Comparison of Prognostic Scores in Chronic Myeloid Leukemia (Cml) Patients with Bcr-Abl Mutation Types B3a2 and B2a2 in Dr. Sardjito General Hospital

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Abstract

Background. Chronic Myeloid Leukemia (CML) is a myeloproliperative malignancy that is caused by reciprocal translocation between chromosomes 9 and 22 which form the BCR-ABL fusion gene. Most CML patients have a major type of BCR-ABL mutation. There are b3a2 and b2a2 types, which produce different oncoproteins in 25 amino acid elements. The expression of different proteins is thought to cause differences in clinical manifestations, laboratory, and prognosis. In CML, there are several prognostic scoring systems, including Sokal, Hasford, and EUTOS scores which combine clinical and laboratory parameters. The effect of this genomic breakpoint location on clinical and biological characteristics is still controversial.
Aims. The aim of this study was to determine the comparison of prognostic scores between CML patients with b3a2 and b2a2 BCR-ABL mutation types in Dr. Sardjito General Hospital.
Methods. This study was a cross sectional retrospective study used secondary data from medical records of Dr. Sardjito General Hospital, from March 2014 to April 2016. The prognostic score of Sokal, Hasford, and EUTOS was calculated in the BCR-ABL mutation type groups b3a2 and b2a2. Data were analyzed by Chi Square test.
Results. A total of 113 CML patients were analyzed with 74 (65.5%) b3a2 mutation type groups and 39 (34.5%) b2a2 mutation type groups. Hemoglobin levels, leukocytes, platelets, basophils, and eosinophils did not differ significantly between the two groups of mutation types. Meanwhile, the statistical test for the phase of disease when the patient was first diagnosed in both types of mutations showed a significant difference ($p = 0.005$). More patients with types of b2a2 mutations came in the acceleration and blast crisis phases than b3a2 types. However, Sokal, Hasford, and EUTOS prognostic scores in the b3a2 mutation type group were not significantly different from the b2a2 group ($p > 0.05$).
Conclusions. There was no significant difference in prognostic scores of CML patients with the b3a2 BCR-ABL mutation type compared with the b2a2 mutation type in Dr. Sardjito General Hospital Yogyakarta.

Keywords: *Chronic Myeloid Leukemia (CML), type of BCR-ABL mutation, prognostic scores*

Abstrak

***Latar Belakang**. Chronic Myeloid Leukemia (CML) merupakan kelainan keganasan mieloproliperatif yang disebabkan oleh translokasi resiprokal antara kromosom 9 dan 22 yang membentuk gena fusi BCR-ABL. Sebagian besar penderita CML mempunyai tipe mutasi mayor BCR-ABL yaitu jenis b3a2 dan b2a2, yang menghasilkan oncoprotein yang berbeda pada 25 elemen asam aminonya. Ekspresi protein yang berbeda diduga menyebabkan perbedaan manifestasi klinis, laboratoris, dan akhirnya prognosis. Pada CML dikenal beberapa sistem skoring prognostik, diantaranya skor Sokal, Hasford, dan EUTOS yang menggabungkan parameter klinis*

