Hemostatic Parameter of Acute Lymphoblastic Leukemia-L1 with Hyperleukocytosis

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Abstract

Background. The increasing rate of morbidity and mortality in leukemic patients are frequently found in hyperleukocytosis state. Hyperleukocytosis is defined by a white blood cell count more than 50.000/mm^3. Hyperleukocytosis still becomes an oncologic emergency that need immediate treatment. The complications that will develop if it is not immediately treated is hemostatic disorder such as intracranial bleeding and pulmonal bleeding.

Objective. This study aimed to assess the differentiation of hemostatic parameters between ALL-L1 with hyperleukocytosis and without hyperleukocytosis

Methods. The study was a cross sectional study at conducted in DR. Wahidin Sudirohusodo Hospital from November 2016 to April 2017 with seventy-two patients aged 1 month–18 years old were diagnosed with ALL-L1 based on BMP result. They were divided into two groups, 31 patients with hyperleukocytosis and 41 without hyperleukocytosis. Then they were assessed by hemostastic parameters laboratory examination (Platelet, PT, APTT, D-Dimer, and Fibrinogen).

Result. There was no significant difference in platelet count, PT, APTT, D-Dimer and fibrinogen levels between ALL-L1 patients with hyperleukocytosis and without hyperleukocytosis. Mean of platelet count with p value = 0.621, mean PT with p value = 0.429, mean APTT with P value = 0.918, mean D-Dimer with p value = 0.882 and mean fibrinogen with p value = 0.455.

Conclusion. There was no significant difference of haemostatic parameters between ALL-L1 patients with hyperleukocytosis and without hyperleukocytosis.

Keywords: *hemostatic parameters, ALL-L1, hyperleukocytosis*

Introduction

Leukemia is a malignancy of the hemopoietic system, that is the maligant transformation of progenitor or prekursor of blood cell which forms a malignant cell clone, marked by uncontrolled proliferation which cause constringency that lead to bone marrow failure and infiltration to other tissues.[1]

Epidemiologically, the incidence of acute leukemia is about 30% to 40 % of entire malignancy in children. There are 2 types of acute leukemia, Acute Lymphoblastic Leukemia (82%) and Acute Myeloblastic Leukemia (17%). approximately 3000 new cases of ALL occurred every year in America, 5000 in Europe, and about 2000-3000 cases in Indonesia. The peak of incidence occurs at age of 2-5 years. The average of incidence in children under 15 years is 4-4.5 cases / 100,000 per year.[2] meanwhile, the most common malignancy in Pediatric Departement of DR Wahidin Sudirohusodo Hospital, Makassar during 2013 was acute leukemia (96%).[3]

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Increased morbidity and mortality in leukemia mostly found in patients with hyperleukocytosis. Hyperleukocytosis can be present in 9-13% of ALL patients. Hyperleukocytosis is defined as an increase of white blood cell in the peripheral blood exceeding 50,000/mm3. This excessive increase is due to disruption of the white blood cell release from the bone marrow. Hyperleukocytosis may cause increased blood viscosity, blast cell aggregation and thrombus occurred in the microcirculation. Beside, due to the size of the blast cells are bigger than mature white blood cell cells, and not easily change the shape of the blast cells cause blast cell easily trapped and cause occlusion on microcirculation. This is called leukocytosis. Leukocytosis will cause poor perfusion and hypoxia, anaerobic metabolism, lactic acidosis, and eventually cause damage to blood vessel walls and bleeding.[4]

Another concept suggests that in leukemia, ineffective phagocytosis of neutrophils may cause the persistence of non-digestible bacteria which lead to inflamation and hidden microcirculation disorder such as extensive tissue hypoxia, endothelial cell damage, coagulation system activation and microcirculatory, and mitochondrial distress syndrome. These factors are important in determining the potential that lead to sepsis, but this condition is not detectable clinically so that the patient shows a stable condition.[5]

In addition, other theories suggest that in leukemia, there is release of procoagulant material from leukemic cell blast. The pathogenesis of this condition is very complex and involves various mechanisms such as coagulation activation by procoagulant substances released by leukemic cells, fibrinolytic path failure, and endothelial changes.[6] Considering hyperleukocytosis can cause various complications in leukemia, it is necessary to prove whether hyperleukocytosis has the potential to cause hemostatic disorder. Therefore, it is important to do the research to find out the impact of hemostatic disorders in children with leukemia who have hyperleukocytosis.

