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Coagulation and Fibrinolysis Profiles of Acute Myeloblastic Leukemia: Preliminary Assessment of Hypercoagulability

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ABSTRACT

Acute Myeloblastic Leukemia is an abnormality of haematological malignancy that often causes bleeding and death. Coagulation and fibrinolysis examinations are screening parameters that need to be reviewed related to the incidence of bleeding events in AML patients in a hypercoagulability assessment.This was a cross-sectional study conducted at Ulin Regional Hospital Banjarmasin on patients with a recent diagnosis of AML patients for the first time using a consecutive sampling method. The measurement of PT, aPTT, fibrinogen, and D-dimer levels were done at the Diponegoro National Hospital using Coalab-1000 tool. The analysis of the correlation between the incidence of bleeding events with coagulation and fibrinolysis parameters used Fisher's exact test.The significance of the correlation test of the incidence of bleeding events with PT, aPTT, Fibrinogen, and D-dimer were 0.731; 0.086; 0.617; 0.587, and 0.731, respectively.The parameters of PT, aPTT, Fibrinogen and D-dimer were not associated with the incidence of bleeding events. The extension of PT and aPTT in most AML patients is suspected due to the deficiency of coagulation factors. Screening tests of PT, aPTT, fibrinogen and D-dimer levels should be performed in AML patients, although the results do not always show abnormalities.

Keywords: Acute Myeloblastic Leukemia (AML), bleeding, hypercoagulability

INTRODUCTION

Leukemia is a disease of hematopoietic tissue malignancy characterized by the replacement of normal bone marrow elements by abnormal blood cells or leukemic cells. Acute Myeloblastic Leukemia (AML) is an acute haematological malignancy dominated by immature myeloid cells[1]. The number of new cases of leukemia is 14.1 cases per 100,000 people per year in the United States and ranks 10th of all newly detected cancers in 2019 with AML dominating the acute leukemia in the adult population (80-90%)[2-4].

One of the clinical manifestations of leukemia is bleeding caused by various abnormalities of hemostasis, where bleeding in AML often causes death[5-7].Leukemia is related to hypercoagulable conditions and high risk for thromboembolic complications[8,4]. Abnormalities of hemostasis can occur in

leukemia in the form of thrombocytopenia, platelet dysfunction, disseminated intravascular coagulation (DIC), coagulation protein defect, primary fibrinolysis and thrombosis[7-10].

Previous studies showed that DIC often occurred in M3 AML (acute promyelocytic leukemia)[11], with a thrombosis incident of only 9.6%. Incidence of DIC was 10-50% in non-M3 AML depended on the subtypes of leukemia, with the incidence of thrombosis of 3.2%[7,12,13]. The recommended examination for DIC is PT, aPTT, fibrinogen, platelet count, fibrin degradation product (FDP), and D-dimer levels[7]. The study of Akanni et al. generally showed that leukemia patients experienced the extension of PT, aPTT, and INR; Increased D-dimer levels; and decreased platelet count[14]. The study of Libourel et al. suggested that high D-dimer levels predicted the incidence of venous and arterial thrombosis in AML[15,16].Some studied suggested that hemostasis profile needed to be examined in leukemia to detect complications immediately.

The number of AML patients in Kalimantan area, especially Banjarmasin, South Kalimantan has increased in the past three years. The incidence of hypercoagulability often occurs in AML patients who need further investigation. This was a preliminary study to determine the profile of hemostasis including PT, aPTT, fibrinogen and D-dimer in patients diagnosed with AML for the first time associated with the hypercoagulability state of bleeding.

METHODS

This was an analytical observational study with a cross-sectional approach. The subject of this study was 13 adult patients diagnosed with AML for the first time from November 2019 to February 2020. AML diagnosis was established by the internist and clinical pathologist based on BMP examination.