dan laboratorium. Pengaruh lokasi breakpoint genomik ini terhadap karakteristik klinis dan biologis ini masih kontroversial.
***Tujuan**. Penelitian ini bertujuan untuk mengetahui perbandingan skor-skor prognostik antara pasien-pasien CML dengan tipe mutasi BCR-ABL b3a2 dan b2a2 di RSUP dr. Sardjito.*
***Metode**. Penelitian ini merupakan penelitian cross sectional retrospective dengan menggunakan data sekunder pemeriksaan klinis dan hasil laboratorium dari rekam medik RSUP dr. Sardjito pada periode Maret 2014-April 2016. Dilakukan perhitungan skor prognostik Sokal, Hasford, dan EUTOS score pada kelompok tipe mutasi BCR-ABL b3a2 dan b2a2. Uji statistik yang digunakan adalah uji Chi Square.*
***Hasil Penelitian.** Sebanyak 113 pasien CML dianalisis, dengan kelompok tipe mutasi b3a2 sebanyak 74 (65,5%) pasien dan b2a2 sebanyak 39 (34,5%) pasien. Kadar Hb, angka leukosit, trombosit, basofil, dan eosinofil tidak berbeda bermakna antara kedua kelompok tipe mutasi. Sedangkan, uji statistik untuk fase penyakit CML saat pertama kali pasien terdiagnosa pada kedua tipe mutasi menunjukkan adanya perbedaan bermakna (p= 0,005). Tipe mutasi b2a2 lebih banyak yang datang pada fase akselerasi dan krisis blast dibanding tipe b3a2. Namun, skor prognostik Sokal, Hasford, dan EUTOS pada kelompok jenis mutasi b3a2 tidak berbeda bermakna dengan kelompok b2a2 (p>0,05).*
***Kesimpulan.** Tidak terdapat perbedaan yang bermakna pada beberapa skor prognostik pasien CML dengan tipe mutasi BCR-ABL b3a2 dibanding tipe mutasi b2a2 di RSUP Dr. Sardjito Yogyakarta.*

***Kata kunci:** Chronic Myeloid Leukemia (CML), tipe mutasi BCR-ABL, skor prognostik*

Introduction

Chronic Myeloid Leukemia (CML) is a myeloproliperative malignancy that caused by reciprocal translocation between chromosomes 9 and 22 which forms the BCR-ABL fusion gene and produces the protein tyrosine kinase that results in uncontrolled proliferation of myeloid cells.[1] The BCR-ABL fusion gene can occur due to the breakpoints at three points, there are major BCR (M-BCR), minor (m-BCR) and micro (μ-BCR). Based on the 3 types of grouping, there are 7 types of breakpoint types in the BCR-ABL fusion gene, there are major b3a2, major b2a2, minor e1a2, micro e19a2, rare type e1a3, rare type e13a3 and rare type e12a1.[2] Each breakpoint type encodes a different type of protein and has a different leukemogenic activity, especially in the activity of the tyrosine kinase enzyme. The expression of different proteins causes different clinical manifestations and prognosis.[3]

Clinical classification of CML is used today to assess prognostic factors. The scoring system prognostic of CML used Sokal, Hasford, and EUTOS scores which in principle combine clinical and laboratory factors. These three prognostic scores generally classify patients into low risk and intermediate-high risk groups.[4]

In the majority of CML patients with positive BCR-ABL, about 95% of patients have b3a2 (e14a2) or b2a2 (e13a2) fusion gene. Both of b3a2 and b2a2 fusion genes encode P210 kDa protein, which is only distinguished by 25 amino acids encoded by exon b3. The effect of this genomic breakpoint location on clinical, laboratory, and prognostic characteristics is still controversial.[5] Therefore, the authors were interested in analyzing the comparison of prognostic scores (Sokal, Hasford, andUTOS scores) between CML patients with b3a2 and b2a2 BCR-ABL mutation types in RSUP dr. Sardjito.

Methods

This study used a retrospective cross-sectional design to determine the comparison of prognostic scores between CML patients

who have BCR-ABL mutation type b3a2 with b2a2 mutation type in Dr. Sardjito General Hospital. The affordable population of the study were CML patients in the outpatient and inpatient installation at the Dr. Sardjito General Hospital in the period March 2014-April 2016. The sample of the study were newly diagnosed CML patients, aged ≥ 18 years, with BCR-ABL mutation types was b3a2 or b2a2, had not received any therapy, and had completed the medical record data. Exclusion criteria in this study were CML patients who had other comorbid diseases, there were chronic kidney disease, diabetes mellitus, chronic liver disease, infections and other malignancies.

Differences between the mean values of the numerical data b3a2 BCR-ABL mutation type group and the b2a2 group were analyzed using the unpaired T test or the Mann-Whitney U test. For differences of Sokal, Hasford, and EUTOS scores, there were the low risk group and the intermediate-high risk group in the b3a2 BCR-ABL mutation type with the b2a2 type were analyzed using the Chi Square test or Fisher's exact test, with P value was significant if less than 0.05 (95% confidence interval).

