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ORIGINAL RESEARCH

Absolute Circulating Leukemic Cells as a Risk Factor for Early Bleeding Events in Patients with Non-High-Risk Acute Promyelocytic Leukemia

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Background: Hemorrhagic complications are the most common cause of early death in patients with APL and remain a major challenge in the management of APL. Early fatal bleeding events occur not only in high-risk but also in non-high-risk acute promyelocytic leukemia (APL) patients with normal or low WBC counts.

Objectives and Methods: To demonstrate the role of the absolute number of circulating leukemic cells in early bleeding events in APL patients. Clinical and laboratory characteristics of 149 patients newly diagnosed with APL were obtained from medical records and retrospectively investigated.

Results: In this study, circulating absolute leukemic cells were positively correlated with the WBC count ($r=0.9813$, $p<0.001$) in all patients with APL, and importantly, they were strongly associated with significant bleeding events in non-high-risk patients. Multivariate logistic regression analysis showed that the absolute number of leukemia cells was an independent risk factor for significant bleeding events in APL patients. A cut-off value of 2.59×10^9/L for circulating leukemic cells to predict significant bleeding events in APL patients was obtained by ROC curve analysis. We further confirmed that the significant bleeding rate of patients with non-high-risk APL was statistically increased when the absolute number of circulating leukemic cells was $\geq2.59\times10^9$/L.

Conclusion: Circulating leukemic cell content has great clinical value for predicting early bleeding events in APL patients, especially in non-high-risk APL.

Keywords: circulating leukemic cells, early fatal bleeding events, non-high-risk acute promyelocytic leukemia

Introduction

Acute promyelocytic leukemia (APL), a unique subtype of acute myeloid leukemia (AML), is characterized by unique morphology of blast cells. The vast majority of APL patients have a specific chromosomal translocation, t (15; 17), forming a fusion transcript between the promyelocytic leukemia (PML) gene and the retinoic acid receptor (RARα) gene, called PML–RARα.[1,2] All-trans retinoic acid (ATRA), which can promote terminal differentiation of malignant promyelocytes into mature granulocytes, has revolutionized the treatment of APL. The combination of ATRA and arsenic trioxide (ATO) or cytotoxic chemotherapy has induced response rates in excess of 90% and long-term leukemia-free survival rates in excess of 80%. APL has evolved from being a deadly to a highly curable disease.[3–13]

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Despite advances in the treatment of APL, early death rates have remained relatively constant. Hemorrhagic complications are the most common cause of early death in patients with APL and remain a major challenge in the management of APL.[11,14–22] It is worth noting that the major risk assessment method based on WBC count was initially used to assess the risk of relapse rather than the risk of early death resulting from fatal hemorrhage.[23–25] Thus, it is critical to identify risk factors for early fatal bleeding events in APL patients. Several laboratory markers and parameters related to major hemorrhage or hemorrhagic death have been investigated. Among all the parameters involved in early fatal bleeding events, WBC count is frequently related to APL disease burden and served as a convenient predictor of fatal hemorrhage in APL at diagnosis in some studies.[13,18,19,26–35] However, other studies have proved that there is no significant association between WBC count and bleeding risk.[36,37] Thus, the value of WBC count as a risk factor for early bleeding events is limited, especially in non-high-risk APL patients with normal or low WBC counts.

The circulating leukemic cell count in APL can reflect the disease burden. The coagulopathy of APL is principally impelled by proteins on the surface of APL cells, such as tissue factor (TF) and Annexin II.[38–42] Therefore, we performed a study to examine the clinical significance of the absolute number of circulating leukemic cells and determine the cut-off value for detecting early significant bleeding in APL patients, especially in non-high-risk APL patients. These experiments were performed with an aim to identify patients at the greatest risk of hemorrhage to ultimately actively prevent hemorrhage.

Materials and Methods

Patients

This retrospective study included 149 patients diagnosed with APL treated at Zhengzhou University People's Hospital and its alliance hospital from December 2016 to September 2020. All patients were newly diagnosed with typical APL, who confirmed t (15;17) and/or PML-RARα fusion genes. M3v type of APL was excluded from our study. This study was conducted in accordance with the Declaration of Helsinki, and was approved by the research ethics committees of each institution. Each participant signed a written informed consent form before enrollment, and a parent or legal guardian of patients under 18 years of age provided informed consent.

