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Clinical Features and Gene Mutations of Disseminated Intravascular Coagulation Patients in Acute Promyelocytic Leukemia

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Abstract

Acute Promyelocytic Leukemia (APL) is a special type of Acute Myeloid Leukemia (AML). Disseminated Intravascular Coagulation (DIC) is a common but life-threatening complication of APL patients, however, the significances of clinical features and gene mutations between DIC and non DIC patients in APL is not well-established. We retrospectively analyzed 73 newly diagnosed APL patients with and without DIC. Of the 73 patients included, there were 28 (38.4%) DIC patients. DIC patients in APL had higher White Blood Cell (WBC) counts ($p<0.001$), higher ECOG performance status ($p=0.021$), higher risk degree ($p<0.001$) and higher Lactate Dehydrogenase (LDH) ($p=0.012$). What's more, DIC patients were associated with higher incidences of Earth Death (ED) ($p=0.009$) and hemorrhage ($p=0.004$). The genetic mutation profiling of DIC patients showed a higher FLT3-ITD mutation ($p=0.038$). Most importantly, DIC patients showed a faster decline of PML-RARα mRNA during induction therapy ($P=0.019$). Our study may provide a new perspective to understand the prediction and treatment of DIC patients in APL.

Keywords: Acute promyelocytic leukemia; Disseminated intravascular coagulation; Clinical features; FLT3-ITD mutation

Introduction

Acute Promyelocytic Leukemia (APL) is a distinct subtype of Acute Myeloid Leukemia (AML) [1-3]. A specific chromosomal translocation of t (15;17) fusing the Promyelocytic Leukemia (PML) gene on chromosome 15 to the Retinoic Acid Receptor α (RARα) gene on chromosome 17 leads to the cell differentiation blockade and insufficiency of apoptosis [4-6]. APL had been considered one of the most fatal types of AML. With the advances in treatment of All-Trans Retinoic Acid (ATRA) [7,8] and Arsenic Trioxide (ATO) [9,10], outcomes and long-term leukemia-free survival rates have drastically improved [11,12] and transform APL from highly fatal to highly curable in AML [13].

Disseminated Intravascular Coagulation (DIC), a life-threatening complication of APL patients, characterized by the excessive activation of coagulation, resulting in fibrin deposition in systemic microvessels, causes severe bleeding owing to the depletion of platelets and coagulation proteins [14]. In addition, annexin 2 expressed by leukemia cells exacerbated the fibrinolysis [15]. The clinical manifestation of DIC patients in APL may be a result of the development of an imbalance in pro-coagulation and anti-coagulation factors.

Previous studies showed that there was a correlation between FLT3 mutation and the occurrence of hemorrhage in APL [16,17], and they also suggested that FLT3-ITD may be an indicator of poor prognosis of APL [18,19]. However, as far as we know, the information of biological characteristics and the cytogenetic features of DIC patients in APL is not clear. In this study, we assessed the clinical characteristics, genetic mutation profiling and cytogenetic features in order to provide a new insight

into the prediction and treatment of DIC patients in APL.

Methods

Study design and patients

A total of 73 newly diagnosed APL patients at the Hematology Department of The First Affiliated Hospital of Soochow University from January 2019 to January 2021 were enrolled in this study. APL diagnosis was confirmed based on the APL morphology and the presence of t (15;17) and/or the PML-RARα fusion gene. Variants patients of APL other than t (15;17) and/or the PML-RARα fusion gene were excluded. All the patients received All-Trans Retinoic Acid (ATRA) and Arsenic Trioxide (ATO) during induction therapy. Complete Response (CR) was defined by the normalization of the white blood cell counts and bone marrow features with less than 5% blast cells. The study was approved by the Institutional Review Board of The First Affiliated Hospital of Soochow University and was performed in agreement with the Declaration of Helsinki.

Diagnosis of DIC

Disseminated Intravascular Coagulation (DIC) scores of APL patients were calculated based on the Chinese DIC Scoring System (CDSS) for hematological malignancies [20]. The diagnosis of DIC was defined by a score greater than or equal to six.

