligodendrocyte reduction, a process accompanied by elevated levels of multiple cytokines. Adverse events of CAR-T therapy all refer to the elevation of cytokines level, while reliable predictive biomarkers remain inadequately characterized [12].

There is currently a lack of systematic clinical studies investigating the relationship between cytokine profiles and ICANS development. Based on the hypothesis that cytokines could be utilized for ICANS prediction, we designed the current investigation. Our study evaluates cytokine kinetic profiles and ranging magnitude for dynamic neurotoxicity prediction, aiming to incorporate parameters within a defined temporal window to optimize the sensitivity and specificity of ICANS prediction. This approach captures the magnitude of change during this critical period rather than relying solely on absolute values to mitigate errors attributable to inter-individual variation through quantification of dynamic trajectories. Although peripheral blood samples may be less accurate than cerebrospinal fluid samples for the progression of neurotoxicity, they are readily available for clinical monitoring. We attempted to predict the occurrence of ICANS adopting the absolute value and magnitude of change in cytokine levels, proposing utilizing kinetic biomarker data to enhance the sensitivity and specificity of diagnostic assays.

Methods

Study population and data collection

This retrospective study analyzed 120 R/R B-ALL patients treated with CAR-T cell therapy at the Children's Hospital of Soochow University between March 2017 and August 2023. Inclusion criteria were: ① Age 28 days to 18 years; ② Confirmed B-ALL relapse involving bone marrow and/or central nervous system (CNS), testicle; ③ Receipt of CAR-T therapy targeting distinct antigens; and ④ Prior lymphodepleting chemotherapy before CAR-T infusion. A final cohort of 120 patients met eligibility requirements. Neurological symptoms were systematically assessed using the Immune Effector Cell-Associated Encephalopathy (ICE) scoring system, per guidelines from the U.S. Department of Health and Human Services. The study protocol adhered to the Declaration of Helsinki and received ethical approval from the Institutional Review Board of the Children's Hospital of Soochow University (Approval No. 2023CS034). Written informed consent was obtained from patients or their guardians.

Cytokines measurement

Peripheral blood cytokines levels were quantified every 24 hours for two consecutive weeks following CAR-T infusion. Serum levels of IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, and IL-17 were measured using a human Th1/Th2/Th17 subset detection kit (flow cytometry fluorescence method; Beckman Coulter, USA).

Statistical analysis

For ICANS patients, cytokines three days prior to symptom onset were analyzed (maximum, minimum, mean, geometric mean (GM), standard deviation (SD), coefficient of variation (CV)). Non-ICANS patients, classified as control group, were analyzed on days 4 - 6. The peak level of cytokines after infusion of the two groups were analyzed as well (Figure 1). Before selecting appropriate statistical tests, the normality of the data was assessed using the Shapiro-Wilk test. Depending on the distribution, continuous variables were analyzed using either the Student's t-test or the Mann-Whitney U test, while categorical variables were compared using the chi-square test. A receiver operating characteristic (ROC) curve analysis was performed to assess the predictive performance for ICANS in CAR-T patients. The optimal cutoff value was determined using the Youden Index method. SPSS 27.0.1 was used for analysis with a significance threshold set at $p < 0.05$ for two-tailed tests. Numeric variables are expressed as quartiles, and counting variables are expressed as percentages.

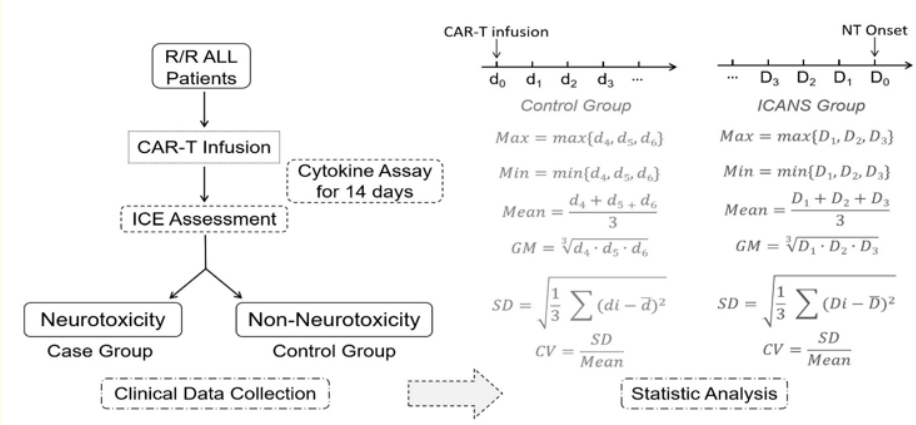


Figure 1: *Design of experiments flowchart. Note: Di denotes day i prior to neurotoxicity onset, and di denotes day i post-infusion.*

