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ORIGINAL RESEARCH

Early Prediction Model for Etoposide-Based Protocols Resistance in Pediatric HLH

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Background: Etoposide-based protocols remain the first-line therapy for pediatric hemophagocytic lymphohistiocytosis (HLH), yet 20–30% of patients exhibit primary resistance. Early identification of non-responders is critical to enable timely salvage therapy. We aimed to develop and validate a predictive model for etoposide-based protocols resistance using readily available clinical and laboratory parameters.

Methods: A retrospective cohort of 79 pediatric HLH patients (median age 3.5 years; 53% male) treated with etoposide-based protocols (HLH-94/2004) at Hunan Children's Hospital (2020–2024) was analyzed. Patients were stratified into refractory (n=20) and responsive (n=59) groups based on 8-week treatment outcomes. Chemosensitive patients were defined as those achieving complete response (CR) or entering maintenance by week 8; chemorefractory patients were defined as those who died during induction, required salvage therapy due to progression, or failed to achieve CR within 8 weeks. CR required normalization of all quantifiable disease markers (sCD25, ferritin, triglycerides, ALT, hemoglobin, neutrophils, platelets). Pre-chemotherapy and early post-chemotherapy (Day 3) variables were compared using *t*-tests, Mann–Whitney *U*-tests, and χ^2 analyses. Multivariable logistic regression and ROC analyses identified predictors of resistance, with internal validation via 1000-bootstrapping.

Results: Pre-chemotherapy IL-10 >131.2 μmol/L (OR=5.1; 95% CI 1.9–13.7; P=0.001) and pre-chemotherapy platelet count $<55.5\times10^9$/L (OR=4.2; 95% CI 1.6–11.3; P=0.004) independently predicted refractoriness. A combined model incorporating these parameters achieved an AUC of 0.822 (sensitivity 81.3%, specificity 75.0%). Post-chemotherapy Day 3 platelet count (OR=1.02 per 10^9/L increase; P=0.01) and lymphocyte count (OR=2.5 per 10^9/L increase; P=0.03) further refined prediction (AUC 0.880). Calibration curves and decision curve analysis confirmed clinical utility.

Conclusion: Pre-chemotherapy IL-10 and platelet count reliably identify pediatric HLH patients at high risk of etoposide-based protocols resistance. Integration of Day 3 post-chemotherapy parameters enables ultra-early and more precise risk stratification, facilitating prompt transition to salvage therapies.

Keywords: hemophagocytic lymphohistiocytosis, etoposide-based protocols, drug resistance, IL-10, platelet count, pediatric

Background

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome driven by dysregulated activation of cytotoxic T cells and NK cells, with its core pathology characterized by the "cytokine storm—excessive macrophage phagocytosis—multi-organ failure" cascade.[1] In untreated pediatric patients, the median survival is less than 2 months,[2] with a long-term survival rate below 5%.[3] Although the etoposide-based protocols HLH-94/2004 regimen has improved the 5-year overall survival rate to 61%, 4.6% of pediatric patients still die within the first 10 days of treatment

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initiation, and 19% succumb before hematopoietic stem cell transplantation.[4] This early mortality peak indicates that the conventional 14-day response assessment window is no longer clinically sufficient, underscoring an urgent need for an ultra-early predictive system capable of identifying high-risk children within 72 hours.

HLH exhibits a "dual-track" etiological spectrum:[5] Primary HLH results from genetic mutations (eg, PRF1, UNC13D, STX11) impairing cytotoxic granule release, while secondary HLH is triggered by hyperinflammatory states induced by Epstein-Barr Virus (EBV) infection, malignancies, autoimmune diseases (MAS in the course of rheumatic diseases) or other less common triggers such as drugs and transplantation.[6] Significant variations in etoposide-based protocols sensitivity and drug toxicity profiles across different etiologies indicate that a "one-size-fits-all" fixed treatment regimen fails to meet individualized therapeutic needs. The 2022 and 2024 Chinese HLH guidelines introduced for the first time the concept of "stratified therapy", advocating for earlier incorporation of salvage regimens (eg, ruxolitinib, emapalumab) based on etiology and early treatment response,[6,7] However, the lack of objective thresholds for determining "when" to switch from first-line regimens results in some pediatric patients missing optimal treatment windows while receiving ineffective chemotherapy.

Previous adult studies have identified age >30 years, disseminated intravascular coagulation (DIC), interleukin-10 (IL-10) >400 μmol/L, elevated lactate dehydrogenase (LDH), hypoalbuminemia, and thrombocytopenia as predictors of poor prognosis.[8–10] However, pediatric HLH demonstrates more rapid progression, lower metabolic reserve, and a higher EBV-driven proportion (up to 80%).[5,11,12] The cutoff values, temporal windows, and biological significance of these indicators remain unvalidated in children. Verkamp et al[13] reported that insufficient platelet or Soluble interleukin-2 receptor (sCD25) recovery within 7 days could predict pre-transplant mortality, but this 7-day waiting period remains inadequate for rapidly deteriorating pediatric cases.

The rapid progression and high mortality of HLH necessitate early identification of patients at risk of severe outcomes. Interleukin-10 (IL-10), traditionally viewed as an anti-inflammatory cytokine, has recently been shown to exhibit paradoxical pro-inflammatory effects, particularly in synergy with interleukin-18 (IL-18). Preclinical models demonstrate that co-overexpression of IL-10/IL-18 can induce lethal HLH-like syndromes, reversible by anti-IL-10 antibodies.[14] Clinically, elevated IL-10 (>400 μmol/L) strongly correlates with ICU admission and mortality in adult cohorts,[8] but its early predictive utility in pediatric HLH remains unvalidated. Similarly, platelets (PLT) are not merely consumption markers but active inflammatory modulators, releasing PF4 from α-granules to amplify circuits.[15] Their dynamic changes may reflect real-time inflammatory, though this has not been systematically investigated.

While previous studies have identified prognostic factors such as thrombocytopenia and elevated IL-10 levels associated with poor overall survival in HLH, their specific utility in predicting resistance to etoposide-based protocols remains unclear. This study extends these findings by demonstrating that these markers can serve as early predictors of chemotherapy resistance, enabling ultra-early risk stratification and timely transition to salvage therapies—a novel contribution that transforms known prognostic indicators into actionable tools for real-time treatment guidance. Based on these findings, we enrolled 79 pediatric HLH patients uniformly treated with the HLH-94/2004 protocol to investigate pre-chemotherapy and day 3 post-chemotherapy IL-10 levels, platelet counts, lymphocyte counts, and metabolic parameters. This study aims to: 1) validate the predictive value of these variables for etoposide-based protocols resistance; 2) develop and validate a risk-stratification model capable of guiding treatment modification within 72 hours; and 3) provide evidence-based support for implementing "data-driven" individualized therapy in clinical practice.

Materials and Methods

Study Design and Ethics

This single-centre, retrospective cohort study consecutively enrolled pediatric patients diagnosed with HLH at Hunan Children's Hospital, Changsha, China, between 1 January 2020 and 29 February 2024. The study protocol was approved by the institutional ethics committee (HCHLL-2018) and conducted in accordance with the 1964 Declaration of Helsinki (as revised in 2013). The requirement for written informed consent was waived owing to the retrospective nature of the analysis and use of de-identified data.

Patients and Definitions

Inclusion Criteria

Children who fulfilled the HLH-2004 diagnostic criteria:[16]

1. Definitive molecular diagnosis (pathogenic variants in PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST, SH2D1A, BIRC4, ITK, AP3B1, MAGT1, CD27 or other recognised loci).
2. At least five of the following eight clinical-laboratory criteria:

Fever: Peak temperature >38.5°C persisting for ≥7 days.

Splenomegaly: Palpable spleen ≥3 cm below left costal margin.

Cytopenias: Affecting at least two of three lineages in peripheral blood (haemoglobin <90 g/L, platelets $<100 \times 10^9$/L, neutrophils $<1.0 \times 10^9$/L).

Hypertriglyceridaemia and/or hypofibrinogenaemia: Fasting triglycerides ≥3 mmol/L, and/or fibrinogen ≤1.5 g/L.

Haemophagocytosis: Evidence in bone marrow, spleen, cerebrospinal fluid, or lymph nodes.

Low or absent NK-cell activity: Below 10% of reference range per local laboratory.

Hyperferritinaemia: Ferritin ≥500 μg/L.

Elevated soluble CD25: sIL-2Rαl≥2400 U/mL (age-adjusted reference).

All patients received etoposide-based protocols (HLH-94 or HLH-2004).

Exclusion Criteria

(1) Prior etoposide-based protocols at an outside facility with incomplete baseline data; (2) relapsed HLH after previous complete remission; (3) concomitant ruxolitinib use; and (4) <8 weeks follow-up or lost-to-follow-up precluding outcome ascertainment.

Outcome Assignment

Chemorefractory group HLH (n=20) was defined by at least one of: (i) death during induction, (ii) switch to salvage therapy because of progression, or (iii) failure to achieve complete response (CR) within 8 weeks. CR required normalisation of all quantifiable disease markers (sCD25, ferritin, triglycerides, glutamic-pyruvic transaminase (ALT), hemoglobin (Hb), absolute neutrophil count (Ne), PLT).[17] Chemosensitive group HLH (n=59) comprised patients who achieved CR or entered maintenance therapy by week 8.

