Evaluation in Hematology and BCR-ABL Molecular Profiles in Patients with Chronic Myeloid Leukemia Undergoing Tyrosine Kinase Inhibitor Therapy

Muhammad Ihza Lisan Shidqi[1], *Budi Santosa[1], Muhamad Muslim[2], Haitami[3]

[1]Department of Clinical/Medical Laboratory Science, Universitas Muhammadiyah Semarang, Indonesia. [2]Department of Medical Laboratory Technology, Universitas Borneo Lestari, Indonesia. [3]Department of Medical Laboratory Technology, Poltekkes Kemenkes Banjarmasin, Indonesia.
*Email: budisantosa@unimus.ac.id

Abstract: Chronic myeloid leukemia (CML) is a myeloproliferative malignancy due to the formation of the BCR-ABL fusion gene in chronic myeloid leukemia. This condition causes excessive cell proliferation, resulting in an increase in the number of leukocytes. Tyrosine Kinase Inhibitor (TKI) is a first-line therapy that helps reduce the percentage of the Breakpoint Cluster Region–Abelson (BCR-ABL) fusion gene in patients with chronic myeloid leukemia. This study was conducted to determine evaluation in the hematological profiles (hemoglobin levels, leukocyte counts, platelet counts) and molecular BCR-ABL in patients with chronic myeloid leukemia before and after 12 months of tyrosine kinase inhibitors therapy. This analytic observational study was administered using a cross-sectional design to in analyzing the medical records of CML patients who underwent TKI therapy at the Sub Specialist Polyclinic of Internal Medicine Hematology Oncology Ulin Banjarmasin Indonesia Regional Hospital from March 2021-April 2022. Statistical test was performed which analysis results showed that 12-month tyrosine kinase inhibitor therapy could increase the hemoglobin levels, decrease leukocyte counts, platelet counts as well as decreasing the percentage of BCR-ABL gene fusion in patients with chronic myeloid leukemia. In conclusion, evaluation of tyrosine kinase inhibitor therapy in patients with chronic myeloid leukemia obtained significant differences in the hematological profiles and the molecular BCR-ABL. Further researchers are recommended to compare the type of tyrosine kinase inhibitor therapy between Imatinib and Nilotinib on the hematological and molecular profiles of BCR-ABL in patients with chronic myeloid leukemia with a larger sample count.

Keywords: Breakpoint cluster region-abelson; chronic myeloid leukemia; hematology profiles; tyrosine kinase inhibitor.

INTRODUCTION

Chronic myeloid leukemia (CML) is a common myeloproliferative malignancy that occurs due to reciprocal translocation between chromosomes 9 and 22 that produces a cimeric oncogene called Breakpoint Cluster Region– Abelson (BCR-ABL). BCR-ABL is a protein tyrosine kinase product that can cause uncontrolled proliferation of myeloid cells (Menon, 2013). Chronic myeloid leukemia mostly occurs at the age of 40-60 years with a peak incidence of 53 years. There have been more male patients suffering from CML than women with a ratio of 3:2. As many as 30-40% of CML patients are asymptomatic. However, clinical manifestations and symptoms of CML

Corresponding Author: Budi Santosa
Department of Clinical/Medical Laboratory Science, Universitas Muhammadiyah Semarang, Indonesia
Email: budisantosa@unimus.ac.id

patients are associated with leukocytosis, splenomegaly or anemia (Reksodiputro, 2014).

At the early diagnosis, CML patients are often diagnosed in the chronic phase. However, unless proper treatment is administered, the disease can progress to an accelerated phase to a blast crisis phase within 3-5 years (Brümmendorf et al., 2020). The chronic phase is usually asymptomatic, yet in the accelerated phase and the blast crisis phase, the symptoms will become more severe as characterized by anemia and a platelet count below 100,000/mm3 or above 1,000,000/mm3 (How et al., 2021).

