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Dynamic monitoring of lymphocyte subsets at different disease stages can predict the prognosis of acute myeloid leukemia especially in complete remission status

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Acute myeloid leukemia (AML) lacks effective prognostic markers. While lymphocyte subsets are recognized as valuable predictive indicators in hematologic malignancies, their role in AML remains largely unexplored, particularly during different stages of AML. Our study analyzed the levels and changes of lymphocyte subsets in AML patients at newly diagnosed (ND) and first complete remission (CR) status, and explored the correlation between lymphocyte subsets and prognosis in different disease stages. Flow cytometry detected peripheral blood lymphocyte subsets in 145 ND AML patients, 125 CR AML patients, and 47 healthy controls (HCs). Dynamic testing was conducted on 28 AML patients at both ND and CR status. Our study found significant differences in lymphocyte subsets between ND, CR, and HCs, with notable changes in $CD3^+$T, $CD4^+$T, $CD8^+$T, effector T (Teff), B, and natural killer (NK) cells between ND and CR status. Low frequencies of $CD8^+$T below HCs thresholds and high regulatory T cell (Treg) frequency above HCs thresholds in the ND group, were independent risk factors for non-response to treatment. ROC curves evaluated the prognostic value of lymphocyte subsets and established cutoff values. Lymphocyte subsets in the ND group were not significantly associated with relapse or survival. Low absolute counts of $CD3^+$T, B, and NK cells in the CR group were linked to AML relapse, and a low NK cell count was an independent predictor of overall survival (OS). Lymphocyte subsets can act as prognostic biomarkers, and their dynamic monitoring predicts treatment response, relapse, and survival in AML.

Keywords Lymphocyte subsets, Acute myeloid leukemia, Prognosis

Acute myeloid leukemia (AML) is a clonal hematopoietic malignancy characterized by disordered differentiation and abnormal proliferation of hematopoietic stem cells. It is typically managed with chemotherapy, targeted therapy, immunotherapy, and hematopoietic stem cell transplantation (HSCT). After initial induction chemotherapy (IC), 60–80% of patients can achieve complete remission (CR). However, relapse is common and the median overall survival (OS) after relapse is only 4–6 months[1,2]. The high relapse rate and poor outcomes of AML remain pressing clinical challenges, there is an urgent demand to identify new diagnostic, therapeutic and prognostic biomarkers for AML. Currently, cytogenetic and molecular genetic markers are commonly used indicators for risk stratification in the prognosis of newly diagnosed (ND) AML[3]. Specific chromosomal abnormalities and characteristic gene mutations, such as TP53 and FLT3, are associated with poor prognosis in AML[4]. The abnormal expression of non-coding RNAs, such as miR-29 and miR-125, is also closely associated with AML prognosis[5–7]. Nonetheless, these markers are only used at the time of initial diagnosis to assess prognosis, with changes after treatment and their significance remaining adequately unevaluated. Additionally, the monitoring of leukemic stem cells (LSC) is also used to assess AML prognosis due to their drug resistance and relapse-initiating properties. However, detection levels and sensitivity vary significantly across different laboratories[8]. Overall, these tests are time-consuming, require advanced equipment and specialized personnel. There is a pressing need for more accurate, efficient, and faster prognostic markers in clinical practice.

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Lymphocyte subsets, including T cells, B lymphocytes, and natural killer (NK) cells, are crucial indicators of cellular immunity. The $CD3^+$T cell population includes two major subsets, $CD4^+$T cells and $CD8^+$T cells, among which regulatory T cells (Treg) and activated effector T cells (Teff) are important functional subpopulations within the $CD4^+$T cell compartment. Immune escape of tumor cells is a key factor in the relapse of AML and lymphocyte subsets play crucial roles in the mechanism of immune escape[9]. Changes in quantity, immune phenotype, and functional imbalance of lymphocyte subsets promote relapse and progression of AML. Lymphocyte subsets are associated with the prognosis of a variaty of solid tumors, such as lung cancer[10–13], breast cancer[14,15], ovarian cancer[16], and cholangiocarcinoma[17]. In terms of hematopoietic malignancy, lymphocyte subsets also have prognostic value in lymphoma[18,19] and chronic lymphocytic leukemia (CLL)[20]. In CLL, changes in lymphocyte subsets at initial diagnosis and after treatment are monitored, and variations in specific subsets are closely associated with prognosis, which underscores the significance of dynamic monitoring[20–23]. Previous studies have shown that the levels of T cells and NK cells in the peripheral blood of ND AML patients are correlated with the maintenance of long-term remission and survival[24,25]. However, the lymphocyte subsets as important indicators to reflect the patients' immune status, still lack of comprehensive evaluation in AML. Additionally, the lymphocyte subsets of leukemia patients are influenced by various factors, including blasts, chemotherapy, and immunotherapy. Previous studies indicate differences in immunological recovery after chemotherapy in AML, which suggest the alterations of lymphocyte subsets are heterogeneous and may be related to the prognosis[26,27]. Therefore, in this study, we conducted dynamic monitoring of peripheral blood lymphocyte subsets in AML, which is beneficial for clarifying the immune status of patients precisely, evaluating prognosis, and guiding follow-up treatment.

Our study explored the changes in the proportion and quantity of peripheral blood lymphocyte subsets in AML patients at initial diagnosis and remission, as well as their correlation with prognosis. We identified lymphocyte subset indicators that are closely associated with prognosis across various disease stages, demonstrating the significance of dynamically monitoring lymphocyte subsets for AML prognosis.

Materials and methods

Study population

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board at the Second Affiliated Hospital of Anhui Medical University. A total of 145 ND and 125 CR adult AML patients from January 2017 to December 2023 at the Second Affiliated Hospital of Anhui Medical University were enrolled. Lymphocyte subsets were evaluated in ND patients before initiating treatment, and CR patients were assessed within one month of attaining their first CR, which contains CR and CR with incomplete hematologic recovery (CRi) according to the response criteria of Chinese guidelines for the diagnosis and treatment of adult AML[28]. 28 patients underwent continuous monitoring of lymphocyte subsets at initial diagnosis and during remission. All patients were stratified diagnostically according to the Chinese guidelines for the diagnosis and treatment of adult AML (excluding acute promyelocytic leukemia, APL) and the Chinese guidelines for the diagnosis and treatment of APL[28,29]. All patients received standard IC according to the guidelines, followed by consolidation therapy or HSCT. The efficacy was evaluated as CR or non-response (NR). NR means non-response after two IC cycles, referring to refractory cases. All patients were followed up to death or the deadline for the research. 47 sex- and age-matched healthy controls (HCs) recruited from the physical examination center were included.

Detection of lymphocyte subsets in peripheral blood by flow cytometry

3-4 mL of venous blood was collected from AML patients and HCs and loaded into heparin sodium anticoagulated vacuum collection tubes (BD Biosciences, USA, Cat# 367525). 100μL of peripheral blood was taken within 3 h of collection and incubated with fluorescence-labeled specific monoclonal antibodies (mAbs) for 15 min in the dark. Isotype controls were also prepared. After staining, 900 μl of red blood cell lysing solution (Solarbio, China, Cat# R1011) was added, mixed, and incubated for 10 min. The mixture was then centrifuged at 400 g for 5 min, washed with PBS (Biosharp, China, Cat# BL302 A), and prepared for flow cytometry. The antibodies used in the experiment are listed in Table S1. The samples were tested using the CytoFLEX instrument and analyzed with CytExpert Software (version 2.0, Beckman Coulter, USA). The gating strategy for lymphocyte subsets is illustrated in Fig S1. The frequencies of $CD3^+$T, $CD4^+$T, $CD8^+$T, B, and NK cells are expressed as a proportion of the total lymphocyte population. Treg (defined as $CD4^+CD25^+CD127^{low}$) and activated effector T cells (defined as $CD4^+CD25^+CD127^{high}$ and abbreviated as Teff in this study) were expressed as proportions of $CD4^+$T cells.