Data Collection and Laboratory Assays

Electronic health records were interrogated for demographics, underlying aetiology (EBV-driven, genetic, autoimmune, malignancy-associated), Peripheral blood laboratory indicators, time from symptom onset to chemotherapy, and imaging. Splenic and hepatic size were quantified by ultrasonography as the maximal subcostal distance (mm) reported by a single pediatric radiologist blinded to outcome.

Laboratory values were extracted at four prespecified time-points:

(1) Baseline: worst recorded value (pre-treatment peak), and final pre-chemotherapy value ("baseline");
(2) Post-chemotherapy: days 3, 6, 9, and 14 (±1 day for days 3–9; ±2 days for day 14).

Statistical Analysis

Continuous and categorical variables were compared using appropriate tests (*t*-test, Mann–Whitney U, the chi-square (χ^2) test, or Fisher's exact test). Variables with $P < 0.10$ in univariable analysis were included in a multivariable logistic regression model with backward elimination. Model performance was assessed by the area under the receiver operating characteristic curve (AUC), calibration plots (bootstrapped with 1000 samples), and the Hosmer-Lemeshow test. Decision-curve analysis quantified clinical utility. Analyses were performed using SPSS 25.0 and R 4.3.1, with a two-sided $P < 0.05$ considered significant.

Result

Baseline Characteristics and Early Mortality Overview

A total of 79 pediatric patients meeting the HLH-2004 criteria were consecutively enrolled (Table 1). The median age was 3.5 years, and 53% (n=42) were male. The refractory group comprised 20 patients, six (30%) of whom died within the first week of diagnosis. No significant differences were observed between the two groups in terms of age, sex, or etiological distribution. However, in the refractory group, the time from symptom onset to chemotherapy initiation exceeded 2 weeks in 50% of the patients (P=0.031). Additionally, a significantly higher proportion of patients in the refractory group had a splenic inferior margin greater than 20.5 mm compared to the treatment-responsive group (72% vs 36%, P=0.008).

Pre-Chemotherapy IL-10 and Platelet Count: Strong Independent Predictors

Comparison of baseline inflammatory cytokine profiles (including IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α) between the Chemorefractory group and Chemosensitive group revealed a significant difference in serum IL-10 levels (Table 2). The median pre-chemotherapy IL-10 concentration was significantly higher in the refractory group than in the sensitive group (530 μmol/L [IQR 69–2500] vs 98 μmol/L [IQR 30–303], P < 0.001). Receiver operating characteristic (ROC) curve analysis indicated that an IL-10 level >131.2 μmol/L predicted chemotherapy resistance (AUC = 0.72, 95% CI

Table 1 Analysis of Clinical Characteristics Between Chemorefractory and Chemosensitive Children

Variable	Total (n=79)	Chemorefractory Group (n=20)	Chemosensitive Group (n=59)	*P value*
Age (month)	41.50 (20.50, 74.00)	48.00 (19.00, 98.75)	39.50 (22.50, 70.25)	0.97
Sex				0.22
Female	37 (47.40%)	7 (35.00%)	30 (50.80%)	
Male	42 (53.20%)	13 (65.00%)	29 (49.20%)	
Etiology				
Infection	78 (98.7%)	20 (100.00%)	58 (98.3%)	
EBV	65 (82.30%)	17 (85.00%)	48 (81.40%)	0.98
Malignancy				0.29
Confirmed	5 (6.3%)	3 (10.00%)	3 (5.20%)	
Suspected	16 (20.30%)	6 (30.00%)	10 (16.90%)	
Excluded	58 (73.4%)	12 (60.00%)	46 (78.00%)	
Rheumatic disease	2 (2.50%)	1 (5.00%)	1 (1.70%)	1.00
Infectious mononucleosis	13 (16.50%)	3 (15.00%)	10 (16.90%)	1.00
Primary HLH	4 (5.20%)	1 (5.30%)	3 (5.20%)	1.00
Other	8 (10.4%)	6 (10.30%)	2 (10.50%)	
Time from onset to chemotherapy				0.031
≤1 week	13 (16.70%)	0 (0.00%)	13 (22.40%)	
1-2 weeks	37 (47.40%)	10 (50.00%)	27 (46.60%)	
>2 weeks	28 (35.90%)	10 (50.00%)	18 (31.00%)	
Liver size (mm below costal margin)	26.00 (16.00, 35.00)	27.00 (24.00, 50.00)	25.00 (14.00, 33.00)	0.15
>49.00	7 (9.5%)	5 (26.3%)	2 (3.6%)	0.014
≤49.00	67 (96.4%)	14 (73.7%)	52 (96.4%)	
Spleen size (mm below costal margin)	19.00 (0.00, 30.00)	26.50 (18.00, 40.25)	17.00 (0.00, 30.00)	0.022
>20.5	33 (45.2%)	13 (72.2%)	20 (36.4%)	0.008
≤20.5	40 (53.8%)	5 (27.8%)	35 (63.6%)	
Hemophagocytosis	72 (92.30%)	18 (94.70%)	54 (91.50%)	1.00
Bone marrow	62 (79.50%)	17 (89.50%)	45 (76.30%)	0.36
Lymph node	45 (81.80%)	8 (72.70%)	37 (84.10%)	0.66

Notes: Data are presented as median (interquartile range) or n (%). Liver and spleen sizes were measured by ultrasonography as the distance from the inferior margin of the liver/spleen to the lower edge of the costal arch.
Abbreviation: EBV, Epstein-Barr virus.

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Table 2 Comparison of Worst Pre-Chemotherapy Values Between Chemorefractory and Chemosensitive Groups

Variable	Chemorefractory Group (n=20)	Chemosensitive Group (n=59)	P value
IL-2 (umol/L)	2.51 (<2.50, 4.43)	3.88 (<2.50, 4.63)	0.50
IL-4 (umol/L)	<2.50 (<2.50, 3.89)	2.66 (<2.503.91)	0.69
IL-6 (umol/L)	36.81 (17.28, 99.21)	19.76 (9.12, 58.21)	0.16
IL-10 (umol/L)	530.47 (68.67, >2500)	97.85 (29.50, 302.72)	0.024
IFN-γ (umol/L)	741.83 (224.99, >2500)	318.92 (27.65, 1806.48)	0.08
TNF-α (umol/L)	<2.50 (<2.50, 6.44)	<2.50 (<2.50, <2.50)	0.24
NK Activity (%)	3.15 (1.73, 3.28)	2.80 (1.75, 3.38)	0.94
sCD25 (U/mL)	17998.50 (10,083.50, 39,360.00)	10,023.31 (5061.00, 20,984.75)	0.046

[0.56–0.88]). Further analysis of routine blood parameters showed a significant baseline difference in platelet counts between the two groups. ROC analysis revealed that thrombocytopenia was significantly associated with chemotherapy resistance, with an AUC of 0.76 (95% CI [0.65–0.87]) at a cutoff value of 55.5×10^9/L. IL-6 and NK cell activity showed no statistically significant differences between the Chemorefractory group and Chemosensitive group ($P > 0.05$).

Multivariate logistic regression analysis confirmed that both IL-10 (OR = 0.21, 95% CI [0.05–0.90], P = 0.050) and platelet count (OR = 1.014, 95% CI [1.00–1.03], P = 0.036) were independent predictors of chemotherapy resistance (Table 3). A combined model incorporating both IL-10 and platelet count significantly improved predictive performance (AUC = 0.82, 95% CI [0.72–0.93]) (Table 4). These results suggest that the pre-chemotherapy "IL-10–platelet axis" may facilitate early identification of treatment-resistant children with hemophagocytic syndrome.

Day 3 Cell Counts: Early Indicators of Treatment Response

Comparative analysis between the chemorefractory and chemosensitive groups identified consistent differences in platelet and absolute lymphocyte counts (both $P < 0.01$; Table 5 and Figure 1). To evaluate their predictive utility, we subsequently assessed the discriminatory power of these parameters. As shown in the ROC analysis and AUC heatmap (Table 6 and Figure 2), both indicators showed good predictive value for refractory HLH (AUC > 0.7). Among them, a platelet count $<109.5\times10^9$/L on day 3 of chemotherapy demonstrated the highest predictive accuracy (AUC = 0.822, 95% [0.723–0.921]). The predictive performance was further improved by combining this with a day 3 lymphocyte count $<0.55\times10^9$/L (AUC = 0.731, 95% [0.601–0.861]), yielding a combined AUC of 0.845 (sensitivity 89%, specificity 71%) (Tables 7–9). This combined model enables the early identification of children with a poor therapeutic response within 72 hours, providing a critical time window for timely adjustment of treatment strategies.

Table 3 Binary Logistic Regression Analysis of Platelet Count and IL-10 Dichotomous Variable for Predicting Chemorefractoriness

Variable	β Coefficient	Standard Error	Wald χ^2	P value	OR	95% CI for OR
PLT_{bf}	0.014	0.007	3.837	0.050	1.014	1.000–1.029
IL-10 $_{Dichotomized}$	−1.578	0.752	4.410	0.036	0.206	0.047–0.900

Notes: Data are results of multivariate binary logistic regression analysis. PLT_{bf}, baseline platelet count before chemotherapy; IL-10 (Dichotomized), dichotomous variable of interleukin-10 level (1 = IL-10 > 131.20 r margin of the liver/spleeμmol/L). For the pre-chemotherapy model, the initial variables entered included: IL-10, platelet count, ferritin, IFN-γ, sCD25, lymphocyte count, and neutrophil count.