Results

General information of patients

A total of 120 pediatric patients receiving CAR-T therapy were enrolled in this study, with 31 (25.8%, 31/120) developed ICANS. No significant differences were observed between ICANS and non-ICANS groups in age (99.2 vs. 79.1 months, P = 0.315), gender (male proportion: 65.2% vs. 74.2%, P = 0.419), treatment response of chemotherapy (relapsed ALL proportion: 93.2% vs. 87.1%, P = 0.548), CAR-T cell dose (1.8 vs. 1.7 × 10^6 cells/kg, P = 0.891), or HSCT rate (6.7% vs. 9.7%, P = 0.839) (Table 1). Notably, 8 patients exhibited CNS infiltration at relapse, of whom 3 developed ICANS compared to 2 in the non-ICANS cohort, demonstrating statistical significance (P < 0.001). Neither tumor burden nor HSCT before CAR-T therapy demonstrated statistically significant associations with ICANS development, while the application of steroid shows satisfying efficiency. All patients underwent lymphodepletion with an FC regimen (fludarabine 50 mg/m^2/day and cyclophosphamide 500 mg/m^2/day for 3 days) prior to CAR-T infusion. Among the 31 ICANS cases, severity was stratified as grade 1 (16 patients, 13.3%), grade 2 (11 patients, 17.5%), grade 3 (3 patients, 2.5%), and grade 4 (1 patient, 0.8%). CRS occurred in 110 patients (91.7%, 110/120), while CRS incidence did not correlate with ICANS occurrence (P = 0.138), a strong association was

observed between CRS and ICANS severity (P < 0.001), with higher CRS grades linked to exacerbated neurotoxicity (P < 0.001 for linear trend; Figure 2A).

	Non-ICANS	ICANS	P
Age (month)	99.2 (71.1, 146.7)	79.1 (59.3, 134.7)	0.315
Gender			0.419
Male	58 (65.2%)	23 (74.2%)	
Female	31 (34.8%)	8 (25.8%)	
Treatment response			0.548
Relapse	82 (93.2%)	27 (87.1%)	
Refractory	6 (6.8%)	4 (12.9%)	
Infusion dosage (10^6/kg)	1.8 (1.1, 3.0)	1.7 (0.9, 3.40)	0.891
HSCT			0.839
Yes	6 (6.7%)	3 (9.7%)	
No	83 (93.3%)	28 (90.3%)	
CNS infiltration	5 (4.2%)	3 (2.5%)	<0.001
BM blast (%)	15 (3, 56)	11 (3.75, 59.5)	0.289
MRD (%)	7.95×10^{-2} (3.21×10^{-3}, 3.64×10^{-1})	6.57×10^{-2} (5.05×10^{-3}, 4.04×10^{-1})	0.332
IV steroid			0.001
Yes	36 (40.9%)	24 (77.4%)	
No	52 (59.1%)	7 (22.6%)	
IT steroid			0.001
Yes	0	20 (64.5%)	
No	88 (100%)	11 (35.5%)	

Table 1: *General information of patients.*

Note: HSCT: Hematopoietic Stem Cell Transplantation; CNS: Central Nervous System; MRD: Minimal Residual Disease; IV: Intravenous Injection; IT: Intrathecal Injection.