Results

The flow of research shown in Figure 1. Basic characteristics data include age of first visit, sex, type of BCR-ABL mutation, phase of CML disease, hemoglobin level, leukocyte count, platelet count, basophil percentage, eosinophil percentage, blast cell count, and spleen size in cm, are explained in table 1.

The results of this study showed that 74 (65.5%) CML patients had b3a2 mutation type, while the b2a2 mutation type were 39 (34.5%) patients. In the data based on the BCR-ABL mutation type, subjects with the b3a2 mutation type had an average younger age of 42.0 ± 14.2 years compared to those of the b2a2 mutation type 46.7 ± 17.9 years, but were not significantly different (p = 0,132), as shown in table 2.

From the statistical analysis based on BCR-ABL mutation type, the phase of CML disease when the subject was first diagnosed, that showed significant difference between b3a2 and b2a2 types (p = 0,005). Blast cell count in CML patient with b3a2 mutation type compared to b2a2 type was significantly

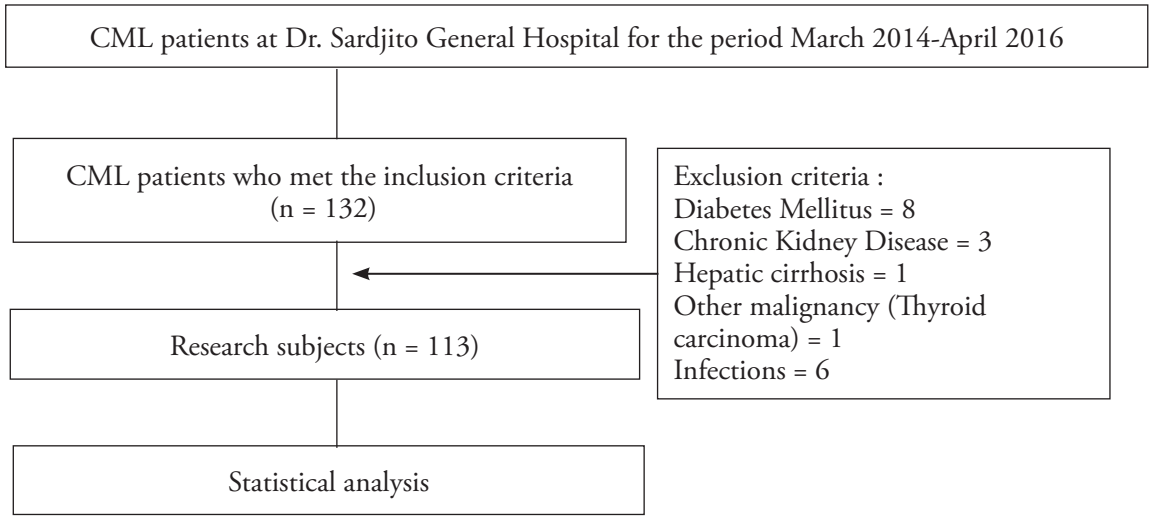

Figure 1. Flow of research subjects

Table 1. Basic characteristics of the research subjects (113 subjects)

Parameter		n	%	Mean	SD	Med	Min	Max
Age (year)				43.6	15.7	44	18	80
Sex	Male	67	59.3					
	Female	46	40.7					
Type of mutation	b3a2	74	65.5					
	b2a2	39	34.5					
Phase of disease	Chronic	100	88.5					
	Acceleration	8	7.1					
	Blast crisis	5	4.4					
Hemoglobin (g/dl)				9.6	2.5	9.2	5.0	21
Leukocytes ($x10^3/mm^3$)				197.3	169.4	155	4.4	741.7
Platelets ($x10^3/mm^3$)				586.3	503	474	28	3368
Basophils (%)				2.6	3.1	1.3	0	12,3
Eosinophils (%)				2.9	2.7	2.3	0	12
Blast (%)				5.6	10.6	3	0	85
Spleen size (cm) from below left costal margin				15.1	7.3	16	0	32