Previous study by Faranita et al.[7] found the hemostatic system is maintained by interactions between endothelial cells, platelets, coagulation proteins and fibrinolytic systems. In injury, these four elements play a role in coagulation system. Hematological malignancies can alter plasma molecule levels including in terms of coagulation and fibrinolysis, such as fibrinogen, PT, APTT and D-Dimer. There is an evidence of increased activation of the coagulation system in acute leukemia patients, although its pathogenesis remains unclear.

Hyperleukocytosis is one of the oncologic emergencies that require prompt treatment. If this condition is not handled appropriately and immediately, this may have a detrimental effect on leukemia patients due to complications.

Material and Method

Design and research variable

This cross-sectional study was conducted in DR Wahidin Sudirohusodo Hospital Makassar, during November 2016 until April 2017. The blood sampling was conducted at the laboratory of DR Wahidin Sudirohusodo Hospital. The research variables consist of: independent variable (hyperleukocytosis), dependent variable (hemostatic parameters (number of platelets, PT, APTT, Fibrinogen level and D-Dimer level)), confounding variable (sepsis), control variable (LLA-L1), intermediary variable (Hyperleukocytosis process in ALL-L1 causing impaired hemostatic parameters), and random variables (age, sex, genetics, nutritional status)

Population and sample

Accessible populations are all patient aged 1 month - 18 years old with ALL-L1 admitted to child care unit of Dr. Wahidin Sudirohusodo Hospital. Samples were all accessible populations who meet the inclusion and exclusion criteria.

Method and data collection

Data collection was obtained from the patient diagnosed with leukemia aged 1 month - 18 years old who meet the inclusion criteria. and then register number, age, sex, nutritional status, vital signs (blood pressure, pulse, temperature and breathing) was recorded. Blood samples were drawn for platelet, PT, APTT, Fibrinogen levels and D-Dimer levels. Bone marrow examination was also done. The results observed were platelet count, PT and APTT, Fibrinogen level, and D-Dimer level in patients diagnosed with ALL-L1 based on Bone Marrow

Punction.

Data Analysis

All data obtained are recorded in the research data form, and then grouped by purpose and data type. The following statistical methods were chosen: 1) univariate analysis and 2) bivariate analysis.

Result

Of 93 patients with a diagnosis of leukemia that meets the inclusion criteria, there were 72 ALL-L1 patients consists of 31 patients with hyperleukocytosis and 41 patients without hyperleukocytosis. There were 22 patients (5 patients with Aplastic Anemia, 4 patients with ANLL-M2, 1 patient with ANLL-M3, 1 patient with ANLL-M4, 5 patients with CML, and 6 patients with MDS).

Based on the characteristics, most of the patients were male (65.3%) aged <10 years old (70.8%), with good nutritional status (38.9%). (Table 1)

Characteristics of hemostatic parameters in ALL-L1 showed that platelet count had a range 3,000-129,000 with mean 37916.67 ± 36100.31. White blood cell was range from 1,100-423,000 with mean 53480.69 ± 74780.86. The range of Hemoglobin level was 2.5-10.1 and mean of 37916.67 ± 36100.31. The range of PT, APTT, D-Dimer, and Fibrinogen were 10.1-90.0 (mean 13.068 ± 9.40), 23.1-180.0 (mean 32.41 ± 18.68), and 0.6-20.6 (mean 2.85 ± 2.83), 63.6-477.2 (mean 284.79 ± 87.47) respectively. (Table 2)

Sex distribution based on white blood cell count in ALL-L1 showed that in the hyperleukocytosis group there were more male (71%) than female (29%), and so does the non-hyperleukocytosis group with percentage were 61% and 39% respectively. Statistical analysis in the table above shows that there is no significant difference of sex distribution based on white blood cell count in patients with ALL-L1 with p = 0.527 (Table 3).

