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ORIGINAL RESEARCH

Effect of Age, Sex and Season on Acute Myeloid Leukemia Clinical Characteristics: A Retrospective Study

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Background: Acute myeloid leukemia (AML) is a lethal malignancy of the bone marrow, characterized by rapid proliferation of immature myeloid cells, leading to insufficient hematopoiesis and immune activities. It is well known that AML is closely associated with various molecular and cytogenetic abnormalities. In addition, the long-standing view that non-genetic factors, including age, sex and season, are also associated with the occurrence and development of AML. However, effects of these factors on AML clinical characteristics remain incompletely understood. During clinical practice, we perceived an imbalance distribution of clinical characteristics (including FAB classification, gene mutations, lymphocyte-associated cytokine levels and lymphocyte-subset proportions) in different age, sex and season groups. In order to elucidate the correlations between these factors, we performed a comprehensive data collection and analysis of AML patients in our hospital from 2013 to 2023.

Methods: Totally, 2798 newly diagnosed AML patients and 220 relapsed AML patients who were admitted to our hospital from January 1, 2013 to December 31, 2023 were included for analysis. Chi-square test was conducted to analyze the correlation between categorical variables. T-tests and one-way ANOVA were employed to compare mean values across two and multiple groups respectively. Mann–Whitney *U*-tests and Kruskal–Wallis *H*-tests were employed to compare mean values across two and multiple groups respectively, when data did not show normal distribution. Logistic regression was used to analyze the correlation between dependent and independent variables. Log rank test was applied for survival analysis. Waterfall diagram and chord diagram of mutated genes were created using R4.3.3 and RStudio tools.

Results: Overall, the distribution of age, sex and season in AML patients were unbalanced. The relationships among various mutated genes had two sides, co-existence or mutual exclusivity. Additionally, the FAB classification and gene mutation status varied significantly across the subgroups. The levels of cytokines and lymphocyte subsets altered significantly in AML patients, and were associated with prognosis and gene mutations.

Conclusion: Age, sex and season have shown partial correlations with AML clinical characteristics, including FAB classification, gene mutations status, lymphocyte-associated cytokine levels and lymphocyte subset proportions. We hope these findings can contribute to a deeper understanding of AML.

Keywords: acute myeloid leukemia, correlation analysis, mutated genes, FAB classification, cytokine, lymphocyte subset, age, sex, season

Introduction

Acute myeloid leukemia (AML) is a highly heterogeneous hematological malignancy, characterized by clonal proliferation of primitive cells in the bone marrow or extramedullary tissues, leading to insufficient hematopoiesis and immune activities.[1] An epidemiological investigation revealed that the global incidence of AML nearly doubled from 1990 to

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2017, and China had the second highest number of AML patients after India.[2] Therefore, it is imperative to explore the pathogenesis and epidemiology of AML and find the potential preventive measures.

It is well known that AML is closely associated with various molecular and cytogenetic abnormalities. In addition, the long-standing view that non-genetic factors, including age, sex and season are also associated with the occurrence and development of AML. For example, epidemiological studies showed that age was an important risk factor for AML, and the overall incidence of people aged over 65 years was 10 times higher than that of those aged under 65 years (20.1 per 100,000 person-years vs 2.0 per 100,000 person-years).[3] Moreover, for AML patients who achieved complete remission (CR), the likelihood of recurrence within the first 3 years increased approximately 17% for each 10-year increase in age.[4] Regarding sex, studies showed that the lifetime risk of AML for males was approximately 1.6 times higher than that of females (5.42 per 100,000 person-years vs 3.47 per 100,000 person-years), but there seemed to be little differences in CR rates, relapse rates and survival outcomes between male and female patients.[3,5] Interestingly, some recent studies suggested that the incidence of AML varied with seasonal changes and the variedness of immune cells, inflammatory factors, and related enzymatic activity in different seasons may increase susceptibility to AML.[6–8] For instance, a study from Spain reported that the incidence of AML was significantly higher in January compared to other months, and this seasonal effect was consistent in different age and sex subgroups.[6] Another research from Pakistan also indicated that the incidence of AML displayed seasonal variability with the highest incidence observed during the southwest monsoon period, and the lowest incidence during the retreating period.[7] However, the correlation between season and AML remains largely unreported.

Although age, sex and season were reported to be associated with the incidence and development of AML, their correlation with clinical characteristics of AML remained unclear. During long-term clinical practice, we perceived an imbalance distribution of clinical characteristics (including FAB classification, gene mutation status, lymphocyte-associated cytokine levels and lymphocyte subset proportions) in different age, sex and season groups. In order to elucidate the correlations between these factors, we collected information from 2798 newly diagnosed AML (ND-AML) patients and 220 relapsed AML (RE-AML) patients between January 1, 2013, and December 31, 2023 at a single center in Southwest China to explore the potential relationships among age, sex, season and AML clinical characteristics.

Materials and Methods

Study Participants

2798 newly-diagnosed AML patients and 220 relapsed AML patients who were admitted to the Hematology Center of the Second Affiliated Hospital of the Army Medical University in Chongqing, China, between January 1, 2013, and December 31, 2023 were included for analysis. And 80 healthy people served as the controls. All data analyzed were unpaired (samples independent from each other). All patients were diagnosed through bone marrow aspiration cytology and classified according to the FAB classification system. Patients' clinical information, including age, sex, primary diagnosis date, FAB classification, gene mutation status, lymphocyte-associated cytokine levels, and lymphocyte-subset proportions were recorded. Age was divided into 5 groups (<18 years, 18~30 years, 31~45 years, 46~65 years, >65 years). Sex was dichotomized into male and female. Season were categorized into four groups according to the onset date of AML, including spring (months March through May), summer (months June through August), autumn (months September through November), and winter (months December through February). FAB subtypes were categorized into seven subtypes (M0/M1, M2, M3, M4, M5, M6, M7).

Next-Generation Sequencing

Bone marrow samples were collected from 647 ND-AML patients at initial diagnosis. The DNA sample was prepared using conventional library construction methods. The library, hybridization probe, Cot DNA, blocker, and other necessary components were added to the hybridization buffer system for overnight hybridization. The hybridized DNA was captured by streptomycin magnetic beads. The target library was eluted in an elution buffer and amplified. The final library was sequenced on the DNBSEQ-T7 platform. FastQC (v0.11.8) and Trimmomatic (v0.39) softwares were used for quality control (QC). High-quality reads were aligned to the human genome (Genome Reference

Consortium Human Reference 37, GRCh37) using the BWA aligner (v0.7.17) with the BWA-MEM algorithm and default parameters, generating SAM files. The SAM files were converted to compressed BAM files using Samtools (v1.9) and sorted according to chromosome coordinates. Local realignment of the BAM files at intervals with indel mismatches was performed using the Genome Analysis Toolkit (GATK, v4.1.9.0), and base quality scores of the reads in the BAM files were recalibrated.