The BCR-ABL fusion gene activates various intracellular signaling pathways which increase the proliferation of hematopoietic stem cells, decreased apoptosis of hematopoietic stem cells and weaken the myeloid cell attachment to the bone marrow stroma. As the results, immature myeloid cells are released into the blood circulation (Sholikah, 2017).

CML treatment includes the use of non-specific agents such as busulfan, hydroxyurea, interferon-alpha treatment. This treatment can control and monitor the number of leukocytes in the chronic phase but it does not reduce the percentage of BCR-ABL cells (Bintoro, 2019). The percentage of the BCR-ABL fusion gene can be reduced through a newly-developed CML therapy called Tyrosine Kinase Inhibitor (TKI) (Huang et al., 2012).

The main goal of TKI therapy in CML predicts the success of the therapy. The 12-month of therapy monitoring is critical in determining patients' (Branford, 2020). Approximately 93.3% of CML patients showed hematological profile with anemia condition shown in the hemoglobin level of 7-10 g/dL, leukocytes counts that increased between 100-250 x 10^3 /uL (52.2%), and platelet count (26.6%) showing thrombocytosis >450 x 10^3/uL (Kumar et al., 2019).

In a previous study at the West Nusa Tenggara Provincial Hospital, explained that CML patients experienced symptoms of moderate anemia, leukocytosis, and thrombocytosis (Sugiharta & Anggoro, 2020). But in this study, it is not yet known how the state of BCR-ABL in chronic myeloid leukemia patients. Meanwhile, research by Khazaal et al. (2019) conducted a BCR-ABL examination qualitatively obtained more variants of the b3a2 subtype than the b2a2 subtype in chronic myeloid leukemia patients. Quantitative BCR-ABL examination is necessary to evaluate CML patients during therapy.

Based on the explanation mentioned earlier, the researchers attempted to examine the evaluation in hematological profiles (hemoglobin levels, leukocyte counts, and platelet counts) and molecular BCR-ABL in patients with chronic myeloid leukemia with 12-month tyrosine kinase inhibitor therapy.

MATERIALS AND METHODS

This study is an analytic observational study using cross-sectional design in April 2022. The population in this study were patients with chronic myeloid leukemia. The samples patients with chronic myeloid leukemia patients who underwent tyrosine kinase inhibitor therapy from March 2021 - April 2022 at the Internal Disease Sub Specialist Polyclinic at Ulin Hospital Banjarmasin.

Hematology profile examination which includes hemoglobin levels, leukocyte count, and platelet count is examined using the hematology analyzer Sysmex XN-1000. Meanwhile, BCR-ABL molecular examination is examined using the Xpert® BCR-ABL Ultra method. Patients' medical record data on the hematological and molecular profiles of BCR-ABL were collected then analyzed in the statistical analysis software. The Kolmogorov-Smirnov normality test was performed to ensure the

normality of the data distribution. The differences in hematological profile were tested in paired T-test and the molecular BCR-ABL was examined in a McNemar test. A P-value of <0.05 was considered statistically significant.

This study has received research ethics eligibility from the Research Ethics Commission of the Banjarmasin Ulin Regional General Hospital with letter number 51/V-Reg Research/RSUDU/22.

RESULTS AND DISCUSSION

The data of this study were collected from 53 patients with CML who underwent the tyrosine kinase inhibitor therapy from March 2021 to April 2022.
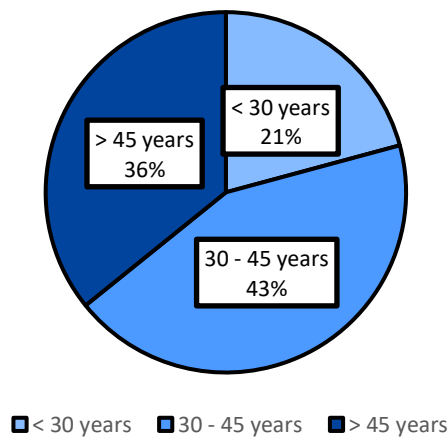

