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# The elevation of red blood cell distribution width is an independent prognostic factor for juvenile myelomonocytic leukemia

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**Abstract**

Juvenile myelomonocytic leukemia (JMML) is a disorder characterized by the simultaneous presence of myeloproliferative and myelodysplastic features, primarily affecting infants and young children. Due to the heterogeneous genetic background among patients, the current clinical and laboratory prognostic features are insufficient for accurately predicting outcomes. Thus, there is a pressing need to identify novel prognostic indicators. Red cell distribution width (RDW) is a critical parameter reflecting the variability in erythrocyte size. Recent studies have emphasized that elevated RDW serves as a valuable predictive marker for unfavorable outcomes across various diseases. However, the prognostic role of RDW in JMML remains unclear. Patients with JMML from our single-center cohort between January 2008 and December 2019 were included. Overall, 77 patients were eligible. Multivariate Cox proportional hazard models showed that patients with red cell distribution width coefficient of variation (RDW-CV) >17.35% at diagnosis were susceptible to much worse overall survival rate (hazard ratio [HR] = 5.22, confidence interval [CI] = 1.50–18.21, *P* = .010). Besides, the combination of RDW elevation and protein phosphatase non-receptor type 11 (PTPN11) mutation was likely to predict a subgroup with the worst outcomes in our cohort. RDW is an independent prognostic variable in JMML subjects. RDW may be regarded as an inexpensive biomarker to predict the clinical outcome in patients with JMML.

**Key Words:** Independent prognostic factor; Juvenile myelomonocytic leukemia; Red cell distribution width

## 1. INTRODUCTION

Juvenile myelomonocytic leukemia (JMML) is a distinct and aggressive hematopoietic disease caused by overproduction of monocytic and granulocytic cells.[1] It presents characteristics overlapping myeloproliferative neoplasms and myelodysplastic syndromes (MDSs) and predominantly affects infants and young children.[2] The pathogenetic mechanism in at least 90% of patients with JMML involves constitutive activation of the Ras signal transduction pathway, which is caused by at least 1 of 5 canonical driver Ras pathway mutations (protein phosphatase non-receptor type 11 [PTPN11], NRAS, KRAS, NF1, or CBL) in leukemic cells.[3,4] The disease is associated with a spectrum of diverse outcomes ranging from spontaneous resolution to transformation to acute myeloid leukemia (AML), which is generally fatal.[5] Previous studies showed that elder age at diagnosis (≥2 years), severe thrombocytopenia ($33 \times 10^9$/L), and elevated age-adjusted hemoglobin F (HbF) levels were prognostic variables that associated with poor outcomes (reduced event-free survival [EFS] and overall survival [OS]) after hematopoietic stem cell transplantation (HSCT).[6–8] Molecular risk factors, including the likelihood of having secondary clonal aberrations, PTPN11 mutation and a hypermethylated DNA profile, characterize patients with a worse prognosis.[9,10]

The red cell distribution width (RDW) refers to the degree of variation in erythrocyte size and reflects anisocytosis. For many years, RDW has been specifically used for the differential diagnosis of anemias.[11] Over the past decades, RDW has been proposed as an adverse biomarker in a number of human disorders including cardiovascular disease,[12] venous thromboembolism,[13] diabetes,[14] kidney disease,[15] and liver disease.[16] RDW is also an independent prognostic factor in massive solid cancers and a variety of hematologic malignancies such as natural killer/T-cell lymphoma,[17] diffuse large B-cell lymphoma (DLBCL),[18] MDS,[19] multiple myeloma (MM),[20] Hodgkin lymphoma (HL),[21] and chronic myeloid leukemia (CML).[22]

Regarding the present advances in this field, we hypothesized that RDW may hold prognostic value in patients with JMML. However, there are limited data on the association. Therefore, we conducted a retrospective investigation to evaluate the potential prognostic role of RDW and search for new markers to stratify the risk of patients with JMML.

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## 2. MATERIALS AND METHODS

### 2.1. Patients and clinical variables

In this retrospective study, a total of 77 newly diagnosed patients with JMML from the Department of Pediatrics, Institute of Hematology & Blood Diseases Hospital, China, between January 2008 and December 2019 were included. Eligibility criteria included a diagnosis of JMML according to the 2016 revision of the World Health Organization classification of myeloid neoplasms and acute leukemia.[4] Exclusion criteria included JMML-like myeloproliferative diseases such as Noonan syndrome (NS) or only germline RAS pathway gene mutations. Splenomegaly and hepatomegaly were defined as an enlargement of the spleen and liver beyond 3 cm below the costal margin. RDW values were measured using a hematology analyzer system (XN-9000, Sysmex, Japan). Sixty-two of 77 JMML children underwent chromosome karyotype analysis using the G-banding method. The study was approved by the Ethics Committee and Institutional Review Board at the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College (project number: IIT2021009-EC-1), and conducted in accordance with the Declaration of Helsinki. All legal guardians of pediatric patients signed written informed consent before participation in this trial. All procedures performed in this study adhered to ethical standards of this research committee.

### 2.2. Genetic mutation analysis

The genetic mutation status of all patients was analyzed at the time of initial diagnosis. Seventy patients received genetic examination in our clinic. Genetic sequencing was conducted on the remaining 7 patients at an external hospital, and the accuracy of their report were checked and validated by Drs Wenyu Yang and Drs Weiru Liang. DNA from bone marrow samples and oral epithelial cells was extracted and prepared for sequencing to determine whether the mutation was somatic or germline. Fifty-one patients received high-throughput sequencing targeting hotspot mutations of JMML, while 19 patients diagnosed after 2017 underwent next-generation sequencing with a 112 Gene Panel closely related to hematological diseases. The details of the gene sequencing are described in the Supplemental Methods, http://links.lww.com/BS/A86.

### 2.3. Statistical analysis

Qualitative variables were expressed as frequencies and percentages, while quantitative variables were expressed as the medians and interquartile ranges (25th–75th). Comparisons between qualitative variables were made using Fisher exact test or the chi-square test. Comparisons between quantitative variables were performed using nonparametric tests (Mann–Whitney *U* or Kruskal–Wallis).