Laboratory Characteristics

All clinical characteristics were obtained from medical records, including age, sex, WBC count, circulating leukemic cell percentage and absolute number, bone marrow leukemic promyelocyte (BMP) percentage, platelet (PLT) count, hemoglobin (HB), prothrombin time (PT) (normal range: 11–17 s), activated partial thromboplastin time (APTT) (normal range: 23–43.5 s), fibrinogen (FIB) (normal range: 2–4 g/L), D-dimer (normal range: 0–0.5 mg/L), lactate dehydrogenase (LDH) (normal range: 120–250 U/L), and creatinine (CREA) (normal range: 58–110 μmol/L). Circulating leukemic cells, also termed peripheral blood promyelocytes, are atypical hypergranular cells and classified as blasts in APL. In typical APL blood smears, the morphologic features of such cells are characterized by a uniform morphology, unevenly sized granules in the cytoplasm, and Auer rods can be seen in some cells (Figure 1). The number of absolute circulating leukemic cells was obtained by multiplying the WBC count from the routine blood test by the percentage of circulating leukemic cells in the blood smear, and the two detection samples were obtained at the same time.

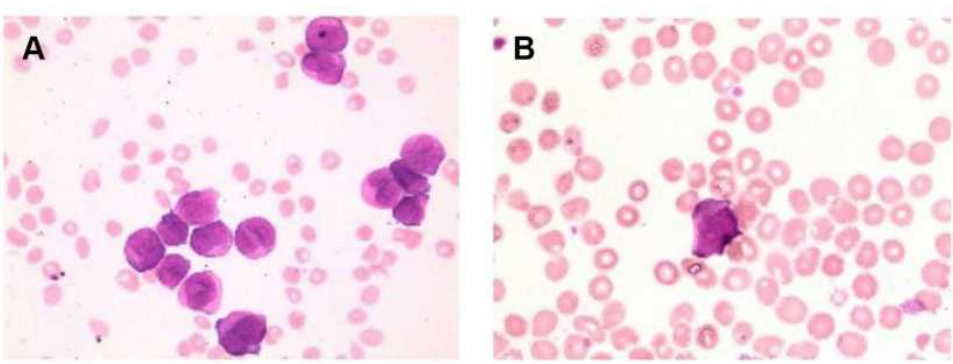

Figure 1 Circulating leukemic cells from peripheral blood smears of different patients (A and B).

Definitions

To evaluate the severity of bleeding manifestations of APL, the widely used World Health Organization (WHO) severity grading system and the modified WHO scale by Kaufman were used in this study.[43–45] A summary of the revised WHO bleeding scale is provided in Supplementary Table 1. According to the bleeding grade, the APL patients were divided into two groups: the nonsignificant bleeding group (grades 0, 1, and 2) and the significant bleeding group (grades 3 and grade 4). Early death (ED) was defined as death from any cause within 30 days after diagnosis. Hemorrhage-related ED (HED) was defined as death due to bleeding events within 30 days. According to the WBC count (< or ≥10×10^9/L) at APL diagnosis, the patients were stratified into a non-high-risk group and a high-risk group.[23]

Treatment Strategies

After diagnosis of APL, ATRA (25 mg/m2/day) combined with ATO (0.16 mg/kg/day) was administered until the patients achieved complete remission (CR). On the second day of treatment in patients with high-risk APL or when WBC counts were ≥10×10^9/L in the non-high-risk group during induction therapy, additional chemotherapy (idarubicin 8 mg/m^2/day for 3–4 days or daunorubicin 45 mg/m^2/day for 3–4 days) was administered to control hyperleukocytosis. If patients were diagnosed with differentiation syndrome (DS) during therapy, ATRA was temporarily suspended, and dexamethasone was given until the clinical symptoms resolved. PLT transfusion was performed to maintain a PLT count in the range of 30–50×10^9/L. Fresh-frozen plasma and cryoprecipitate were infused to maintain fibrinogen levels at 1.5 g/L and the PT and APTT near the normal range.

Statistical Analysis

The chi-square test or Fisher's exact test was used to compare categorical variables. For continuous variables, the Mann–Whitney *U*-test was used to compare the characteristics of the baseline data for each group. A logistic regression model was used for multifactorial analysis. Using the receiver operating characteristic curve (ROC) method, the optimal cutoff values of circulating leukemic cells to predict significant bleeding events in APL patients was determined. All statistical analyses were performed using SPSS version 24.0, and graphs were drawn with GraphPad Prism version 8.0, and $p<0.05$ was considered to indicate statistical significance.

Results

Clinical and Laboratory Characteristics of the Patients

This study retrospectively analyzed the clinical and laboratory characteristics of 149 newly diagnosed APL patients (76 males and 73 females). The median age was 40 years (range 3–83 years). The baseline characteristics are shown in Table 1. Patients were stratified into a non-high-risk group (WBC count < 10×10^9/L, 94 patients, 63.1%) and a high-risk group (WBC count ≥ 10×10^9/L, 55 patients, 36.9%) according to the WBC count at the time of initial diagnosis. Based on the bleeding grade, the APL patients were divided into two groups: the nonsignificant bleeding group (grades 0, 1 and 2, 102 patients, 68.5%) and the significant bleeding group (grades 3 and 4, 47 patients, 31.5%). Among the 149 patients with APL, 2 patients died before treatment, 58 patients received ARTA + ATO treatment, and 89 patients received ATRA + ATO + IDA/DNR treatment. ED occurred in 12 patients (8.1%), of which 9 (75%) died from bleeding events, making them the leading cause of ED. The main causes of HED were intracranial hemorrhage (8 patients) and alveolar hemorrhage (1 patient).