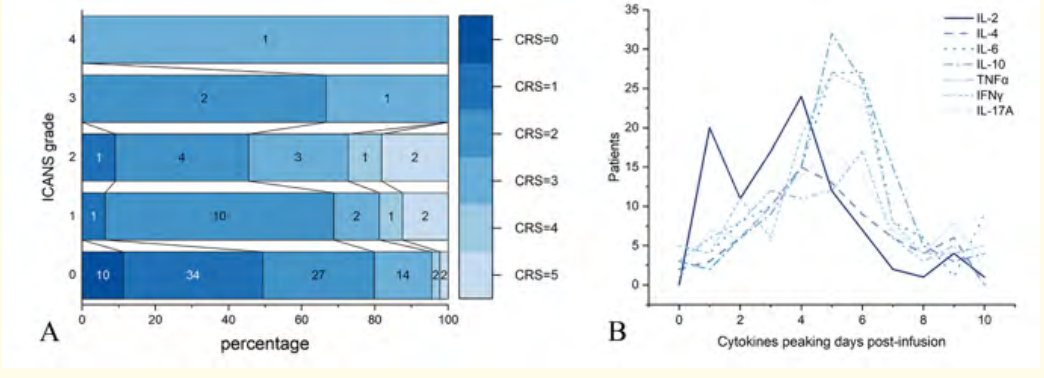
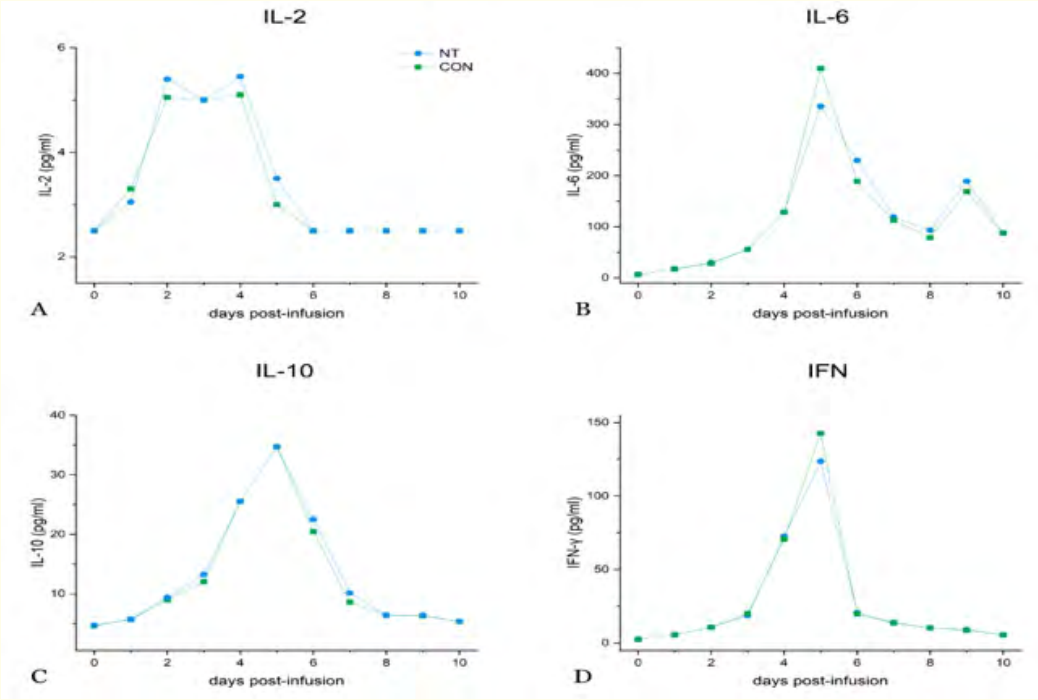


Figure 2: *(A) Percent stacked bar chart for CRS and ICANS. Note: The numeric value is absolute count in figure. (B) Time to peak cytokines in total cohort. Note: The values in the figure represent the number of patients whose corresponding cytokines reached the peak after CAR-T infusion on that day.*

Cytokines dynamics and predictive value for ICANS

The manifestation of peak cytokine concentrations within this cohort was most densely observed during the period of day 4 - 6 post-infusion (Figure 2B). This distinct temporal profile served as the principal reason for focusing our control group analyses on this critical time frame, as the trends of cytokine changes in the two groups of patients were almost identical (Supplementary figure 1). IL-2, IL-4, IL-6, IL-10, TNF-α displayed feasible predictive performance for ICANS development. Cytokines above exhibited statistically significant differences in both CV (P = 0.005, 0.001, 0.001, 0.019, < 0.001 for IL-2, IL-4, IL-6, IL-10 and TNF-α respectively) and peak levels post-infusion (P = 0.002, 0.001, 0.004, 0.003, < 0.001 respectively) between ICANS and control groups. Elevated GM (P < 0.001), mean (P < 0.001), and Min (P < 0.001) of IL-10 suggested potential ICANS development. During the first three days post-infusion, increased SD (P = 0.011, 0.002, and <0.001 respectively), maximum values (P = 0.010, 0.001, <0.001 respectively), and mean (P = 0.034, 0.002, <0.001 respectively) levels of IL-2, IL-4, and TNF-α were associated with ICANS occurrence. Both Max (P = 0.046) and Min (P = 0.025) values of IL-6 demonstrated significant differences between the two groups. Fluctuations in cytokines levels within the three days before the onset of neurotoxicity demonstrated optimal predictive utility (Table 2).