Patient outcome data updated on December 31, 2021, were utilized. Survival analysis was conducted using OS, which was defined as the period from the date of diagnosis to the date of any cause of death or the last follow-up. Survival rates were calculated and compared using Kaplan–Meier methods and log-rank tests. Univariate and multivariate analyses were carried out according to the Cox proportional hazard regression model. For quantitative variables, receiver operating characteristic curve (ROC) analysis was employed to determine the optimal cutoff point for group division. All reported *P* values were 2-sided, with statistical significance defined at $P < .05$. The statistical analysis was performed in R version 4.1.1 and RStudio Version 1.4.1717.

## 3. RESULTS

### 3.1. Clinical and biological features at diagnosis

The clinical and biological features of the 77 patients are summarized in Table 1 and Figure 1. Of the 77 cases, 60 were male; the median age at diagnosis was 24 (11.00–36.00) months, while 45 cases were less than 24 months. 61.04%, 44.16%, and 71.43% of the total patients showed rash, hepatomegaly, and splenomegaly, respectively. The number of white blood cells (WBC) at diagnosis was 28.09 (13.46–52.46) $\times 10^9$/L, the hemoglobin count was 93.00 (77.00–102.00) g/L, the platelet (PLT) count was 37.00 (21.00–81.00) $\times 10^9$/L, and the monocyte number was 4.20 (2.19–5.94) $\times 10^9$/L. The mean red cell distribution width standard deviation (RDW-SD) and red cell distribution width coefficient of variation (RDW-CV) count were 53.30 (47.85-60.30) fl and 18.30 (16.75–20.00) %, respectively. The mean reticulocyte count (Ret) at diagnosis was 3.26 (2.18–4.96) $\times 10^9$/L. The percentage of HbF at diagnosis was 16.35 (5.62–47.23) %, with an increase observed for age in 47 (61.04%) patients. Sixty-two cases underwent chromosome karyotype analysis, among which 55 had normal karyotypes. There were 3 patients with monosomy 7 and 4 cases with other abnormalities.

High-throughput sequencing or targeted gene sequencing was performed in 70 cases. Among them, there were 32 patients with PTPN11 mutation, 16 with NRAS mutations, 9 with KRAS mutations, 6 with NF1 mutations, 1 with CBL mutation. Additionally, there were 14 patients with other mutations that did not belong to the five classical RAS-signaling mutations mentioned previously. Eight patients presented more than one classical Ras pathway mutation. All mutations in our cohort were somatic mutations. Among the 19 patients with targeted gene sequencing, 14 (73.68%) patients had secondary mutations. The canonical and secondary gene mutations, including some epigenetic modification genes, are summarized in Figure 2 and Table S1, http://links.lww.com/BS/A86.

According to the optimal cut-point of RDW-CV analyzed by ROC, the patients were divided into low and high RDW-CV groups (**Fig. 3**). There were 25 patients in the low RDW-CV group (≤17.35%) and 52 patients in the high RDW-CV group (>17.35%). The high RDW-CV group displayed a higher WBC count ($P = .014$), a higher nucleated red blood cell count, a higher RET count ($P = .018$), a lower mean corpuscular hemoglobin concentration (MCHC) level ($P < .001$), and a lower MCH level ($P = .010$). No significant differences were observed in other variables (Table 2). Twenty-eight patients received cytokine testing; patients in the high RDW-CV group displayed relatively elevated levels of Interleukin (IL)-1b, IL-2R, IL-6, IL-8, and tumor necrosis factor (TNF)-α, although no significant differences were observed (Table S2, http://links.lww.com/BS/A86).

When comparing the baseline characteristics of patients with or without PTPN11 mutation, we also found that the mutant group exhibited a significantly older age ($P = .045$), a higher proportion of cases with HbF elevation ($P < .001$) and splenomegaly ($P = .045$), a lower PLT count ($P = .001$), a lower MCHC level at diagnosis ($P = .014$) (Table S3, http://links.lww.com/BS/A86).

HSCT was recommended for all children with NF1, somatic PTPN11 or KRAS mutations, and the majority of patients with somatic NRAS mutations. A small fraction of patients with somatic NRAS or CBL mutations were closely monitored and not immediately offered HSCT. In our study, 53 patients did not receive HSCT due to financial or physical reasons, and a "watch and wait" strategy was applied to 3 patients. Twenty-one cases underwent allogeneic HSCT, with 1 patient receiving transplantation 4 years after diagnosis because of disease progression. The remaining 56 patients did not undergo transplantation. Among these cases, only 2 underwent several courses of decitabine therapy and 1 case received recorded chemotherapy. After a median follow-up of 12 (0–134) months, 35 children were still