Patient with Significant Bleeding Events

Of the 149 patients, 47 patients (31.5%) had significant bleeding (grades 3 and 4), which mainly occurred in the oropharyngeal, nasal, mucosa, skin, lung, and brain regions. A total of 33 patients (22.1%) had grade 3 bleeding, including bleeding requiring red blood cell transfusion in addition to routine transfusion needs and bleeding associated with moderate hemodynamic instability. Fourteen patients (9.4%) had grade 4 bleeding, including bleeding associated with severe hemodynamic instability, fatal bleeding and central nervous system (CNS) bleeding on imaging with or without dysfunction. The median time to grade 4 bleeding events was 6 days (0–25 days). Among the 14 patients with grade 4 bleeding, 10 patients had intracranial hemorrhage, and 2 patients had alveolar hemorrhage. All grade 4 bleeding patients also had skin and mucous membrane bleeding, gum bleeding, and epistaxis. The basic clinical characteristics of the patients with grade 4 bleeding are shown in Table 2.

Table 1 Patient Demographics and Baseline Characteristics

Clinical Characteristics	Normal Range	Median (Range)/N (%)
Age		40 (3–83)
Sex (n)		
Male		76 (51%)
Female		73 (49%)
WBC (×10^9/L)	3.5–9.5	4.13 (0.37–258.01)
Circulating leukemic cell percentage (%)		57 (0–98.5)
Absolute number of circulating leukemic cells (×10^9/L)		2.08 (0–250.27)
BMP (%)		83 (13–95.4)
PLT (×10^9/L)	125–350	25 (1–319)
HGB (g/L)	130–175	82 (35–136)
PT (s)	11–17	14.5 (9.8–27.4)
APTT (s)	28–43.5	28.9 (15.3–54.1)
FIB (g/L)	2–4	1.20 (0.19–6.01)
D-Dimer (mg/L)	0–0.05	16.04 (0.71–152.53)
LDH (U/L)	120–250	306 (113–7836)
CREA (μmol/L)	58–110	57 (22–181)
Risk groups (n)		
Standard-risk group		94 (63.1%)
High-risk group		55 (36.9%)
Bleeding (n)		
Significant bleeding		47 (31.5%)
Nonsignificant bleeding		102 (68.5%)
Thrombosis		
Yes		13 (8.7%)
No		136 (91.3%)
DS		
Yes		50 (33.6%)
No		99 (66.4%)
ED		
Yes		12 (8.1%)
No		137 (91.9%)

Abbreviations: WBC, white blood cell; BMP, bone marrow leukemic promyelocyte; PLT, platelets; HB, hemoglobin; PT, prothrombin time; APTT, activated partial thromboplastin time; FIB, fibrinogen; LDH, lactate dehydrogenase; CREA, creatinine; DS, differentiation syndrome; ED, early death.

Risk Factors for Hemorrhage

The clinical characteristics of patients in the significant bleeding group (47 patients) were compared to those of patients in the nonsignificant bleeding group (102 patients). The results showed that WBC count (p=0.013, Figure 2A), circulating leukemic cell

Table 2 Clinical and Laboratory Characteristics of Patients with Grade 4 Bleeding

Patient	Age/Sex	WBC (×10^9/L)	Circulating Leukemic Cell Percentage (%)	Absolute Number of Circulating Leukemic Cells (×10^9/L)	BMP (%)	PLT (×10^9/L)	HB (g/L)	PT (s)	APTT (s)	FIB (g/L)	D-Dimer (mg/L)	LDH (U/L)	CREA (μmol/L)	Bleeding Sites	Days from Diagnosis to Bleeding
1	25/M	0.9	14	0.13	73.6	31	71	13.0	30.5	1.15	55.18	187	36	Lung	12
2	44/M	64.21	77	49.44	81.2	17	101	18.7	26.9	0.57	12.62	605	93	Brain	2
3	40/M	131.93	98	129.29	93.2	43	67	13.1	27.6	0.87	54.07	903	60	Brain	0
4	7/F	161.04	74	119.17	89.6	22	49	17.7	30.7	0.60	38.20	448	30	Brain	3
5	3/M	258.01	97	250.27	89.2	40	109	19.4	26.2	1.48	21.55	1650	53	Brain	2
6	22/F	121.83	91	110.87	89.2	18	50	14.6	19.3	2.46	22.41	1252	55	Brain	3
7	64/M	13.69	89	12.18	86	21	91	19.4	28.7	0.53	26.23	490	70	Brain	9
8	67/F	122.49	92	112.69	93.2	19	63	21.0	26.1	0.62	9.66	NA	75	Brain	0
9	43/M	80.07	91	72.86	87.6	11	62	17.0	32.0	0.65	6.50	607	83	Lung	6
10	61/F	3.12	90	2.81	88	26	57	12.1	34.0	1.30	12.83	307	49	Brain	25
11	48/F	1.94	66	1.28	89.6	41	87	13.1	20.5	0.44	17.18	398	52	Brain	6
12	56/M	1.66	67	1.11	64.8	47	63	17.1	49.4	1.50	10.00	381	54	Brain	15
13	53/M	1.25	56	0.70	89.6	1	72	18.5	26.6	1.17	6.19	207	51	Brain	17
14	74/M	21.41	92	19.69	92	10	82	20.2	34.5	0.35	26.39	2810	181	Brain	12