Flow Cytometry Analysis

Peripheral blood mononuclear cells were obtained by density gradient with Ficoll from peripheral blood specimens. Lymphocyte-associated cytokine levels were measured by Human Th1/Th2/Th17 Cytokine Detection Kit (P110700102, CELLGENE BIOTECH, Jiangxi, China), and specimens from 217 ND-AML patients and 28 RE-AML patients were tested. The lymphocyte subsets were detected by BD MultiTEST IMK Kit (662966, BD Biosciences, USA), and specimens from 43 ND-AML patients and 13 RE-AML patients were tested. Samples collected from 80 healthy subjects were used as controls. Flow cytometry was performed using the FACSCanto II (BD Biosciences, USA) and analyzed with FACSDiva software (BD Biosciences, USA).

Statistical Analysis

SPSS 25.0 software was used for statistical analysis. Chi-square test was conducted to analyze the correlation between categorical variables. T-tests and one-way ANOVA were employed to compare mean values across two and multiple groups respectively. Mann–Whitney *U*-tests and Kruskal–Wallis *H*-tests were employed to compare mean values across two and multiple groups respectively, when data did not show normal distribution. Logistic regression was used to analyze the correlation between dependent and independent variables. Log rank test was applied for survival analysis. Waterfall diagram and chord diagram of mutated genes were created using R4.3.3 and RStudio tools. $P<0.05$ indicated statistically significant.

Result

Clinical Information Statistics

In total, 2798 ND-AML patients from January 1, 2013, to December 31, 2023 were included for analysis. Among ND-AML patients, the proportion of males was higher than that of females (Male: 1504, 53.75%; Female: 1294, 46.25%) (Figure 1A). The season distribution exhibited roughly balanced (Spring: 732, 26.16%; Summer: 715, 25.55%; Autumn: 667, 23.84%; Winter: 684, 24.45%) (Figure 1B). For FAB classification of ND-AML, M2 subtype exhibited the highest prevalence, while M7 subtype was the least common (M0/M1: 297, 10.61%; M2: 793, 28.34%; M3: 334, 11.94%; M4: 604, 21.59%; M5: 656, 23.45%; M6: 91, 3.25%; M7: 23, 0.82%) (Figure 1C). The median age of all ND-AML patients was 50 years, with 50 years for male patients and 50.5 years for female patients (Figure 1D). The majority of ND-AML patients were middle-aged and elderly groups (<18 years: 203, 7.26%; 18~30 years: 322, 11.51%; 31~45 years: 541, 19.34%; 46~65 years: 1151, 41.14%; >65 years: 581, 20.76%) (Figure 1E).

Moreover, a total of 220 RE-AML patients from January 1, 2013, to December 31, 2023 were included for analysis. Among RE-AML patients, the proportion of males and females was approximately equivalent (Male: 111, 50.45%; Female: 109, 49.55%) (Figure 2A). The higher incidence of relapse was observed during autumn and winter (Spring: 51, 23.18%; Summer: 45, 20.45%; Autumn: 62, 28.18%; Winter: 62, 28.18%) (Figure 2B). For FAB classification of RE-AML, M2 subtype still exhibited the highest prevalence (M0/M1: 10, 4.55%; M2: 75, 34.09%; M3: 5, 2.27%; M4: 51, 23.18%; M5: 73, 33.18%; M6: 6, 2.73%; M7: 0, data on M7 subtype were not collected) (Figure 2C). And the proportion of M3 subtype was significantly lower in RE-AML patients compared with ND-AML patients ($P<0.001$), benefiting from the combination therapy of all-trans retinoic acid and arsenic trioxide. The median age of all RE-AML patients was 48 years, with 47 years for male patients and 49 years for female patients (Figure 2D). It was worth noting that patients with RE-AML tended to be younger than those with ND-AML (<18 years: 18, 8.18%; 18~30 years: 31, 14.09%; 31~45 years: 50, 22.73%; 46~65 years: 109, 49.55%; >65 years: 12, 5.45%) (Figure 2E).

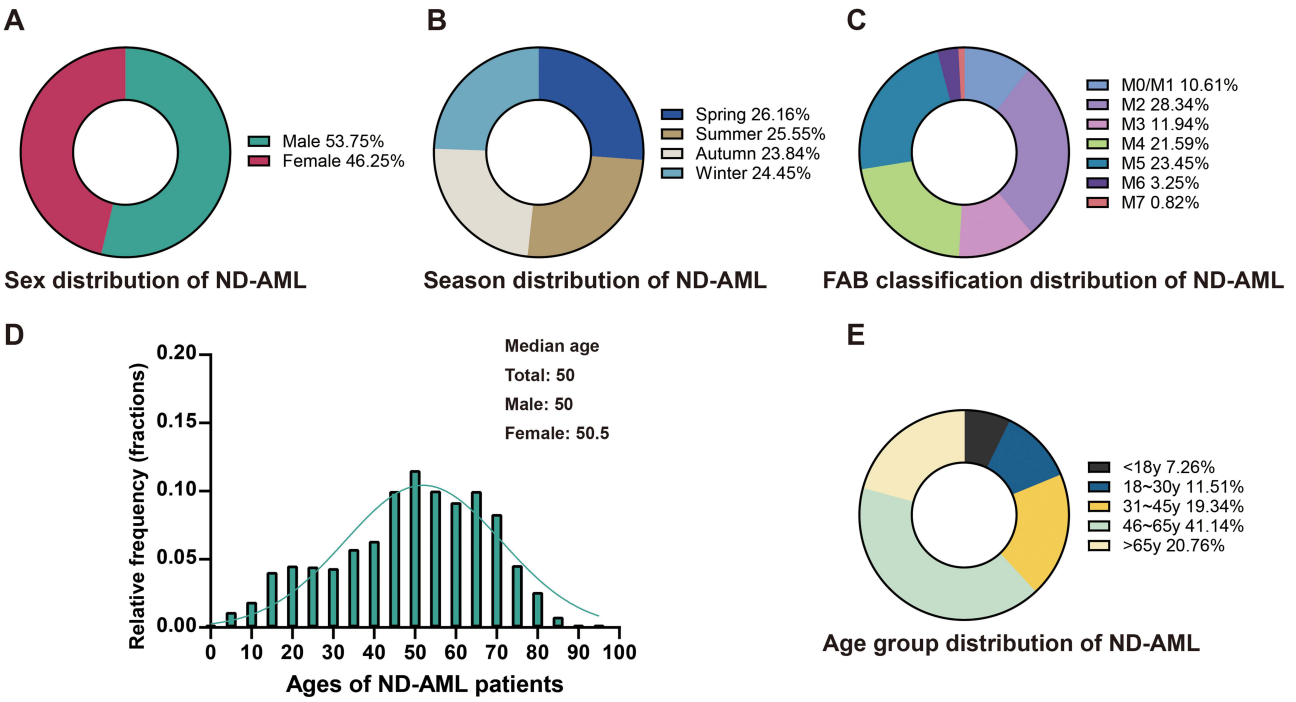

Figure 1 General information of ND-AML patients. (**A**) Sex distribution of ND-AML. (**B**) Season distribution of ND-AML. (**C**) FAB classification distribution of ND-AML. (**D**) Age distribution of ND-AML. (**E**) Age group distribution of ND-AML.