**Table 1**

**Clinical and laboratory features of patients with JMML.**

| Variable | Value | Total cases (N) |
|---|---|---|
| Median patient age at diagnosis, mo | 24.00 [11.00, 36.00] | 77 |
| Gender, male | 60 (77.92) | 77 |
| Rash | 47 (61.04) | 77 |
| Hepatomegaly | 34 (44.16) | 77 |
| Splenomegaly | 55 (71.43) | 77 |
| Median WBC count at diagnosis, $\times 10^9$/L | 28.09 [13.46, 52.46] | 77 |
| Median count of hemoglobin at diagnosis, g/L | 93.00 [77.00, 102.00] | 77 |
| Median platelet count at diagnosis, $\times 10^9$/L | 37.00 [21.00, 81.00] | 77 |
| Median monocyte count at diagnosis, $\times 10^9$/L | 4.20 [2.19, 5.94] | 77 |
| Median NRBC count at diagnosis, $\times 10^9$/L | 0.33 [0.08, 0.98] | 77 |
| Median MCHC count at diagnosis, % | 310.00 [299.00, 320.25] | 77 |
| Median MCV count at diagnosis, fl | 83.20 [78.00, 89.30] | 77 |
| Median MCH count at diagnosis, pg | 26.10 [23.40, 28.20] | 77 |
| Median reticulocyte count at diagnosis, $\times 10^9$/L | 3.26 [2.18, 4.96] | 77 |
| Median percentage of HbF at diagnosis, % | 16.35 [5.62, 47.23] | 77 |
| HbF elevated for age, n (%) | 47 (61.04) | 77 |
| Median RDW-CV count at diagnosis, % | 18.30 [16.75, 20.00] | 77 |
| Median RDW-SD count at diagnosis, fl | 53.30 [47.85, 60.30] | 77 |
| Median LDH count at diagnosis, U/L | 338.00 [248.60, 606.20] | 77 |
| Erythroblast, % (median [IQR]) | 1.55 [0.80, 5.20] | 77 |
| Karyotype | | 62 |
| Normal karyotype | 55 (88.71) | |
| Monosomy 7 | 3 (4.94) | |
| Other abnormalities | 4 (6.45) | |
| RAS-signaling genes | | 70 |
| PTPN11 | 32 (45.71) | |
| NRAS | 16 (22.86) | |
| KRAS | 9 (12.86) | |
| NF1 | 6 (2.86) | |
| CBL | 1 | |
| Non-Ras-signaling genes | 14 (20.00) | |
| PTPN11 mutation with additional alterations | 8 (11.43) | 70 |
| ≥2 Ras mutation | 25 (35.71) | 70 |
| Number of secondary mutations | | 19 |
| 0 or 1 | 5 (26.32) | |
| 2 or more | 14 (73.68) | |
| HSCT | 20 (25.97) | 77 |

HbF = hemoglobin F, HSCT = hematopoietic stem cell transplant, LDH = lactate dehydrogenase, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, NRBC = nucleated red blood cells, RDW-CV = red cell distribution width coefficient of variation, RDW-SD = red cell distribution width standard deviation, WBC = white blood cell.

alive. According to the Kaplan–Meier method, the estimated 1-year OS was 50.81%. The details of pre-transplantation and HSCT are summarized in Table S4 and Figure S1, http://links.lww.com/BS/A86.

### 3.2. Assessment of RDW for significant prognostic variable

We then analyzed the relevant factors related to OS. Patients with an elevation in RDW-CV showed significantly lower OS (hazard ratio [HR] = 4.332, 95% confidence interval [CI] = 1.824–10.290, $P$ = .001) and this remained significant in the multivariate analysis (HR = 5.264, 95% CI = 2.068–13.400, $P$ < .001). Additionally, PTPN11 mutation (HR = 2.338, 95% CI = 1.034–5.284, $P$ = .041) and HSCT (HR = 0.315, 95% CI = 0.151–0.817, $P$ = .015) were also remained independently prognostic factors in our cohort (Table 3). By introducing the above 3 independent predictors, a prognostic nomogram was developed and is presented in Figure S2, http://links.lww.com/BS/A86.

In addition, we carried out a stratified analysis to explore the correlation between RDW and all-cause mortality according to possible modifying factors, including gender, age, PLT count, Hb level, HbF level, PTPN11 mutation, and HSCT. We observed that an increase in RDW-CV was associated with a higher risk of all-cause mortality across various subgroups without any observed interaction effect among them (**Fig. 4**).

### 3.3. Survival analysis

With a median follow-up of 10 (0–103) months, patients with low RDW-CV showed a higher survival rate ($P$ = .0003, **Fig. 5**), and this significance remained in the subgroup without HSCT ($P$ = .0018, **Fig. 6**). We further investigated the OS of patients with PTPN11 mutation and an elevated RDW-CV. The Kaplan–Meir curve showed that patients with both PTPN11 mutation and RDW-CV elevation displayed the worst survival rate, while the subgroup without either PTPN11 mutation or RDW elevation presented the best survival (**Fig. 7**).

We also compared the survival rate of patients with RDW-CV elevation or not in different subgroups. Patients with high RDW-CV showed lower survival rate in the subgroup of without HSCT/PTPN11$^{mut}$ and without HSCT/non PTPN11$^{mut}$ (Figure S3, http://links.lww.com/BS/A86).

As for the relationship of NRAS/KRAS mutations and the RDW level, the survival analysis showed that in patients without NRAS/KRAS mutation, a low RDW-CV level displayed a better survival in comparison with a higher RDW-CV level ($P$ = .0019, Figure S4, http://links.lww.com/BS/A86).

## 4. DISCUSSION

Our findings demonstrated that the baseline RDW level was associated with poor prognosis in patients with JMML. We found that patients with relatively high RDW levels at