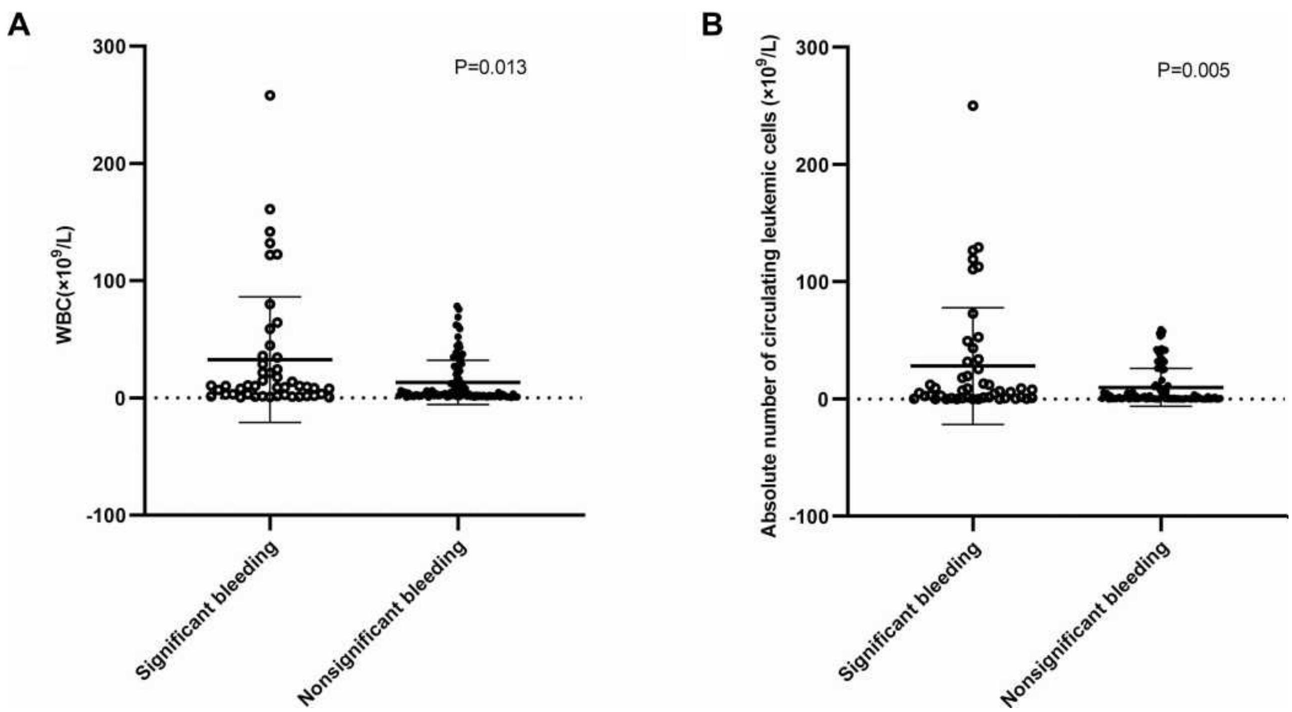

Figure 2 Laboratory data showed that there were significant differences in WBC count (**A**) and absolute number of circulating leukemic cells (**B**) between patients with or without significant bleeding.

percentage (p=0.002), absolute number of circulating leukemic cells (p=0.005, Figure 2B), BMP (p=0.003), PT (p <0.001), FIB (p=0.009), D-dimer (p <0.001) and LDH (p=0.03) were obviously associated with significant bleeding (Table 3).