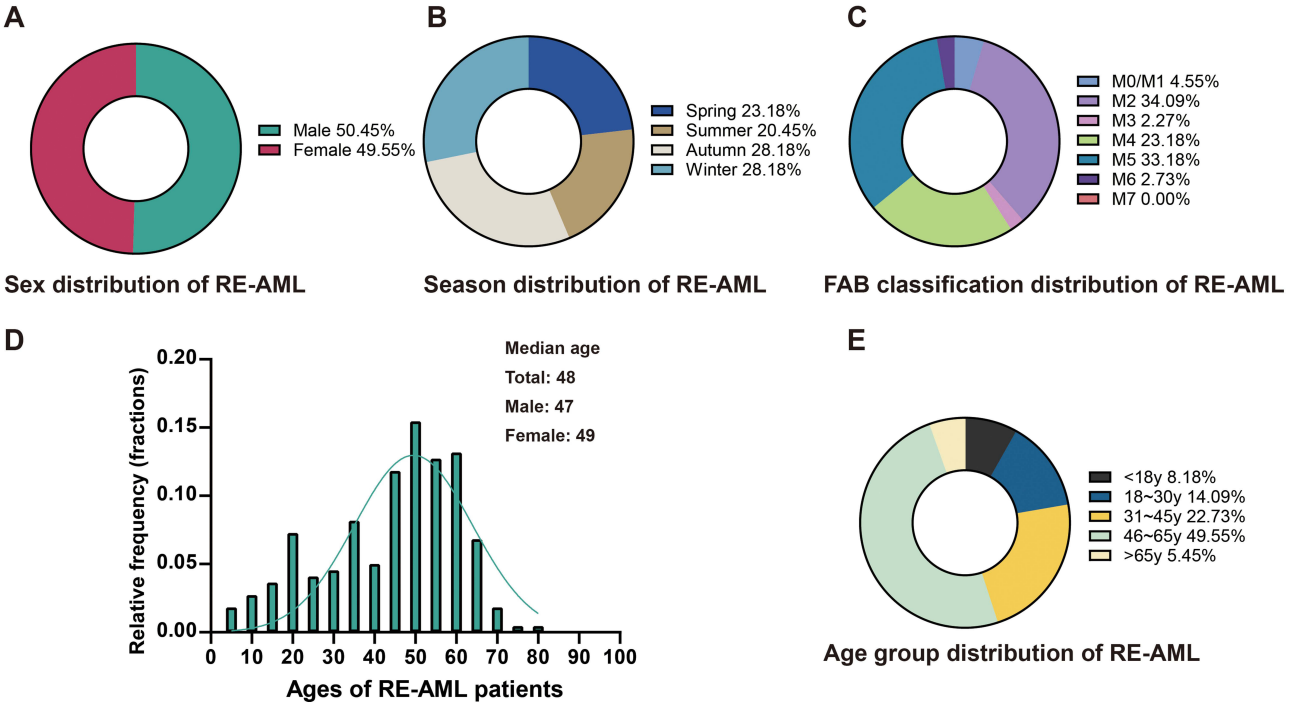

Figure 2 General information of RE-AML patients. (**A**) Sex distribution of RE-AML. (**B**) Season distribution of RE-AML. (**C**) FAB classification distribution of RE-AML. (**D**) Age distribution of RE-AML. (**E**) Age group distribution of RE-AML.

The Gene Mutation Landscape of AML Patients

In our results, over 80% of AML patients (with NGS results) harbored mutated genes. Among them, 167 patients (25.81%) had one mutated gene, 137 patients (21.17%) had two simultaneous mutated genes, 98 patients (15.15%) had three simultaneous mutated genes, 68 patients (10.51%) had four simultaneous mutated genes, 50 patients (7.73%) had more than or equal to five simultaneous mutated genes, and 127 patients (19.63%) did not have mutated genes (Figure 3A). The landscape of mutated genes was presented by a waterfall diagram (Figure 3B). The co-existence relationship between mutated genes was shown by a chord diagram (Figure 3C). Our results revealed that 7 pairs of mutated genes exhibited co-existence relationships (TET2 and DNMT3A, P=0.003; TET2 and NPM1, P<0.001; FLT3-ITD and DNMT3A, P<0.001; FLT3-ITD and NPM1, P<0.001; ASXL1 and RUNX1, P=0.006; DNMT3A and NPM1, P<0.001; NRAS and RUNX1, P=0.024), and 3 pairs of mutated genes exhibited mutually exclusive relationships (FLT3-ITD and ASXL1, P=0.001; ASXL1 and NPM1, P=0.001; NRAS and TP53, P=0.034) (Table 1).