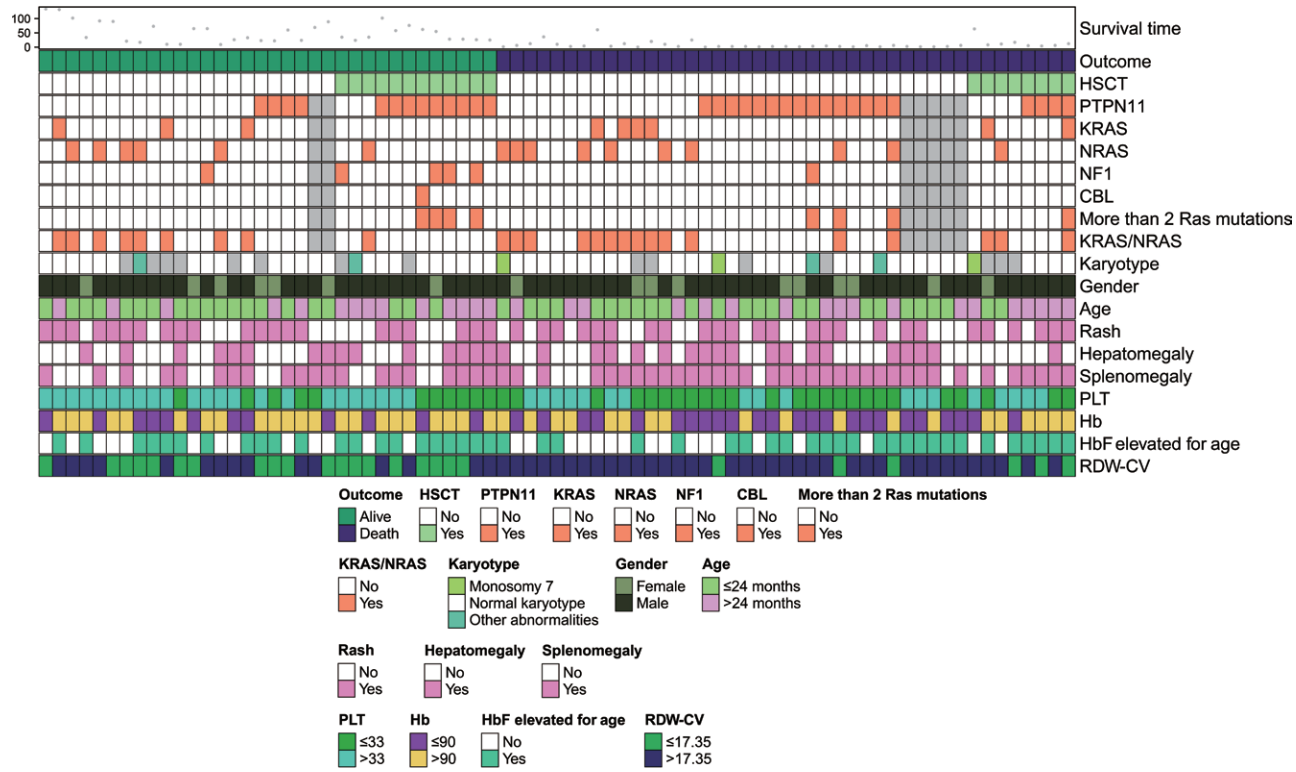


**Figure 1.** Clinical and genetic profiles of the 77 patients. HbF = hemoglobin F, HSCT = hematopoietic stem cell transplantation, PLT = platelet, PTPN11 = protein phosphatase non-receptor type 11, RDW-CV = red cell distribution width coefficient of variation.

diagnosis experienced shorter OS times than patients with low RDW levels. The Cox multivariate regression model confirmed our hypothesis that the baseline RDW value was an independent parameter to predict the survival of patients with JMML. Moreover, the survival analysis also demonstrated that cases with both PTPN11 mutation and RDW elevation at diagnosis had the worst survival in comparison with other combinations of the 2 parameters. For this group of patients, a more aggressive treatment regimen should be adopted, such as HSCT.

The RDW was a simple and inexpensive parameter. In recent years, evidence has shown that an increased RDW is a negative predictive factor in several kinds of benign and malignant

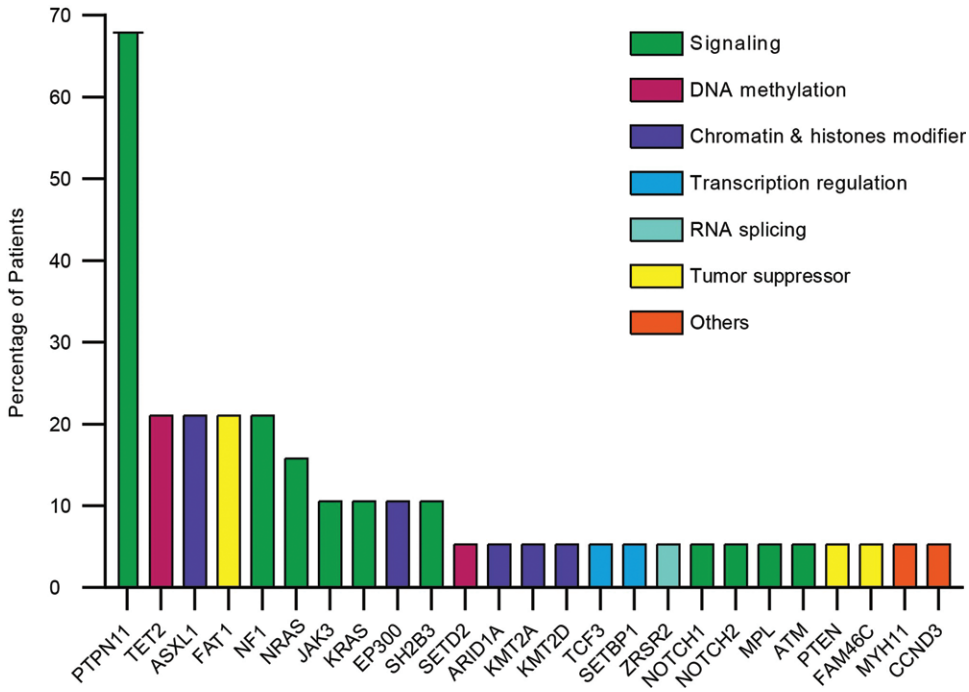


**Figure 2.** Canonical and secondary mutated genes in 19 JMML patients undergoing NGS. JMML = juvenile myelomonocytic leukemia, NGS = next generation sequencing.