Statistical analysis

Data analysis was conducted using SPSS Statistics Version 27.0 (IBM Corporation, USA) and GraphPad Prism Version 8.3 (GraphPad Software, USA). Descriptive statistics were reported as median (interquartile range, IQR). Pearson's Chi-square test was used to compare categorical variables between groups. For numerical variables, statistical analysis was performed based on the data distribution type. ANOVA or Kruskal-Wallis H-test was used to compare multiple groups, while t-test or Mann-Whitney U-test was used to compare two groups. The predictive power of the variable was evaluated by calculating the area under the receiver operating characteristic (ROC) curve. The optimum cutoff value was determined using Youden's index (J = max[sens + spec-1]). Univariate logistic regression analysis was performed to assess treatment response. Variables with.

$p < 0.2$ in the univariate analysis were included in the multivariate model, which also employed Firth's correction to address data sparsity and separation issues. The prognostic value was evaluated using the Kaplan-Meier log-rank test. Relapse-free survival (RFS) was defined as the time from the date of first CR until the date of relapse or the end of follow-up. OS was defined as the time from the date of diagnosis until death or the end

of follow-up. Meanwhile, univariate Cox regression analysis was used to assess RFS and OS, and variables with $p < 0.2$ were included in multivariate Cox analysis.

Results

Variability of lymphocyte subsets in AML patients across different disease status

We observed significant differences in lymphocyte subsets between AML patients and HCs, as well as alterations in lymphocyte subsets across different AML disease status (Fig. 1). The basic characteristics of 145 ND, 125 CR AML patients and 47 HCs are shown in Table S2.

The frequency and absolute count of $CD3^+T$, $CD4^+T$, $CD8^+T$ cells, and B lymphocytes showed no difference between ND group and HCs but revealed discrepancies between the CR group and HCs ($p < 0.01$). The absolute count of $CD8^+T$ cells in the CR group is lower than the HCs ($p < 0.05$), while the proportion increased significantly compared with the HCs group ($p < 0.01$). Treg, Teff, and NK cells in the ND and CR groups differ from HCs in both frequency and absolute count ($p < 0.05$). The $CD4^+T/CD8^+T$ ratio and Treg/Teff ratio showed no statistical difference among the three groups. Notably, the frequency of T cell subsets, including $CD3^+T$, $CD4^+T$, and $CD8^+T$ cells, in the CR group was higher than in the ND group ($p < 0.01$), while the absolute count of $CD3^+T$ and $CD4^+T$ decreased in the CR group compared to the ND group ($p < 0.05$) and the absolute count of $CD8^+T$ cells also showed a decline but without statistical significant. The absolute count of B and NK cells in the CR group also decreased compared to the ND group ($p < 0.01$).

Lymphocyte subset indicators for predicting treatment response in newly diagnosed AML patients

Clinical characteristics and lymphocyte subsets between the CR and NR groups were compared and presented in Table 1. The results showed that the age of the NR group was higher than that of the CR group [60 (50, 72.25) vs. 53 (41, 64), $p = 0.0042$]. The proportion of B cells in the NR group was reduced compared to the CR group [9.10 (5.50, 17.95) vs. 13.40 (8.30, 18.40), $p = 0.037$]. There was no difference in other lymphocyte subsets between the CR and NR groups.

To accurately identify AML patients whose lymphocyte subsets differ from HCs, we defined the 2.5% and 97.5% quantiles of HCs as the normal value. In ND AML patients, for $CD3^+T$, $CD4^+T$, $CD8^+T$, Teff, B, and NK cells, values less than the lower limits of normal value were defined as the low group, and vice versa were the high group; for Treg and Treg/teff, values greater than the upper limits of normal value were defined as the high group, and vice versa for the low group. Although both $CD4^+$ and $CD8^+$ T cells can mediate antileukemic effects, the significant reduction in the $CD4^+/CD8^+$ ratio commonly observed in AML may indicate a predominance of the immunosuppressive microenvironment. To identify high-risk patient subgroups with severely dysregulated immune states, we used the 2.5 th percentile of the $CD4^+/CD8^+$ T cell ratio in the healthy population as the cutoff value. Patients with values below the lower limit of the normal range were classified as the low group, while those with values above it were classified as the high group. Univariate and multivariate logistic regression analyses with Firth's correction assessed associations between clinical features, lymphocyte subsets, and treatment response, as shown in Table 2. Table S3 presents the original results from traditional maximum likelihood estimation for comparison with the Firth-adjusted models reported in Table 2.

The analyses revealed high white blood cell (WBC) counts at diagnosis served as an independent protective factor for treatment response in multivariate analysis [OR = 0.210 (0.050, 0.882), $p = 0.032$], though this association was not statistically significant in univariate modeling [OR = 0.994 (0.987, 1.002), $p = 0.140$]. Multivariable analyses identified elevated $CD8^+T$ cell frequency as an independent protective factor for treatment response [OR = 0.150 (0.040, 0.560), $p = 0.005$], whereas higher Treg levels emerged as a significant risk factor for non-response [OR = 12.452 (4.500, 34.200), $p < 0.001$]. These findings persisted in univariate analyses [$CD8^+T$: OR = 0.060 (0.019, 0.191), $p < 0.001$; Treg: OR = 18.330 (4.150, 80.910), $p < 0.001$]. Monitoring baseline $CD8^+T$ and Treg frequencies may therefore provide clinically relevant biomarkers for predicting AML treatment outcomes.

Correlation between lymphocyte subsets and prognosis of newly diagnosed AML patients

The correlation between lymphocyte subsets in ND AML patients and prognosis was examined. It was found that lymphocyte subsets at diagnosis were not significantly associated with AML relapse; however, a higher proportion of $CD4^+T$ cells was associated with longer OS (Fig. 2).

Of the 145 ND AML patients, 112 cases achieved CR after treatment; 47 (42.0%) cases relapsed, and 65 (58.0%) cases remained in continuous CR at follow-up. ROC curves were used to assess the ability of lymphocyte subsets to predict relapse, as shown in Fig. 2A. The proportion of B lymphocytes showed the highest discriminative value but with a low AUC of 0.602 ($p = 0.066$), followed by the absolute count of B lymphocytes (AUC = 0.591) and Teff cells (AUC = 0.559). The predictive efficacy of lymphocyte subsets is presented in Fig S2. The above results suggested that lymphocyte subsets detected at initial diagnosis showed poor efficacy in predicting relapse. Similarly, we used ROC curves to assess the efficacy of lymphocyte subsets in predicting survival outcomes. The proportion of $CD4^+T$ cells showed the highest predictive efficiency with an AUC of 0.627 ($p = 0.009$), followed by the $CD4^+T/CD8^+T$ ratio (AUC = 0.591) and $CD8^+T$ frequency (AUC = 0.562). Figure 2B shows ROC curves assessing the ability of lymphocyte subsets to predict survival outcomes. The performance of each indicator in predicting the survival of AML patients is presented in Fig S3. The proportion of $CD4^+T$ cells showed best predictive power, as shown in Fig. 2C. According to Youden's index, the optimal cutoff value of $CD4^+T$ frequency was 36.35%. The high $CD4^+T$ frequency group was defined as 36.35% or greater, and the low group was defined as less than 36.35%. Kaplan-Meier survival curves showed the median OS of low $CD4^+T$ frequency group was shorter than the high group (14.9 versus 55.1 months), as shown in Fig. 2D ($p = 0.027$).