Table 2. Basic characteristicsof the research subjects based on the type of BCR-ABL mutation

Variable		Mean ± SD Median (Min - Max)		*P*
		b3a2	b2a2	
Age (year)		42.0 ±14.2	46.7± 17.9	0.132*
Sex	Male n (%)	48 (64.9)	19 (48.7)	0.144**
	Female n (%)	26 (35.1)	20 (51.3)	
Phase of disease	Chronic n (%)	70 (94.6)	30 (76.9)	0.005***
	Acceleration n (%)	3 (4.1)	5 (12.8)	
	Blast crisis n (%)	1 (1.4)	4 (10.3)	
Hemoglobin (g/dl)		9.7 ± 2.6	9.2 ± 2.3	0.365*
Leukocytes ($x10^3/mm^3$)		161 (4.6-741.7)	146.4 (4.4-602.5)	0.833***
Platelets ($x10^3/mm^3$)		606 ± 560.9	548 ± 375.8	0.612*
Basophils (%)		2.1 ± 2.5	3.6 ± 3.9	0.381*
Eosinophils (%)		2.8 (0-12)	2.2 (0-12)	0.493***
Blast (%)		3 (0-30)	3 (1-85)	0.036***
Spleen size (cm) from below left costal margin		16(0-32)	16(0-32)	0.04***

** Unpaired T test , ** Chi square, ***Mann-Whitney U test,* Significance : P < 0.05

different (p = 0.036). However, the levels of hemoglobin, leukocytes, platelets, basophils and eosinophils are not different in the two types of mutations. Based on statistical analysis for the spleen size, there is a significant difference that splenomegaly in the b3a2 type is greater than b2a2 (p = 0.04).

The research subjects on b3a2 BCR-ABL mutation types group and b2a2 group, were grouping based on risk class of Sokal, Hasford, and EUTOS scores. The statistical analysis used Chi square test as shown in table 3. In the three prognostic scores, the proportion of subjects included in the low risk group and intermediate-high risk group in patients with b3a2 BCR-ABL mutation type compared to b2a2 mutation type showed no significant difference.

Table 3. Comparison of Sokal, Hasford, and EUTOS scores on b3a2 and b2a2 BCR-ABL mutation types.

Variable		BCR-ABL mutation types		P
		b3a2	b2a2	
Sokal score n (%)	*Low risk*	3 (4.1%)	2 (5.1%)	0.566*
	Intermediate - High risk	71 (95.9%)	37 (94.9%)	
Hasford score n (%)	*Low risk*	6 (8.1%)	4 (10.3%)	0.471*
	Intermediate - High risk	68 (91.9%)	35 (89.7%)	
EUTOS score n (%)	*Low risk*	42 (56.8%)	25 (64.1%)	0.579**
	High risk	32 (43.2%)	14 (35.9)	

** Fisher, **Chi square,* Significance : P < 0.05

Discussion

In this study, the number of subjects with b3a2 BCR-ABL mutation type is higher than b2a2 mutation type, which is 65.5% compared to 34.5%. The results are different from the research of Deb *et al*. in India which had 56.25% subjects with b3a2 mutation type, 41.25% b2a2 mutation type and 2.5% other types.[6] The study of Jain *et al*. also found different results, there were 41% b3a2 mutation type, 42% b2a2 type, and 18% both of b3a2 and b2a2 type.[7] In the study of Luke *et al*. obtained 55% subjects with b3a2 mutation type and 45% b2a2 type.[8] Subjects with b2a2 mutation type in this study more to be excluded because they came with other comorbid diseases, for example infections due to clinical conditions which initially were worse than the b3a2 mutation type. This causes the comparison of the number of mutations in this study is different from other studies.