Figure 1. The Data Distribution on the Age of Patients with CML in Ulin Banjarmasin Regional Hospital from March 2021 – April 2022

As presented in Fıgure 1,11 patients aged < 30 years (21%), 23 patients aged 30-45 (43%) and 19 patients aged > 45 years (36%). The average age is 40 years with the median score of 38 years and age range between 19 to 66 years.
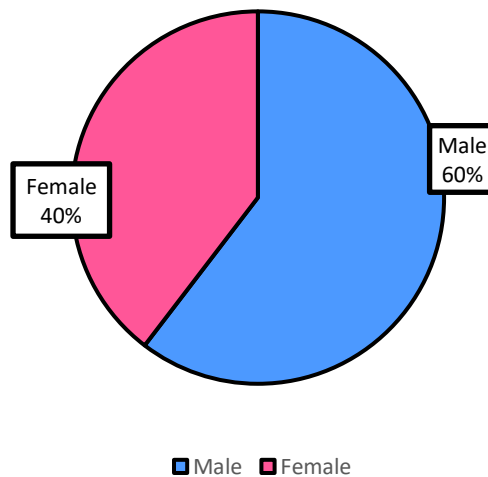

Figure 2. The Data Distribution on the Sex of Patients with CML in Ulin Banjarmasin Regional Hospital from March 2021 – April 2022

Figure 2 shows that 32 patients are male (60%) and 21 patients were female (40%).

Table 1. The Hematology Profile of Patients with CML in Ulin Banjarmasin Regional Hospital from March 2021 – April 2022

Hematology Profile	Before Therapy		After Therapy	
	Mean	Std Deviation	Mean	Std Deviation
Hemoglobin Level	9,96	1,91	12,25	2,28
Leukocytes Counts	193.934	184.208	8679	4508
Platelet Counts	422.000	286.781	254.987	161.572

Based on Table 1, the hemoglobin level before therapy is averagely 9.96 with standard deviation of 1.91 which then increased to 12.25 and a standard deviation of 2.28 after therapy. The leukocytes count before therapy is 193,934 with a standard deviation of 184,208 which then decreased to 8679 with a standard deviation of 4508 after the therapy. The platelet counts also decreased to 254.987 with a standard deviation of 161.572 from 422,000 with a 286.781.
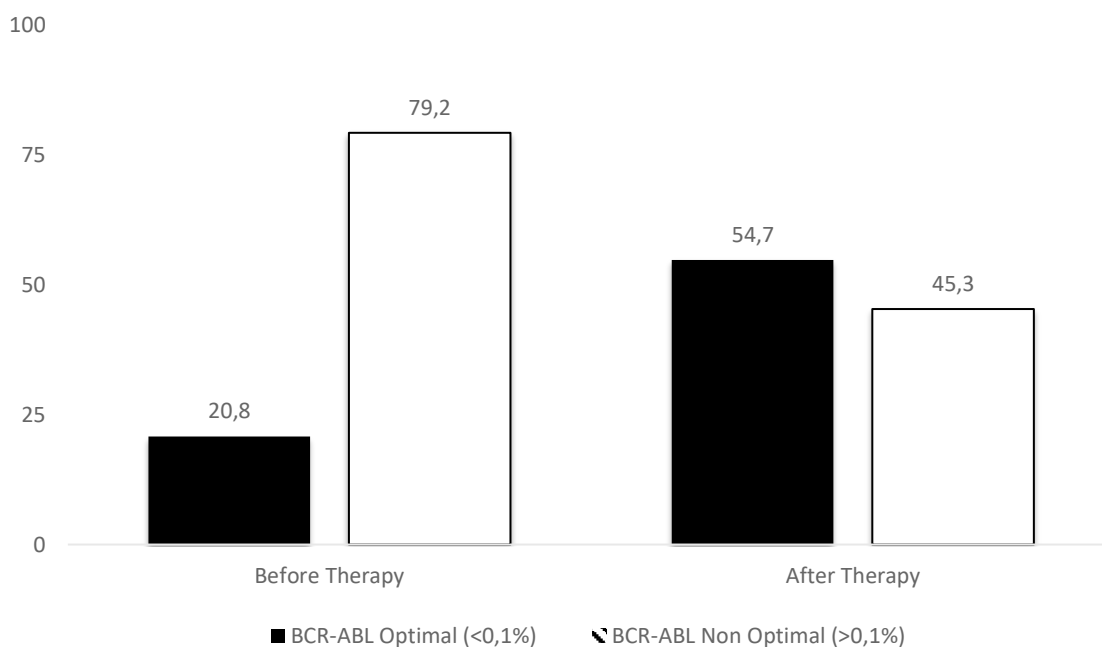