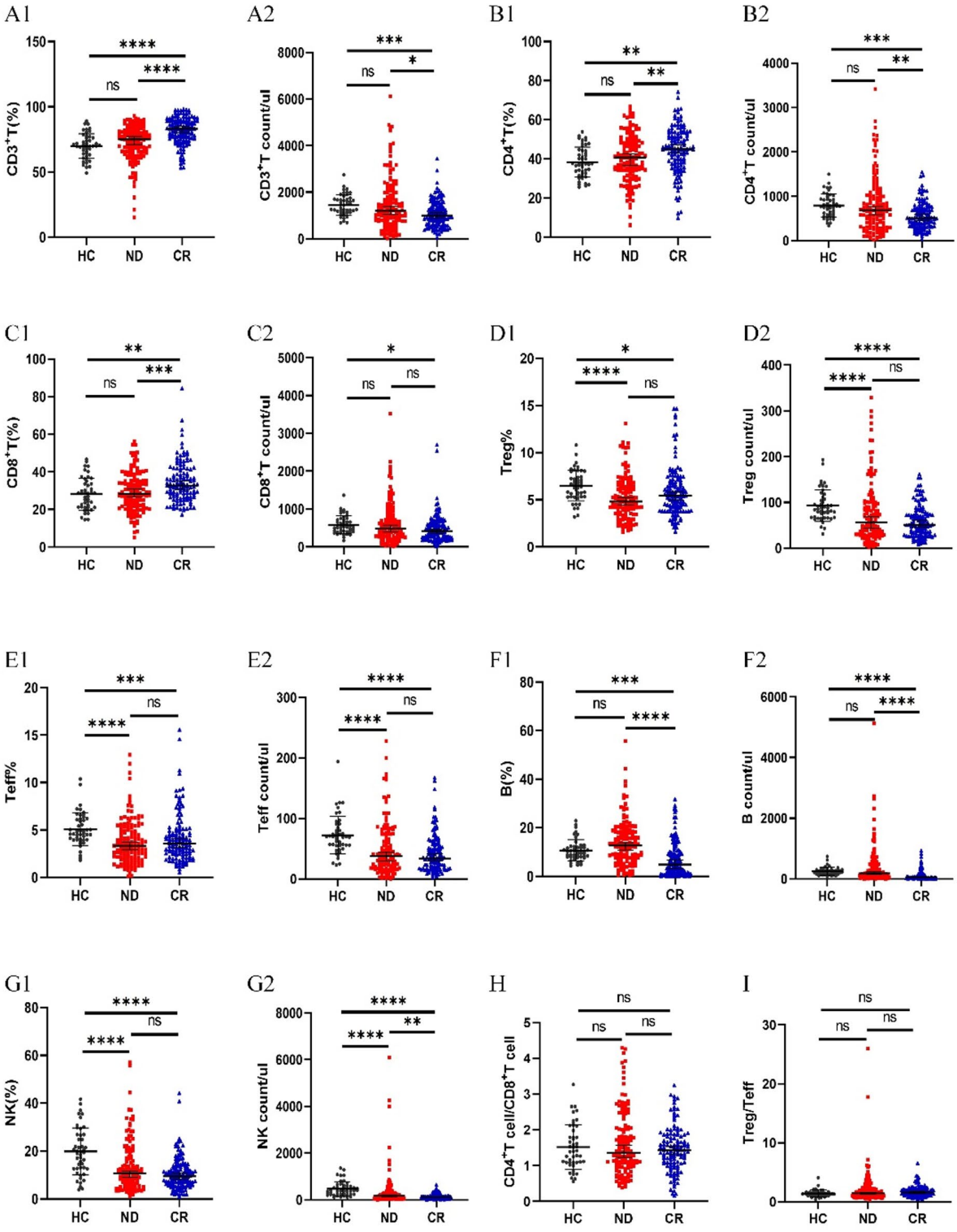

Fig. 1. Comparison of lymphocyte subsets among AML newly diagnosed(ND), complete remission(CR) patients and healthy controls(HC). (**A1-G1**) Frequency of lymphocyte subsets; (**A2-G2**) Absolute count of lymphocyte subsets; (**H**) CD4 + T/CD8 + T ratio; (**I**) Treg/teff ratio. Statistical analysis was performed using Kruskal-Wallis H-test. $^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

Changes of lymphocyte subsets between newly diagnosed and complete remission status in AML patients

In AML patients, characteristic changes in lymphocyte subset distribution are observed upon achieving CR compared to the ND stage (Fig. 3). Although the absolute counts of $CD3^+$T cells, $CD4^+$T cells, $CD8^+$T cells,

Characteristics	CR	NR	*p*-value
AML, n	99	46	
Age, years, median (IQR)	53(41,64)	60(50,72.25)	0.004
Sex			0.594
Male, n (%)	60	30	
Female, n (%)	39	16	
MDS/MPN history			0.273
Yes	9	7	
No	90	39	
Treatment-related/Secondary AML			0.516
Yes	4	3	
No	95	43	
Infection status			0.561
Yes	80	39	
No	19	7	
Molecular cytogenetic stratification			0.393
Low-medium risk	57	23	
High risk	42	23	
Extramedullary diseases			0.397
Yes	5	4	
No	94	42	
BM blasts (%)	67.00(37.50,88.00)	56.25(28.88,79.13)	0.068
WBC,$\times 10^9$/L	9.66(3.07,55.00)	7.78(1.76,38.54)	0.231
Lymphocyte,$\times 10^9$/L	2.81(1.24,7.58)	2.14(0.90,6.09)	0.378
HB, g/L	79.00(61.00,93.00)	69.00(53.75,91.00)	0.362
PLT,$\times 10^9$/L	34.00(20.00,61.00)	32.00(12.00,62.25)	0.452
$CD3^+T$ (%)	74.70(65.40,80.50)	75.00(63.90,83.05)	0.928
$CD4^+T$ (%)	40.70(34.40,48.00)	37.10(28.40,49.33)	0.352
$CD8^+T$ (%)	28.30(21.70,34.60)	28.15(21.58,38.93)	0.660
$CD4^+T/CD8^+T$	1.39(1.07,2.00)	1.31(0.83,2.41)	0.406
Treg (%)	4.61(3.55,6.25)	4.85(3.45,6.68)	0.606
Teff (%)	3.27(2.04,5.21)	3.40(2.12,4.81)	0.905
Treg/Teff	1.38(0.99,2.24)	1.53(0.90,2.23)	0.907
B (%)	13.40(8.30,18.40)	9.10(5.50,17.95)	0.037
NK (%)	10.70(6.00,14.00)	10.80(6.30,21.13)	0.614
$CD3^+T$ count/ul	1285.0(791.0,2058.0)	1066.0(669.3,1989.0)	0.394
$CD4^+T$ count/ul	743.0(405.0,1098.0)	581.0(320.8,973.8)	0.193
$CD8^+T$ count/ul	519.0(262.0,771.0)	406.5(261.8,820.8)	0.642
B count/ul	219.0(104.0,471.0)	151.0(53.8,536.3)	0.202
NK count/ul	194.0(76.0,371.0)	154.0(80.0,243.0)	0.603
Treg count/ul	56.0(29.0,99.0)	50.0(29.8,97.3)	0.724
Teff count/ul	39.0(19.0,75.0)	37.0(17.8,65.8)	0.633

Table 1. Comparison of lymphocyte subsets and clinical characteristics at diagnosis between non-response and complete remission groups in newly diagnosed AML patients undergoing induction chemotherapy.

Teff, and NK cells were significantly lower in the CR group compared to the ND group (all $p < 0.05$), their relative proportions within the lymphocyte population showed an inverse trend. Specifically, the proportions of $CD3^+T$ cells, $CD4^+T$ cells, and $CD8^+T$ cells were significantly higher in the CR group than in the ND group (all $p < 0.01$). Notably, B lymphocytes exhibited a dual decline in the CR stage, with both their absolute counts and proportions being significantly reduced compared to the ND group ($p < 0.01$ and $p < 0.05$, respectively). In contrast, parameters related to Treg cells (including frequency, absolute counts, and the Treg/Teff ratio), the $CD4^+/CD8^+T$ cell ratio, and NK cell frequency showed no statistically significant differences between the two groups (all $p > 0.05$). This indicates that these immune characteristics remain relatively stable across different disease stages.