Table 4 ROC Analysis of the Combined Predictive Model Using Pre-Chemotherapy Platelet Count and IL-10

Variable	AUC	AUC 95% CI	P value	Cut-Off Value	Sensitivity (%)	Specificity (%)	Youden's Index
Logit P	0.822	0.715–0.930	<0.001	>1.215	81.25%	75.00%	0.563

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Table 5 Pre- and Post-Treatment Parameters: Refractory vs Sensitive Groups

Variable	Group	Wrs	BL	3day	6day	9day	14day
PLT (10^9/L)	R	27.50 (9.50, 37.75)	41.50 (23.00, 59.50)	45.00 (21.50, 77.25)	41.50 (20.00, 125.00)	92.50 (42.25, 128.00)	197.18±112.91
	S	60.00 (31.00, 106.00)	91.00 (42.00, 168.00)	147.00 (68.25, 259.25)	221.00 (90.00, 389.00)	235.00 (125.00, 417.00)	303.27±157.86
	P	0.003	0.001	<0.001	<0.001	0.002	0.037
LY (10^9/L)	R	0.96 (0.38, 1.46)	1.20 (0.47, 4.22)	0.50 (0.34, 0.83)	0.63 (0.29, 1.00)	0.54 (0.40, 0.84)	0.88 (0.64, 1.42)
	S	0.92 (0.42, 1.83)	1.23 (0.61, 2.37)	0.94 (0.61, 1.86)	1.20 (0.68, 2.11)	1.59 (0.89, 2.36)	1.81 (1.12, 2.91)
	P	0.72	0.82	0.003	0.001	0.001	0.005
TC (mmol/L)	R	2.25 (1.81, 2.95)	2.46 (2.02, 3.26)	3.54±1.05	3.67±0.75	4.30±1.62	4.88±1.99
	S	2.73 (1.87, 3.59)	3.05 (2.38, 3.79)	4.32±1.47	4.76±1.02	5.20±11.32	5.78±1.61
	P	0.22	0.15	0.11	0.007	0.16	0.15
LDL (mmol/L)	R	0.83 (0.13, 1.24)	1.20 (0.13, 1.34)	1.55 (1.35, 2.18)	2.07±0.55	2.53±0.96	2.46±1.22
	S	0.71 (0.20, 1.27)	1.13 (0.39, 2.05)	2.31 (1.47, 2.76)	2.82±0.94	3.21±1.13	3.28±1.21
	P	0.79	0.48	0.07	0.024	0.18	0.0715
Alb (g/L)	R	29.28±5.68	30.86±4.84	32.16±5.31	33.62±5.91	36.01±7.77	38.16±7.60
	S	29.18±3.97	31.59±4.54	34.85±4.05	37.20±4.72	37.65±4.57	40.38±4.15
	P	0.94	0.56	0.049	0.039	0.54	0.25
	Group	Wrs	BL	3day	6day	9day	14day
AST (U/L)	R	349.90 (224.20, 541.20)	315.20 (101.10, 537.40)	165.20 (70.10, 598.50)	155.80 (74.98, 914.33)	43.65 (21.10, 112.25)	30.40 (25.30, 62.20)
	S	381.10 (164.60, 681.90)	183.80 (80.10, 454.10)	146.20 (71.95, 319.90)	61.75 (34.80, 98.08)	42.60 (28.80, 107.65)	31.85 (27.45, 45.85)
	P	0.87	0.37	0.45	0.020	0.63	0.71
ALT (U/L)	R	212.00 (118.30, 371.60)	137.00 (63.50, 264.90)	93.40 (43.60, 441.70)	176.15 (67.05, 384.08)	98.60 (41.88, 229.45)	72.40 (35.90, 27.90)
	S	224.00 (94.00, 403.00)	129.10 (49.70, 288.50)	142.20 (72.80, 274.10)	78.60 (35.33, 166.50)	61.30 (34.70, 144.10)	47.50 (27.25, 71.33)
	P	0.78	0.78	0.88	0.043	0.43	0.17
LDH (U/L)	R	1112.00 (744.00, 1793.00)	995.00 (682.00, 1436.00)	808.00 (404.00, 1306.00)	1176.50 (486.50, 1927.75)	308.00 (209.00, 820.00)	274.00 (188.00, 311.00)
	S	1156.00 (843.00, 1913.00)	861.00 (540.00, 1687.00)	529.50 (363.75, 1240.50)	285.00 (218.00, 764.00)	322.00 (220.50, 417.75)	245.00 (201.00, 337.50)
	P	0.81	0.47	0.38	0.004	0.91	0.95
SF (ng/mL)	R	5215.70 (2223.20, 10,016.90)	6246.90 (2134.50, 12,013.80)	4582.40 (2927.58, >15,000)	4903.90 (612.55, 11,333.05)	1007.00 (547.30, 6341.40)	494.05 (355.38, 1654.80)
	S	4321.50 (1785.00, 11,191.50)	6236.20 (1833.70, >15,000)	1687.75 (925.78, 5717.95)	1305.95 (659.03, 5733.13)	976.50 (409.28, 2124.43)	456.50 (214.30, 806.50)
	P	0.19	0.91	0.036	0.34	0.78	0.34

Note: Data are presented as median (interquartile range) / n (%) / mean ± standard deviation.

Abbreviations: Wrs, worst value before chemotherapy; BL, baseline value (the last measurement before chemotherapy initiation); 3day, value on day 3 of chemotherapy; 6day, value on day 6 of chemotherapy; 9day, value on day 9 of chemotherapy; 14day, value on day 14 of chemotherapy. PLT, platelets; LY, lymphocytes; TC total cholesterol; LDL, low density lipoprotein; Alb, albumin; AST, glutamic oxalacetic transaminase; ALT, glutamic-pyruvic transaminase; LDH, lactate dehydrogenase; SF, serum ferritin.

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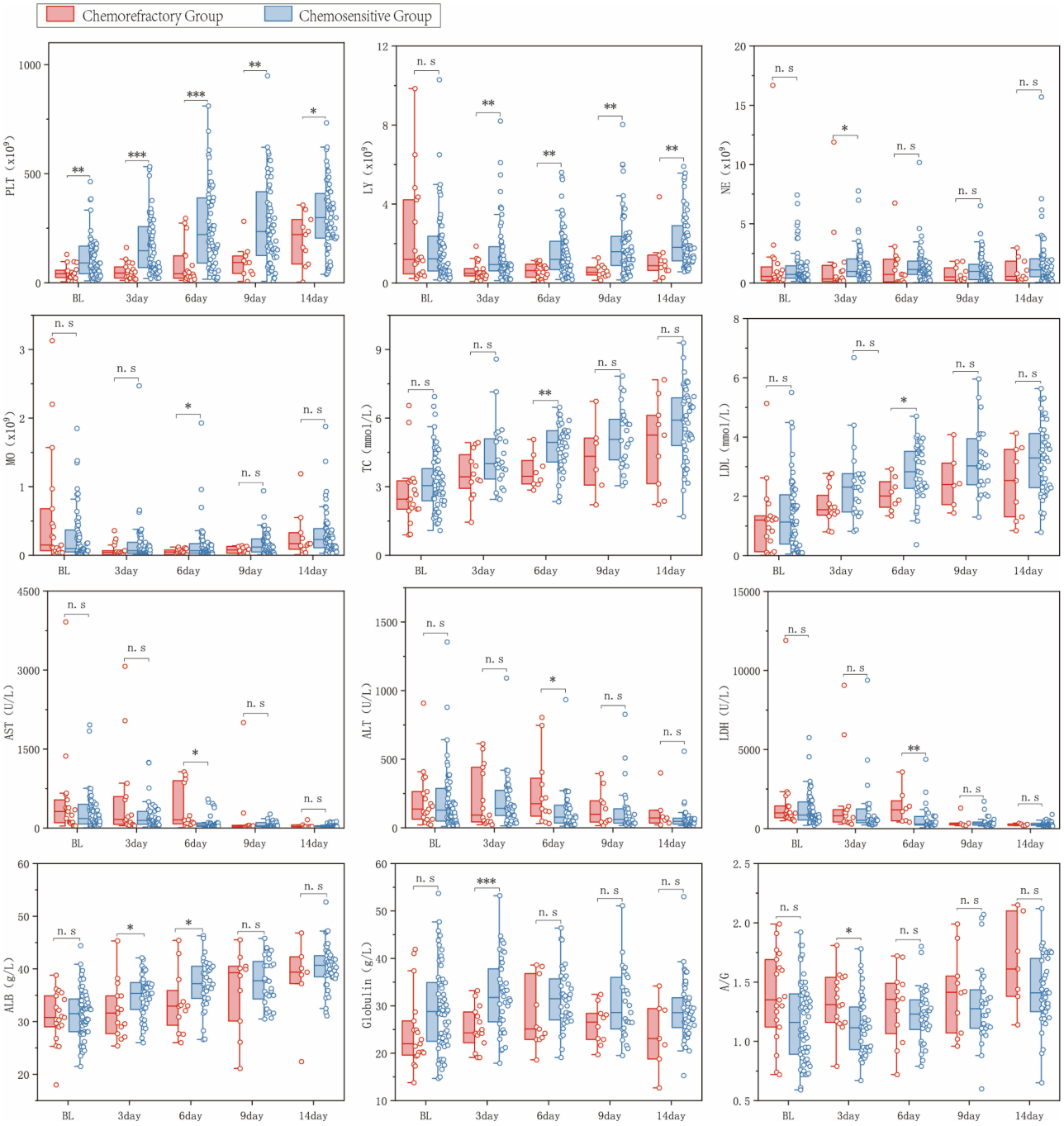

Figure 1 Comparison Between the Chemorefractory and Chemosensitive Groups at Different Treatment Stages.
Notes: The figure presents box plots (left) with overlaid scatter plots (right) comparing laboratory parameters between the Chemorefractory Group and Chemosensitive Groups before and after treatment. Data are presented as median with inter-quartile range. *P < 0.05, **P < 0.01, ***P < 0.001, n.s. (not significant), P ≥ 0.05.