Supplementary figure 1: *Daily cytokines levels, IL-2(A), IL-6(B), IL-10(C), IFN-γ(D). Note: NT: Neurotoxicity; CON: Control Group. The median is exhibited in the figure, IL-4, TNF-α, IL-17A have remained baseline value all the time, so not included.*

Value		IL-2		IL-4		IL-6		IL-10		TNF-α		IFN-γ		IL-17A	
		P	Value	P	Value	P	Value	P	Value	P	Value	P	Value	P	
Mean	ICANS	12.2 (8.0, 57.5)	0.034	3.2 (2.5, 6.2)	0.002	1070.2 (38.6, 3705.73)	0.201	26.8 (20.8, 67.4)	0.014	2.5 (2.5, 6.1)	<0.001	293.3 (18.55, 929.37)	0.25	10.0 (10.0, 14.8)	0.986
	CON	3.9 (2.5, 11.1)		2.5 (2.5, 2.8)		33.0 (10.1, 135.1)		8.2 (4.1, 19.4)		2.5 (2.5, 2.6)		11.5 (4.5, 68.6)		10.0 (10.0, 12.8)	
GM	ICANS	10.1 (4.6, 24.9)	0.366	4.2 (2.5, 5.2)	0.002	237.8 (33.7, 824.8)	0.245	18.5 (9.7, 50.1)	<0.001	3.5 (2.5, 5.3)	<0.001	112.1 (12.7, 349.4)	0.457	10.2 (10.0, 14.0)	0.986
	CON	3.6 (2.5, 10.3)		2.5 (2.5, 2.8)		23.7 (8.7, 101.5)		7.7 (4.1, 18.2)		2.5 (2.5, 2.6)		9.5 (4.4, 51.3)		10.0 (10.0, 12.9)	
SD	ICANS	10.9 (3.6, 44.1)	0.011	2.5 (0.0, 4.6)	0.002	1040.7 (26.7, 4226.6)	0.065	22.2 (11.1, 43.2)	0.217	2.2 (0.0, 4.3)	<0.001	323.1 (12.7, 972.0)	0.165	0.3 (0.0, 44.3)	0.746
	CON	1.6 (0.2, 5.6)		0.0 (0.0, 0.9)		21.8 (3.5, 110.5)		4.0 (1.2, 10.2)		0.0 (0.0, 0.4)		8.3 (1.7, 84.3)		0.0 (0.0, 4.2)	
CV	ICANS	0.7 (0.5, 1.1)	0.005	0.4 (0.0, 1.1)	0.001	1.2 (0.7, 1.6)	0.001	0.6 (0.4, 0.9)	0.019	0.5 (0.0, 1.3)	<0.001	0.9 (0.5, 1.5)	0.067	0.1 (0.0, 0.6)	0.323
	CON	0.4 (0.1, 0.7)		0.0 (0.0, 0.2)		0.6 (0.3, 1.1)		0.5 (0.2, 0.8)		0.0 (0.0, 0.1)		0.7 (0.4, 1.1)		0.0 (0.0, 0.3)	
MAX	ICANS	24.7 (11.8, 103.2)	0.010	5.7 (2.5, 12.0)	0.001	2725.8 (57.5, 7926.6)	0.046	62.7 (31.6, 109.9)	0.074	4.8 (2.5, 9.8)	<0.001	543.9 (31.2, 1761.6)	0.155	10.0 (10.0, 24.5)	0.815
	CON	5.6 (2.5, 15.5)		2.5 (2.5, 3.4)		48.6 (13.0, 262.5)		12.0 (5.7, 27.6)		2.5 (2.5, 2.9)		19.7 (5.2, 131.5)		10.0 (10.0, 15.9)	
MIN	ICANS	4.3 (2.5, 6.1)	0.255	2.5 (2.5, 2.5)	0.798	24.3 (8.2, 157.4)	0.025	9.5 (3.6, 32.9)	<0.001	2.5 (2.5, 2.5)	0.146	9.6 (4.1, 130.5)	0.528	10.0 (10.0, 10.0)	0.481
	CON	2.5 (2.5, 4.7)		2.5 (2.5, 2.5)		13.4 (4.3, 31.875)		4.1 (2.5, 9.4)		2.5 (2.5, 2.5)		3.2 (2.5, 9.6)		10.0 (10.0, 10.0)	
Peak level post -infu-sion	ICANS	26.3 (14.8, 165.3)	0.002	12.1 (3.6, 22.5)	0.001	7926.6 (1166.5, 18378.7)	0.004	196.6 (72.2, 380.0)	0.003	9.8 (2.6, 43.1)	<0.001	2829.6 (825.1, 10234.2)	0.01	25.1 (11.6, 96.5)	0.329
	CON	8.9 (3.6, 35.7)		2.9 (2.5, 8.8)		1044.7 (54.3, 7902.6)		76.9 (18.6, 231.2)		3.0 (2.5, 8.2)		341.1 (24.0, 3383.7)		19.2 (10.0, 48.1)	