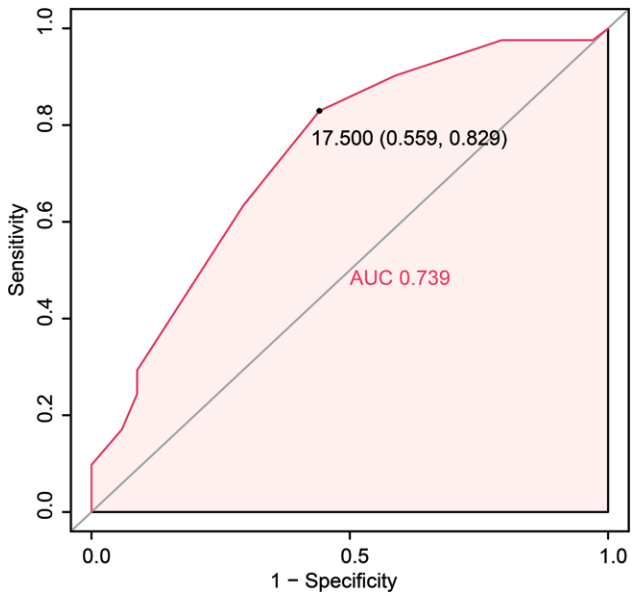


Figure 3. ROC analysis for determining the optimal cutoff value in predicting OS for RDW-CV. OS = overall survival, RDW-CV = red cell distribution width coefficient of variation, ROC = receiver operating characteristic curve.

hematological diseases and conveys important information for risk stratification and treatment. Regarding myeloid malignancies, RDW was described as an independent predictive factor to diagnose MDS, and increased RDW might reflect dyserythropoiesis.[23,24] RDW elevation was described as an independent prognostic variable in MDS patients with bone marrow blasts <5%.[19] Higher-than-normal RDW levels have been linked to worse EFS in CML and can predict treatment response to TKIs.[25] RDW elevation also predicts a worse outcome in AML patients.[26] However, the exact mechanism underlying the associations of RDW elevation with these hematological diseases has not been clearly explained.

We hypothesized that the association between RDW elevation and poor prognosis in malignancies may results from inflammatory microenvironment, hypoxic state, and malnutrition. Inflammation plays a significant role in cancer and contributes to carcinogenesis, cell differentiation, and the development and advancement of tumors.[27] Several studies have demonstrated the correlations between RDW and high-sensitivity C-reactive protein (CRP), erythrocyte sedimentation rate, and other inflammatory factors.[28,29] Inflammation might alter iron homeostasis and lead to erythropoietin resistance, thus causing a mixed RBC population in the blood circulation.[30] A recent investigation has shown that JMML cells impose inflammatory stress on normal stem cells by overproduction of IL-1b, thus leading HSCs to lose quiescence and become exhausted and normal hematopoietic cell development to be suppressed.[31] We analyzed several cytokines that reflected the status of the inflammatory response and patients in the high RDW group showed relative high cytokine levels, although there was no statistical significance (Table S2, http://links.lww.com/BS/A86). A larger JMML cohort is needed to further verify our hypothesis. Other studies have demonstrated that an increased RDW level results in a difference in the oxygen-carrying capacity, leading to microcirculation hypoperfusion, hypoxia, and oxidative stress. These factors may induce treatment resistance of tumor cells and are likely to be critical factors in JMML progression.[32,33] Malignant tumors also lead to malnutrition, which is associated with RDW elevation, is another reason for lower survival rates. Malnourished patients would be less resistant to several complications and cytotoxic chemotherapy.[34] In summary, the mechanisms by which RDW elevation results in poor prognosis of JMML were still unclear, and additional studies were needed.

PTPN11 somatic mutations are the most common genetic drivers of JMML, associated with an aggressive clinical course and poor disease outcome and was the only unfavorable factor for relapse after HSCT.[7] In our study, patients with PTPN11 mutation did not show significant worse survival rate in the HSCT group (Figure S3, http://links.lww.com/BS/A86), which may due to small sample size of that subgroup. However, patients with both PTPN11 mutation and elevated RDW-CV

**Table 2**

**Comparison of RDW-CV$^{low}$ and RDW-CV$^{high}$ groups.**

| Variable | RDW-CV$^{low}$ (n = 25) | RDW-CV$^{high}$ (n = 52) | *P* value |
|---|---|---|---|
| Age, mo (median [IQR]) | 36.00 [9.00, 48.00] | 21.00 [12.00, 36.00] | .487 |
| Gender, male n (%) | 19 (76.00) | 41 (78.85) | 1.000 |
| Rash, n (%) | 16 (64.00) | 30 (57.69) | .779 |
| Hepatomegaly, n (%) | 9 (36.00) | 25 (48.08) | .451 |
| Splenomegaly, n (%) | 15 (60.00) | 40 (76.92) | .204 |
| WBC, $\times 10^9$/L (median [IQR]) | 18.88 [11.31, 32.29] | 28.94 [16.94, 58.23] | *.014* |
| Hb, g/L (median [IQR]) | 98.00 [81.00, 106.00] | 86.50 [77.00, 101.25] | .061 |
| PLT, $\times 10^9$/L (median [IQR]) | 43.00 [25.00, 121.00] | 34.00 [21.00, 64.50] | .294 |
| Mo, $\times 10^9$/L (median [IQR]) | 3.54 [1.74, 5.54] | 4.58 [2.68, 7.24] | .119 |
| NRBC (median [IQR]) | 0.16 [0.02, 0.26] | 0.47 [0.21, 1.57] | *.001* |
| MCHC, % (median [IQR]) | 323.00 [305.00, 337.00] | 305.50 [294.50, 312.25] | *<.001* |
| MCV, fl (median [IQR]) | 87.40 [80.50, 89.50] | 82.95 [77.60, 88.62] | .234 |
| MCH, (median [IQR]) | 27.30 [25.20, 30.10] | 25.45 [22.95, 27.30] | *.010* |
| RET, $10^9$/L (median [IQR]) | 2.93 [1.39, 4.12] | 3.82 [2.30, 5.60] | *.018* |
| HbF (median [IQR]) | 26.75 [6.30, 57.37] | 14.75 [5.62, 45.75] | .304 |
| HbF elevated for age, n (%) | 17 (68.00) | 30 (57.69) | .536 |
| LDH, U/L (median [IQR]) | 263.00 [239.00, 603.00] | 375.00 [263.50, 615.28] | .079 |
| PTPN11 mutation, n (%) | 13/25 (52.00)* | 19/45 (42.22)* | .380 |
| Kras or Nas mutation, n (%) | 7/25 (28.00)* | 19/45 (42.22)* | .248 |
| ≥2 Ras mutation (%) | 6/25 (24.00)* | 2/45 (4.44)* | .051 |

Hb = hemoglobin, HSCT = hematopoietic stem cell transplant, LDH = lactate dehydrogenase, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, Mo = monocyte, NRBC = nucleated red blood cells, PLT = platelet, RDW-CV = red cell distribution width coefficient of variation, RDW-SD = red cell distribution width standard deviation, WBC = white blood cell.