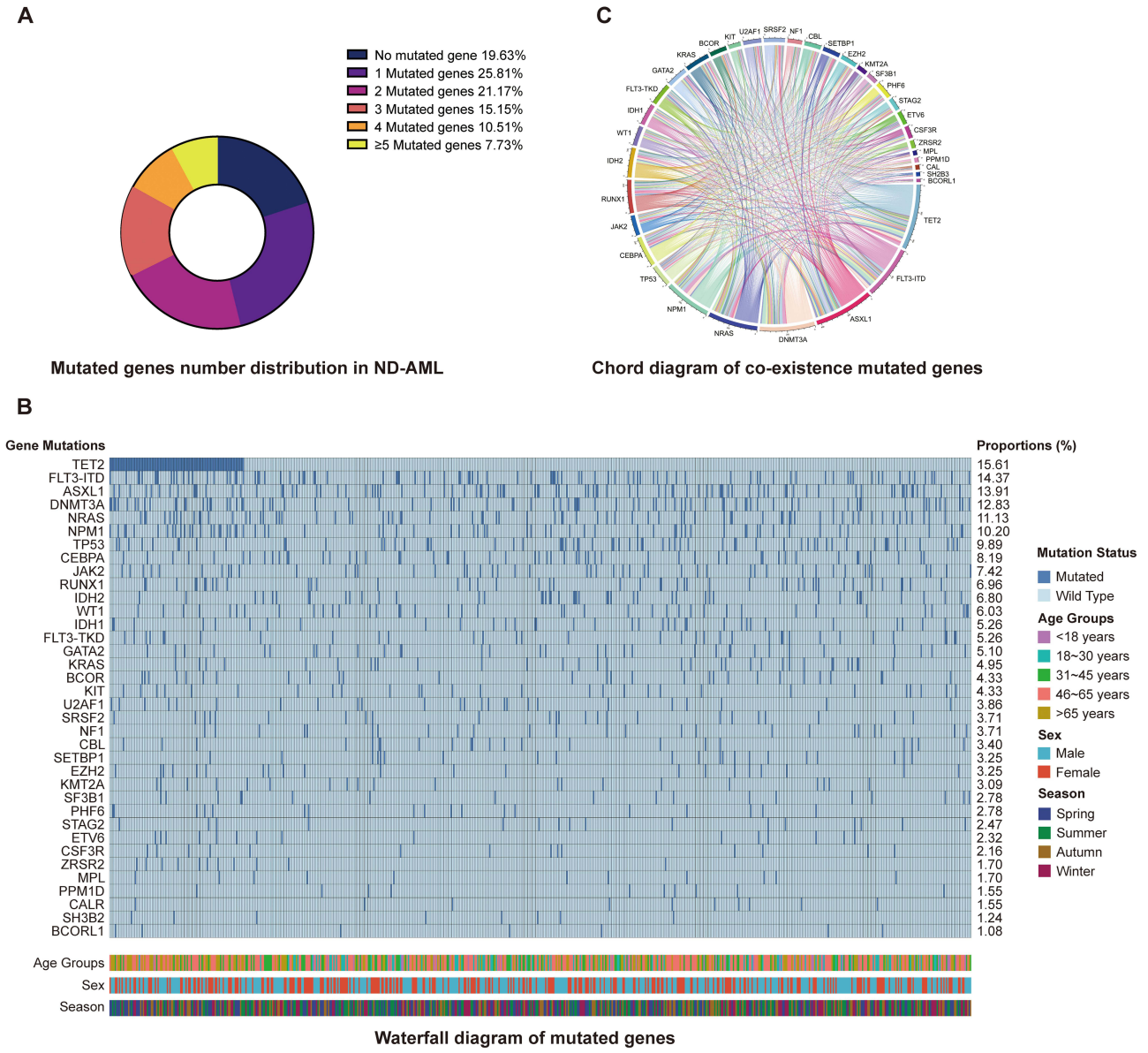

Figure 3 Landscape of gene mutations in 647 ND-AML patients. (**A**) Mutated genes number distribution in ND-AML. (**B**) Waterfall diagram of mutated genes. (**C**) Chord diagram of co-existence mutated genes.

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Table 1 Co-Existence and Mutual Exclusivity of Mutated Genes in ND-AML

Gene A	Gene B	M/M (n)	M/W (n)	W/M (n)	W/W (n)	C	P value	Correlation
TET2	DNMT3A	23	78	60	486	0.127	0.003	Co-existence
TET2	NPM1	23	78	43	503	0.176	<0.001	Co-existence
FLT3-ITD	ASXL1	3	90	87	467	0.126	0.001	Mutual exclusivity
FLT3-ITD	DNMT3A	24	69	59	495	0.157	<0.001	Co-existence
FLT3-ITD	NPM1	30	63	36	518	0.286	<0.001	Co-existence
ASXL1	NPM1	1	89	65	492	0.120	0.001	Mutual exclusivity
ASXL1	RUNX1	13	77	32	525	0.118	0.006	Co-existence
DNMT3A	NPM1	28	55	38	526	0.286	<0.001	Co-existence
NRAS	TP53	2	70	62	513	0.084	0.034	Mutual exclusivity
NRAS	RUNX1	10	62	35	540	0.096	0.024	Co-existence

Notes: Attention: "M/M" means gene A and gene B are mutated; "M/W" means gene A is mutated and gene B is wildtype; "W/M" means gene A is wildtype and gene B is mutated; "W/W" means gene A and gene B are wildtype; "C" means the coefficient of contingency.

Subsequently, we also analyzed the distribution of AML gene mutations across different age, sex and season subgroups. Concerning age, we found that most AML-related gene mutations exhibited a higher prevalence in middle-aged or elderly individuals, as shown in Figure 4A. For example, compared with other groups, >65y group exhibited the highest mutation rate of TET2 (χ^2=16.682, P<0.001), ASXL1 (χ^2=30.049, P<0.001), DNMT3A (χ^2=12.062, P=0.001), TP53 (χ^2=10.289, P=0.001), SRSF2 (χ^2=26.851, P<0.001), SETBP1 (χ^2=9.734, P=0.002), SF3B1 (χ^2=9.829, P=0.002) and STAG2 (χ^2=12.799, P<0.001) mutations, and 46~65y group exhibited the highest mutation rate of FLT3-ITD (χ^2=6.415, P=0.011), NPM1 (χ^2=4.132, P=0.042), IDH2 (χ^2=5.109, P=0.024), CBL (χ^2=4.033, P=0.045), and PHF6 (χ^2=4.303, P=0.038) mutations (Figure 4A and Supplement Table 1). Moreover, patients in 31~45y group exhibited lower mutation rate of ASXL1 (χ^2=9.199, P=0.002), and higher mutation rate of WT1 (χ^2=8.891, P=0.003) and KMT2A (χ^2=3.987, P=0.046) (Figure 4A and Supplement Table 1). Among 18~30y group, the mutation rate of KIT (χ^2=5.928, P=0.015) was significantly higher than that of other groups (Figure 4A and Supplement Table 1). As for sex, our results indicated that male patients had higher mutation rate of TP53 (χ^2=11.516, P<0.001), and female patients had higher mutation rate of DNMT3A (χ^2=3.976, P=0.046) and IDH2 (χ^2=7.483, P=0.006) (Figure 4B and Supplement Table 2). In terms of season, our results indicated that the mutation rate of TET2 (χ^2=6.088, P=0.014) and FLT3-TKD (χ^2=4.335, P=0.037) were significantly higher in spring than those observed in other seasons (Figure 4C and Supplement Table 3). Besides, summer-diagnosed patients displayed unusually elevated mutation rate in NF1 (χ^2=6.001, P=0.014), CBL (χ^2=18.272, P<0.001) and SETBP1 (χ^2=4.157, P=0.041) (Figure 4C and Supplement Table 3). Among autumn-diagnosed AML patients, the NRAS (χ^2=4.916, P=0.027) mutation was rare (Figure 4C and Supplement Table 3). Furthermore, we observed a higher prevalence of RUNX1 (χ^2=10.916, P<0.001) and GATA2 (χ^2=3.893, P=0.048) mutations among winter-diagnosed AML patients (Figure 4C and Supplement Table 3).

