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Original Article

Activation of coagulation system and d-dimer levels in children with acute leukemia

Harun Wijaya, Novie Amelia Chozie, Badriul Hegar

Abstract

Background D-dimer is a molecule as result of breaking down of excessive fibrin formation from the activation of coagulation system. There is evidence of increased activation of coagulation in patients with acute leukemia which was showed by the increment of d-dimer levels.

Objective To evaluate the incidence of activation of coagulation system in children with acute leukemia before receiving chemotherapy.

Method This cross-sectional study was performed at Dr. Cipto Mangunkusumo Hospital. All newly-diagnosed children with acute leukemia were included in this study, prior to their receiving any chemotherapy treatment. Blast count, prothrombin time (PTT), activated partial thromboplastin time (APTT), and D-dimer levels were examined after the diagnosis was confirmed by morphology and immunophenotyping studies on bone marrow specimens.

Results Out of 22 subjects, 13 subjects had increased D-dimer values. The median D-dimer level of this elevated group was 1,000 (range 500-14,700) ng/mL. In the acute myeloblastic leukemia (AML) patients, activation of coagulation was found in 7 out of 8 subjects. The median D-dimer levels was 950 (range 100-14,700) ng/mL. In the acute lymphocytic leukemia (ALL) patients, 6 out of 14 subjects had increased activation of coagulation with median D-dimer level of 300 (range 100-3,800) ng/mL. Nine out of 10 subjects with blast cells on peripheral blood smear had a median D-dimer level of 1,000 (range 500-3,800) ng/mL. Both PT and APTT were found normal in all subjects.

Conclusion Activation of coagulation system occurs at the time of diagnosis as shown by increased D-dimer levels. The characteristics of activation of coagulation system are different between ALL and AML subjects, as well as between subjects with positive and negative blast counts on peripheral blood smears. Despite the increased activation of coagulation, PT and APTT remain normal. **[Paediatr Indones. 2014;54:227-31.]**

Keywords: *coagulation, acute leukemia, D-dimer, prothrombin time, activated partial thromboplastin time*

There is evidence of increased activation of the coagulation system in patients with acute leukemia, although its pathogenesis remains unclear.[1-9] Malignant cells[8-19] and chemotherapeutic agents[20-34] used to treat acute leukemia are considered to have an important role in activating the coagulation system.

In addition to abnormal levels of coagulation factors such as fibrinogen, factor VIII, von Willebrand factor, factor XIII-A, and plasminogen-activator inhibitor-1,[1,2,7,9,16] several investigators have documented abnormal expression of tissue factor on blast cells and circulating cancer procoagulant that is associated with the activation of coagulation system and increased thrombin generation in patients with acute leukemia.[9,10,13,15-19] Several markers have been evaluated to document thrombin generation. Among these, the thrombin-antithrombin (TAT) complex and D-dimer have been used more often, with the former considered to be more specific.[1,2,4-8,10,13]

Despite these observations in patients from several countries, no study has been done to

From the Department of Child Health, University of Indonesia Medical School/Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Reprint requests to: dr. Harun Wijaya, Child Health Department, University of Indonesia Medical School/Dr. Cipto Mangunkusumo Hospital, Jl. Diponegoro No.7, Jakarta. Mobile phone: +62 815 85069872. E-mail: mikael_harun@yahoo.com.

evaluate the activation of coagulation system in acute leukemia patients in Indonesia. The aim of this study was to assess for activation of coagulation system in children with acute leukemia.

Methods

This prospective, cross-sectional study was conducted at the Pediatric Hematology and Oncology Division, Child Health Department, Dr. Cipto Mangunkusumo General Hospital, between September to November 2012. All newly-diagnosed cases of acute leukemia from neonates up to 18-year-old children were eligible for the study. Patients with a history of steroid therapy or chemotherapy, any medication known to affect hemostatic systems, or those who had received fresh frozen plasma or cryoprecipitate in the preceding 3 days were ineligible to participate in the study.

Patients' blood specimens were collected only once and divided into two blood collecting tubes (*BD Vacutainer®* plus plastic citrate tube and *BD Vacutainer® Plastic Whole Blood* tube with spray-coated K_2EDTA; *Becton, Dickinson and Company*) for hemostatic examinations (PT, APTT and D-dimer) and complete blood counts (including peripheral blood smears for blast counts), respectively. All specimens were analyzed at the 24-hour Laboratory of Dr. Cipto Mangunkusumo General Hospital. *Sysmex® CA-1500* (*Sysmex Corporation, Siemens Healthcare Diagnostic Inc., Germany*) was used to analyze PT and APTT, while *Nycocard D-Dimer® reader II* (*Axis-Shield PoC AS, Norway*) was used to measure D-dimer levels.

D-dimer level was considered abnormal if above 300 ng/mL, while PT and APTT were considered prolonged if the result increased more than 1.5-fold of the highest range of normal value. Continuous data were described using median and range (minimum – maximum) and categorical data were described using counts.

Results

A total of 22 children were diagnosed with acute leukemia over the period of the study and consecutively enrolled. Fourteen new cases of ALL and eight cases of AML were diagnosed. The median age of subjects was 7 years and 4 months with a range of 2–16 years. Subjects consisted of 13 boys and 9 girls, as shown in **Table 1**. The median hemoglobin and hematocrit levels, leukocyte and platelet counts were 9.6 (range 6–17) g/dL, 28.3 (range 17.3–49.6)%, 4.34 (range 1.0–384.0) x 10^3/mm^3, and 22.9 (range 5.0 x 10^3–146.0) x 10^3/mm^3, respectively. Of 22 subjects, only 10 subjects showed blast cells in their peripheral blood smears, with a median of 35% and range of 4 – 97% (**Table 2**).