Next-generation sequencing (NGS)

At the time of initial diagnosis of APL patients, genomic DNA was extracted from bone marrow. Targeted ultradeep sequencing of 51 hot spot genes in the hematological malignancy was performed by the Ion Torrent S5 system in 54 APL patients.

Statistical analysis

All of the statistical tests were performed using the SPSS statistics 23.0 (IBM Co., Armonk, NY, USA). Continuous variables were compared using the nonparametric rank test, and categorical variables were compared using the chi-square or the Fisher's exact test. Data were presented as median (range) or percent, respectively. P<0.05 was considered statistically significant.

Results

Clinical and laboratory characteristics of DIC patients in APL

This study was enrolled 73 newly diagnosed APL patients. In all cases, the incidence of DIC was 38.4% (28/73). The clinical and laboratory characteristics are shown in Table 1. Clinical features revealed that no significant differences were seen in age, sex, BMI or comorbidities between APL patients with and without DIC. In contrast, there were significant differences in ECOG performance status (p=0.021), risk degree (p<0.001) and complications of hemorrhage (p=0.004) between the two groups. Most importantly, the early death rate in the DIC group was significantly higher than that in the non DIC group (P=0.009). For laboratory characteristics, there were significantly differences in White Blood Cell (WBC) counts (p<0.001) and Lactate Dehydrogenase (LDH) (p=0.012) between the two groups. There was no significant association between DIC and hemoglobin, platelet, albumin or total cholesterol.

Genetic mutation profiling of DIC patients in APL

In order to reveal the molecular characteristics of DIC patients in APL, we selected 54 APL patients, including 22 DIC and 32 non DIC for next-generation sequencing, which contained 51 hot spot genes in the hematological malignancy. As detailed in Figure 1, the rate of gene variants was 86.4% (19/22), most of which were involved in activation signal pathway related genes and transcription factor related genes (Figure S1A). In addition, our study revealed that the top three common gene mutations were FLT3-ITD, FLT3-TKD and