The age distribution based on white blood cell count in ALL-L1 showed that in the hyperleukocytosis group there were 51.6% of patients <10 years old and 48.4% were ≥10 years old. While in the non-hyperleukocytosis group there were 85.4% of patients <10 years old and 14.6% were ≥10 years old. Statistical analysis in table 4 showed that there was a significant difference of age distribution based on white blood cell count in ALL-L1 with p = 0.003, while the percentage of ≥10 years was higher (48.4%) in ALL-L1 Hyper leukocytosis (Table 4).

Comparison of hemostatic parameters based on the number of white blood cell in patients with ALL-L1 showed that there was no significant difference in mean platelet count between hyperleukocytosis and non-hyperleukocytosis with p = 0.621. There was no significant difference of PT, APTT, D-dimer, and fibrinogen level between hyperleukocytosis and non-hyperleukocytosis with p value were 0.429, 0.918, 0.882, and 0.455 respectively. (Table 5).

Table 1. Characteristics of subjects (no=72)

Variable	Category	n	%
ALL-L1	Hyperleucocytosis	31	43.1
	Non-hyperleucocytosis	41	56.9
Sex	Male	47	65,3
	Female	25	34,7
Age	<10 years	51	70,8
	≥10 years	21	29,2
Nutritional state	Malnutrition	14	19,4
	Less nutrition	25	34,7
	Good nutrition	28	38,9
	Overweight	3	4,2
	Obesity	2	2,8

Table 2. Characteristic of Hemostatic Parameter in ALL-L1

Variable	mean±SD	Minimum	Maximum
Platelet	37916.67±36100.31	3000	129000
WBC	53480.69±74780.86	1100	423000
Hb	7.124±1.93	2.5	10.1
PT	13.068±9.40	10.1	90.0
APTT	32.41±18.68	23,0	180.0
D-DIMER	2.85±2.83	0.6	20.6
Fibrinogen	284.79±87.47	63.6	477.2

Table 3. Sex distribution on WBC in ALL-L1

Sex		ALL-L1		Total
		Hyperleucocytosis n (%)	Non- hyperleucocytosis n (%)	n (%)
	Male	22 (71.0%)	25 (61.0%)	47 (65.3%)
	Female	9 (29.0%)	16 (39.0%)	25 (34.7%)
Total		31 (100.0%)	41 (100.0%)	72 (100.0%)

Chi Square p=0.527 (p>0.05)

Tabel 4. Age distribution based on WBC in ALL-L1

Age		ALL-L1		Total
		hyperleucocytosis n (%)	Non- hyperleucocytosis n (%)	n (%)
	<10 years	16 (51.6%)	35 (85.4%)	51 (70.8%)
	≥10 years	15 (48.4%)	6 (14.6%)	21 (29.2%)
Total		31 (100.0%)	41 (100.0%)	72 (100.0%)

Chi Square p=0.003 (p<0.05)

Table 5. Comparison of Hemostatic Parameters Based on WBC in ALL-L1

Hemostatic Parameters		LLA -L1	
		hyperleucocytosis (n=31)	**Non hyperleucocytosis (n=41)**
Platelet	Minimum	3.000	3.000
	Maximum	121.000	129.000
	Mean	36096,77	39292.68
	SD	31809.17	39368.29
	p = 0.621b		
PT	Minimum	10.2	10.1
	Maximum	19,9	90
	Mean	12.45	13.54
	SD	2.50	12.31
	p = 0.429b		
APPT	Minimum	23	23,2
	Maximum	50.4	180
	Mean	30.56	33.82
	SD	6.72	24.10
	p = 0.918b		
D-Dimer	Minimum	0.6	0.6
	Maximum	5,5	20.6
	Mean	2.57	3.06
	SD	1.57	3.51
	p = 0.882b		
Fibrinogen	Minimum	128.5	63.6
	Maximum	453.5	477.2
	Mean	275.86	291.54
	SD	70.67	98.62
	p = 0.455a		

[a]*Independen T Test* [b]*Mann-Whitney*

Discussion

This study showed no significant difference in mean platelet count, PT, APTT, D-dimer levels, and fibrinogen levels among patients with hyperleukocytosis and non-hyperleukocytosis LLA-L1 patients with p value of 0.621 platelet mean, PT 0.429 mean APTT 0.918, mean D-dimer 0.882, and mean fibrinogen 0.455.