Item	Univariate analysis		Multivariate analysis[d]	
	OR (95%CI)	*P*	OR (95%CI)	*P*
Sex (Male/Female)	1.219(0.588,2.525)	0.595		
Age (≥ 60y/<60y)	2.284(1.117,4.672)	**0.024**		
MDS/MPN history (Yes/No)	1.795(0.624,5.164)	0.278		
Treatment-related/Secondary AML (Yes/No)	1.657(0.355,7.727)	0.520		
Infection status (Yes/No)	1.323(0.513,3.412)	0.562		
Molecular cytogenetic stratification (High risk/Low-medium risk)	1.357(0.673,2.739)	0.394		
Extramedullary diseases (Yes/No)	1.790(0.458,7.005)	0.403		
BM blasts (≥ 50%/<50%)	0.680(0.334,1.384)	0.089		
High WBC at newly diagnosis[a] (Yes/No)	0.994(0.987,1.002)	0.140	0.210 (0.050,0.882)	**0.032**
Lymphocyte,$\times10^9$/L	0.988(0.965,1.012)	0.327		
HB, g/L	0.997(0.984,1.011)	0.702		
PLT,$\times10^9$/L	1.001(0.995,1.007)	0.756		
LDH, U/L	1.000(1.000,1.001)	0.276		
$CD3^+T(\%)^b$ (≥ 50.1/<50.1)	0.799(0.222,2.878)	0.731		
$CD4^+T(\%)^b$ (≥ 25.7/<25.7)	0.171(0.069,0.424)	< **0.001**		
$CD8^+T(\%)^b$ (≥ 14.6/<14.6)	0.060(0.019,0.191)[d]	< **0.001**	0.150 (0.040,0.560)	**0.005**
$CD4^+T/CD8^+T^b$ (≥ 0.6/<0.6)	0.152(0.045,0.514)	**0.002**		
Treg(%)[c] (> 10.6/≤10.6)	18.330(4.150,80.910)[d]	< **0.001**	12.452 (4.500,34.200)	< **0.001**
Teff(%)[b] (≥ 1.8/<1.8)	1.175(0.493,2.801)	0.717		
Treg/Teff[c] (> 3.8/≤3.8)	0.952(0.277,3.269)	0.938		
B(%)[b] (≥ 4.5/<4.5)	0.084(0.034,0.206)	< **0.001**		
NK(%)[b] (≥ 3.9/<3.9)	1.240(0.414,3.710)	0.701		
$CD3^+T$ count/ul[b] (≥ 690/<690)	0.717(0.316,1.630)	0.428		
$CD4^+T$ count/ul[b](≥ 347/<347)	0.731(0.336,1.593)	0.431		
$CD8^+T$ count/ul[b] (≥ 181/<181)	1.190(0.430,3.298)	0.737		
B count/ul[b] (≥ 85/<85)	0.309(0.143,0.666)	**0.003**		
NK count/ul[b] (≥ 107/<107)	0.892(0.431,1.849)	0.759		
Treg count/ul[c] (> 191/≤191)	0.517(0.105,2.537)	0.416		
Teff count/ul[b] (≥ 23/<23)	0.707(0.338,1.479)	0.357		

Table 2. Treatment response was analyzed by univariate and multivariate logistic regression according to different lymphocyte subsets of ND AML patients. [a]Indicates the absolute count of white blood cell greater or equal to 100^*10^9/L. [b]The cutoff value was the lower limit of the normal range in healthy controls. [c]The cutoff value was the upper limit of the normal range in healthy controls. [d]Variables marked with a superscript 'd' in the univariate analysis indicate results adjusted by Firth' s correction due to data sparsity; multivariate analysis uniformly applied Firth' s correction to address collinearity and separation issues.

The predictive efficacy of lymphocyte subsets for the prognosis of complete remission AML patients

Lymphocyte subsets with significant differences between the CR and ND groups were included in subsequent prognostic analysis and the subgroup parameters included in the analysis were all measured at CR time points. The absolute count of lymphocyte subsets predicts relapse more effectively than the proportion according to the area under the ROC curve, as shown in Fig. 4 and Fig S4. The absolute count of $CD3^+T$ cells showed the highest predictive efficiency with an AUC of 0.634 followed by Teff count (AUC = 0.618), $CD8^+T$ count (AUC = 0.616), B cell count (AUC = 0.604), NK cell count (AUC = 0.603), and $CD4^+T$ count (AUC = 0.601). The cutoff values for the absolute count of $CD3^+T$, Teff, $CD8^+T$, B, and NK cells were calculated based on Youden's index, while the p value for the area under the ROC curve of $CD4^+T$ count was slightly greater than 0.05, the cutoff value for $CD4^+T$ count was the median of the group. Greater than or equal to the cutoff value was the high group and less than was the low group for the above indicators. The Kaplan-Meier curves showed the difference in survival between high and low levels of lymphocyte subsets (Fig. 5).

For CR AML patients, the median RFS in patients with low $CD3^+T$ count (< 840/uL), $CD4^+T$ count (< 509/uL), $CD8^+T$ count (< 449/uL), Teff count (< 25/uL), B lymphocyte count (< 146/uL), NK count (< 61/uL)was significantly shorter than that in patients with high $CD3^+T$ count, $CD4^+T$ count, $CD8^+T$ count, Teff count, B lymphocyte count and NK count, respectively ($p < 0.05$). Patients of the low $CD3^+T$ count, $CD8^+T$ count, Teff count and NK count group also showed a shorter median OS than the high $CD3^+T$ count, $CD8^+T$ count, Teff count and NK count group, respectively ($p < 0.05$), while there was no significant difference in OS between the high and low $CD4^+T$ cell and B cell groups.