*P* values <.05 are shown in italics.

*This test excluded those with missing data.

**Table 3**

**The relationship between RDW-CV and OS of JMML patients.**

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| **Variable** | **HR (95% CI)** | ***P* value** | **HR (95% CI)** | ***P* value** |
| Gender (male) | 1.339 (0.659–2.720) | .419 | 0.722 (0.342–1.527) | .395 |
| Age >24 mo at diagnosis | 1.043 (0.568–1.915) | .892 | 1.087 (0.512–2.305) | .828 |
| PLT <33 × $10^9$/L at diagnosis | 2.206 (1.191–4.085) | *.012* | 0.925 (0.427–2.006) | .844 |
| HbF elevated for age | 1.153 (0.628–2.117) | .647 | 0.931 (0.433–2.001) | .855 |
| HSCT | 0.466 (0.216–1.006) | .052 | 0.315 (0.151–0.817) | *.015* |
| PTPN11 mutation | 2.725 (1.200–6.191) | *.017* | 2.338 (1.034–5.284) | *.041* |
| RDW-CV >17.35% | 4.332 (1.824–10.290) | *.001* | 5.264 (2.068–13.400) | *<.001* |

CI = confidence interval, HbF = hemoglobin F, HSCT = hematopoietic stem cell transplantation, HR = hazard ratio, JMML = juvenile myelomonocytic leukemia, OS = overall survival, PLT = platelet count, PTPN11 = protein phosphatase non-receptor type 11, RDW-CV = red cell distribution width coefficient of variation.

*P* values <.05 are shown in italics.

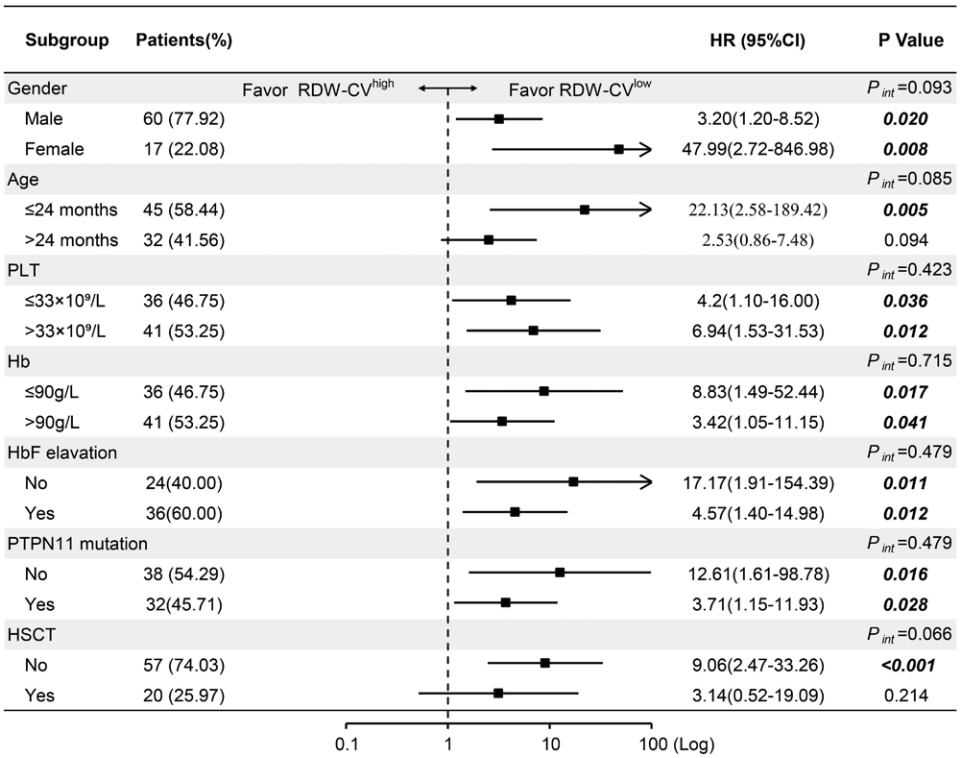


**Figure 4.** Subgroup analysis for all-cause mortality in different subgroups. CI = confidence interval, Hb = hemoglobin, HbF = hemoglobin F, HR = hazard ratio, HSCT = hematopoietic stem cell transplantation, PLT = platelet, PTPN11 = protein phosphatase non-receptor type 11, RDW-CV = red cell distribution width coefficient of variation.

displayed the worst survival rate. Recent studies have discovered that intracellular levels of reactive oxygen species (ROS), which were byproducts of mitochondrial oxidative phosphorylation, were elevated in Ptpn11$^{E76K/+}$ cells. The gain of function of the Shp2 phosphatase enhanced mitochondrial respiration.[35] Therefore, we hypothesized that the combination of PTPN11 mutation and RDW elevation may enhance oxidative stress and lead to a hypoxic tumor microenvironment, resulting in resistance to conventional therapies and poor prognosis for JMML.

In our study, the baseline RDW value was negatively correlated with the PLT level. Previous studies have provided evidence that a lower PLT count is a clear prognostic indicator in patients with JMML.[2] The strong association of the elevation of RDW with lower PLT count would explain its prognostic role to some extent. We also found that the average RDW-CV of patients with JMML in our cohort was generally above the normal level, indicating an increase in the proportion of nucleated RBCs.[36] This finding is consistent with previous observation that reticulocytes are usually evident in peripheral blood smear in JMML patients.