The Lymphocyte-Associated Cytokines Levels and Lymphocyte Subsets Distribution of AML Patients

Compared with healthy people, the levels of IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL-17A were greatly increased in ND-AML and RE-AML patients (Figure 5A-5G). Moreover, there was a significant increase in the percentage of T cells in comparison with healthy humans, but significant reductions in B cells and $CD4^+T/CD8^+T$ cell ratio (Figure 5H-5J). Survival analysis showed that patients with high IL-6 levels exhibited a poorer 3-year overall survival (OS) than those with low IL-6 levels (Figure 5K). While other cytokines did not have impact on prognosis (Supplement Figure 1). Besides, we also assessed whether cytokines levels at initial diagnosis were associated with gene mutations in AML patients. We found that IL-17A levels were positively correlated with NRAS mutation (P=0.012) (Figure 5L). All cytokines and lymphocyte subset proportions showed no differences among subgroups of age, sex and season in ND-AML (Supplement Figure 2–4).

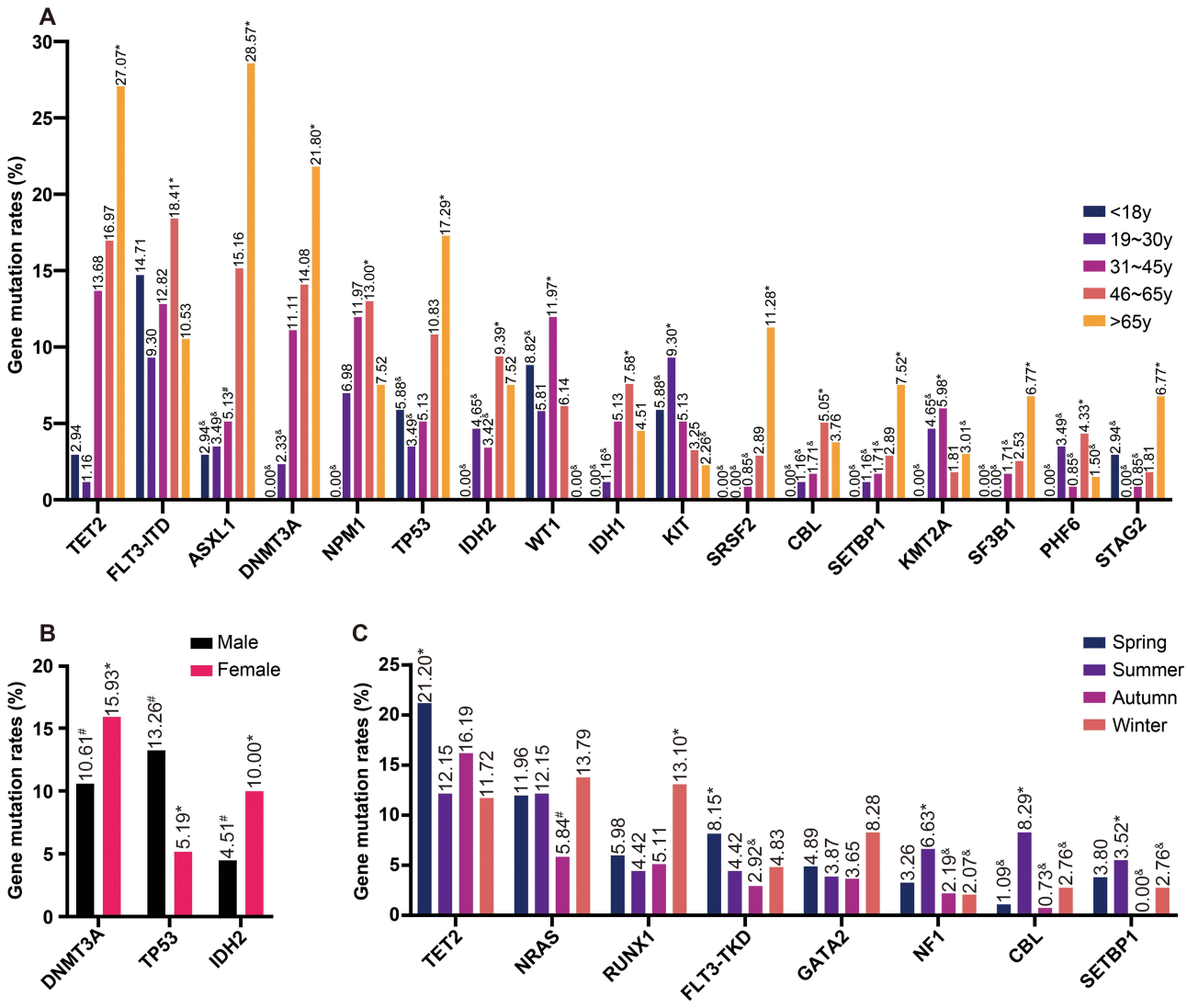

Figure 4 Correlation analysis between age, sex, season and mutated genes. (**A**) Gene mutation rate distribution in different age groups. (**B**) Gene mutation rate distribution in different sex groups. (**C**) Gene mutation rate distribution in different season groups. * represents significantly higher proportion (P<0.05). # represents significantly lower proportion (P<0.05) and indicates that the sample size of this group is insufficient for analysis (n<5).

Correlation Analysis Between Age, Sex, Season, and FAB Classification

Herein, we presented the correlations between pairs of clinical characteristics, including age, sex, season and FAB classification. However, there was no statistically correlation between age and season in either ND-AML or RE-AML.

Correlation Analysis Between Age and Sex

Among ND-AML, the proportion of female patients were higher in 46~65y group than that of other age groups (χ^2=7.994, P=0.005) (Figure 6A and Supplement Table 4). Among RE-AML, the sex distribution did not exhibited statistical differences between age groups (Figure 6B and Supplement Table 5).

Correlation Analysis Between Age and FAB Classification

Among ND-AML, patients with M0/M1 subtype were more likely to be distributed in the >65y group (χ^2=8.553, P=0.003) and notably rare in the <18y group (χ^2=4.090, P=0.043) (Figure 6C and Supplement Table 6). Moreover, patients with M3 subtype exhibited a distribution skewed toward younger groups including <18y group (χ^2=19.744, P<0.001), 18~30y group (χ^2=15.522, P<0.001) and 31~45y group (χ^2=11.957, P<0.001), but absolutely not >65y group (χ^2=52.395, P<0.001) (Figure 6C and Supplement Table 6). In addition, patients with M4 subtype (χ^2=5.435, P=0.020) and M7 subtype (χ^2=4.754, P=0.029) tended