The subjects were collected by consecutive sampling based on the inclusion and exclusion criteria. The inclusion criteria were 1) a minimum age of 18 years old, 2) male and female sex, 3) steroid-free, and 4) normal menstruation according to age. Patients with a history of drugs consumption leading to coagulation abnormalities, or anticoagulant and antiplatelet agents (e.g., aspirin, heparin, warfarin and clopidogrel), a history of autoimmune diseases, history or suffering from coronary heart disease and stroke or other vascular abnormalities, history or suffering from renal abnormalities, trauma, history or suffering from liver abnormalities and history or suffering from malignancy other than leukemia were not included in the study.

Patients who met the inclusion and exclusion criteria underwent blood sampling. 6 ml of blood from each patient was taken and divided into two namely 3 ml of EDTA blood for a complete haematology assessment and 3 ml of sodium citrate 3.6% blood to determine PT, aPTT, fibrinogen, and D-dimer levels. We perform a complete haematology assessment in the laboratory of Ulin Regional Hospital Banjarmasin, coagulation and fibrinolysis assessments in Diponegoro National Hospital Semarang. We analyzed the citrated blood samples in the laboratory of Ulin Regional Hospital Banjarmasin to obtain

citrate plasma samples. The citrate plasma samples were stored in temperatures of -20 ºC for 2 weeks and taken to the laboratory of Diponegoro National Hospital of Semarang for coagulation and fibrinolysis parameters assessment with a preservative method. The data on illness history and physical examination were taken from the patient's medical records and confirmed directly to the patient.

We performed a haematology assessment with Hematological Analyzer XS-800i. The measurement of PT, aPTT, fibrinogen levels used the CoaLab 1000 tool by LABiTec®, Germany. PT reagent used TEClot PT-S, aPTT reagent used TEClot APTT-S, fibrinogen levels used TEClot FIB, D-dimer levels used Blue D-dimer.

We performed coding, entry, cleaning, and editing the data of the study using a computer statistics program. We also performed a univariate analysis as a characteristic of the study subject. Bivariate analysis of the correlation between the incidence of bleeding with the parameters of PT, aPTT, fibrinogen as well as D-Dimer performed using the Fisher's exact test. The analysis of this correlation used the cut-off value for PT of 12 seconds, aPTT of 37.5 sec, fibrinogen of 200 mg/dl, D-dimer of 500 µg/L and platelet of 150,000/µl with the p-value of <0.05 was considered significant.

This study was approved by the Medical and Health Research Ethics Committees of Dr. Kariadi Hospital, Semarang. All subjects of the study gave their written informed consents. The researchers bore all research costs and guaranteed the confidentiality of the research.

RESULTS

A total of 13 AML patients were diagnosed for the first time when they experienced the symptoms and visited the hospital in Banjarmasin, South Kalimantan. The age of the patient ranged from 18 to 67 years. The number of female patients was seven and the male patients were six. The characteristic of the patients shown in Table 1. Diagnosis of AML established from the history taking, physical examination, and laboratory examination. We found various clinical symptoms such as malaise, fatigue, fever, coughing up blood, vomiting blood, and bleeding gum. From the physical examination, we found pale skin, bleeding manifestations, skin lesions, organomegaly, lymphadenopathy, and gingival hypertrophy. Eight patients (61.5%) experienced manifestations of bleeding looked like melena, hematochezia, hematemesis, bleeding gum, and menometrorrhagia. This showed that bleeding is an important concern in the treatment of patients.

Complete blood count showed various results. The results showed leukocytosis in 61.5% of AML patients (8 of 13 patients), but an increase of more than 100,000 cells/mm^3 obtained only in 1 patient. It also found decreasedleukocytes in 4 patients (30.8%). Cytopenia is a sign of bone marrow failure contributed to the symptoms and signs of AML. Normocytic normochromic anemia found very dominant in almost entire patients (12 patients; 92.3%). The patients with normocytic normochromic anemia also had neutropenia (8 patients; 66.7%) and thrombocytopenia. Six of them had severe thrombocytopenia

(below 50,000 cells/mm^3). Patients with severe thrombocytopenia experienced manifestations of bleeding such as melena, hematochezia, hematemesis, bleeding gum as well as menometrorrhagia.