Examination of longitudinal ferritin changes revealed a time-dependent effect: while baseline levels were comparable between groups, a significant difference emerged on day 3 post-chemotherapy (P =0.046), with higher levels in the Chemorefractory group. This difference was not sustained at later time points, suggesting that day 3 ferritin may serve as an adjunctive early indicator despite its transient nature.

Interestingly, analysis of liver function-related indicators at various time points revealed that on Day 6 of chemotherapy, the refractory group exhibited a distinct liver function profile characterized by lower total cholesterol, LDL, and albumin, alongside elevated AST and LDH (Table 5): total cholesterol 3.67 mmol/L vs 4.76 mmol/L (P=0.007), low

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Table 6 Predictive Performance of Various Indicators for Etoposide-Resistant HLH

Variable	AUC	AUC 95% CI	P value	Cut-Off Value	Sensitivity (%)	Specificity (%)	Youden's Index
PLT_{wrs}	0.727	0.604–0.850	0.003	<31.50 x10^9/L	70.00	74.58	0.446
PLT_{bf}	0.759	0.649–0.869	<0.001	<55.50 x10^9/L	75.00	69.48	0.445
PLT_{3day}	0.822	0.723–0.921	<0.001	<109.50 x10^9/L	94.44	56.90	0.513
PLT_{6day}	0.814	0.701–0.927	<0.001	<55.50 x10^9/L	66.67	88.14	0.548
PLT_{9day}	0.808	0.683–0.932	0.002	<145.00 x10^9/L	90.00	72.88	0.629
PLT_{14day}	0.688	0.541–0.835	0.049	<336.50 x10^9/L	90.91	44.07	0.350
$PLT\text{-}d_{3day}$	0.717	0.598–0.837	0.006	<31.50 x10^9/L	88.89	55.17	0.441
$PLT\text{-}d_{6day}$	0.754	0.628–0.879	0.001	<32.00 x10^9/L	72.22	81.36	0.536
$PLT\text{-}d_{9day}$	0.731	0.585–0.876	0.020	<69.50 x10^9/L	80.00	69.49	0.495
$PLT\text{-}d_{14day}$	0.560	0.399–0.720	0.53	<234.00 x10^9/L	90.91	36.21	0.271
WBC_{3day}	0.702	0.537–0.866	0.010	<1.200 x10^9/L	61.11	82.76	0.439
WBC_{6day}	0.697	0.550–0.845	0.014	<1.705 x10^9/L	64.71	72.88	0.376
WBC_{9day}	0.804	0.691–0.918	0.002	<2.365 x10^9/L	90.00	69.49	0.595
WBC_{14day}	0.700	0.504–0.895	0.037	<1.485 x10^9/L	54.55	93.22	0.478
LY_{3day}	0.731	0.601–0.861	0.003	<0.5500 x10^9/L	66.67	81.03	0.477
LY_{6day}	0.758	0.647–0.869	0.001	<1.1700 x10^9/L	100.00	50.85	0.509
LY_{9day}	0.836	0.736–0.936	0.005	<0.9150 x10^9/L	90.00	74.58	0.646
LY_{14day}	0.767	0.604–0.929	0.006	<1.5555 x10^9/L	90.91	59.32	0.502
NE_{wrs}	0.610	0.459–0.763	0.14	<0.3900 x10^9/L	65.00	67.80	0.328
NE_{3day}	0.679	0.516–0.843	0.022	<0.2650 x10^9/L	50.00	93.10	0.431
MO_{6day}	0.690	0.555–0.825	0.018	<0.1250 x10^9/L	100.00	37.29	0.373
TP_{3day}	0.762	0.625–0.898	0.003	<59.35 g/L	73.33	80.00	0.533
TP_{6day}	0.696	0.503–0.890	0.044	<58.90 g/L	58.33	91.43	0.498
TP_{14day}	0.638	0.413–0.863	0.24	<60.40 g/L	42.86	91.30	0.342
Alb_{3day}	0.690	0.520–0.860	0.031	<35.05 g/L	80.00	57.50	0.375
Alb_{6day}	0.717	0.522–0.912	0.026	<33.20 g/L	66.67	85.71	0.524
Glo_{3day}	0.781	0.658–0.904	0.002	<30.20 g/L	86.67	52.50	0.492
A/G_{3day}	0.739	0.592–0.886	0.007	>1.14	86.67	60.00	0.467
TC_{6day}	0.813	0.669–0.956	0.006	<3.935 mmol/L	75.00	78.38	0.534
LDL_{6day}	0.757	0.602–0.911	0.024	<3.030 mmol/L	100.00	45.95	0.460
ALT_{6day}	0.697	0.522–0.872	0.043	<209.80 U/L	50.00	86.11	0.361
AST_{6day}	0.727	0.530–0.924	0.020	<111.70 U/L	66.67	83.33	0.500
LDH_{6day}	0.835	0.707–0.962	0.004	>405.00 U/L	100.00	67.74	0.677
TBA_{6day}	0.763	0.614–0.912	0.007	>11.30 mmol/L	83.33	65.71	0.490
SF_{3day}	0.746	0.577–0.914	0.036	>1535.00 g/mL	100.00	50.00	0.500
$IL\text{-}10_{wrs}$	0.720	0.562–0.878	0.008	>131.22 umol/L	81.25	65.38	0.466
$Spleen_{wrs}$	0.679	0.535–0.823	0.024	>20.50 mm	72.22	63.64	0.359
$sCD25_{wrs}$	0.672	0.510–0.834	0.046	>12,528.00 U/mL	75.00	60.00	0.350

Notes: Data are presented as median (interquartile range) or n (%).

Abbreviations: Wrs, worst value before chemotherapy; Bf, last value before chemotherapy; 3day, value on day 3 of chemotherapy; 6day, value on day 6 of chemotherapy; 9day, value on day 9 of chemotherapy; 14day, value on day 14 of chemotherapy; -d, difference between pre- and post-chemotherapy values (post- minus pre-); PLT, platelets; WBC, white blood cell; LY, lymphocytes; NE, neutrophil count; MO, monocyte count; TP, total protein; Alb, albumin; Glo, globulin; A/G, albumin-to-globulin ratio; TC total cholesterol; LDL, low density lipoprotein; ALT, glutamic-pyruvic transaminase; AST, glutamic oxalacetic transaminase; LDH, lactate dehydrogenase; TBA, total bile acid; SF, serum ferritin.

density lipoprotein (LDL) 2.07 vs 2.82 mmol/L (P=0.024), albumin (Alb) 33.6 g/L vs 37.2 g/L (P=0.039); AST 155.80 U/L vs 61.75 U/L (P=0.020), lactate dehydrogenase (LDH) 1176.50 U/L vs 285.00 U/L (P=0.004). ROC curve analysis further confirmed that total cholesterol (AUC=0.813, 95% [0.669–0.956]), LDL (AUC=0.757, 95% [0.602–0.911]), albumin (AUC=0.717, 95% [0.522–0.912]), LDH (AUC=0.835, 95% [0.707–0.962]) demonstrated strong discriminatory ability for chemotherapy-refractory HLH (Table 6). Notably, AST (AUC=0.727, 95% [0.530–0.924]) showed superior

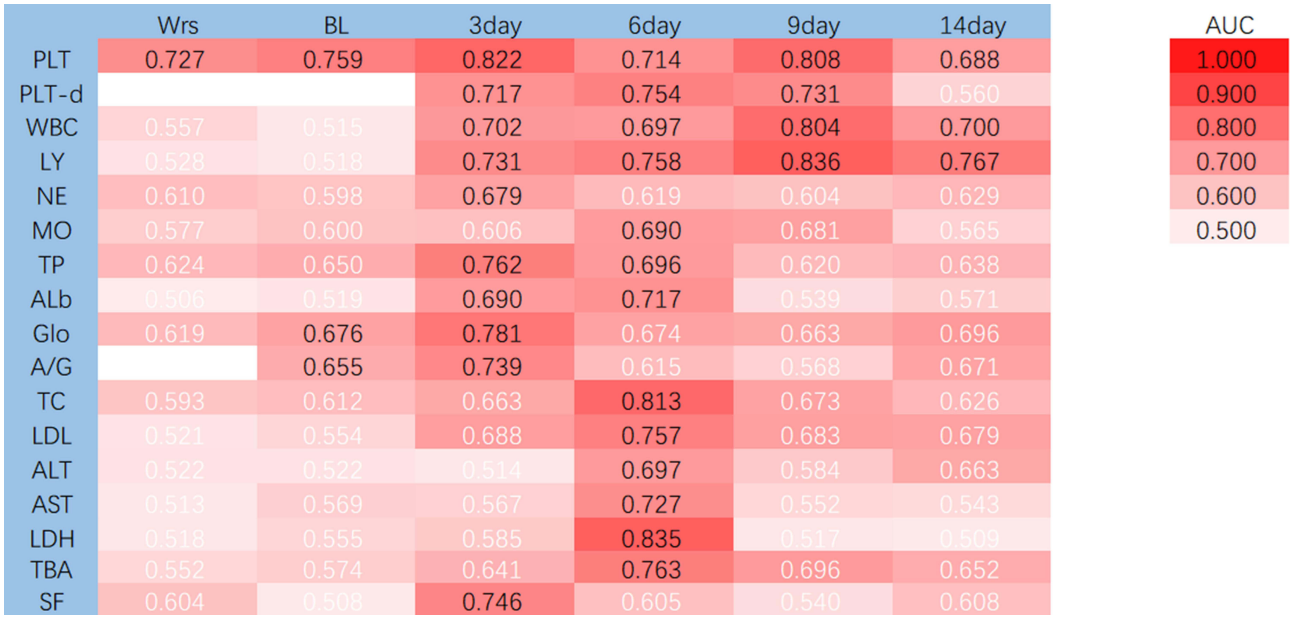

Figure 2 Heatmap of the Area Under the Curve (AUC) for Various Indicators.
Note: White font color indicates P ≥ 0.05.
Abbreviations: Wrs, worst value before chemotherapy; Bf, last value before chemotherapy; 3day, value on day 3 of chemotherapy; 6day, value on day 6 of chemotherapy; 9day, value on day 9 of chemotherapy; 14day, value on day 14 of chemotherapy; -d, difference between pre- and post-chemotherapy values (post- minus pre-).