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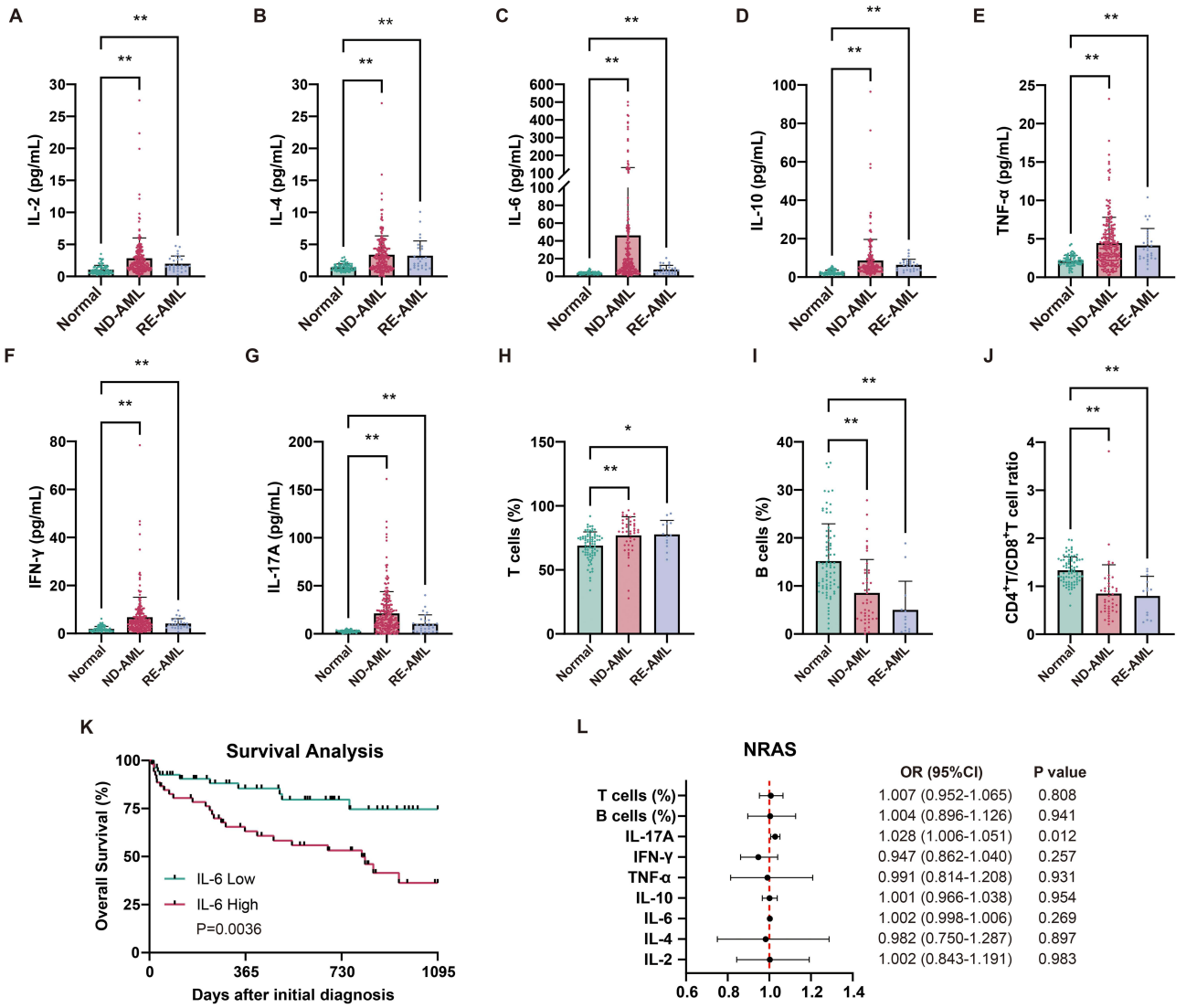

Figure 5 Lymphocyte-associated cytokines and lymphocyte subsets analysis. (**A**) IL-2 concentrations. (**B**) IL-4 concentrations. (**C**) IL-6 concentrations. (**D**) IL-10 concentrations. (**E**) TNF-α concentrations. (**F**) IFN-γ concentrations. (**G**) IL-17A concentrations. (**H**) T cell percentages. (**I**) B cell percentages. (**J**) $CD4^+T/CD8^+T$ cell ratios. (**K**) The overall survival in IL-6 high patients and IL-6 Low patients. The cut-off value of cytokines was defined as >75% percentile (High) and <25% percentile (Low). (**L**) The correlation between immune factors and NRAS mutation. * P<0.05, ** P<0.01.

Abbreviations: ND, Newly Diagnosed; RE, Relapsed; OR, Odds Ratio; CI, Confidence Interval.

to be in the >65y group (Figure 6C and Supplement Table 6). Finally, the highest proportion of patients with M6 subtype was observed in 31~45y group (χ^2=7.884, P=0.005) (Figure 6C and Supplement Table 6). Among RE-AML, patients with M2 subtype exhibited the broadest distribution within >65y group (χ^2=5.994, P=0.0.014) (Figure 6D and Supplement Table 7).

Correlation Analysis Between Sex and Season

Among ND-AML patients, the proportion of female patients in spring was higher than that of other seasons (χ^2=4.456, P=0.035) (Figure 6E and Supplement Table 8). However, there was no statistical difference between sex and season in RE-AML patients (Figure 6F and Supplement Table 9).

Correlation Analysis Between Sex and FAB Classification

Among ND-AML patients, the proportion of patients with M0/M1 subtype in females was significantly higher than that in males (χ^2=4.718, P=0.030) (Figure 6G and Supplement Table 10). Nevertheless, there was no statistical difference in sex between FAB subtypes among RE-AML patients (Figure 6H and Supplement Table 11).