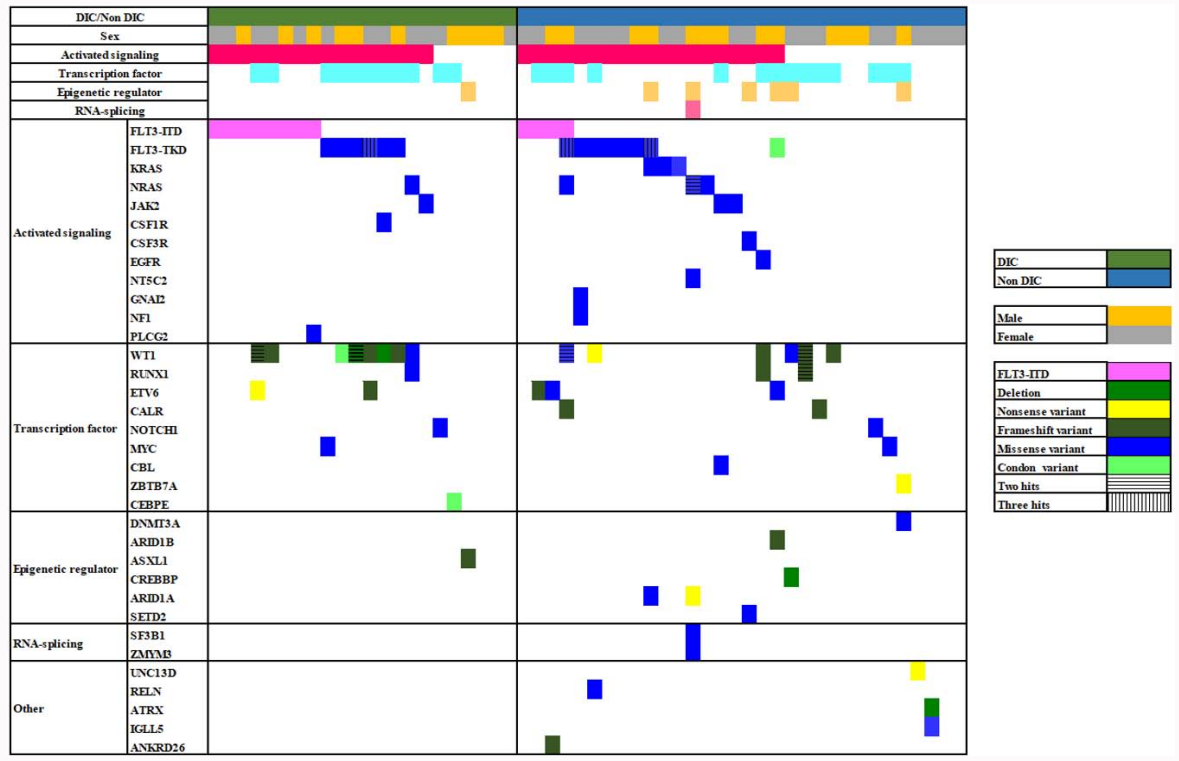

Figure 1: Genetic mutation profiling in APL patients with and without DIC.

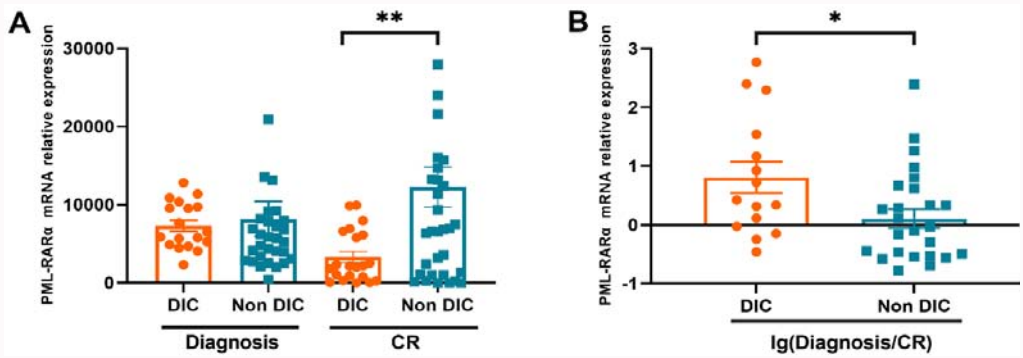

Figure 2: PML-RARα mRNA relative expression at diagnosis and Complete Response (CR) (A), and the decrease of PML-RARα mRNA relative expression during one induction therapy (B). *p<0.05, **p<0.01.

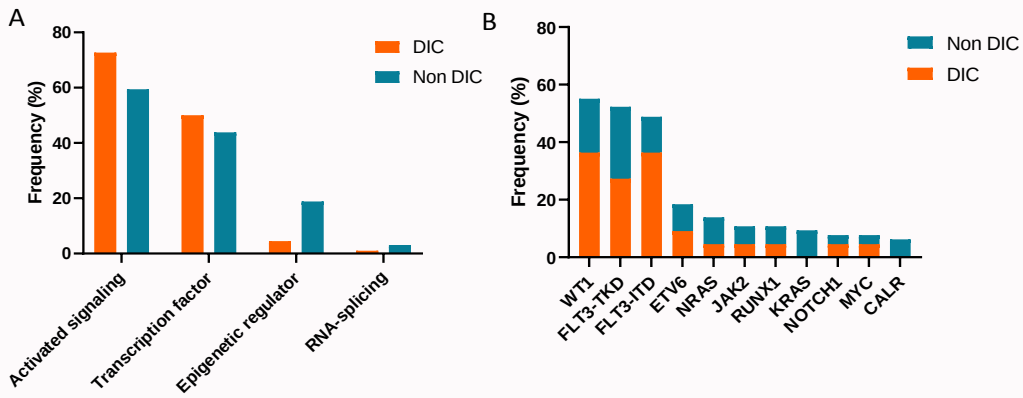

Figure S1: Different pathways involved in genetic mutations of APL patients with and without DIC (A). The frequency of gene variants in APL patients with and without DIC (B).