RDW has been used for the differential diagnosis of anemias. It is generally accepted that RDW elevation tends to be associated with nutritional deficiencies (such as iron, folate, or vitamin B12).[11] In our cohort, the patients' serum iron and folic acid levels were within the normal range (Table S5, http://links.lww.com/BS/A86). However, the high-RDW group was associated with lower MCHC, which was an indicator of iron deficiency or impaired iron acquisition and higher serum iron level. It was confusing. There were too many missing data for these items. More number of cases are needed to verify our results.

Allogeneic HSCT is the only curative treatment option for patients with JMML.[3] In our cohort, patients with RDW elevation, which is an independent risk factor proven in this study, achieved longer survival after receiving allogeneic HSCT, either from a histocompatible sibling or from an unrelated donor (Table S4, http://links.lww.com/BS/A86). According to our

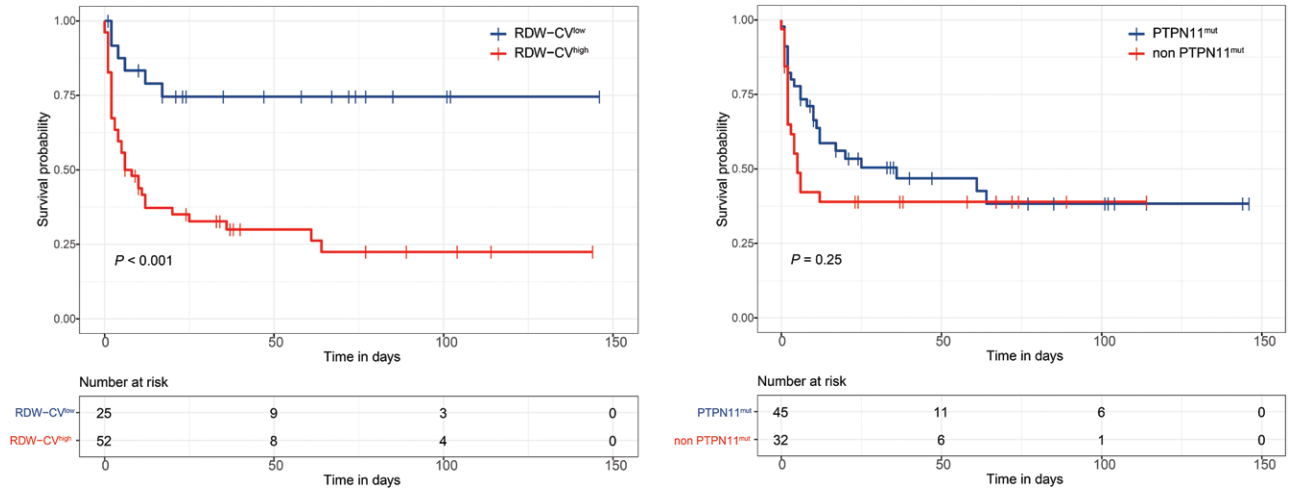


**Figure 5.** Kaplan–Meier curves for overall survival of JMML patients according to RDW-CV. JMML = juvenile myelomonocytic leukemia, PTPN11 = protein phosphatase non-receptor type 11, RDW-CV = red cell distribution width coefficient of variation.