Although the PT and APTT levels of all subjects

Table 1. Demographic characteristics of subjects

Characteristics	ALL (n=14)	AML (n=8)	Total (n=22)
Age			
• Median	6 years 7 months	8 years	7 years 4 months
• Range	2-16 years	2-15 years	2-16 years
Gender			
• Male	10	3	13
• Female	4	5	9

Table 2. Hematologic characteristics of subjects

Characteristic	Median (range)
Hemoglobin level (n=22)	9.6 (6-17) g/dL
Hematocrit level (n=22)	28.3 (17.3-49.6) %
Platelet counts (n=22)	22,900 (5,000-146,000) /mm^3
Leukocyte counts (n=22)	4,340 (1,000-384,000) /mm^3
Blast cells in peripheral blood smear (n=9)	35 (4-97) %

Table 3. Hemostatic characteristics of subjects

Characteristics	ALL (n=14)	AML (n=8)	Blast cells		Total (n=22)
			Present (n=10)	Absent (n=12)	
PT (range), sec	11.8 (10.3 - 17.9)	12.5 (10.7 - 14.4)	12.6 (11.1 - 14.4)	11.6 (10.3 - 17.9)	12.1 (10.3 - 17.9)
Prolongation of PT (range), fold higher	0.9 (0.8 - 1.3)	0.9 (0.8 - 1.1)	0.9 (0.8 - 1.1)	0.9 (0.8 - 1.3)	0.9 (0.8 - 1.3)
APTT (range), sec	37.0 (28.8 - 50.9)	40.7 (25.9 - 47.7)	34.9 (28.8 - 47.7)	39.9 (25.9 - 50.9)	38.7 (25.9 - 50.9)
Prolongation of APTT (range), fold higher	1.1 (0.8 - 1.5)	1.2 (0.7 - 1.4)	1.0 (0.8 - 1.4)	1.1 (0.7 - 1.5)	1.1 (0.8 - 1.5)
Median D-dimer levels (range), ng/mL	300 (100 - 3,800)	950 (100 - 14,700)	1,000 (100 - 3,800)	300 (100 - 14,700)	650 (100 - 14,700)

were within normal ranges, 13 of 22 subjects had increased D-dimer levels, with a median of 1,000 (range 500-14,700) ng/mL. Further classification of subjects based on their diagnosis showed activation of coagulation in 7 of 8 subjects with AML, and 6 of 14 subjects with ALL. The median D-dimer levels of AML patients was 950 (range 100-14,700) ng/mL and of ALL patients was 300 (range 100-3,800) ng/mL. Among subjects with blast cells in their peripheral blood smears, 9 of 10 subjects had increased D-dimer levels, with a median of 1,000 (range 500-3,800) ng/mL. Only 4 of 12 subjects without blast cells in their peripheral blood smears had increased D-dimer levels, with a median of 300 (range 100-14,700) ng/mL (**Table 3**).

Discussion

In this study, we found elevated D-dimer levels in acute leukemia patients at initial diagnosis. These are indicative of activated coagulation systems in acute leukemia patients, as reported by authors from several developed countries.[1,2,5-7] Athale *et al*. reported a mean D-dimer level of 2,766 (SD 2,385.8) ng/mL in newly diagnosed case of ALL in children while Giordano *et al*. reported a mean of 299 (SD 32) ng/mL in children with ALL.[1,2] Comparing the incidence of elevated D-dimer level in ALL, our finding was lower than Athale's but almost similar with the result of Giordano *et al*. Another studies reported that 80% of their subjects with ALL had elevated D-dimer levels.[5,7] Chojnowski *et al*. also found that 85% of AML subjects had elevated D-dimer levels.[5]

A previous study reported a significant correlation between the presence of blast cells in peripheral blood smears and higher D-dimer levelss.[9] Although our study also showed more subjects with elevated D-dimer levels in the subgroup with blast cells compared those without blast cells, but this study was not designed to confirm that finding.

One patient had an extremely elevated D-dimer levels of 14,700 ng/mL. This patient had been diagnosed with a ventricular septal defect, patent foramen ovale, patent ductus arteriosus and Down syndrome prior to the AML diagnosis. Several genetic mutations have been reported to alter coagulation systems, such as mutation of the MTHFR TT 677

gene, the prothrombin G20210A variant and the FV G1691A gene, causing hyperprothrombinemia once triggered.[8,35] We did not assess the genetic basis of activated coagulation in this patient.

This study was cross-sectional in design with a small sample size. We did not evaluate a cause-and-effect relationship between diagnosis and the blast count subgroup. Despite this limitation, our study provides a foundation for future studies in our country.

In conclusion, we find activation of coagulation system in children at the time of diagnosis of acute leukemia. More than half of patients with acute leukemia in our study show increased D-dimer levels. Despite the increased of D-dimer levels, PT and APTT values are within the normal range. As such, adequate mechanisms compensated for the increased thrombin generation. Further studies are necessary to establish a relationship among findings not confirmed by this study.

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