WT1 in DIC patients (Figure S1B). Most importantly, as shown in Table 2, FLT3-ITD mutation in the DIC patients was significantly higher than that in the non DIC patients (p=0.038).

Cytogenetic features of DIC patients in APL

To further study cytogenetic features of DIC patients in APL, we compared the PML-RARα relative mRNA expression of APL patients with and without DIC at diagnosis and Complete Response (CR). Figure 2A showed that there was no significant difference between DIC and non DIC patients at diagnosis. However, the PML-RARα mRNA relative expression was significantly higher in non DIC patients at CR (p=0.009). During one induction therapy, the PML-RARα mRNA relative expression decreased faster in DIC patients than that in non DIC patients (p=0.019, Figure 2B).

Discussion

DIC is a well-known and potentially lethal complication in APL. The mechanism of DIC in APL is the generation or activation of procoagulants, cytokines, tissue Plasminogen Activator (tPA) and urokinase-type Plasminogen Activator (u-PA), which leads to the hypercoagulability, hyperfibrinolysis, and endothelial cell damage [21,22]. Although there are many studies discussing optimal treatment of DIC, there is a paucity of information on the molecular characteristics and the cytogenetic features of DIC patients in APL.

In this study, by analyzing all the 73 newly diagnosed APL patients, we found 28 patients met criteria for DIC and the rate of DIC was 38.4%. Compared to non DIC patients, DIC patients showed a higher WBC count. An increased WBC counts was already known to be associated with a poor prognosis of APL, and several reports had noted that a higher total WBC counts or peripheral blast counts associated with an increased risk of severe bleeding during induction [23,24]. Our study revealed that DIC patients in APL were more likely to develop hemorrhage complications. The release of procoagulant enzymes from APL blasts granules trigger the coagulopathy or the interaction of APL cells with the endothelium participate in the fibrinolytic activity are thought to cause DIC.

LDH is another reflection of malignant cells in APL. Previous studies identified LDH as a significant predictor of hemorrhage [25,26]. Our data demonstrated that there was a significantly difference between APL patients with and without DIC. Increasing of LDH may be correlated with increases of the WBC counts. Besides, DIC patients in APL showed a poor ECOG performance status. 14.3% of DIC patients developed Early Death (ED), which defined as occurring within 1 month of APL diagnosis and is a common cause of death in APL patients [27-29]. While there was no ED occurred in non DIC patients. It has been reported that ECOG performance status was related with higher rate of Early Death (ED) [30].

The presence of Internal Tandem Duplication (ITD) of the FMS-Like Tyrosine Kinase 3 (FLT3) gene was frequently detected in the normal karyotype AML and was an adverse prognostic factor for AML [31,32]. Previous studies revealed that FLT3 mutation associated with immature immunophenotype and the occurrence of early death in APL [17,33,34]. Here, we found that the presence of FLT3-ITD

Table 1: Clinical and laboratory characteristics at diagnosis of APL patients with and without DIC.