Hyperleukocytosis is one of the oncologic emergencies that prompt immediate treatment.8 Increased morbidity and mortality in leukemia patients are often found in hyperleokocytosis. If this condition is not treated promptly and correctly, it can lead to

death due to intracranial and/or pulmonary hemorrhage, as well as metabolic disturbance due to leukemia cell lysis.9

Patients with ALL-L1 were mostly male, which is similar to a study by Ramadhina et al.5 in Cipto Mangunkusumo Hospital, and Permatasari et al.10, with incidence percentage were 61% and 64.4% respectively.

Most subjects were <10 years (68.1%), the youngest age was 2 months old and the oldest age was 16 years old 7 months old. The same thing was also found in a study by Budiyanto et al.11 and Falakh et al.12, with percentage of patients aged <10 years were more than 60%.

Furthermore, for nutritional status, in this study most people with ALL-L1 was well nourished (38.9%). This suggests that the rate of diagnosis greatly influences disease progression and nutritional status. So that is necessary to have knowledge about leukemia diagnosis. One of the clinical manifestations of leukemia is bleeding. The most common bleeding manifestations include petechiae, purpura or ecchymosis, occurring in 40-70% of patients with acute leukemia at the time of diagnosis. The most common sites of bleeding are the skin, eyes, nasal mucous membranes, gingiva and gastrointestinal tract. Life-threatening hemorrhages usually occur in the gastrointestinal tract and the central nervous system. This bleeding manifestation arises as a result of various hemostatic disorders.6

The hemostatic system is maintained by interactions between endothelial cells, platelets, coagulation proteins and fibrinolytic systems. At the injury state, all four elements work together in the coagulation system. Haematological malignancies can alter plasma molecule levels including in terms of coagulation and fibrinolysis, such as fibrinogen, PT, APTT and D-Dimer. There is an evidence of increased activation of the coagulation system in acute leukemia patients, although its pathogenesis remains unclear.7

In this study there was no significant difference of distribution and mean of PT and APTT in patients with ALL-L1 hyperleukocytosis and non-hyperleukositosis. This suggests that blast cells infiltrated to the liver will cause a decrease in synthesis of coagulation factors. Then in hyperleukocytosis, leukocytosis is easily occurs that will result in the occurrence of anaerobic metabolism and endothelial damage of blood vessels and will activate the coagulation system.

Wijaya et al.13 in a study on the activation of coagulation system and D-Dimer levels in acute leukemia patients at Dr. Cipto Mangunkusomo Hospital found that there was an increase in D-Dimer levels in all ALL-L1 patients at the time of initial diagnosis but PT and APTT were within normal limits. In Ichikawa et al.14 study, it was mentioned that D-Dimer levels in the hyperleukocytosis group were higher than in the non-hyperleukocytosis group. Likewise, a study by Athale et al (2010) reported mean D-Dimer levels 2,766 (SD 2,385) ng / ml in the hyperleukocytosis group.

Leukemia and other malignancies are associated with high-risk hyper coagulant conditions for thrombohemorrhagic complications. Clinical complications range from localized thrombosis to severe hemorrhagic due to DIC. In leukemia, DIC complications occur due to the release of procoagulant material (thromboplastin-like substances) from blast cells. The procoagulant material is such a tissue factor that will form a complex with factor VIIa thus activating the coagulation cascade through the extrinsic pathway that forms the fibrin systemically. Ongoing coagulation will decrease plasma antithrombin III levels which are important inhibitors for the coagulation process. Then inhibit fibrinolytic system due to the maximum coagulation activation. This inhibition is caused by an increase of plasminogen activator inhibitor type 1 (PAI1) as the major inhibitor in the fibrinolytic system.

There were no significant differences in distribution and mean of fibrinogen in the hyperleukocytosis and non-hyperleukositosis group. Most fibrinogen was normal in both groups. However, there are also some patients who have elevated fibrinogen levels. Increased fibrinogen levels show that in leukemia, the risk of infection cannot be ignored and fibrinogen is an acute phase reactant released during infection even though this condition is not clinically detectable and the patient's condition as if stable.5

There was no significant difference between patients who had thrombocytopenia with high PT and APTT between hyperleukocytosis and non hyperleukocytosis group. This suggests that in leukemia infiltration of

blast cells to the liver and cause synthesis of coagulation factors.