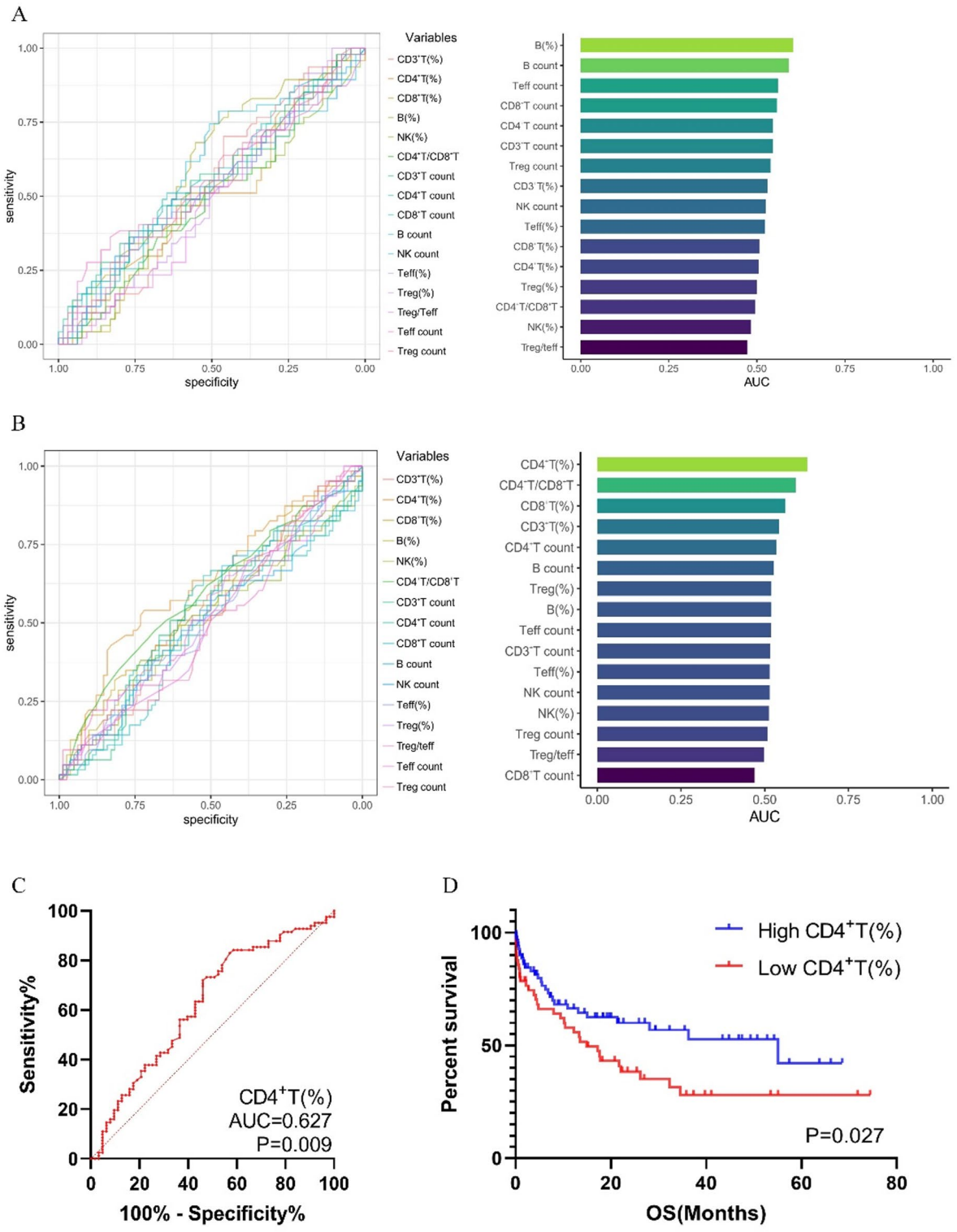

Fig. 2. Receiver operating characteristic (ROC) curves assess lymphocyte subsets of ND patients in predicting the relapse (**A**) and survival (**B**) of AML. (**C**) ROC curves assess CD4 + T(%) of ND patients in predicting the survival of AML. (**D**) KM analysis of overall survival (OS) according to high and low groups determined by cutoff value of CD4 + T(%). The mortality rate for low CD4 + T frequency was 59.60%, whereas for high CD4 + T frequency it was 33.00%.The median OS in the low CD4 + T frequency group was 14.9 months, shorter than 55.1 months in the high CD4 + T frequency group ($p = 0.027$).

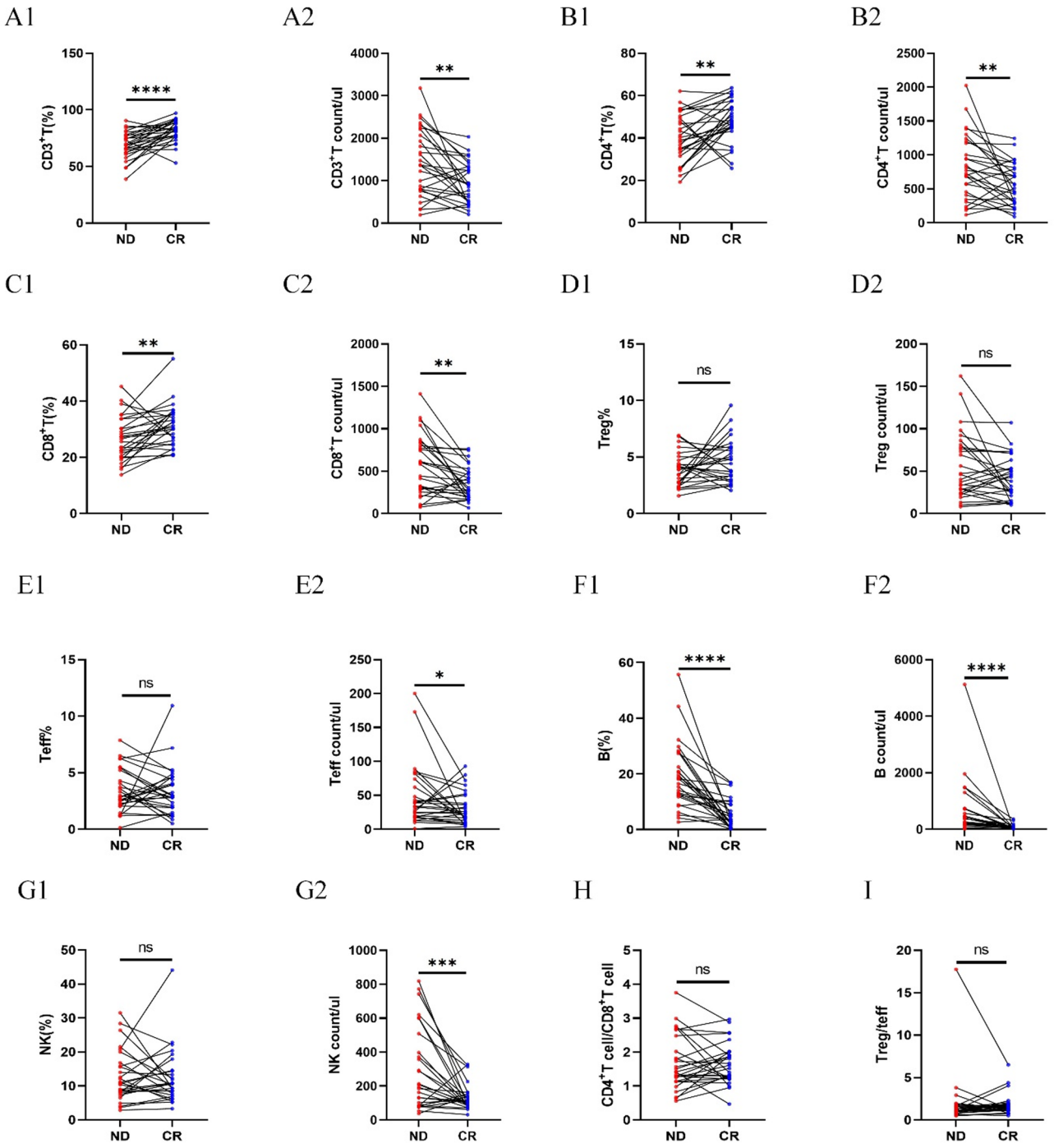

Fig. 3. Dynamic changes of lymphocyte subsets in 28 AML patients at ND and CR status. (**A1-G1**) Frequency of lymphocyte subsets; (**A2-G2**) Absolute count of lymphocyte subsets; (**H**) CD4 + T/CD8 + T ratio; (**I**) Treg/teff ratio. Statistical analysis was performed using Wilcoxon matched-pairs signed rank test. $^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

Correlation between lymphocyte subsets and prognosis of complete remission AML patients

Age, bone marrow minimal residual disease (BM-MRD), and the absolute counts of $CD3^+$T cells, B cells, and NK cells at CR status were identified as valuable predictors of AML relapse through univariate and multivariate Cox regression analyses. Additionally, advanced age, low hemoglobin levels, and a low NK cell count during CR were associated with poor OS.

Tables 3 and 4 present the results of the Cox analyses for RFS and OS, respectively. The final results showed that advanced age (univariate analysis: HR = 1.424 [0.830–2.442], $p = 0.199$; multivariate analysis: HR = 2.057 [1.158–3.653], $p = 0.014$) and positive BM-MRD (univariate analysis: HR = 2.382 [1.349–4.207], $p = 0.003$; multivariate analysis: HR = 2.745 [1.504–5.012], $p = 0.001$) were prognostic risk factors for relapse. High levels of