discriminatory capacity compared to ALT (AUC=0.697, 95% [0.522–0.872]), currently the standard for HLH treatment efficacy and disease assessment.

IL-10 and 7-Day Mortality: Early Warning

Studies revealed (Tables 1, 2 and 5) IL-10 levels of 530.47 μmol/L vs 97.85 μmol/L (P=0.024), sCD25 17,998.50 U/mL vs 10023.31 U/mL (P=0.046), spleen volume 26.50 mm vs 17.00 mm (P=0.022), Pre-chemotherapy worst platelet count

Table 7 Binary Logistic Regression Analysis of Day 3 Platelet and Lymphocyte Counts for Predicting Chemorefractoriness

Variable	β Coefficient	Standard Error	Waldχ^2	*P value*	OR	95% CI for OR
PLT_{3day}	0.018	0.007	6.655	0.010	1.019	1.004–1.033
LY_{3day}	0.926	0.613	2.281	0.13	2.526	0.759–8.405

Note: Data are results of multivariate binary logistic regression analysis. For the day-3 model, the initial variables included: day 3 platelet count, day 3 lymphocyte count, day 3 ferritin, and day 3 neutrophil count, day 3 total protein; day 3 albumin; day 3 globulin, day 3 albumin-to-globulin ratio; day 3 total cholesterol; day 3 serum ferritin.
Abbreviations: PLT_{3day}, platelet count on day 3 of chemotherapy; LY_{3day}, lymphocyte count on day 3 of chemotherapy; OR, odds ratio; CI, confidence interval.

Table 8 Binary Logistic Regression Analysis of Pre-Chemotherapy IL-10 and Day 3 Platelet/Lymphocyte Counts for Predicting Chemorefractoriness

Variable	β Coefficient	Standard Error	Waldχ^2	*P value*	OR	95% CI for OR
PLT_{3day}	0.015	0.008	3.964	0.046	1.015	1.000–1.031
LY_{3day}	1.152	0.762	2.285	0.13	3.165	0.710–14.104
dich. IL-10	−1.479	0.819	3.257	0.07	0.228	0.046–1.136

Notes: PLT_{3day}, platelet count on day 3 of chemotherapy; LY_{3day} lymphocyte count on day 3 of chemotherapy; dich. IL-10, interleukin-10 level categorized as high (>131.20 μmol/L) or low (≤131.20 μmol/L), assigned a value of 1 or 0, respectively.

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Table 9 ROC Analysis of Day 3 Platelet Count, Lymphocyte Count, and Combined Predictive Model

Variable	AUC	AUC 95% CI	*P value*	Cut-off value	Sensitivity (%)	Specificity (%)	Youden's Index
Logit (P1)	0.845	0.752–0.938	<0.001	<0.9050	88.89%	70.69%	0.596
Logit (P2)	0.880	0.796–0.963	<0.001	<1.8350	100.00%	66.67%	0.667

Notes: Logit (P1), prediction model constructed with day 3 lymphocyte count and platelet count; Logit (P2), prediction model constructed with peak pre-chemotherapy IL-10 level, day 3 lymphocyte count, and platelet count.

27.50×10^9/L vs 60.00×10^9/L (P=0.003) and baseline platelet count 41.50×10^9/L vs 91.00×10^9/L (P=0.001), among others, showed pre-chemotherapy differences between the two groups; To determine whether these changes correlated with disease severity, we regrouped the samples: patients dying within 1 week were classified as the early death group (n=6), and patients surviving beyond one week as the non-early death group (73 cases). Comparing these indicators between groups (Table 10) revealed that the median IL-10 level in the early death group was >2500 μmol/L, significantly higher than the median IL-10 level of 107.44 μmol/L in the non-early death group (P=0.034), with a single-marker AUC of 0.763 (sensitivity 83%, specificity 77%) (Table 11), indicating elevated IL-10 as an independent risk factor for early mortality. One-week mortality outcomes correlated with early marker changes. Early screening—particularly within the first week, even before chemotherapy and 1–3 days post-treatment—proves beneficial for assessing patient condition and treatment efficacy.

Validation of Predictive Models and Clinical Tools

Preliminary data analysis revealed missing data in the chemotherapy-refractory group due to early mortality. To minimize experimental errors caused by missing data while achieving the research objective of early prediction of etoposide-based protocols efficacy, the time points of pre-chemotherapy and Day 3 post-chemotherapy were selected for the predictive model. Following data screening, IL-10 + platelets were ultimately incorporated into the pre-therapy model. Of note, although IFN-γ and sCD25 showed associations in univariate analyses, their explanatory power was weaker than that of IL-10 after adjusting for confounders; to maintain model parsimony and avoid overfitting, they were excluded from the final model. Analysis via ROC curves (Figure 3), calibration curves (Figure 4), and decision curves (Figure 5) demonstrated optimal predictive value for HLH chemotherapy resistance (AUC = 0.822, 95% [0.715–0.930]), confirming the model's stability and clinical significance. With excellent stability in 1000 Bootstrap resampling iterations (mean AUC 0.761 ± 0.083) (Figure 6).

Table 10 Analysis of Pre-Chemotherapy Indicators and Their Association with 1-Week Mortality

Variable	Early Mortality Group (n=6)	Non-Early Mortality Group (n=73)	P value
Spleen size (mm)	30.00 (0.00, 48.00)	19.00 (0.00, 30.00)	0.409
IL-10 (umol/L)	>2500.00 (296.87, >2500.00)	107.44 (31.98, 371.22)	0.034
sCD25 (U/mL)	39,360.00 (12,560.00, -)	12,230.00 (5076.00, 21,700.00)	0.102
PLT_{wrs} (x10^9/L)	22.50 (10.25, 43.00)	48.00 (26.00, 100.00)	0.084
PLT_{bf} (x10^9/L)	42.00 (17.75, 64.75)	76.00 (34.50, 150.00)	0.076

Notes: Data are presented as median (interquartile range), n (%), or mean ± standard deviation; Spleen size was measured by ultrasonography as the distance from the inferior margin of the spleen to the costal margin.
Abbreviations: Wrs, worst value before chemotherapy; Bf, last value before chemotherapy.

Table 11 Predictive Performance of IL-10 for Early Mortality

Variable	AUC	AUC 95% CI	P value	Cut-Off Value	Sensitivity (%)	Specificity (%)	Youden's Index
$IL\text{-}10_{wrs}$	0.763	0.491–1.000	<0.001	>388.3 1umol/L	83.33	77.42	0.608

Note: Data are presented as median (interquartile range) or n (%) as appropriate.
Abbreviation: IL-10wrs, peak interleukin-10 level before chemotherapy.

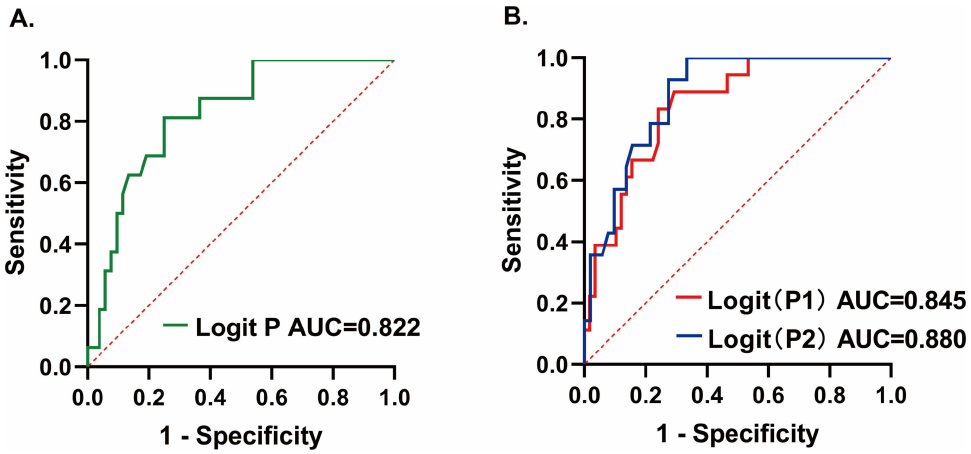

Figure 3 Receiver Operating Characteristic (ROC) Curves of the Pre-Chemotherapy and Day-3 Models. (**A**) Green curve: ROC for the Pre-chemotherapy Model (Maximum IL-10 and Baseline Platelets). (**B**) Red curve [Logit (P1)]: ROC for the Day-3 Model (Platelet and Lymphocyte Counts). Blue curve [Logit (P2)]: ROC for the Comprehensive Combined Model (Dichotomized IL-10, Day-3 PLT and LY Counts).