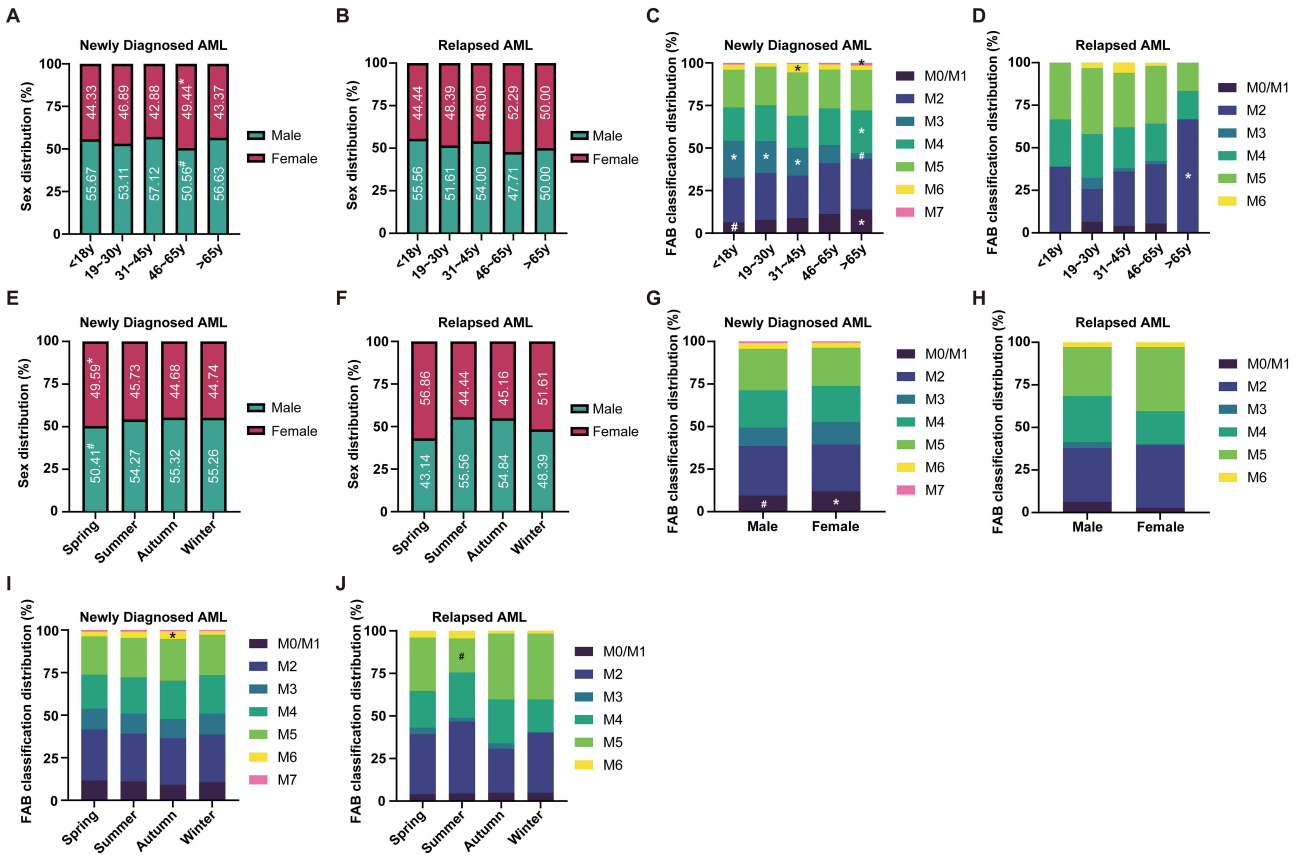

Figure 6 Correlation analysis between age, sex, season and FAB classification. (**A**) The sex distribution in age subgroups among ND-AML. (**B**) The sex distribution in age subgroups among RE-AML. (**C**) The FAB classification distribution in age subgroups among ND-AML. (**D**) The FAB classification distribution in age subgroups among RE-AML. (**E**) The sex distribution in season subgroups among ND-AML. (**F**) The sex distribution in season subgroups among RE-AML. (**G**) The FAB classification distribution in sex subgroups among ND-AML. (**H**) The FAB classification distribution in sex subgroups among RE-AML. (**I**) The FAB classification distribution in season subgroups among ND-AML. (**J**) The FAB classification distribution in season subgroups among RE-AML. * represents significantly higher proportion (P<0.05). # represents significantly lower proportion (P<0.05).

Correlation Analysis Between Season and FAB Classification

Among ND-AML patients, the proportion of patients with M6 subtype diagnosed in autumn was higher than that in other seasons (χ^2=4.317, P=0.038) (Figure 6I and Supplement Table 12). Among RE-AML patients, the proportion of patients with M5 subtype diagnosed in summer was significantly lower than that in other seasons (χ^2=4.434, P=0.035) (Figure 6J and Supplement Table 13).

Correlation Analysis Between FAB Classification and Age, Sex, Season: Based on Logistic Regression

We further constructed a multivariate Logistic regression model to analyze the correlation of FAB classification with age, sex and season. For the disordered multinomial variables, FAB classification and season, we used M0/M1 subtype and winter as reference categories. As shown in Table 2, in ND-AML, M2 and M5 subtypes were negatively correlated with age, and positively correlated with sex male; M3 subtype was negatively correlated with age; and M6 subtype was positively correlated with autumn. In RE-AML, the multivariate Logistic regression was not applicable due to insufficient data volume.

Discussion

Although great advances have been made in the treatment of AML in recent decades, such as targeted therapy, cell-based therapy, allogeneic or autologous hematopoietic stem cell transplantation therapy, AML still remains an important lethal disease and the 5-year relative survival rate is only 29.5%.[9–13] Apart from cytogenetic and molecular factors, non-genetic factors also potentially influence the disease characteristics of AML, and exploring their relationship can enhance our

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Table 2 The Logistic Regression Analysis in ND-AML

FAB Classification	Variables	B	P value	OR (95% CI)
M2	Age	−0.008	0.040	0.992 (0.985–1.000)
	Sex: male	0.288	0.035	1.333 (1.020–1.743)
	Season: spring	−0.010	0.958	0.990 (0.684–1.433)
	Season: summer	−0.045	0.817	0.956 (0.656–1.394)
	Season: autumn	0.112	0.579	1.119 (0.752–1.665)
M3	Age	−0.037	<0.001	0.964 (0.956–0.973)
	Sex: male	0.024	0.883	1.024 (0.746–1.407
	Season: spring	−0.042	0.850	0.959 (0.618–1.487)
	Season: summer	−0.070	0.758	0.932 (0.597–1.456)
	Season: autumn	0.046	0.847	1.047 (0.656–1.672)
M4	Age	−0.005	0.178	0.995 (0.987–1.002)
	Sex: male	0.256	0.072	1.292 (0.978–1.708
	Season: spring	−0.206	0.298	0.814 (0.552–1.199)
	Season: summer	−0.106	0.595	0.899 (0.609–1.329)
	Season: autumn	0.140	0.501	1.150 (0.765–1.731)
M5	Age	−0.011	0.007	0.989 (0.982–0.997)
	Sex: male	0.310	0.028	1.363 (1.035–0.796)
	Season: spring	−0.119	0.543	0.888 (0.606–1.302)
	Season: summer	−0.060	0.760	0.942 (0.640–1.386)
	Season: autumn	0.172	0.407	1.187 (0.792–1.781)
M6	Age	−0.010	0.132	0.990 (0.977–1.003)
	Sex: male	0.361	0.136	1.435 (0.892–2.306)
	Season: spring	0.152	0.687	1.165 (0.555–2.442)
	Season: summer	0.466	0.199	1.593 (0.782–3.247)
	Season: autumn	0.859	0.017	2.360 (1.163–4.791)
M7	Age	0.017	0.196	1.017 (0.991–1.044)
	Sex: male	0.542	0.222	1.719 (0.721–4.099)
	Season: spring	0.405	0.532	1.500 (0.421–5.339)
	Season: summer	0.470	0.468	1.601 (0.449–5.701)
	Season: autumn	0.394	0.570	1.482 (0.381–5.770)

understanding of AML. In this study, we meticulously collected and analyzed the clinical data from AML patients in the latest 10 years of our hospital. Subsequently, we performed a statistical analysis of the correlation between age, sex, season and AML characteristics, including FAB classification, gene mutation status, lymphocyte-associated cytokine levels and lymphocyte-subset proportions.