Table 2: *Dynamic predictors of ICANS occurrence.*

Note: GM: Geometric Mean; SD: Standard Deviation; CV: Coefficient of Variation; Max: Maximum; Min: Minimum.

IL-10 as a robust biomarker for ICANS

ROC curve analysis was performed using derived parameters of IL-2, IL-4, IL-6, IL-10, and TNF-α, revealing clinically satisfactory predictive utility for IL-6, IL-10, and TNF-α. Within the 72-hour observation window, the MAX of IL-6 concentration exceeding 471.85 pg/mL (AUC = 0.744, Specificity = 0.776, Sensitivity = 0.696) substantially increased ICANS risk, while mean values of IL-10 (>20.55 pg/mL, AUC = 0.765, Specificity = 0.777, Sensitivity = 0.783) and TNF-α (>3.12 pg/mL, AUC = 0.724, Specificity = 0.839, Sensitivity = 0.611)

levels similarly demonstrated predictive capability. Reflecting the non-linear kinetics characteristic of cytokine dynamics, GM of IL-10 (>9.16 pg/mL, AUC = 0.730, Specificity = 0.596, Sensitivity = 0.826) and TNF-α (>3.00 pg/mL, AUC = 0.730, Specificity = 0.839, Sensitivity = 0.611) additionally exhibited discriminatory power. Despite systematic evaluation of multiple combinatorial models, no parameter combination surpassed the predictive accuracy achieved by individual biomarkers (Figure 3). These findings underscore the prognostic value of cytokines kinetics in anticipating severe neurotoxicity, while the mean level of IL-10 has the most satisfactory predictive valve of ICANS.