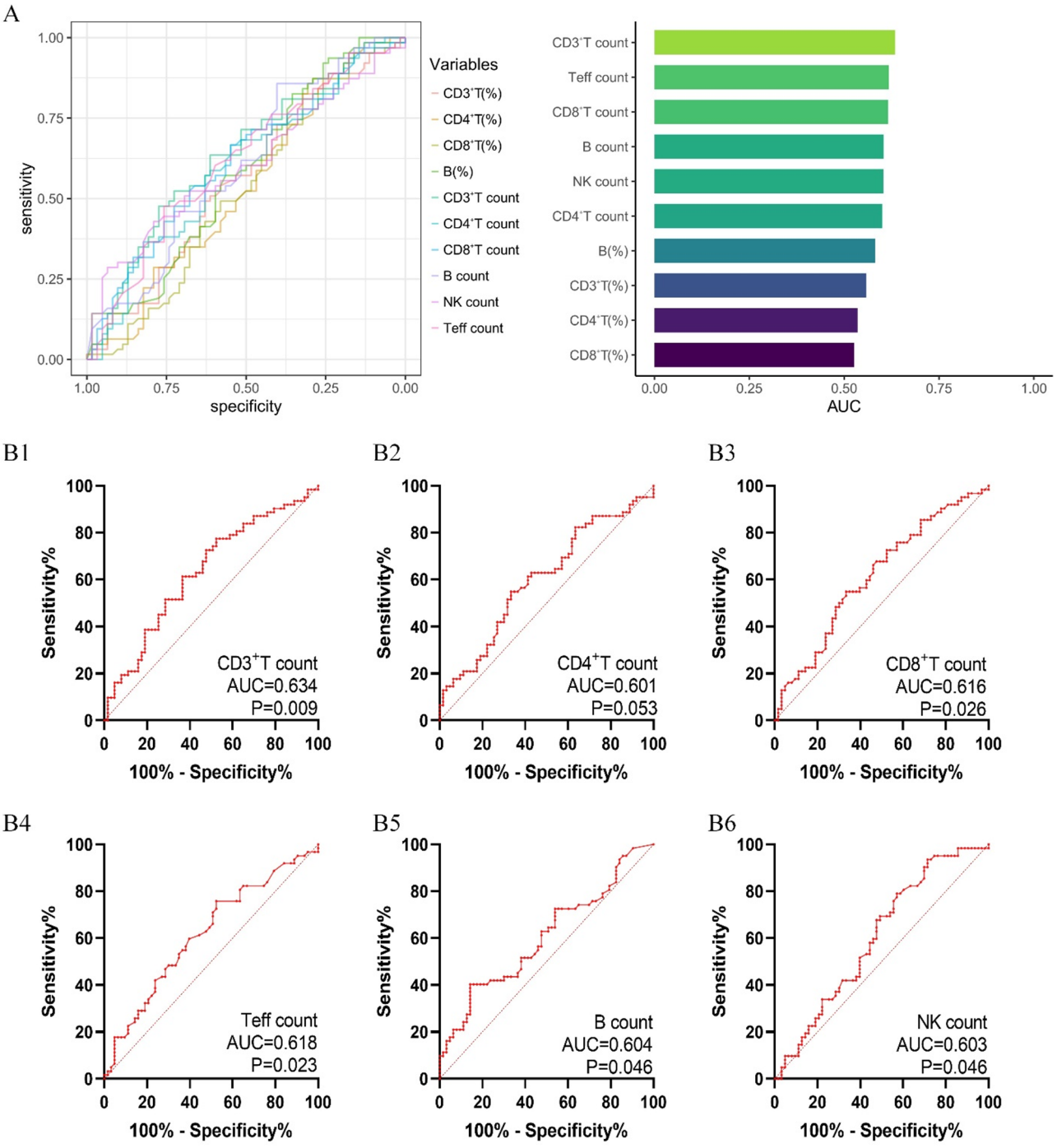

Fig. 4. (**A**) ROC curves assess lymphocyte subsets of CR stages in predicting the relapse of AML. (**B**) The ROC curve analysis for the prediction of relapse using the absolute count of lymphocyte subsets in CR stages.

$CD3^+$T count(univariate analysis: HR = 0.347 [0.210–0.572], $p < 0.001$; multivariate analysis: HR = 0.488 [0.285–0.835], $p = 0.009$), B lymphocyte count (univariate analysis: HR = 0.248 [0.122–0.504], $p < 0.001$; multivariate analysis: HR = 0.298 [0.142–0.628], $p = 0.001$), and NK cell count (univariate analysis: HR = 0.303 [0.174–0.530], $p < 0.001$; multivariate analysis: HR = 0.423 [0.233–0.767], $p = 0.005$) were independent protective indicators for relapse. Cox regression analysis of OS was also performed using the same method. Clinical characteristics indicated that advanced age (univariate analysis: HR = 2.025 [1.080–3.800], $p = 0.028$; multivariate analysis: HR = 3.309 [1.664–6.581], $p = 0.001$) and low hemoglobin (univariate analysis: HR = 0.299 [0.155–0.578], $p < 0.001$; multivariate analysis: HR = 0.350 [0.165–0.741], $p = 0.006$) were predictive risk factors for OS. Among lymphocyte subsets, a low level of NK cell count (univariate analysis: HR = 0.301 [0.154, 0.591], $p < 0.001$; multivariate analysis: HR = 0.351 [0.172, 0.716], $p = 0.004$) was an independent prognostic risk factor for OS.

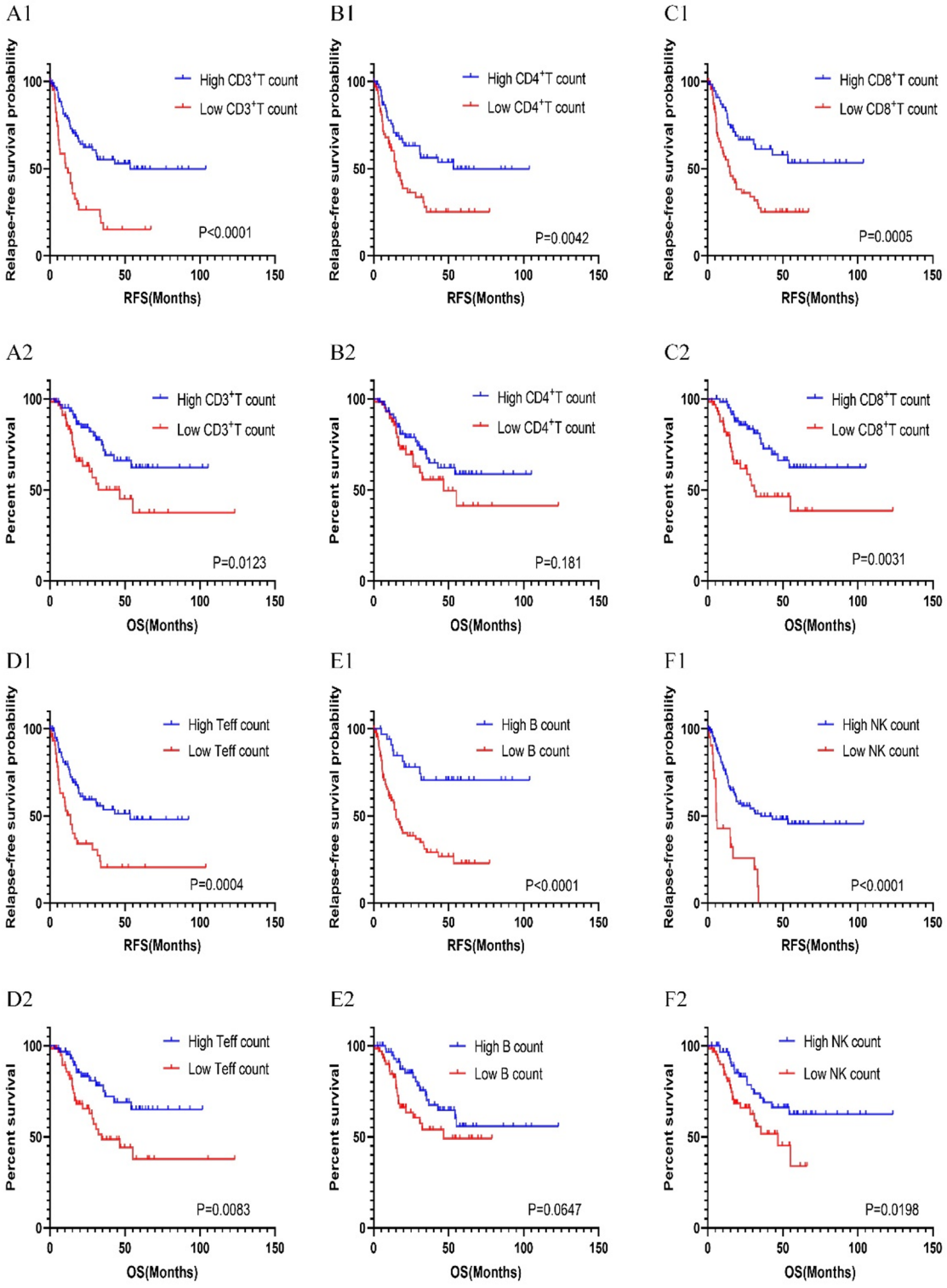

Fig. 5. Absolute count of lymphocyte subsets of CR patients is associated with the prognosis of AML. KM analysis of RFS and OS according to high and low groups determined by cutoff value of CD3 + T, CD4 + T, CD8 + T, Teff, B lymphocyte, NK cell count. (**A1**-**F1**) Relapse-free survival(RFS) in AML patients with different absolute count of lymphocyte subsets. (**A2**-**F2**) Overall survival (OS) in AML patients with different absolute count of lymphocyte subsets.