Correlation Between the Absolute Number of Circulating Leukemic Cells and the WBC Count

As both the WBC count and the absolute number of circulating leukemic cells were associated with bleeding events, we next analyzed the correlation of the WBC count with the circulating leukemic cell percentage and the absolute number of circulating leukemic cells in APL patients. We found that the circulating leukemic cell percentage was not correlated with the WBC count (Figure 3A). In contrast, the absolute number of circulating leukemic cells was positively correlated with the WBC count (r=0.9813, p <0.001; Figure 3B). Additionally, we further analyzed the correlation of the WBC count with the circulating leukemic cell percentage and the absolute number of circulating leukemic cells in the non-high-risk and high-risk groups. Similarly, the circulating leukemic cell percentage was not evidently correlated with the WBC count in either high-risk or non-high-risk group patients (Figure 3C and E). As expected, the absolute number of circulating leukemic cells was positively correlated with the WBC count in the high-risk group (r=0.9727, p <0.001, Figure 3D). In the non-high-risk group, there was also a positive linear correlation between the absolute number of circulating leukemic cells and the WBC count (r=0.8788, p <0.001, Figure 3F).

Multivariate Logistic Regression Analysis of Risk Factors for Significant Bleeding Events in APL Patients

The bleeding events in APL patients was considered as the dependent variable, and the absolute number of leukemia cells, BMP, PT, FIB, D-dimer and LDH were included in logistic regression model (WBC count, the circulating leukemic cell percentage and the absolute number of circulating leukemic cells were correlated in APL, so only the absolute number of circulating leukemic cells was included in logistic regression model). The result showed that the absolute number of leukemia cells (OR=1.017, 95% CI: 1.000–1.013, p=0.045) was an independent risk factor for significant bleeding events in APL patients (Table 4).

Table 3 Main Characteristics of Patients with or without Significant Bleeding

Clinical Characteristics	Significant Bleeding (n=47)	Nonsignificant Bleeding (n=102)	*p*-value
Age	44 (3–74)	40 (8–83)	0.426
Sex (n)			0.487
Male	22	54	
Female	25	48	
WBC (×10^9/L)	10.00 (0.49–258.01)	3.11 (0.37–78.19)	0.013
Circulating leukemic cell percentage (%)	77 (0–98.5)	41 (0–97.5)	0.002
Absolute number of circulating leukemic cells (×10^9/L)	6.78 (0 −250.27)	1.20 (0 −59.21)	0.005
BMP (%)	86.8 (13–95.4)	81.1 (29.5–94.4)	0.003
PLT (×10^9/L)	22 (1–84)	27 (4–319)	0.117
PT (s)	17 (11–27.4)	13.55 (9.8–25.4)	<0.001
APTT (s)	28.5 (19.3–49.4)	28.9 (15.3–54.1)	0.967
FIB (g/L)	1.02 (0.31–3.33)	1.35 (0.19–6.01)	0.009
D-Dimer (mg/L)	20.00 (4.17–81.2)	10.93 (0.71–152.53)	<0.001
LDH (U/L)	381 (136–2810)	278 (113–7836)	0.03
CREA (μmol/L)	54 (25–181)	58 (22–154)	0.202

Limitations of the WBC Count for Predicting Hemorrhage

In the ATRA era, the prognostic stratification of patients with APL is based on the WBC count at initial diagnosis, with WBC count ≥10×10^9/L categorizing patients into the high-risk group. Studies have identified that a WBC count over 10 × 10^9/L is more associated with bleeding than a WBC ≤10 × 10^9/L. Consistent with this idea, our results demonstrated that the incidence of significant bleeding was obviously higher in patients with WBC ≥10×10^9/L (p=0.015, Figure 4). The data indicated that patients in the high-risk group had a higher risk of significant bleeding than patients in the non-high-risk group. However, in the non-high-risk group (WBC <10×10^9/L), there were still some patients with significant bleeding. Thus, the WBC count has limitations in predicting early bleeding events in the non-high-risk group, which had normal or low WBC counts.

The Role of the Absolute Number of Circulating Leukemic Cells in Predicting Bleeding Events

Since the absolute number of circulating leukemic cells was identified as a risk factor for bleeding events in APL, we next investigated the potential roles of the absolute number of circulating leukemic cells in predicting hemorrhage events in APL patients. The cut-off value for the absolute number of circulating leukemic cells to predict significant bleeding events was obtained by ROC curve analysis (2.59×10^9/L). Then, patients were divided into two groups: the absolute number of circulating leukemic cells ≥2.59×10^9/L group and the absolute number of circulating leukemic cells <2.59×10^9/L group. The rate of significant bleeding was remarkably different between the two groups (p=0.002, Figure 5A), implying that patients with an absolute number of circulating leukemic cells ≥2.59×10^9/L are more likely to have significant bleeding than those with an absolute number of circulating leukemic cells <2.59×10^9/L. In addition, WBC count (p <0.001), circulating leukemic cell percentage (p=0.002), BMP percentage (p <0.001), PT (p <0.001), APTT (p=0.004), FIB (p <0.001), D-dimer (p=0.016), LDH (p <0.001), and CREA (p=0.016) were significantly different between the two groups (Table 5). More importantly, when patients in the non-high-risk group were divided by the absolute number of circulating leukemic cells, the incidence of significant bleeding was much higher in patients with an absolute number of circulating leukemic cells ≥2.59×10^9/L than in those with an absolute number of circulating leukemic cells <2.59×10^9/L (p=0.021, Figure 5B). These results strongly demonstrated