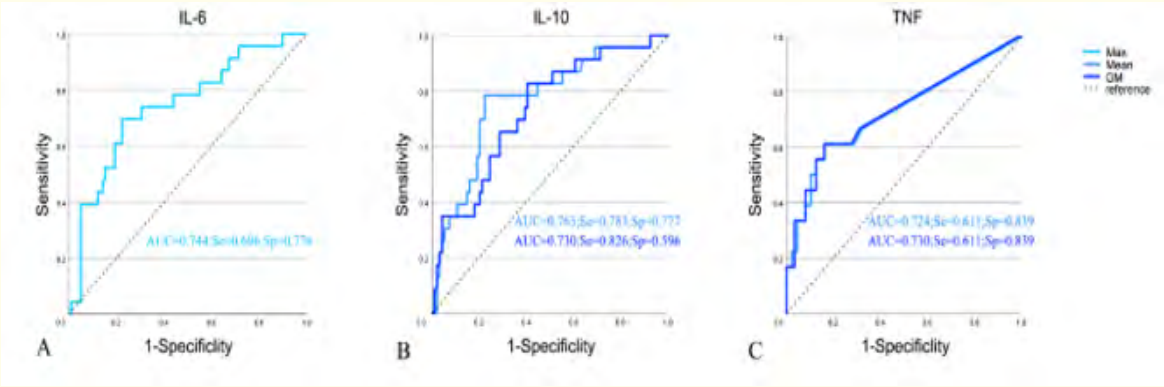


Figure 3: *(A) The predicting value of IL-6 for the incidence of ICANS. Data in the figure is the maximum value of IL-6 within 72 hours before the onset of the ICANS. (B) The predicting value of IL-10 for the incidence of ICANS. Note: Data in the figure is the mean value and geometric mean value of IL-10 within 72 hours before the onset of the ICANS; GM, geometric mean. (C) The predicting value of TNF-α for the incidence of ICANS. Note: Data in the figure is the mean value and geometric mean value of IL-10 within 72 hours before the onset of the ICANS; GM: Geometric Mean.*

Discussion

Microglial activation, oligodendrocyte injury, and elevated cytokines/chemokines constitute integral components of the ICANS pathological cascade [12], with studies suggesting associations with intracranial inflammation and blood-brain barrier disruption [13]. Activation of monocyte-macrophage system signifies non-infiltrative elevations of IL-1β, IL-6 and TNF-α, concomitant with vascular endothelial injury and systemic inflammatory cascade amplification [14]. Although CSF cytokine levels theoretically represent biomarkers with superior specificity, the clinical feasibility of dynamic monitoring remains limited. Consequently, we have redirected our focus toward peripheral blood cytokine profiling.

Current treatment options are mainly focused on intrathecal or intravenous steroids. Delayed steroid administration may lead to refractory ICANS, while early systemic steroid use risks impairing CAR-T cell efficacy and increasing tumor relapse rates [15]. Early intrathecal steroid administration has shown potential for improving outcomes, consistent with the results of our experiments, though timing remain empirically guided [16]. As ICANS is currently manageable, early recognition and intervention are critical for better outcome. Previous studies have proposed that fluctuations in conventional inflammatory markers such as IL-6, ferritin, and CRP may indicate the onset of ICANS, with early-onset fever also serving as a potential warning signal [17-19]. Unfortunately, ferritin and CRP did not demonstrate satisfying predictive performance in our cohort. Serum neurofilament light chain levels have also shown some effect in the prediction of ICANS, but not clinically feasible currently [20]. While models incorporating temperature, CRP, and WBC have been

proposed for adult lymphoma cohorts, these lacked predictive utility in p-ALL patients [21]. Existing studies indicate that IFN-γ may increase blood-brain barrier permeability to T cells, potentially contributing to ICANS [22]. Perhaps there are many influencing factors for IFN-γ production, the specificity is poor.

The selection of the day 4 - 6 timeframe for the control group was guided by the observation that peak expression of the seven cytokines most frequently occurred during this period, which also coincided with the highest incidence of ICANS. Our aim was to determine whether cytokine profiles within this critical window have sufficient predictive value for the development of ICANS. Longitudinal cytokine profiling spanning 14 days post-infusion was conducted, with subsequent delineation of dynamic trajectories through analysis of concentration variability, maximum values, average levels, and baseline values while also employing these parameters to predict the occurrence of neurotoxicity. The selection rationale for these statistical measures derives from their analytical utility in data characterization. We've tried day-to-day ratios/differences, but the result was not satisfactory.

Some studies have reported an association between CAR-T cell infusion dosage and ICANS occurrence, though our experiments failed to replicate this finding, which may relate to the absence of CD28 costimulatory domain in the CAR construct [23]. In our cohort, cytokines peaks were concentrated on days 4 - 6 post-infusion. For patients without ICANS, cytokines during this window were analyzed. For ICANS cases, cytokines levels from the 72 hours preceding neurotoxicity onset were evaluated, with adjacent 2 - 3 days intervals used for statistical analysis, enabling dynamic profiling compared to prior studies [24].

Although CRS and ICANS occurrence were not directly linked, severe CRS often coincided with higher-grade ICANS. CRS and ICANS share an established risk factor, rapid CAR-T cell expansion, which may explain the positive correlation in their severity. In this study, the onset of CRS and ICANS demonstrated temporal dissociation. This phenomenon may be attributed to differential IL-6 production by CNS microglia versus peripheral monocyte-macrophage systems upon CAR-T cell activation. Furthermore, the reciprocal influence on severity potentially stems from bidirectional trafficking of elevated IL-6 across the compromised BBB between systemic circulation and the central nervous system. Compared to the management of CRS, the use of tocilizumab led to a significant increase in both the incidence and severity of ICANS, worsening the progression of neurotoxicity [25]. In contrast, siltuximab, as an IL-6 antagonist, did not significantly affect the course of ICANS [26]. This phenomenon may be related to the fact that after tocilizumab blocks the IL-6 receptor, serum free IL-6 titers increase and infiltrate the CNS, leading to aggravated CNS inflammation. Siltuximab directly reduces serum IL-6 concentrations, thus no significant changes in neurotoxicity were observed.