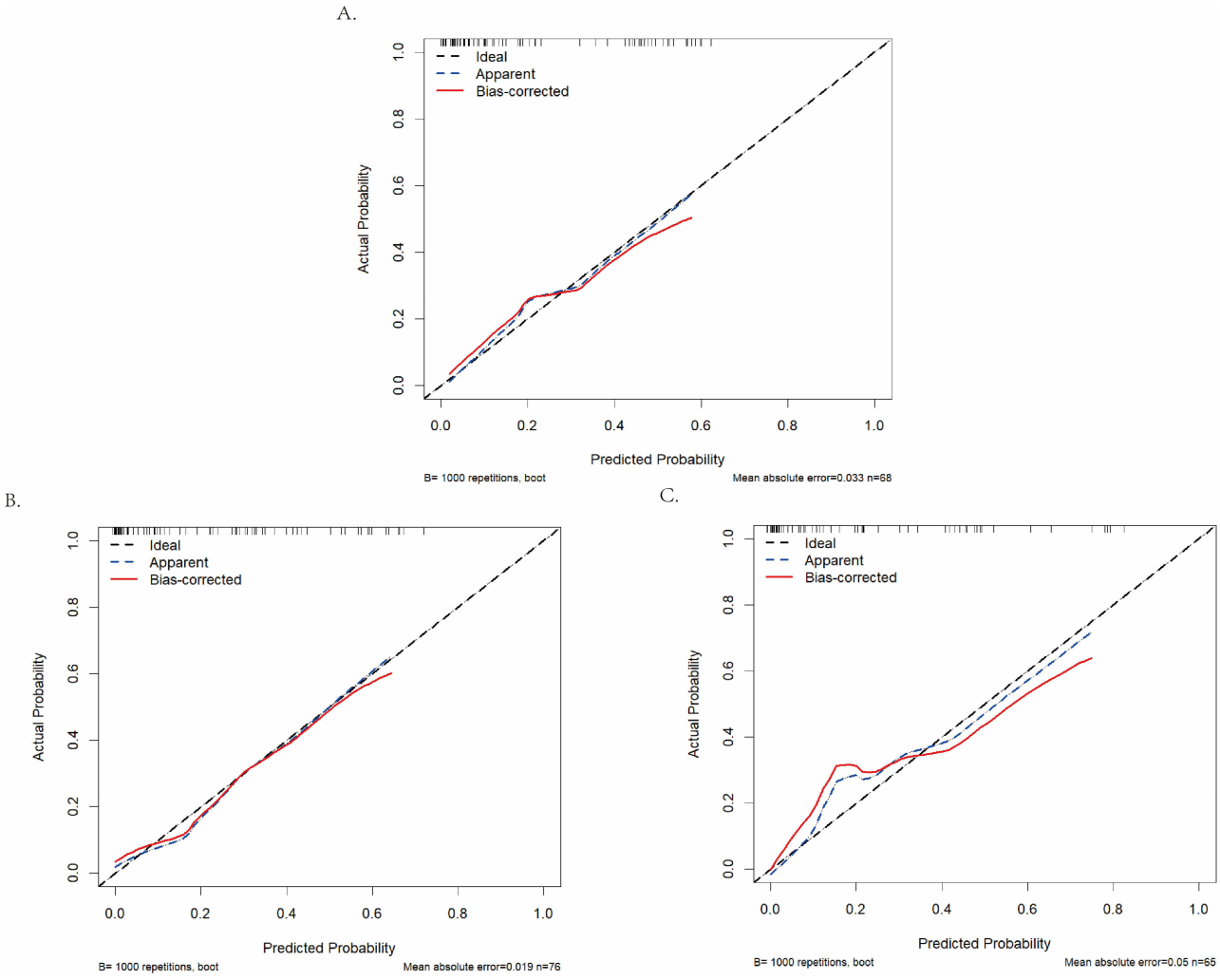

Figure 4 Calibration Curves of the Predictive Models. (**A**) Calibration Curve of the Pre-chemotherapy Combined Model [Maximum IL-10 + Baseline Platelet Count]. (**B**) Calibration Curve of the Day 3 Combined Model [Platelet Count + Lymphocyte Count]. (**C**) Calibration Curve of the Comprehensive Combined Model [Dichotomized IL-10 + Day 3 PLT and LY Counts].

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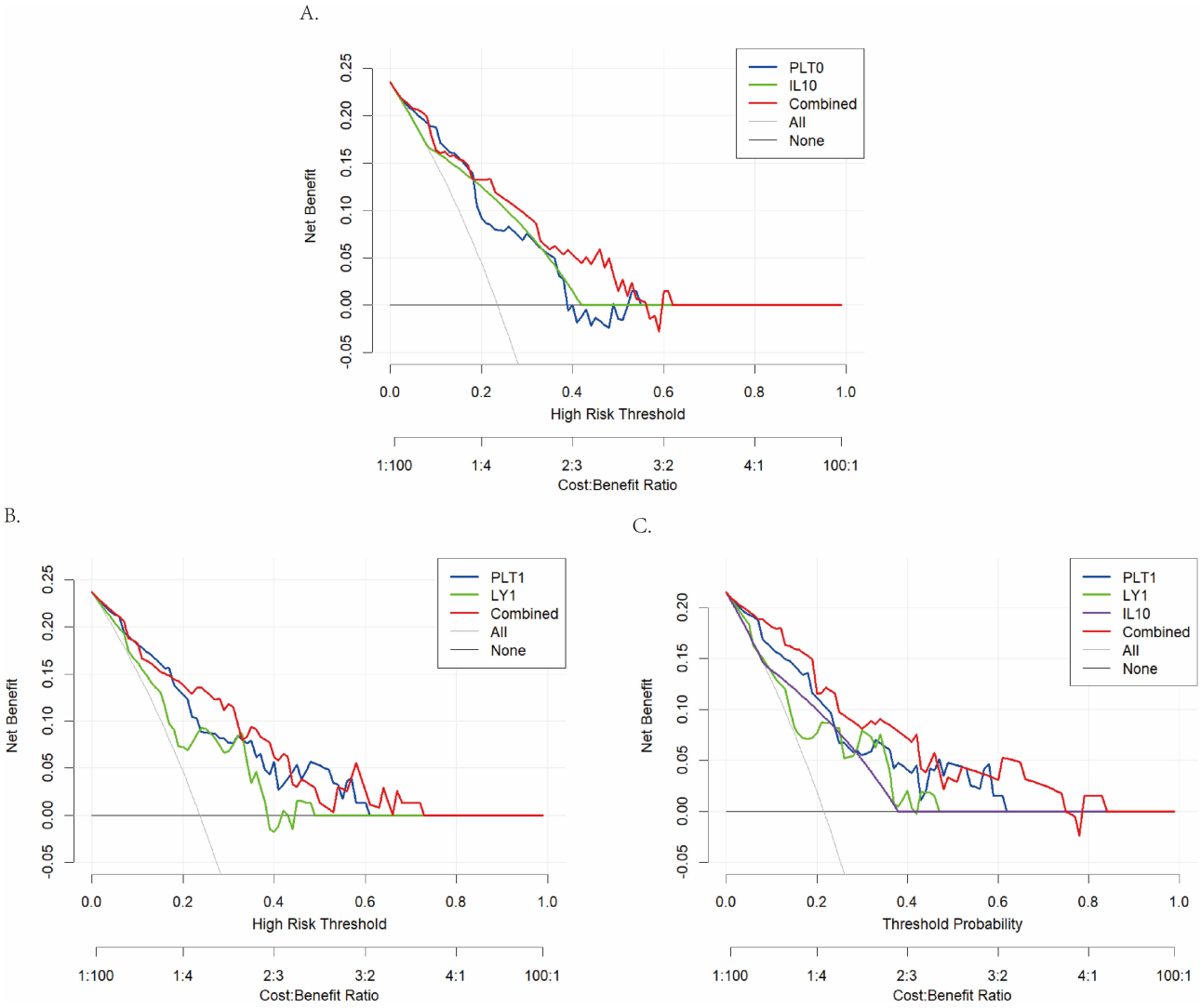

Figure 5 Decision Curve Analysis (DCA) of the Predictive Models. (**A**) Decision Curve Analysis of the Pre-chemotherapy Combined Model [Maximum IL-10 + Baseline Platelet Count]. (**B**) Decision Curve Analysis of the Day 3 Combined Model [Platelet Count + Lymphocyte Count]. (**C**) Decision Curve Analysis of the Comprehensive Combined Model [Dichotomized IL-10 + Day 3 PLT and LY Counts].

The combined model of Day 3 post-chemotherapy platelets (PLT) and lymphocytes (LY) demonstrated good predictive performance (AUC = 0.845, 95% [0.752–0.938] sensitivity 88.89%, specificity 70.69%) (Table 9). Considering IL-10's predictive value for refractory patients, particularly those at early mortality risk, a new day-3 model (platelet + lymphocyte ± IL-10) was constructed by adding pre-chemotherapy IL-10 > 131.2 μmol/L (AUC = 0.72, 95% [0.562–0.878]) to the prior model (PLT + LY prediction model) (Table 8). This enhanced predictive performance (AUC = 0.880, 95% [0.796–0.963], sensitivity 100%, specificity 66.67%) (Table 9). The decision curve demonstrated significant net benefit within the 0.1–0.7 threshold range, with excellent stability after 1000 Bootstrap resamples (mean AUC 0.837 ± 0.085) (Figure 6).