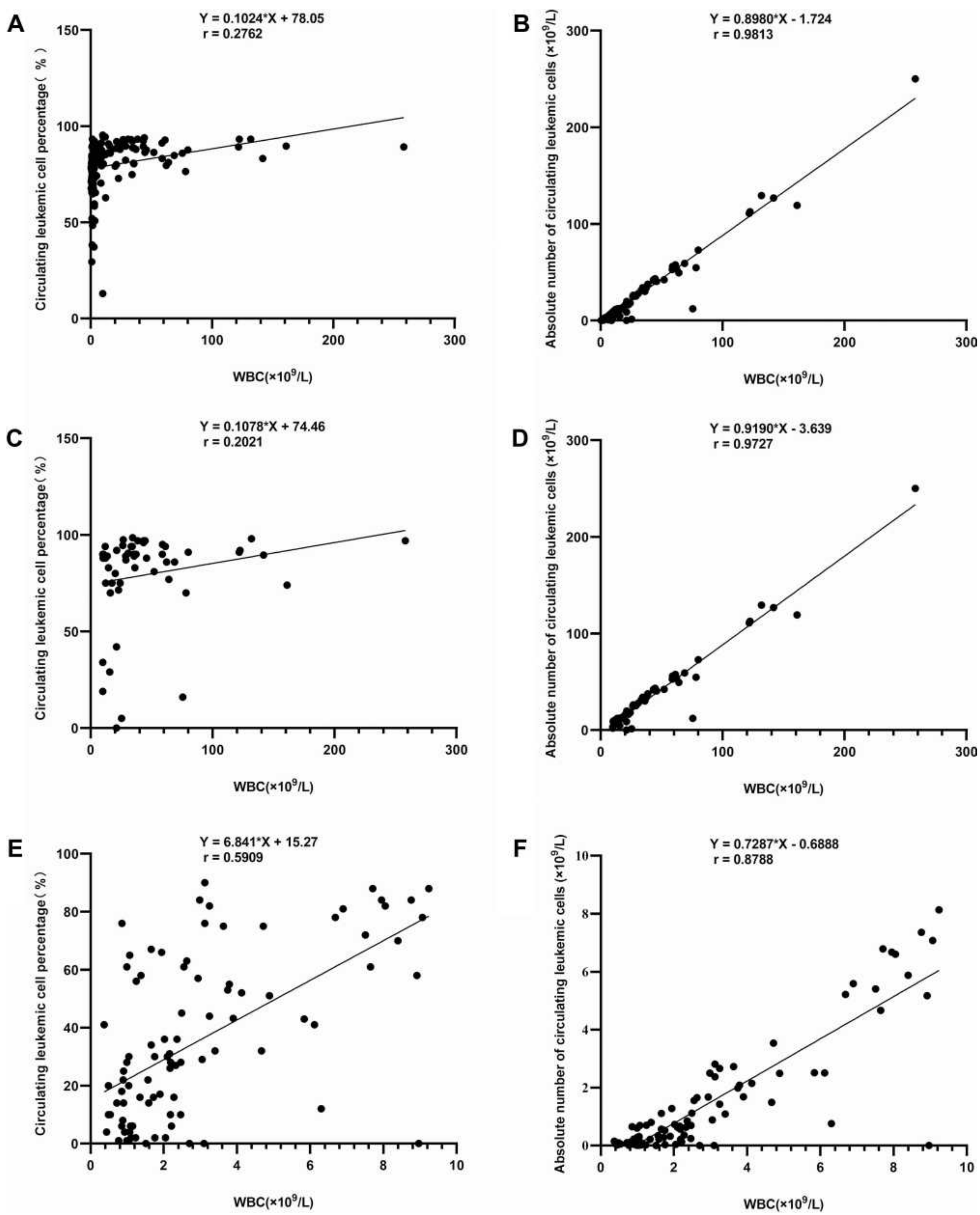

Figure 3 Correlation of WBC count with circulating leukemic cell percentage and absolute number of circulating leukemic cells. There was no evident correlation between circulating leukemic cell percentage and WBC count in all APL patients (**A**), the high-risk group (**C**) or the non-high-risk group (**E**). The absolute number of circulating leukemic cells was positively correlated with the WBC count in all APL patients (**B**), the high-risk group (**D**) and the non-high-risk group (**F**).