In this study, the majority of patients exhibited progressively increasing cytokine levels with substantial fluctuations prior to ICANS onset, which is a pattern consistent with established clinical observations. Peripheral blood IL-6, IL-10, and TNF-α emerged as viable predictors of ICANS onset. While minimum levels of IL-10 and TNF-α showed no significant differences between groups, which indicates comparable baseline concentrations, elevated GM and SD of both cytokines were significantly associated with ICANS development. The combined increase in absolute cytokine production and variability magnitude may link to rapid immune response progression, predicting ICANS manifestation. Although IL-2 and IL-4 demonstrated statistically significant intergroup differences, their predictive utility was suboptimal, likely attributable to their primary association with T-cell activation rather than microglial engagement. Given microglial activation constitutes the central pathological driver in ICANS, this may explain the superior predictive performance of microglia-derived IL-6, IL-10, and TNF-α.

Limitations of the Study

In pediatric patients, the manifestation of classic ICANS clinical signs exhibits delayed presentation. Furthermore, language dysfunction - a hallmark neurotoxicity feature - poses diagnostic challenges in differentiating between age-appropriate developmental variations and true neurotoxicity. Concurrently, the ICE assessment tool adopted in this study carries inherent subjectivity, potentially introducing measurement bias. As a retrospective investigation, these findings necessitate validation through prospective cohort studies with optimized pediatric-specific instrument.

Conclusion

Significant quantitative difference were observed in circulating levels of IL-2, IL-4, IL-6, IL-10, and TNF-α between the ICANS group and the control group. Critical thresholds predictive of potential ICANS emergence include: IL-6 concentrations exceeding 471.85 pg/ml; geometric mean IL-10 levels > 20.55 pg/ml coupled with standard deviation > 9.16 pg/ml; and concurrent elevations in TNF-α mean concentrations > 3.12 pg/ml with geometric mean > 3.00 pg/ml. These biomarker signatures should raise heightened clinical vigilance for neurotoxicity development.

Acknowledgements

Ruixin Zhu and Rongzhou Xiao both authors contributed equally to this article.

Authors' Contributions

Ruixin Zhu, Rongzhou Xiao: Acquisition of data and draft the work; Huaqing Liu: Analysis and interpretation of data; Conglian Qiu, Chunyi Zhou, and Luyao Dai: Acquisition of data and chart edition; Shuiyan Wu: Designed and drafted the work and substantively revised it for content; Zhenjiang Bai, Shaoyan Hu, and Jun Lu: Made contributions to the conception. All authors have approved the submitted version and agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work.

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Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The study was approved by the Research Ethics Committee of Children's Hospital Affiliated to Soochow University (2023CS034). Informed written consent to participate was obtained from the parents/guardians of the minors included in this study.

Competing Interests

The authors declare no competing interests.

Bibliography

1. Tuong PN., *et al*. "Relapsed childhood acute lymphoblastic leukemia: a single-institution experience". *Cureus* 12.7 (2020): e9238.
2. Hunger SP and Mullighan CG. "Acute lymphoblastic leukemia in children". *New England Journal of Medicine* 373.16 (2015): 1541-1552.
3. Bailey LC., *et al*. "Bone-marrow relapse in paediatric acute lymphoblastic leukaemia". *Lancet Oncology* 9.9 (2008): 873-883.
4. Pui CH., *et al*. "Childhood acute lymphoblastic leukemia: progress through collaboration". *Journal of Clinical Oncology* 33.27 (2015): 2938-2948.

5. Goyco Vera D., *et al*. "Approved Car-T therapies have reproducible efficacy and safety in clinical practice". *Human Vaccines and Immunotherapeutics* 20.1 (2024): 2378543.