Pre-therapy model = 0.947 + 0.014 × XPLT-bf - 1.578 × XIL-10 binary classification

Day-3 model = −0.377 + 0.015 × XPLT-3day + 1.152 × XLY-3day - 1.479 × XIL-10 binary classification

To visualize the predictive model for clinical application, a model column chart integrating the three indicators was created using R Studio (Figure 7). This enables real-time bedside risk calculation, facilitating implementation in primary care settings.

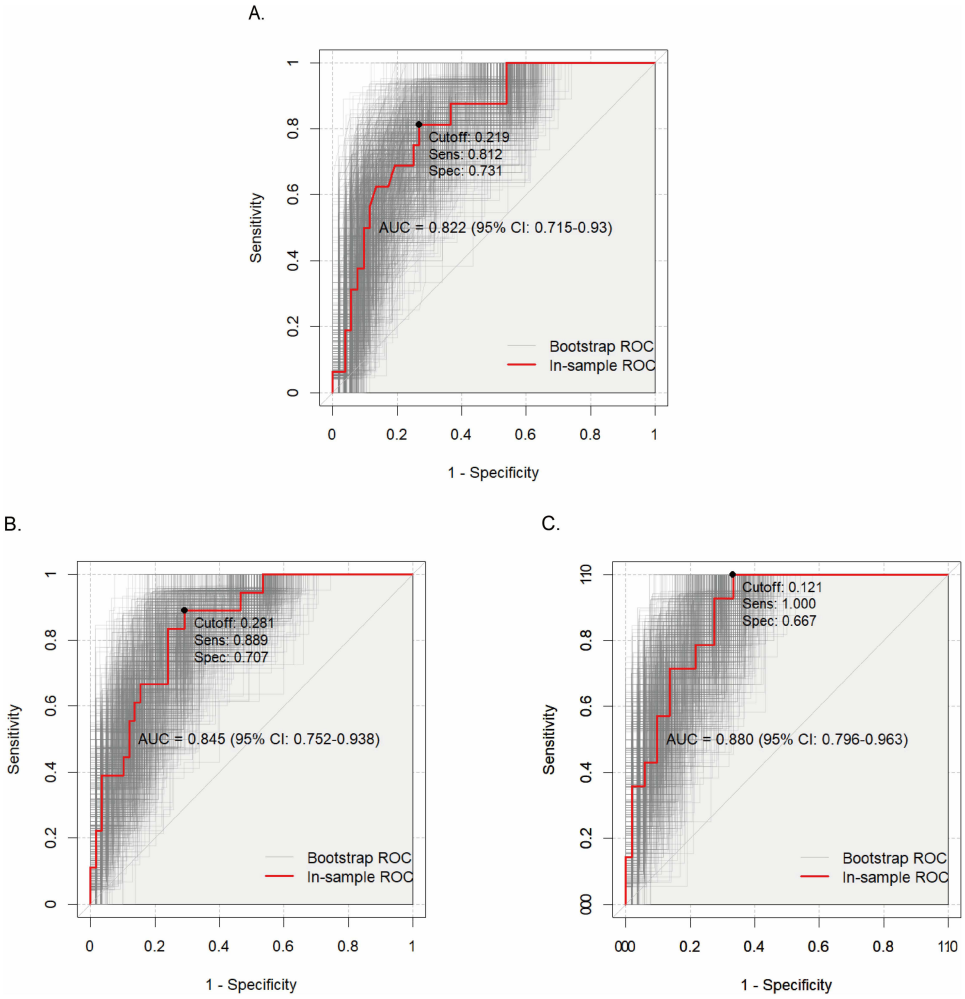

Figure 6 Internal Validation of the Predictive Models Using Bootstrap Resampling. (**A**) Internal Validation ROC Curve of the Pre-chemotherapy Model (Maximum IL-10 and Baseline Platelets). (**B**) Internal Validation ROC Curve of the Day-3 Model (Platelet and Lymphocyte Counts). (**C**) Internal Validation ROC Curve of the Comprehensive Combined Model (Dichotomized IL-10, Day-3 PLT and LY Counts). In each panel, the red curve represents the ROC curve of the training set, and the gray curves represent ROC curves generated from each bootstrap sample. The displayed AUC value, 95% confidence interval (95% CI), optimal cutoff, sensitivity (Sens), and specificity (Spec) correspond to the parameters of the training set ROC curve (red).

Discussion

Although etoposide-based protocols remain the primary cornerstone treatment for HLH. In children,[18] primary resistance[19] and early mortality persist as stubborn challenges in clinical practice. Our study observed a significantly longer interval from onset to chemotherapy in the refractory group, attributed to a combination of disease severity, early diagnostic challenges, and referral pathways. However, this starkly reveals a key paradox: the sickest children, who require urgent treatment the most, are paradoxically those most likely to experience treatment delays and miss the optimal therapeutic window.

Based on continuous observation of a retrospective cohort of 79 pediatric patients, this study first identified that pre-chemotherapy IL-10 > 131.2 μmol/L combined with platelets < 55.5 × 10^9/L independently predicted resistance risk, with an AUC of 0.822. Furthermore, incorporating IL-10 >131.2 μmol/L (AUC=0.72, 95% [0.562–0.878]) into the two dynamic indicators on Day 3 of chemotherapy— "platelet count <109.50×10^9/L" and "lymphocyte count <0.55×10^9/L"—elevated model performance to 0.880. Both Bootstrap internal validation and decision curve analysis demonstrated stable clinical net benefit. This study compressed the traditional 2-week efficacy evaluation window to 72 hours, providing a feasible pathway for early precision intervention. Importantly, all parameters included in our models, including IL-10, platelet count, and lymphocyte count, are routine laboratory tests. Platelet and lymphocyte counts are

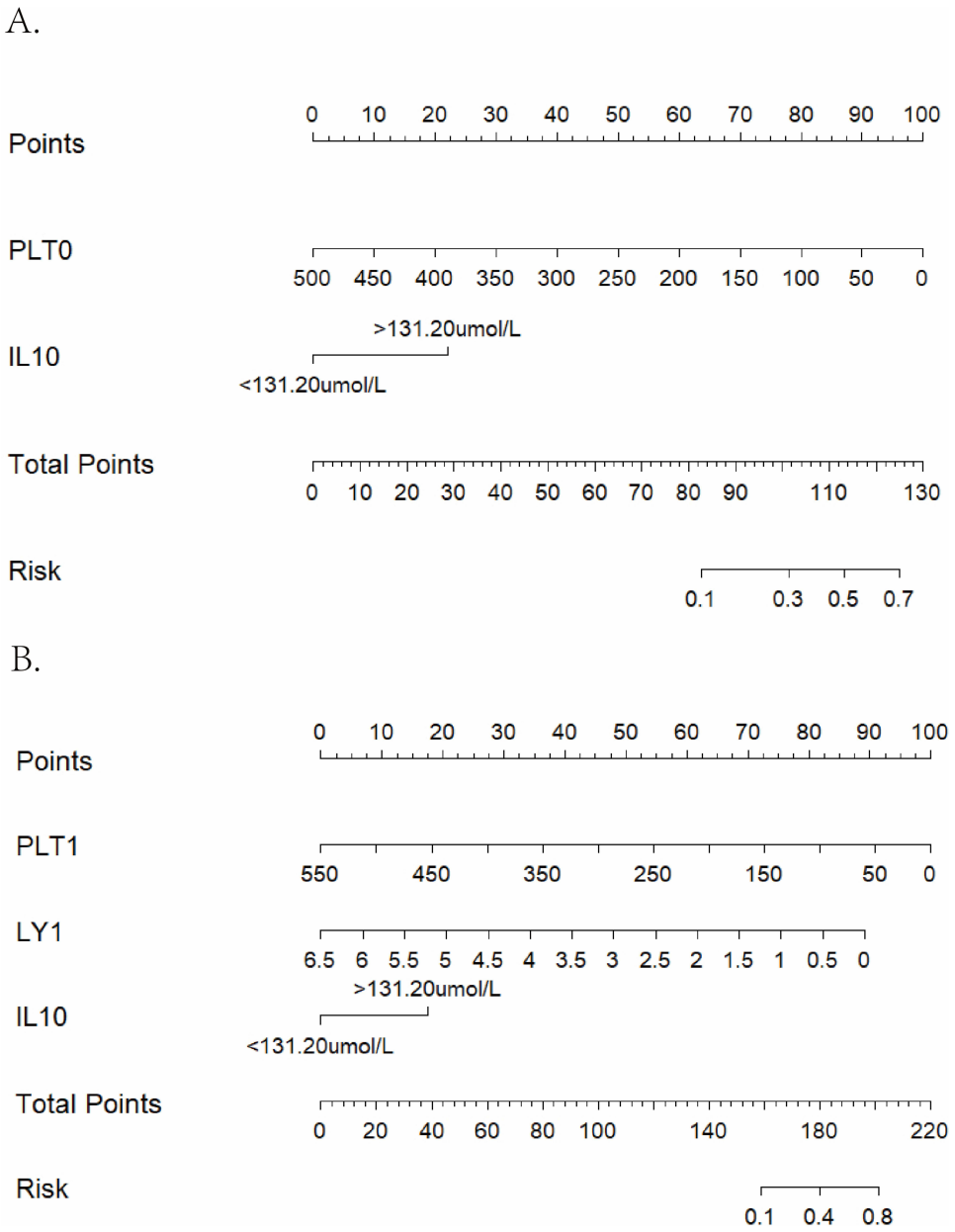

Figure 7 Nomogram of Prediction Models Constructed with Pre-Chemotherapy and Day 3 Indicators. (**A**) Nomogram of the pre-chemotherapy combined model [maximum IL-10 + baseline platelet count]. (**B**) Nomogram of the comprehensive combined model [dichotomized IL-10 + day 3 PLT and LY counts].

standard components of the complete blood count, universally available in all hospitals. IL-10 measurement, while not available in every primary care setting, is routinely performed in most tertiary referral centers where HLH patients are typically managed. For settings where IL-10 is unavailable, the combination of day 3 platelet and lymphocyte counts alone offers a practical alternative with robust predictive performance (AUC = 0.845).