The age of the CML patients when first diagnosed for the b2a2 mutation type was 46.7 years, that was older than the b3a2 mutation type which was 42 years, but it was not statistically significant different (p = 0.132). These results are similar to the study of Jain *et al*. and De Almeida Filho *et al*.[7,9], which described that patients with CML with the mutation types b3a2 and b2a2 does not mean any different. However, in a study in India by Deb et al in 2014 was found that the age of patients with CML type B2A2 mutation was older and significantly different.

In this study, the hemoglobin level of the subjects with b2a2 mutation type, was lower at 9.2 g/dl compared to the subjects with b3a2 type which was 9.7 g/dl, but it was not statistically significant (p = 0.365). Leukocytes count of subjects with b3a2 mutation type is almost similar to subjects with b2a2 mutation type (p = 0.833). The platelet count in subjects with b3a2 type was slightly higher (mean = $606x10^3/mm^3$) compared to the b2a2 mutation type (mean = $548x10^3/mm^3$), but it was not statistically significant (p = 0.612). The hematological profile of the subjects in this study is similar to the results of Bazatlenko *et al*. study which showed that was no significant difference between hemoglobin levels, leukocytes count and platelets count in CML patients with b3a2 BCL-ABL transcript compared to b2a2. However, different things were found in the study of Vasconcelos *et al*. which showed that the b3a2 BCR-ABL transcript is associated with a higher platelet count (thrombocytosis), whereas the b2a2 transcript shows a higher leukocyte count.[10,11]

Based on the phase of CML disease, it was found that the b2a2 mutation type group was more in the acceleration and blast

crisis phase compared to the b3a2 mutation type, and this was significantly different (p = 0.005). These results are similar to the study of Polampalli *et al*. at India with 202 research subjects.[12] However, the different results were obtained in Vanconceles *et al.* study at Brazil.[11]

The percentage of blast cells in b2a2 mutation type was also higher compared to b3a2 mutation type and it was significantly different (p = 0.036). This is certainly related to the number of patients who come in acceleration and blast crisis phases, which is more commonly found in b2a2 mutation type compared to b3a2 type. It also shows a higher leukemogenic activity in the b2a2 mutation type.

Based on BCR-ABL mutation type, basic characteristics of subjects showed that subjects with the b2a2 mutation type had a clinical profile that tended to be worse than the b3a2 mutation type, as evidenced by the CML disease phase when the patient was first diagnosed and blast cell counts.

On the spleen palpation, the same median was obtained between the type of mutation b3a2 and b2a2 which is 16 cm. However, after statistical analysis, there were significant differences in the types of b3a2 and b2a2 (p = 0.04). The type of b3a2 mutation had a greater spleen than b2a2 type. This result might because the number of b3a2 mutations is higher and the average CML patients who come to RSUP dr. Sardjito tends to be late, with most of their main complaints being an enlarged abdomen, due to splenomegaly.

In this study, from the calculation of Sokal, Hasford, and EUTOS prognostic scores showed results that did not differ significantly between the b3a2 and b2a2 types of BCR-ABL mutations. These results are same as the study of De Almaida *et al*. at Brazil which also compared the three prognostic scores in both of types of mutations.[9] However, Deb *et al*. at India showed CML patients with b2a2 BCR-ABL transcripts type had higher Sokal, Hasford and European Treatment and Outcomes Study scores than b3a2 transcripts type, or worse prognostic scores.[6] The results of researchs regarding the relationship of BCR-ABL mutation types with prognostic score of CML patients is still varied, so it is still controversial.

The absence of differences between the haematological, clinical profile and prognostic score in CML patients with b3a2 and b2a2 mutation types is the number of samples with b2a2 mutation type fewer than b3a2 type. In this study, CML patients with the b2a2 mutation type were more excluded because they came with other comorbidities, such as an infection that might be related to the clinical condition or phase of the disease when the patient arrived. In addition, differences in the spleen size between both of mutation types, the results obtained that the type b3a2 has a larger spleen size, which will affect the calculation of prognostic scores.

Conclusion

There was no significant difference in prognostic scores of CML patients with the b3a2 BCR-ABL mutation type compared with the b2a2 mutation type in Dr. Sardjito General Yogyakarta.

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