6. Schroeder T., *et al*. "Management of chimeric antigen receptor T (Car-T) cell-associated toxicities". *Intensive Care Medicine* 50.9 (2024): 1459-1469.

7. Dziedzic-Jankowska K., *et al*. "Association of subclinical inflammation markers with primary hypertension in children-a systematic review with meta-analysis". *Journal of Clinical Medicine* 14.7 (2025): 2319.

8. Shimabukuro-Vornhagen A., *et al*. "Cytokine release syndrome". *Journal for ImmunoTherapy of Cancer* 6.1 (2018): 56.

9. Schuster SJ., *et al*. "Grading and management of cytokine release syndrome in patients treated with tisagenlecleucel in the Juliet trial". *Blood Advances* 4.7 (2020): 1432-1439.

10. Lee DW., *et al*. "Astct consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells". *Biology of Blood and Marrow Transplantation* 25.4 (2019): 625-638.

11. Karschnia P., *et al*. "Clinical presentation, management, and biomarkers of neurotoxicity after adoptive immunotherapy with Car T cells". *Blood* 133.20 (2019): 2212-2221.

12. Geraghty AC., *et al*. "Immunotherapy-related cognitive impairment after Car T cell therapy in mice". *Cell* 188.12 (2025): 3238-3258. e3225.

13. Schulenburg A., *et al*. "Retrospective analysis: S100 as marker for immune effector cell-associated neurotoxicity syndrome". *Oncology* 103.10 (2025): 930-933.

14. Norelli M., *et al*. "Monocyte-derived Il-1 and Il-6 are differentially required for cytokine-release syndrome and neurotoxicity due to Car T cells". *Nature Medicine* 24.6 (2018): 739-748.

15. Kagimoto T., *et al*. "Detection of pyrimidine 5'-nucleotidase deficiency using 1h- or 31p-nuclear magnetic resonance". *Experientia* 42.1 (1986): 69-72.

16. Zurko JC., *et al*. "Use of early intrathecal therapy to manage high-grade immune effector cell-associated neurotoxicity syndrome". *JAMA Oncology* 8.5 (2022): 773-775.

17. Amidi Y., *et al*. "Forecasting immune effector cell-associated neurotoxicity syndrome after chimeric antigen receptor T-cell therapy". *Journal for ImmunoTherapy of Cancer* 10.11 (2022): e005459.

18. Zandaki D., *et al*. "Easix and M-easix predict Crs and Icans in pediatric and Aya patients after Cd19-Car T-cell therapy". *Blood Advances* 9.2 (2025): 270-279.

19. Santomasso BD., *et al*. "Management of immune-related adverse events in patients treated with chimeric antigen receptor T-cell therapy: Asco guideline". *Journal of Clinical Oncology* 39.35 (2021): 3978-3992.

20. Larue M., *et al*. "Neurofilament light chain levels as an early predictive biomarker of neurotoxicity after Car T-cell therapy". *Journal for ImmunoTherapy of Cancer* 12.9 (2024): e009525.

21. Sharma N., *et al*. "Cytopenia after Car-T cell therapy-a brief review of a complex problem". *Cancers (Basel)* 14.6 (2022): 1501.

22. Sonar SA., *et al*. "Ifn-Γ promotes transendothelial migration of Cd4(+) T cells across the blood-brain barrier". *Immunology and Cell Biology* 95.9 (2017): 843-853.

23. Angelillo P., *et al*. "Severe Icans after Car T-cell therapy and assessment of prevention with levetiracetam for seizure prophylaxis following Car T-Cell for Dlbcl & Pmbcl in Europe: A survey on behalf of the cellular therapy & immunobiology working party (Ctiwp) of the Ebmt". *Bone Marrow Transplant* 60.1 (2025): 100-102.

24. Rubin DB., *et al*. "Clinical predictors of neurotoxicity after chimeric antigen receptor T-cell therapy". *JAMA Neurology* 77.12 (2020): 1536-1542.

25. Brudno JN and Kochenderfer JN. "Current understanding and management of Car T cell-associated toxicities". *Nature Reviews Clinical Oncology* 21.7 (2024): 501-521.

26. Thompson JA., *et al*. "Management of immunotherapy-related toxicities, version 1.2022, Nccn clinical practice guidelines in oncology". *Journal of the National Comprehensive Cancer Network* 20.4 (2022): 387-405.

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