Gene mutations are well-established driven factors in the pathogenesis of AML. In our results, over 80% of ND-AML patients harbored at least 1 mutation, and the mutation profile was consistent with the report from other regions of China (Nanjing, Taiwan).[14,15] For certain genes, such as FLT3-ITD and NPM1, the mutation frequencies in our study were lower than other countries, such as Egypt and Iran, possibly due to differences in ethnic backgrounds.[16,17] In addition, the co-existence of gene mutations were common in AML. For example, NPM1 mutation frequently co-occurred with the FLT3-ITD mutation, which was correlated with poor prognosis, and our findings corroborated this association.[18] Herein, the FLT3-ITD mutation rate was 45.45% in NPM(+) AML patients, whereas it was 10.84% in NPM(-) patients. Moreover, our findings indicated that ASXL1 mutation and RUNX1 mutation frequently occurred in pairs. Bera et al reported that the co-occurrence of ASXL1 and RUNX1 mutations played a cooperative role in leukemia development and resulted in worse prognosis.[19] There was also a co-existence relationship between FLT3-ITD and DNMT3A mutation, which was associated with poor prognosis.[20] The clinical significance of other co-existence relationships we reported, including TET2/DNMT3A, TET2/NPM1, DNMT3A/NPM1, NRAS/RUNX1, needed further investigation.

In terms of the correlation between age, sex, season and gene mutations, we found some interesting results. Firstly, AML-related genes generally exhibited higher mutation rates in middle-aged or elderly patients (such as TET2, ASXL1,

DNMT3A, TP53, FLT3-ITD, IDH2 et al), compared to younger patients, which was largely due to the accumulation of somatic mutations with aging, and consistent with previous studies.[21] Nextly, TP53 mutation was more frequent in males, and DNMT3A and IDH2 mutations were more frequent in females. Finally, we unveiled the season-related specific high-frequency mutated genes, such as TET2 and FLT3-TKD for spring, NF1, CBL and SETBP1 for summer, RUNX1 and GATA2 for winter. The clinical significances of these associations warrant further research.

Cytokines and lymphocytes dysregulation have often been associated with tumor development.[22–24] Our results revealed that 7 cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, IL-17A) and T cell percentages were significantly increased, while B cell percentages and $CD4^+T/CD8^+T$ cell ratio were significantly decreased in ND-AML and RE-AML patients compared with healthy donors. These findings were consistent with previous studies.[25–32] These immune factors could regulate AML development by modulating inflammatory microenvironment, activating signaling pathways, or altering metabolism.[28,33–35] We also revealed that AML patients with high IL-6 levels exhibited poorer OS rates. In agreement with us, other 2 studies also reported that elevated levels of IL-6 in AML patients were correlated with substantially worse event-free survival (EFS) and OS.[22,36] Moreover, we revealed a positive correlation between IL-17A and NRAS mutations. Searching the literature, we found that NRAS mutations can promote the expansion of $TCR\alpha\beta^+CD4^-CD8^-$ double-negative T cells (αβDNTs) and facilitate their release of IL-17A, ultimately promoting the progression of leukoproliferative disease to leukemia.[37] Another study revealed that the NRAS mutation can lead to IL-17A release by stimulating RAS signaling in rheumatoid arthritis.[38] However, the clinical implications of NRAS mutation accompanied by release of IL-17A and its specific regulatory mechanism are not clear in AML.

As a non-genetic factor, the roles of season were only reported in a limited number of studies due to many variables across countries affecting the assay results, such as ambient temperature, humidity, sunshine duration and so on.[6–8] Herein, most of our patients were from the southwest region of China, which shared similar environmental conditions:[39] terrain dominated by mountains and highlands, subtropical monsoon climate, limited sunshine duration, and ethnic group dominated by Han. We hoped our results about seasons was useful for understanding the situation of AML patients in regions with similar environment. Concretely, our results indicated that the season distribution was balanced in ND-AML patients, but tended to autumn and winter in RE-AML patients. Additionally, the female incidence of AML was higher in spring, rather than other seasons. As for FAB classification, patients with M6 subtype tended to be in autumn among ND-AML, and M5 patients with M5 subtype tended to avoid summer among RE-AML.

Taken together, we conducted the correlation analysis of the age, sex, season and AML characteristics, including FAB classification, frequently mutated genes, lymphocyte-associated cytokines and lymphocyte subsets in AML. Our study provided a depiction of the clinical characteristics of AML patients in a single hospital in Southwest China, which had certain clinical reference significance.

Abbreviations

AML, acute myeloid leukemia; FAB, French-American-British; HD, Healthy Donor; ND, newly diagnosed; CR, complete remission; RE, relapsed; NGS, next generation sequencing; TET2, tet methylcytosine dioxygenase 2; FLT3-ITD, FMS-like kinase 3-internal tandem duplication; ASXL1, ASXL transcriptional regulator 1; DNMT3A, DNA methyltransferase 3 alpha; NRAS, neuroblastoma RAS viral oncogene homolog; NPM1, nucleophosmin 1; TP53, tumor protein P53; CEBPA, CCAAT/enhancer binding protein alpha; JAK2, janus kinase 2; RUNX1, runt-related transcription factor 1; IDH2, isocitrate dehydrogenase (NADP(+)) 2; WT1, Wilms tumor 1; IDH1, isocitrate dehydrogenase (NADP(+)) 2; FLT3-TKD, FMS-like kinase 3-tyrosine kinase domain mutation; GATA2, GATA binding protein 2; KRAS, Kirsten rat sarcoma viral oncogene homolog; BCOR, BCL6 corepressor; KIT, KIT proto-oncogene receptor tyrosine kinase; U2AF1, U2 small nuclear RNA auxiliary factor 1; SRSF2, serine/arginine-rich splicing factor 2; NF1, neurofibromin 1; CBL, Cbl proto-oncogene; SETBP1, SET binding protein 1; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; KMT2A, Lysine (K) methyltransferase 2A; SF3B1, splicing factor 3b subunit 1; PHF6, PHD finger protein 6; STAG2, STAG2 cohesin complex component; ETV6, ETS variant transcription factor 6; CSF3R, colony stimulating factor 3 receptor; ZRSR2, zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2; MPL, MPL proto-oncogene, thrombopoietin receptor; PPM1D, protein phosphatase Mg^{2+}/Mn^{2+} dependent 1D; CALR, calreticulin; SH2B3, SH2B adaptor protein 3; BCORL1, BCL6 corepressor like 1.

Data Sharing Statement

The analyzed data can be found in the article or Supplement data. The unprocessed data are available from the corresponding author on reasonable request.

Ethics

This study was approved by the Medical Ethics Committee of Second Affiliated Hospital of Army Medical University. This study complies with the Declaration of Helsinki.

Consent for Publication

Written informed consent was obtained from all patients.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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