Elevated IL-10 level is an adverse prognostic factor in HLH.[20,21] Notably, elevated IL-10 not only precedes classic pro-inflammatory factors like IFN-γ and TNF-α but also exhibits a significant negative correlation with platelet count. This suggests it may not merely function as a negative feedback protector[22] but could amplify the inflammatory storm by inhibiting NK cell cytotoxicity and antigen presentation. This finding strongly aligns with Tang et al's[14] mouse model demonstrating IL-10/IL-18 synergistic induction of lethal HLH, providing clinical screening rationale for future IL-10 blockade therapy.

The prognostic value of IL-10 extended beyond treatment resistance to early mortality. In our cohort, patients who died within the first week of treatment had markedly higher IL-10 levels (>2500 μmol/L) compared to those who survived (107.44 μmol/L, P=0.034), with IL-10 alone predicting 7-day mortality with an AUC of 0.763. This finding reinforces the central role of IL-10 as a marker of disease severity in pediatric HLH, consistent with previous studies identifying elevated IL-10 as an adverse prognostic factor in both adult and pediatric populations.[20,21] The observation that IL-10 predicts both etoposide resistance and early mortality suggests that extreme IL-10 elevation may identify a subset of patients at imminent risk of death who require immediate, aggressive intervention beyond standard therapy. This further supports the clinical utility of IL-10 as a key biomarker for ultra-early risk stratification.

Equally noteworthy are platelet dynamics. A lower platelet count was inversely associated with mortality in secondary HLH.[23] Although bone marrow hemophagocytosis did not differ between refractory and responsive cases—contrary to the traditional belief that thrombocytopenia results from inadequate marrow production—our study shifts the focus to peripheral mechanisms.[16,24,25] This is further supported by the markedly restricted platelet recovery observed by day 3, suggesting peripheral consumption (eg, in microthrombosis or DIC) as the primary mechanism rather than bone marrow failure. Combined with the fact that platelet α-granule PF4 further amplifies inflammation,[15] we hypothesize that IL-10-PF4-platelet interactions form a vicious cycle accelerating the storm, providing theoretical support for combined antiplatelet or anticoagulant targeted therapy.

Concurrently, lymphocytes exhibited a "deep trough" as early as day 3 of chemotherapy, persisting until day 14. This not only reflects etoposide's cytotoxic effects but may also indicate limited hematopoietic stem cell repair capacity or underlying DNA repair defects. This early bone marrow suppression signal aligns closely with the "7-day recovery delay" pattern reported by Verkamp et al[13] Dziedzic et al reported that low lymphocyte count at diagnosis predicts mortality in adult HLH, consistent with our finding that lymphocyte dynamics serve as important prognostic indicators in children. Building on these observations, our study further demonstrates that lymphocyte assessment at a specific early time point—day 3 of chemotherapy—provides valuable prognostic information for identifying children at risk of etoposide resistance. We observed that a day 3 lymphocyte count $<0.55\times10^9$/L strongly predicted treatment failure (AUC=0.731), extending the utility of lymphocyte monitoring from baseline assessment to the critical early treatment window.[26]

Etoposide exerts its therapeutic effect in HLH by selectively ablating activated T cells,[18] which are the primary drivers of hyperinflammation and an important source of IL-10 production.[27] Consistent with this mechanism, the refractory group with poor outcomes in our cohort had significantly higher baseline IL-10 levels and exhibited more profound lymphocyte depletion by day 3 of chemotherapy, confirming that etoposide achieved its target inhibitory effect. Paradoxically, however, this effective target engagement did not translate into favorable outcomes but was instead associated with poor prognosis. One possible explanation is that treatment-induced rapid, large-scale T cell death may trigger tumor lysis syndrome-like inflammatory sequelae,[28] generating a secondary wave of inflammation that counteracts the therapeutic benefit. These proposed mechanisms remain speculative. Whether these associations reflect direct mechanistic interactions or merely mark a particularly severe disease phenotype warrants further investigation. These proposed mechanisms remain speculative. Whether these associations reflect direct mechanistic interactions or merely mark a particularly severe disease phenotype warrants further investigation.

At the metabolic level, this study found that refractory patients on day 6 of chemotherapy exhibited a distinct profile of lower total cholesterol, LDL, and albumin, accompanied by significantly higher AST and LDH. This combination represents not isolated laboratory abnormalities but a comprehensive decompensation signal of the "mitochondrial-liver-lipid metabolism axis" under acute inflammatory storm in HLH.[29,30] First, both cholesterol (especially LDL) and albumin are synthesized by hepatocytes. Severe hepatic dysfunction impairs serum total cholesterol and LDL synthesis, leading to rapid depletion.[9] Concurrently, capillary leakage and impaired hepatic synthesis further deplete albumin.[31,32] Second, AST is primarily localized in hepatocyte mitochondria, while ALT resides in the cytoplasm. When microthrombi and oxidative stress cause mitochondrial membrane rupture, AST elevation often exceeds that of ALT, forming a "mitochondrial-type" liver injury pattern.[33] Recent studies have collectively demonstrated that elevated levels of ALT and AST are associated with adverse clinical outcomes.[34,35] More specifically, an elevated AST/ALT ratio has been identified as a significant prognostic factor, correlating with poorer overall survival in adult patients with hemophagocytic lymphohistiocytosis (HLH).[33] This study observed that AST demonstrated superior predictive ability for chemotherapy resistance compared to ALT in the refractory

group, alongside abnormalities in total cholesterol and LDL metabolism, suggesting hepatocyte mitochondrial injury. Furthermore, elevated LDH reflects disrupted cell membrane integrity and enhanced glycolysis,[36] jointly forming a comprehensive indicator of systemic metabolic exhaustion alongside decreased cholesterol. Therefore, integrating total cholesterol, LDL, albumin with AST, ALT, and LDH provides a more sensitive detection of early organ dysfunction, offering a metabolic basis for initiating personalized interventions within 72 hours.

A notable finding was that ferritin, a cornerstone marker for HLH diagnosis and treatment monitoring,[35,37,38] also demonstrated significant predictive value (AUC=0.746, 95% [0.577–0.914]). However, it was ultimately excluded from the final model due to statistical instability from small subgroup sample sizes and inconsistent re-evaluation timepoints in clinical practice, which led to substantial missing data. Despite this, its early trend remains clinically valuable for monitoring.

Taken together, our study developed and validated two predictive models that offer practical advantages for clinical implementation. The principal strength of these models lies in its immediate feasibility for widespread adoption, as it relies exclusively on existing, guideline-mandated laboratory parameters, incurring no additional operational or financial burdens. Limitations of this study include its single-center, retrospective design and relatively limited sample size, which may introduce selection bias and affect the stability of estimates, particularly for key indicators like day 3 IL-10 with high missing data rates. Most importantly, the predictive model awaits external validation in multicenter, prospective cohorts to confirm its generalizability and real-world clinical utility.

Conclusion

This study developed and validated a novel "two-step" prediction strategy that seamlessly integrates into the current HLH-94/2004 treatment workflow, enabling early and dynamic risk stratification. The first step, applicable at diagnosis, utilizes a combination of IL-10 > 131.2 μmol/L and platelet count < 55.5×10^9/L which are both routine tests, to identify high-risk patients without requiring additional resources. The second step employs a bedside nomogram at the 72-hour post-chemotherapy mark, incorporating dynamic changes in platelet count and the absolute lymphocyte count to generate individualized risk probabilities. This approach allows for the potential initiation of targeted salvage therapy within a critical window and avoiding the typical two-week therapeutic delay.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy and confidentiality concerns but are available from the corresponding author upon reasonable request. The statistical analyses were performed using R (version 4.3.1; R Foundation for Statistical Computing) and SPSS (version 25.0; IBM Corp.) and other publicly available software. For data access inquiries, please contact Xiulan Lu (13787252674@163.com).

Ethics Approval and Consent to Participate

The study protocol was approved by the institutional ethics committee of Hunan Children's Hospital (Approval No. HCHLL-2018). All procedures were performed in accordance with the 1964 Declaration of Helsinki (as revised in 2013). The requirement for written informed consent was waived by the committee due to the retrospective nature of the study and the use of de-identified data.

Consent for Publication

Not applicable. The requirement for separate written informed consent for publication was also waived by the ethics committee for the reasons stated above.

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Disclosure

The authors report no conflicts of interest in this work.

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