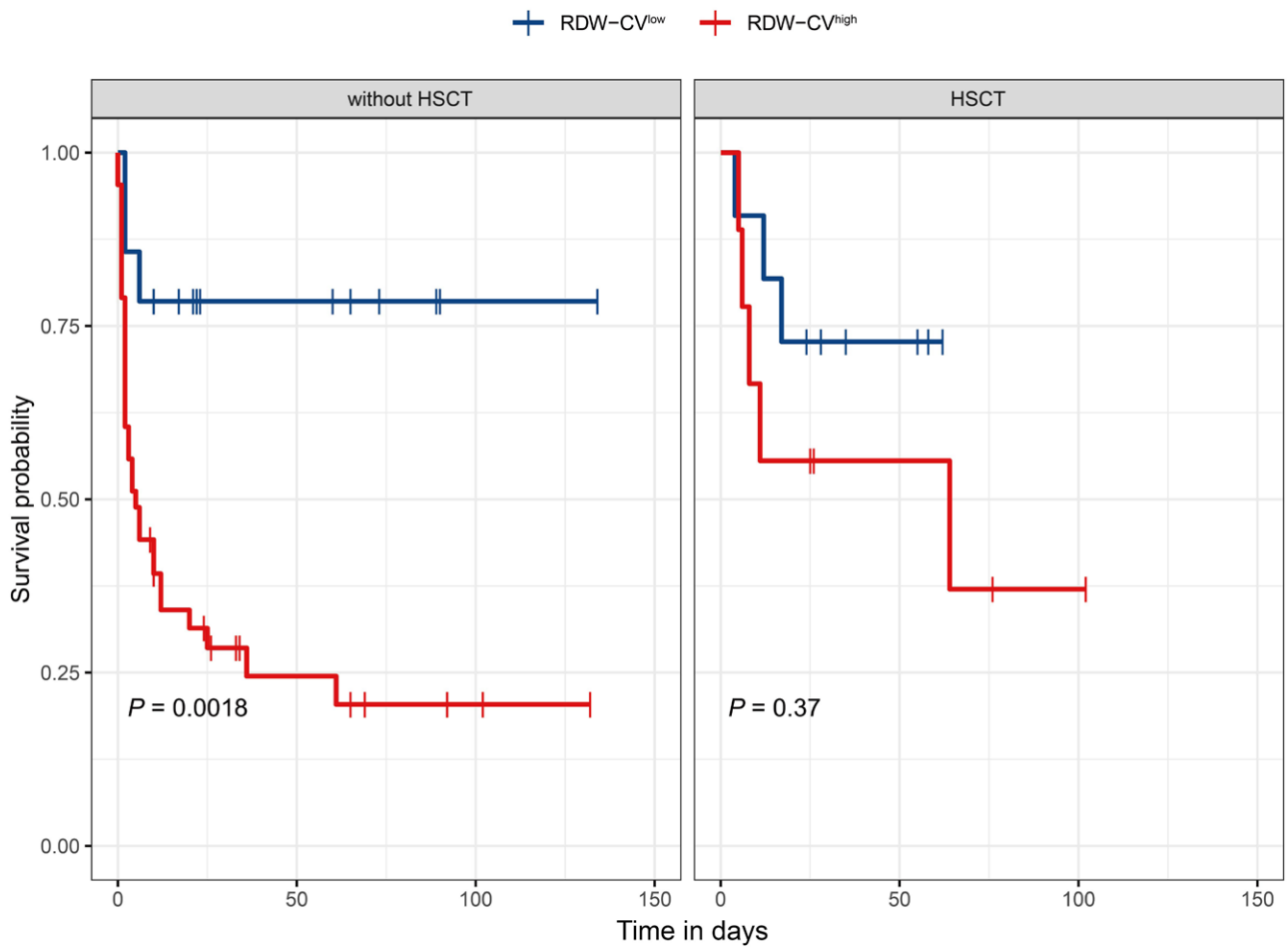


**Figure 6.** Kaplan–Meier curves for overall survival of JMML patients with HSCT or without HSCT according to RDW-CV level. HSCT = hematopoietic stem cell transplantation, JMML = juvenile myelomonocytic leukemia, PTPN11 = protein phosphatase non-receptor type 11, RDW-CV = red cell distribution width coefficient of variation.

results, RDW could be a novel predictive factor and can identify patients who are more in need of transplantation.

There were still some limitations in our study. First, this was a retrospective study in a single center with a limited sample size. There may be potential bias and inaccuracy in the data collection. Second, the treatment regimens and other clinical characteristics were heterogeneous; some patients received demethylating drugs and transplantation, while others did not. Third, we did not find a specific correlation between RDW and inflammatory markers since there were too many missing data for these items. Further investigations are needed to verify the role of inflammatory activity in the progression of JMML disease. Finally, the

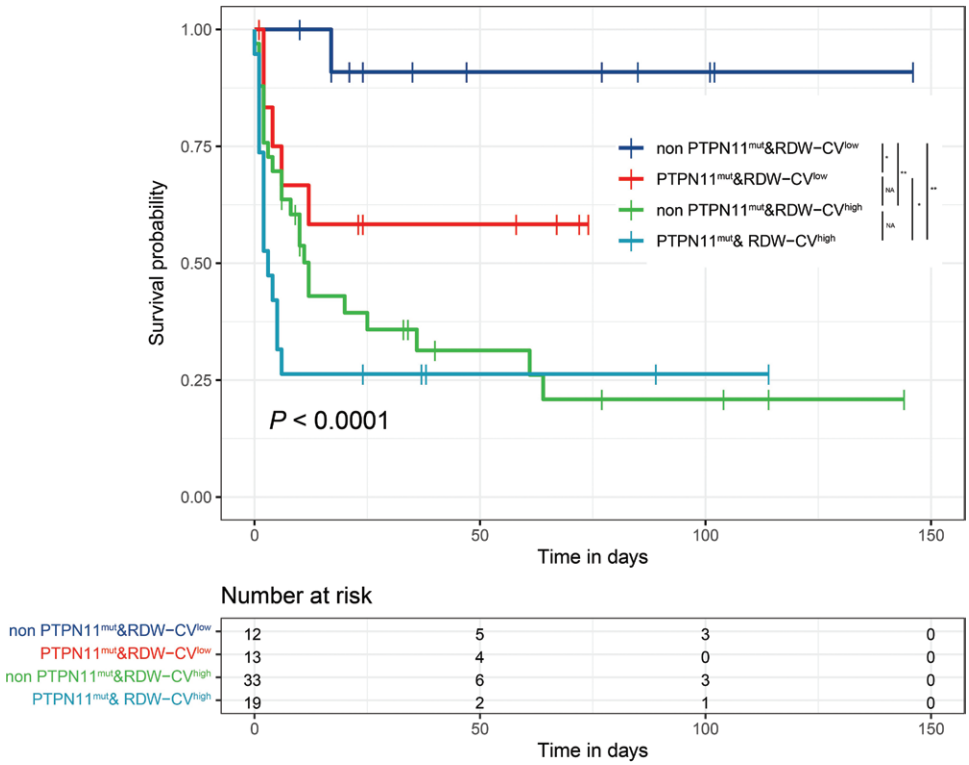


**Figure 7.** Kaplan–Meier curves for overall survival of JMML patients according to the combination of RDW-CV level and PTPN11 mutation. NA $P > .05$, $^{*}P < .05$, $^{**}P < .01$. HSCT = hematopoietic stem cell transplantation, JMML = juvenile myelomonocytic leukemia, RDW-CV= red cell distribution width coefficient of variation.

cutoff value of RDW in the prediction of poor prognosis may not commonly apply to different institutions and populations because the methods used for RDW calculation differ widely among the most commonly used hematological analyzers,[37] and the normal RDW values are slightly different among different ethnic groups.[38,39]

Despite the limitations, to the best of our knowledge, this is the first report showing RDW as an independent prognostic variable in JMML subjects. RDW may be regarded as an inexpensive biomarker to predict the clinical outcome in patients with JMML. However, our data are preliminary, and conclusive results would require analysis of a larger cohort of patients. Further studies are required to specifically ascertain the molecular mechanism(s) linking the increased RDW with poor prognosis of JMML.

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## ETHICAL APPROVAL

The study was approved by the Ethics Committee and Institutional Review Board of Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College (project number: IIT2021009-EC-1), and conducted in concordance with the Declaration of Helsinki. All legal guardians of pediatric patients signed written informed consent before participation in the trial. All procedures performed in the study were in accordance with the ethical standards of this research committee.

## AUTHOR CONTRIBUTIONS

This study was performed in collaboration among all authors. W.Y. and X.Z. designed the study, W.L. wrote the protocol and the first draft of the manuscript. J.Z. managed the analyses of the study. C.L. managed the literature searches and data curation. All co-authors read and approved the final manuscript.

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