Item	Univariate cox		Multivariate cox	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Sex (Male/Female)	1.041(0.634,1.709)	0.875		
Age (≥ 60y/<60y)	1.424(0.830,2.442)	0.199	2.057(1.158,3.653)	**0.014**
MDS/MPN history (Yes/No)	1.444(0.688,3.034)	0.332		
Treatment-related/Secondary AML (Yes/No)	0.523(0.073,3.778)	0.521		
Infection status (Yes/No)	2.077(1.103,3.913)	**0.024**		
Molecular cytogenetic stratification (High risk/Low-medium risk)	1.288(0.777,2.136)	0.327		
Extramedullary diseases (Yes/No)	0.821(0.200,3.365)	0.784		
BM blasts (≥ 50%/<50%)	0.739(0.451,1.213)	0.232		
High WBC at newly diagnosis[a] (Yes/No)	1.846(0.877,3.886)	0.107		
WBC[b],$\times10^9$/L (≥ 4.3/<4.3)	0.422(0.254,0.700)	**<0.001**		
Neutrophil[b],$\times10^9$/L (≥ 2.16/<2.16)	0.469(0.283,0.776)	**0.003**		
Lymphocyte[b],$\times10^9$/L (≥ 2.14/<1.24)	0.427(0.257,0.709)	**<0.001**		
HB[b],g/L (≥ 103/<103)	0.520(0.316,0.858)	**0.011**		
PLT[b],$\times10^9$/L (≥ 171/<171)	0.959(0.585,1.572)	0.868		
LDH[b],U/L (≥ 250/<250)	0.444(0.139,1.417)	0.17		
BM-MRD (Positive/Negative)	2.382(1.349,4.207)	**0.003**	2.745(1.504,5.012)	**0.001**
$CD3^+$T count[c] (≥ 840/<840)	0.347(0.210,0.572)	**<0.001**	0.488(0.285,0.835)	**0.009**
$CD4^+$T count[b] (≥ 509/<509)	0.485(0.292,0.804)	**0.005**		
$CD8^+$T count[c] (≥ 449/<449)	0.404(0.238,0.683)	**<0.001**		
B count[c] (≥ 146/<146)	0.248(0.122,0.504)	**<0.001**	0.298(0.142,0.628)	**0.001**
NK count[c] (≥ 61/<61)	0.303(0.174,0.530)	**<0.001**	0.423(0.233,0.767)	**0.005**
Teff count[c] (≥ 25/<25)	0.420(0.255,0.691)	**<0.001**		

Table 3. RFS was analyzed by univariate and multivariate Cox regression according to different lymphocyte subsets of AML patients in complete remission. [a]Indicates the absolute count of white blood cell greater or equal to 100^*10^9/L. [b]The cutoff value was the median. [c]The cutoff value was calculated based on Youden's index.

Dicussion

The immune evasion of AML blast cells is a critical factor in the initiation and progression of the disease. Lymphocyte subsets play a role in immune evasion, and research has identified certain subsets involved in the mechanisms of immune escape[9]. $CD4^+$T cells express exhaustion markers, resist activation, and contribute to $CD8^+$T cell exhaustion and tumor immune evasion[30–33]. Treg recruitment enhances immunosuppression, aiding AML blast growth[34]. NK cell loss and reduced receptor expression further enable tumor immune escape[35,36]. Therefore, lymphocyte subsets may be closely associated with disease progression and have the potential to serve as prognostic markers, making it essential to comprehensively evaluate this indicator in AML. Previous studies have explored the relationship between peripheral blood lymphocyte subpopulations detected at initial diagnosis and the prognosis of AML, and the results have been heterogeneous. Studies by M. Alcasid[24] and Sang Hyuk Park et al.[37] showed that the proportion of NK cells is correlated with prognosis, while Yumi Park[38] found that NK cells are not independently correlated with survival. These results indicated that T cell subsets and B cells were not significantly associated with the prognosis of AML. Most studies focus only on the role of specific subsets in AML, leading to incomplete evaluation of lymphocyte subsets and neglecting changes in each subset across different disease status. Our study comprehensively assessed the levels and changes of various lymphocyte subsets across different AML disease status and explored their correlation with prognosis under these conditions.

Our study found that the proportions and absolute counts of $CD3^+$T cells, $CD4^+$T cells, and $CD8^+$T cells in AML patients at initial diagnosis were not significantly different from those in HCs. However, the proportions and absolute counts of Teff cells, Treg cells, B cells, and NK cells were generally lower compared to HCs. Using HCs as a reference, low frequencies of $CD4^+$T cells, $CD8^+$T cells, and B cells at initial diagnosis suggested no response to treatment. $CD4^+$T cells and $CD8^+$T cells are the two most important subgroups of $CD3^+$T cells. Sander[25] and Ozkazanc[31] have confirmed the critical role of $CD4^+$T and $CD8^+$T cells in AML, where their depletion and phenotypic exhaustion lead to poor outcomes. Our results also validated a reduction in the frequencies of $CD4^+$T cells and $CD8^+$T cells is detrimental to tumor clearance, especially $CD8^+$T cells. Our results showed that B lymphocytes are important participants in tumor immunity of AML. Infiltrated B cells in solid tumors can recognize tumor antigens and produce IgG antibodies (Abs) to inhibit tumor growth and directly kill tumor cells[39,40]. They can also mediate T cell killing as direct antigen-presenting cells[41]. Nevertheless, B lymphocytes may also promote tumor progression. For instance, specific subtypes of B lymphocytes in B-cell lymphoma facilitate angiogenesis and tumor metastasis[42–44]. The role of B lymphocytes in AML, however, has not yet received much attention.We speculate that the reduction in B cells may weaken T cell-mediated cytotoxicity, potentially leading to treatment failure, though the underlying mechanisms remain to be further explored.

Item	Univariate cox		Multivariate cox	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Sex (Male/Female)	1.005(0.544,1.858)	0.986		
Age (≥ 60y/<60y)	2.025(1.080,3.800)	**0.028**	3.309(1.664,6.581)	**0.001**
MDS/MPN history (Yes/No)	1.326(0.520,3.383)	0.554		
Treatment-related/Secondary AML (Yes/No)	0.884(0.121,6.442)	0.903		
Infection status (Yes/No)	2.303(1.053,5.038)	**0.037**		
Molecular cytogenetic stratification (High risk/Low-medium risk)	1.967(1.064,3.636)	**0.031**		
Extramedullary diseases (Yes/No)	0.509(0.070,3.711)	0.505		
BM blasts (≥ 50%/<50%)	0.808(0.437,1.494)	0.497		
High WBC at newly diagnosis[a] (Yes/No)	1.644(0.644,4.199)	0.298		
WBC[b],$\times10^9$/L (≥ 4.3/<4.3)	0.495(0.265,0.924)	**0.027**		
Neutrophil[b],$\times10^9$/L (≥ 2.16/<2.16)	0.515(0.276,0.961)	**0.037**		
Lymphocyte[b],$\times10^9$/L (≥ 2.14/<1.24)	0.476(0.255,0.890)	**0.020**		
HB[b],g/L (≥ 103/<103)	0.299(0.155,0.578)	< **0.001**	0.350(0.165,0.741)	**0.006**
PLT[b],$\times10^9$/L (≥ 171/<171)	0.817(0.442,1.510)	0.519		
LDH[b],U/L (≥ 250/<250)	1.382(0.540,3.534)	0.500		
BM-MRD (Positive/Negative)	1.723(0.819,3.624)	0.151		
$CD3^+$T count[c] (≥ 840/<840)	0.376(0.201,0.701)	**0.002**		
$CD4^+$T count[b] (≥ 509/<509)	0.657(0.354,1.219)	0.183		
$CD8^+$T count[c] (≥ 449/<449)	0.341(0.173,0.669)	**0.002**		
B count[c] (≥ 146/<146)	0.304(0.134,0.690)	**0.004**		
NK count[c] (≥ 61/<61)	0.301(0.154,0.591)	< **0.001**	0.351(0.172,0.716)	**0.004**
Teff count[c] (≥ 25/<25)	0.382(0.206,0.708)	**0.002**		