Likewise, the distribution of thrombocytopenia with hypofibrinogenemia, there was no significant difference between hyperleukocytosis and non-hyperleukocytosis group. This suggests that in both groups, endothelial vessel damage occurs due to inflammatory cytokines that will lead to activation of the coagulation system.

Research Xiao et al.15 found that the incidence of coagulation disorders (PT, APTT, D-Dimer and Fibrinogen) in ALL patients had correlation with the number of blast cells present in the bone marrow.

In conclusion, there was no significant difference in fibrinogen levels between patients with ALL-L1 hyperleukocytosis and non-hyperleukocytosis. There was no significant difference in D-Dimer levels between people with ALL-L1 who had hyperleukocytosis and who had no hyperleukocytosis. The authors recommend that further study to have larger sample quantities and use prospective cohort study to evaluate the differences in hemostatic parameters between patients with ALL -L1 hyperleukocytosis and non hyperleukocytosis.

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References

1. Permono B, Ugrasena IDG. Leukemia akut. Buku Ajar Hematologi-Onkologi Anak. IDAI 2012;236-246.
2. Roganovic. *Acute Lymphoblastic Leukemia in Children*. 2013. Available at: http://dx.doi.org/10.5772/55655.
3. Daud Dasril. Pendekatan diagnostik leukemia akut di layanan kesehatan primer. Dibawakan di Makassar Pedatric Update 29-30 Agustus 2015.
4. Abdulsalam M. Hiperleukositosis. Buku Ajar Hematologi-Onkologi Anak. IDAI 2012;236-246.
5. Ramadhina N, Windiastuti E., Budiman HI. Hipoksia jaringan dan kadar laktat leukemia limfoblastik akut dengan demam neutropenia dalam fase induksi. Sari Pediatri.2008; 10(3).
6. Dia Rofinda Z. Kelainan hemostasis pada leukemia. Jur Kes Andalas. 2012; 1(2).
7. Faranita T., Trisbawati Y., Lubis M. Gangguan koagulasi pada sepsis.Sari pediatri,2011:226-232.
8. Oymak Yesim. Clinical outcomes of childhood leukemia with hyperleukocytosis, without leukopheresis. Acta medica 2014,3;89-94.
9. Kong SG, Seo JH, Jun SE, Lee BK, Lim YT. Childhood acute lymphoblastic leukemia with hyperleukocytosis at presentation. Blood research. 2014;49(1):29-35. Available at: doi.10.5045/br.2014.49.1.29.
10. Permatasari E, Windiastuti E, Satari HI, Survival and prognostic factors of childhood acute lymphoblastic leukemia, Paediatrica Indonesiana. 2009;11(49).
11. Budiyanto W., Mulatsih S., Sutaryo. Luaran terapi pasien leukemia limfoblastik akut dengan leukosit ≥50.000/µl di RSUP DR. Sardjito.2009.
12. Falakh F., Max F.J Mantik., Adrian U. Analisis kadar kreatinin pada anak dengan leukemia limfoblastik akut di pusat kanker anak estella BLU RSUP Prof. Dr. R.D. Kandou.
13. Wijaya H, Amelia N., Hegar B. Activation coagulation system and d-dimer levels in children with acute leukemia. Peadiatr Indones, 2014,54:227-231.
14. Ichikawa, K., Edahiro, Y., Gotoh, A. Et al. Co-occurance of hyperleukocytosis and elevated fibrin-fibrinogen degradation product levels is a risk factor for early intracranial hemorrhage in patients with de novo acute leukemia. Int J of Hematol, 2016;104:612-20.
15. Xiao FF1, Hu KX, Guo M, Qiao JH, Sun QY, Ai HS et al. Abnormality of Blood Coagulation Indexes in Patients with de Novo Acute Leukemia and its Clinical Significance. 2013 Apr;21(2):300-4. doi: 10.7534/j.issn.1009-2137.2013.02.008.