Table 4 Multivariate Analysis of Risk Factors for Significant Bleeding Events

Clinical Characteristics	p-value	OR	95% CI
Absolute number of circulating leukemic cells (×10^9/L)	0.045	1.017	1.000~1.013
BMP (%)	0.501	1.012	0.978~1.047
PT (s)	0.149	1.115	0.962~1.292
FIB (g/L)	0.168	0.708	0.433~1.159
D-Dimer (mg/L)	0.195	1.014	0.993~1.035
LDH (U/L)	0.160	0.999	0.999~1.000

that the absolute number of circulating leukemic cells is extremely critical for predicting early bleeding events in APL, especially in non-high-risk APL with normal or low WBC counts.

Discussion

Fatal bleeding events and HED, as the most common causes of induction failure, remain of vital concern in the treatment of APL patients. In this study, the significant bleeding rate was 31.5%, and the incidence of early hemorrhagic death was 6%, which is similar to that seen in previous studies.[11,14–19,37] Moreover, the results presented here also demonstrate that the WBC count and routine clotting test parameters (such as PT and FIB) are associated with significant bleeding in APL, which was consistent with previous studies.

Patients with APL exhibited some abnormal coagulation test results, including low FIB levels and elevated PT, APTT and D-dimer. Although these abnormalities were not present at the same time or in all patients, they still endowed a high risk of fatal bleeding.[6,46] Malignant promyelocytes mediate fibrinogenesis, enhance fibrinolysis and induce abnormal bleeding in patients with APL. Routine clotting parameters, such as PT, APTT, fibrinogen and D-dimer, are indirect measures and may not timely reflect the process of fibrinolysis.[11,15] Therefore, coagulation markers appear to be poor predictors of early bleeding events in patients with APL.

Unlike laboratory markers of disseminated intravascular coagulation (DIC), the WBC count is frequently related to APL disease burden and has served as a convenient predictor of fatal hemorrhage in APL at

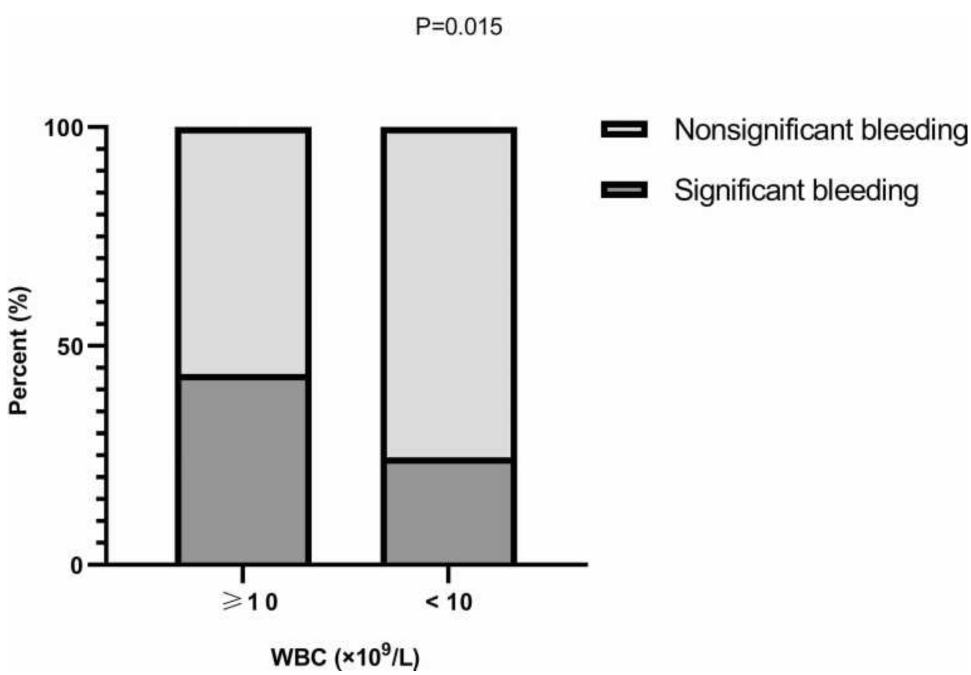

Figure 4 Association between WBC count and bleeding events. The significant bleeding rate was higher in patients with WBC ≥10×10^9/L than in those with WBC <10×10^9/L.