Table 4. OS was analyzed by univariate and multivariate Cox regression according to different lymphocyte subsets of AML patients in complete remission. [a]Indicates the absolute count of white blood cell greater or equal to 100^*10^9/L. [b]The cutoff value was the median. [c]The cutoff value was calculated based on Youden's index.

A high frequency of Treg cells at initial diagnosis was associated with a lower rate of complete response in our study, suggesting a poor prognosis, which is similar to previous studies in AML[34,45,46]. Treg cells are involved in inhibiting anti-tumor immune responses in solid tumors such as lung and breast cancers[47–51], and their elevated levels in hematologic malignancies like non-Hodgkin's lymphoma, CLL, and multiple myeloma are also associated with unfavorable outcomes[23,52–54]. Treg cells accumulate in the immunosuppressive microenvironment of AML in the early stage, dampen effector cell activity via secretion of cytokines and adenosine and increased adenosine triphosphate hydrolysis, which may be the mechanism underlying the association between elevated Treg levels and poor prognosis in AML[55,56]. Our study showed the overall Treg frequency in ND AML patients was lower than that in HCs, consistent with the results of Sang Hyuk Park[37]. However, the Treg frequency in patients included by Wang X[57] and Shenghui[45] was significantly higher than that in HCs. This difference may be related to the different ages of patients included in these single-center studies. The AML patients and HCs included by Sang Hyuk Park[37] were mainly middle-aged and elderly, with a median age of 50 years old, which was higher than that in Wang X[57]. A small meta-analysis showed that Treg levels are affected by age, and the frequency of Treg in the peripheral blood of healthy elderly people increases[58]. This heterogeneity suggests that the immune status of AML patients of different ages may vary, which still needs to be further explored. Overall, our findings further confirm the negative impact of Treg cells on anti-tumor immunity in AML, where patients with elevated Treg frequencies exhibit unfavorable outcomes.

Through dynamic monitoring, we found significant differences in lymphocyte subsets between the ND group and the CR group. Our study is the first to comprehensively evaluate the changes in lymphocyte subsets in AML before and after IC. As Behl D[59] reported, the recovery of absolute lymphocyte count (ALC) after IC is associated with favorable outcomes and the immune recovery of patients after treatment is heterogeneous. The heterogeneity may explain why lymphocyte subsets in the ND state showed no significant correlation with prognosis in this study, as the patients' immune status had changed compared to the pre-treatment phase. The differences between the ND and CR groups also suggest that lymphocyte subsets at ND status do not fully represent the current immune status of AML patients, highlighting the necessity of dynamic monitoring in AML.

Based on the changes in lymphocyte subsets across different disease states of AML, we analyzed the correlation between lymphocyte subsets and prognosis in CR patients. And this is the first report on the association between lymphocyte subsets and AML prognosis in the CR group. While Behl D[59] only broadly discussed the relationship between the absolute count of total lymphocytes and prognosis, our research found that low absolute counts of $CD3^+$T cells, B cells, and NK cells in the CR group were associated with relapse, and the absolute count of NK cells was closely related to OS. The recovery of $CD3^+$ T cells, B cells, and NK cells after chemotherapy is crucial for RFS in AML, with NK cell recovery being particularly associated with better OS. Craddock C[60]

found that the number of $CD3^+$T cells in relapsed AML patients decreases and shows depletion phenotype, and the production of interferon-γ (IFN-γ)/tumor necrosis factor-α (TNF-α) and other cytokines released by $CD4^+$T cells is impaired, suggesting potential mechanisms through which T cells may be related to relapse in AML. $CD3^+$T cells were not significant in the multivariate analysis for OS, possibly due to parts of patients receiving T-cell-based immunotherapy. NK cells are innate lymphocytes of the immune system that monitor and eliminate tumor cells[26]. The AML immunosuppressive microenvironment often results in reductions in NK cell population and/or function, NK cells from patients with AML present with an unfavorable phenotype, including downregulation of natural cytotoxicity receptors, reduced capacity to produce and secrete IFN-γ[61]. Our findings suggest that $CD3^+$T and NK cells are potential biomarkers for predicting AML prognosis and closely related to antitumor immune response in AML.

Overall, our results demonstrate that lymphocyte subsets can serve as prognostic markers for AML. These subsets have predictive value across different disease status, highlighting the clinical importance of dynamic monitoring. Peripheral blood sampling offers distinct advantages of minimally invasive collection and enhanced reproducibility, particularly suitable for post-remission AML patients requiring long-term and high-frequency monitoring, whereas the invasive nature of bone marrow aspiration limits its utility in longitudinal surveillance. Although the bone marrow represents the primary disease site in AML, the composition and functional profiles of peripheral blood immune cells (e.g., T cells, NK cells) more comprehensively reflect systemic anti-tumor immune responses. Furthermore, peripheral blood profiling protocols can be seamlessly integrated into routine clinical workflows (e.g., concurrent with complete blood count analysis), offering superior translational potential. In contrast, the specialized infrastructure requirements for bone marrow sample processing may hinder its broad clinical adoption. However, this study has certain limitations as it was a retrospective single-center study with a limited sample size. Additionally, the prognostic effects of immunotherapy, including HSCT, were not included in the analysis, which may affect our study results. Our study focused on peripheral blood lymphocyte subsets at ND and first CR stages. The impact of subsequent consolidation therapy and additional chemotherapy cycles on lymphocyte subsets remains unknown. While the single time-point analysis identified independent prognostic factors at the CR stage, it did not capture the dynamic trajectories of immune reconstitution during treatment or their influence on relapse. Future research should incorporate continuous monitoring of lymphocyte subset changes across ND, CR, and maintenance therapy stages using time-varying covariate Cox models. Additionally, further studies are needed to investigate the underlying mechanisms driving these immune alterations in AML.

Conclusion

In this study, we identified changes in lymphocyte subsets across different disease stages of AML and their correlation with prognosis. Monitoring lymphocyte subsets at initial diagnosis predict the treatment response to induction therapy, and significant changes were observed in lymphocyte subsets between initial diagnosis and remission status in the same patient. Monitoring lymphocyte subsets at remission has predictive value for relapse and survival in AML.This study demonstrates that dynamic monitoring lymphocyte subsets has significant application value in evaluating the clinical prognosis of AML patients and may provide strategies for immunotherapy selection.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Z.Z. and H.W. conceived and designed the study. X.J., J.Z., J.Z., M.X., Y.D., and H.X. assisted with data acquisition, analysis, and interpretation. X.J., J.Z., and X.L.contributed to the drafting, review, and revision of the manuscript. X.J., Y.L., H.W., and others conducted patient follow-up. All authors contributed to data analysis, drafting, and critical revision of the paper, and agreed to be accountable for all aspects of the work.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Institutional Review Board at the Second Affiliated Hospital of Anhui Medical University(No.20140013).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Additional information

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