Table 1. Characteristics of the AML patients included in this study

Subject characteristics	Frequency (number); Percentage (%)	Median± SE (min; max)
General characteristics		
Age (years)		36 (18;67)
Sex		
Male	6;46,2	
Female	7;53,8	
Complete Blood Count		
Hb (gr%)		8,1 ±0,84 (3,3; 13,2)
Ht (%)		25,3 ±2,63 (10,7; 42)
Erythrocyte count (million/mm^3)		2,73 ±0,34 (1,09; 5,22)
MCV (fL)		88,2 ±2,11 (78,2; 99,4)
MCH (pg)		27,8 ±1,03 (23,9; 34,2)
MCHC (%)		32 ±0,71 (28; 36)
Leukocyte count (cells/mm^3)		12.500 ±16.567,95 (351; 215.500)
Platelet count (cells/mm^3)		42.000 ±15.911,27 (8.000;191.000)
Blast cell count in peripheral blood smear (%)		32±8,16 (11; 93)
Blast cell count in bone marrow (%)		43±7,33 (13; 89)
Clinical manifestations of bleeding		
Yes	8;61,5	
No	5;38,5	

There were 13 AML patients had sub-types as shown in Figure 1 dominated by AML-M1 and the least number of subtypes were AML-M4, AML-M0, and AML-M5b with the same percentage of 7.7%.

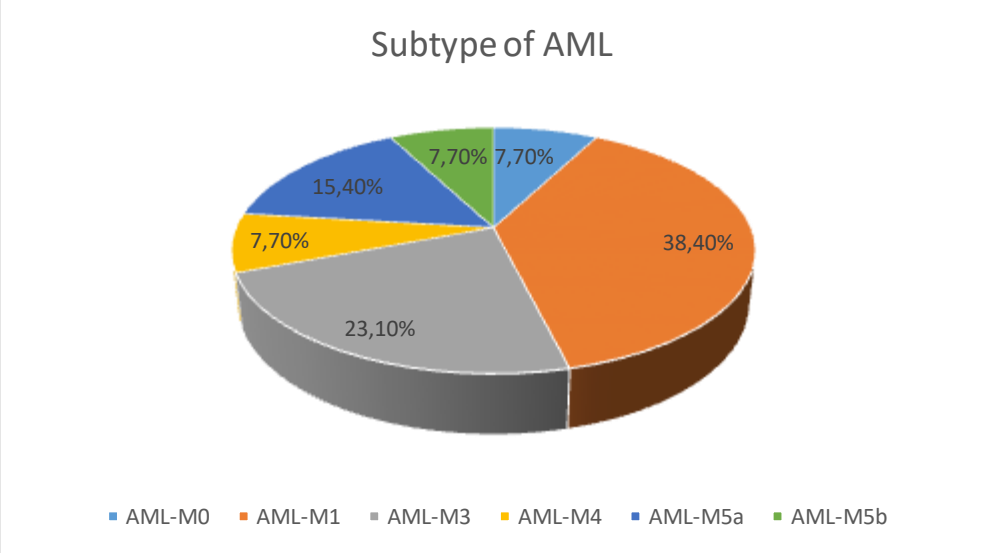

Fig. 1. Subtype of AML in the study

The results of the coagulation and fibrinolysis profiles in AML patients can be seen in table 2.

Table 2. Hemostasisprofile of AML patients

Hemostasis profile	Frequency (number); Percentage (%)	Median± SE (min; max)
PT (seconds)		13,9 ± 0,58 (11,1; 18,8)
9,8-12,0	2;15,4	
>12,0	11;84,6	
aPTT (seconds)		38,2± 3,05 (17,9; 60,9)
26,4-37,5	6;46,2	
>37,5	7;53,8	
Fibrinogen (mg/dL)		225 ± 48,15(26; 568)
<200	6;46,2	
200-450	5;38,4	
>450	2;15,4	
D-dimer (µg/L)		995±4.640,86(77; 61.180)
0-500	6;46,2	
>500	7;53,8	
Platelet count (/µl)		42.000 ±15.911,27 (8.000;191.000)
<150.000	2; 15,4	
≥150.000	11; 84,6	