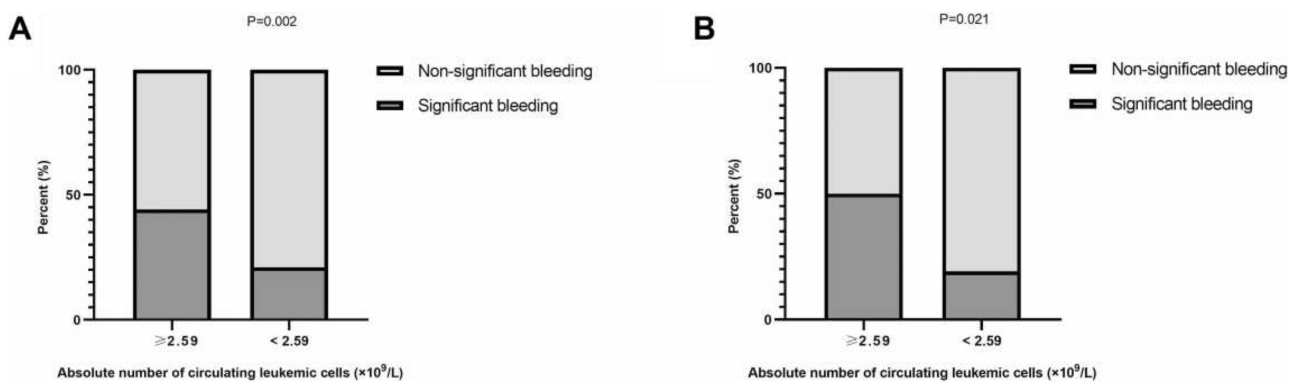

Figure 5 Association between absolute number of circulating leukemic cells and bleeding events. The significant bleeding rate was higher in patients with an absolute number of circulating leukemic cells ≥2.59×10^9/L than in those with an absolute number of circulating leukemic cells <2.59×10^9/L in all patients with APL (**A**). In the non-high -risk group, the significant bleeding rate was higher in patients with peripheral blood promyelocyte counts ≥2.59×10^9/L (**B**).

diagnosis in some studies. As reported, factors associated with high bleeding risk include presenting WBC counts ranging from 10 × 109/L to 30 × 109/L.[13,18,19,26–28,32–35] However, some studies have proved that there is no significant association between WBC count and bleeding risk.[23,36,37] Thus, the value of the WBC count as a risk factor for early bleeding events is limited, especially in non-high-risk APL.

In this study, we focused on investigating the clinical value of the absolute number of circulating leukemic cells for predicting early significant bleeding in APL patients, especially in patients with non-high-risk APL. Our study emphasized that the absolute number of circulating leukemic cells, and not the leukemic cell percentage, in the peripheral blood was positively correlated with the WBC count in both high-risk and 46 non-high-risk group patients. Importantly, the results presented

Table 5 Main Characteristics of Our Series and Comparison of Groups with an Absolute Number of Circulating Leukemic Cells ≥2.59×10^9/L and <2.59×10^9/L

Clinical Characteristics	Absolute Number of Circulating Leukemic Cells ≥2.59×10^9/L (n=68)	Absolute Number of Circulating Leukemic Cells <2.59×10^9/L (n=81)	*p*-value
Age	42 (3–83)	40 (10–81)	0.93
Sex (n)			0.446
Male	37	39	
Female	31	42	
WBC (×10^9/L)	26.57 (3.12–258.01)	1.76 (0.37–25.40)	<0.001
Circulating leukemic cell percentage (%)	88 (16–98.5)	20 (0–84)	<0.001
BMP (%)	86.6 (13–95.4)	78.4 (29.5–93.2)	<0.001
PLT (×10^9/L)	22.5 (6–84)	29 (1–319)	0.254
PT(s)	16.35 (9.8–27.4)	13.1 (10.1–24.4)	<0.001
APTT(s)	27 (15.3–44.5)	30.1 (20.5–54.1)	0.004
FIB(g/L)	0.97 (0.19–4.99)	1.45 (0.25–6.01)	<0.001
D-Dimer (mg/L)	20.00 (3.49–54.07)	11.04 (0.71–152.53)	0.016
LDH (U/L)	506.5 (166–7836)	238 (113–2127)	<0.001
CREA (μmol/L)	61 (28–181)	53.5 (22–154)	0.016

here definitively demonstrate that the absolute number of circulating leukemic cells is strongly associated with significant bleeding events in APL, not only in the high-risk group but also in the non-high-risk group. And the absolute number of leukemia cells was an independent risk factor for significant bleeding events in APL patients.

Another novel contribution of the current study is that a cut-off value for the absolute number of circulating leukemic cells as a risk factor for early bleeding events in patients with APL was obtained by ROC curve analysis. We further confirmed that the significant bleeding rate of patients with non-high-risk APL was statistically increased when the absolute number of circulating leukemic cells was $\geq 2.59\times10^9$/L. Due to the limited number of patients and the relatively short follow-up duration, the results of the current study may be influenced by unrecognized bias. Thus, to further validate the present findings, larger-scale studies need to be performed in the future.

Conclusion

In conclusion, the absolute number of circulating leukemic cells is of great clinical value for identifying patients at the greatest risk of hemorrhage and actively preventing early bleeding events in APL, especially in patients with non-high-risk APL.

Ethics Approval and Consent to Participate

The study protocol was approved by the research ethics committees of Zhengzhou university people's hospital and hospital league. Each participant signed a written informed consent form before enrolment, and a parent or legal guardian of patients under 18 years of age provided informed consent.

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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 report no conflicts of interest in this work.

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