Characteristics	Total (n=73, 100%) Median (range)/ no. (%)	DIC (n=28, 38.4%) Median (range)/ no. (%)	Non DIC (n=45, 61.6%) Median (range)/no. (%)	*P*
Age, years	34(15-70)	33 (15-69)	37 (15-70)	0.348
Sex				0.516
Male	33 (45.2)	14 (50)	19 (42.2)	
Female	40 (54.8)	14 (50)	26 (57.8)	
ECOG performance status	2 (1-4)	2 (1-4)	1 (1-4)	0.021
1-2	60 (82.2)	20 (71.4)	40 (88.9)	
3	6 (8.2)	4 (14.3)	2 (4.4)	
4	7 (9.6)	4 (14.3)	3 (6.7)	
BMI	24.03 (18.77-34.9)	24 (19.38-32.65)	24.09 (18.77-34.9)	0.694
Risk degree				<0.001
High risk	25 (34.2)	18 (64.3)	7 (15.6)	
Low risk	48 (65.8)	10 (35.7)	38 (84.4)	
Comorbidities				
Hypertension	3 (4.1)	1 (3.6)	2 (4.4)	0.855
Diabetes	4 (5.5)	1 (3.6)	3 (6.7)	0.572
Heart disease	2 (2.7)	0 (0)	2 (4.4)	0.258
Pregnant	3 (4.1)	1 (3.6)	2 (4.4)	0.855
Complications				
Infection	40 (54.8)	19 (67.9)	21 (46.7)	0.077
Differentiation syndrome	24 (32.9)	12 (42.9)	12 (26.7)	0.152
Hemorrhage	51 (69.9)	25 (89.3)	26 (57.8)	0.004
Early death	4 (5.5)	4 (14.3)	0 (0)	0.009
WBC (*10^9/L)	4.09 (0.43-200.87)	19.23 (0.97-200.87)	1.89 (0.43-30.34)	<0.001
Hb (g/L)	85 (41-150)	92 (48-150)	80 (41-140)	0.173
PLT (*10^9/L)	32 (5-195)	28.5 (5-158)	32 (7-195)	0.193
ALB (g/L)	39.9 (25.2-52.1)	42.85 (25.2-52.1)	39.6 (30.5-45.5)	0.172
TC (mmol/L)	4.26 (2.65-7.3)	4.5 (3.67-7.3)	4.18 (2.65-5.95)	0.196
LDH (U/L)	250.7 (104.7-832.3)	400.65 (104.7-828)	182.5 (125.3-832.3)	0.012

Abbreviations: Data were presented as median (range) or percent. WBC: White Blood Cells; BMI: Body Mass Index; Hb: Hemoglobin; PLT: Platelet; ALB: Albumin; TC: Total Cholesterol; LDH: Lactate Dehydrogenase

Table 2: Common genetic mutation of APL patients with and without DIC.

Gene variants	Total (n=54), no.%	DIC (n=22), no.%	Non DIC (n=32), no.%	P
FLT3-ITD	12 (22.2)	8 (36.4)	4 (12.5)	0.038
FLT3-TKD	14 (25.9)	6 (27.3)	8 (25)	0.851
WT1	14 (25.9)	8 (36.4)	6 (18.8)	0.147
ETV6	5 (9.3)	2 (9.1)	3 (9.4)	0.972
NRAS	4 (7.4)	1 (4.5)	3 (9.4)	0.506
KRAS	3 (5.6)	0 (0)	3 (9.4)	0.139
JAK2	3 (5.6)	1 (4.5)	2 (6.3)	0.788
RUNX1	3 (5.6)	1 (4.5)	2 (6.3)	0.788
NOTCH1	2 (3.7)	1 (4.5)	1 (3.3)	0.786
MYC	2 (3.7)	1 (4.5)	1 (3.3)	0.786
CALR	2 (3.7)	0 (0)	2 (6.25)	0.232

correlated in a statistically significant relationship with DIC. This may be because FLT3-ITD mutation involved in the coagulopathy pathogenesis of APL [16].

The results of cytogenetics demonstrated that during one induction therapy, PML-RARα mRNA relative expression decreased faster in DIC patients. We hypothesize that immune system may be more active in DIC patients, which causes immune cells to attack more APL cells. In fact, Immune cells and cytokines play an important role

in the development of DIC [35-38]. DIC may be the result of a cross-reaction between the coagulation pathway and the immune system. The balance of the coagulation pathway and the immune system may be important for the treatment of DIC patients in APL. More proofs are needed to clarify this hypothesis.

In summary, we demonstrated that: (1) 38.4% (28/73) of APL patients occurred DIC. WBC counts, LDH, ECOG performance status and FLT3-ITD mutation could be predictors of DIC in APL. (2) Compared to non DIC patients, DIC patients are more likely to develop early death. (3) DIC patients showed a quickly decline of PML-RARα during induction therapy.

To our knowledge, this is the first report showing the significances of FLT3-ITD and the decreasing of PML-RARα of DIC patients in APL. The findings of the present study are beneficial for better understanding the predictors and treatment therapy of DIC. Balancing the coagulation pathway and immune system may provide a new insight to develop personalized treatment of DIC patients in APL.

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