AML patients showed more prevalent of prolonged PT value compared to normal PT value. Meanwhile, the extended aPTT value had a significant difference because the total patients who had an extended aPTT value differ by 1 person with the total patients who had normal aPTT values. Fibrinogen levels in AML patients varied with predominant hypofibrinogenemia and the least hyperfibrinogenemia. D-dimer levels of more than 50% increased compared to normal levels (Table 2).

Table 3.Correlation between bleeding events and hemostasis profiles of AML patients

Hemostasis parameters	Bleeding events		p
	Yes	No	
PT (seconds)			
9,8-12,0	1	1	0,731
>12,0	6	5	
aPTT (seconds)			
≤37,5	1	4	0,086
>37,5	6	2	
Fibrinogen levels (mg/dl)			
<200	3	3	0,617
≥200	4	3	
D-dimer levels (µg/L)			
0-500	3	3	0,587
>500	4	3	
Platelet count (/µl)			0,731

≤150.000	1	1
>150.000	6	5

Table 3 shows that the hemostasis screening parameters of PT, aPTT, fibrinogen, D-dimers and platelets in hypercoagulability state do not have a significant correlation with bleeding events.

DISCUSSION

The results of the study found thrombocytopenia in only two patients, which is usually dominant in AML, in addition to anemia. Thrombocytopenia in leukemia is a result of infiltration of the leukemic cells to the bone marrow causes a decreased number of megakaryocytes and platelet production. Patients often require repeated platelet transfusions which increases the risk of alloimmunization and finally leads to the platelet destruction by alloantibodies[17]. AML patients have low thrombopoietin levels due to c-mpl thrombopoietin receptors in myeloblast[18].

Some subjects had impaired hemostasis profiles in the form of thrombocytopenia, an extension of PT and aPTT, hypofibrinogenemia, and increased levels of D-dimer. These conditions indicated the activation of the hemostatic system as reported in previous studies. Activation of the hemostatic system was due to the expression of tissue factor (TF) and cancer procoagulant (CP) by blast cells activated both extrinsic and intrinsic coagulation pathways. Fibrinolysis was also stimulated by the presence of blast cells activated plasminogen into plasmin and increased fibrinolysis due to the increased coagulation activity[7,19,20]. These abnormalities in coagulation and fibrinolysis indicated a possible DIC occurrence which was a complication of leukemia. However, this study did not find such conditions. The results of the hemostasis examinations of the study were similar to the study of Rashidi et al. (2013) and Lee at al. (2015) which stated that routine coagulation examination found in DIC characterized by the various extension of PT and aPTT, low fibrinogen and platelets as well as increased D-dimer levels. However, this variable was not correlated either with the severity of bleeding or thrombosis[21,22].

AML patients also had normal PT and aPTT values. There was suspicion of deficiency in the coagulation factors involved in the formation of the fibrin clot. It was in line with the study of Elting et al. (2002) and Mansour et al. (2007) which stated that in leukemia, the deficiency of coagulation factors such as the VII, VIII, IX, XII and XI factors could occur. There was no correlation between the parameters of coagulation examination and fibrinolysis with the incidence of bleeding in AML patients[23,24].

We did not examine each coagulation factor in this study. It takes a study with larger samples and a longer period to evaluate coagulation and fibrinolysis parameters which are likely that might have varying results than the present study results.

CONCLUSION

The parameters of PT, aPTT, fibrinogen and D-dimer were not related to the incidence of bleeding, although, in some AML patients, PT, aPTT and D-dimer levels could be extended or increased. The extension of PT and aPTT in most AML patients is suspected due to the deficiency of coagulation factors.Screening tests of PT, aPTT, fibrinogen and D-Dimerlevels should be performed in AML patients, although the results do not always show abnormalities.

CONFLICT OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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