Figure 3. The Results of BCR-ABL Molecular Profile in Patients with CML at Ulin Banjarmasin Regional Hospital from March 2021 – April 2022

As presented in Figure 3, the BCR-ABL before therapy was found optimal in 11 patients (20.8%) and non-optimal in 42 patients (79.2%). After the therapy, 29 patients developed optimal BCR-ABL (54.7%) and 24 patients had non-optimal results (45.3%).

Table 2. The Results of Hematology Profile Test in Patients with CML at Ulin Banjarmasin Regional Hospital from March 2021 – April 2022

	Average (s.d)	Gap (s.d)	p Value Dependent Sample-test
Hemoglobin level before therapy (n=53)	9,96 (1,91)	-2,29 (2,75)	<0,001*
Hemoglobin level after therapy (n=53)	12,25 (2,28)		
Leukocytes count before therapy (n=53)	193934 (184208)	185255 (183699)	<0,001*
Leukocytes count after therapy (n=53)	8679 (4508)		
Platelet count before therapy (n=53)	422000 (286781)	167013 (326407)	<0,001*
Platelet count after therapy (n=53)	254987 (161572)		

*Probability values show significant difference at α = 0.05

The Bivariate Parametric Paired Sample T test on the hematological profile (hemoglobin level, leukocyte count, and platelet count) showed difference values of -2.29, 185.255, and 167.013 with probability values <0.001 . Since 0.001 < 0.05, Ho is rejected, indicating the presence of a significant difference in hematological profiles (hemoglobin levels, leukocyte counts, platelet counts) before and after tyrosine kinase inhibitor therapy in chronic myeloid leukemia patients at the Internal Medicine Sub Specialist Polyclinic at Ulin Hospital Banjarmasin .

Table 3. The Results of BCR-ABL Molecular Statistic Test in Patients with CML at Ulin Banjarmasin Regional Hospital March 2021 – April 2022

		After Therapy		Total	p Value McNemar's Test
		Optimal	Non-Optimal		
Before therapy	Optimal	11 (20.8%)	0 (0.0%)	11 (20.8%)	0.001*
	Non-optimal	18 (34.0%)	24 (45.3%)	42 (79.2%)	
Total		29 (54.7%)	24 (45.3%)	53 (100%)	

*Probability values show significant difference at α = 0.05

As seen in table 3, the results of the non-parametric McNemar molecular BCL-ABL bivariate statistical test in patients with chronic myeloid leukemia showed optimal outcome in 11 patients people (20.8%). 18 patients (34.0%) had non-optimal outcome which then improved to the optimal level after the therapy. Meanwhile, 24 patients (45.3%) still show non-optimal results after the therapy. A probability value of 0.001 was obtained, and Ho is rejected. Hence, there is a significant molecular difference between BCR-ABL before and after tyrosine kinase inhibitor therapy in chronic myeloid leukemia patients at the Internal Medicine Sub Specialist Polyclinic at Ulin Hospital Banjarmasin.

The tyrosine kinase inhibitors therapy effectively improves the hematological profile (hemoglobin level, leukocyte count, platelet count) in the chronic phase of CML. Based on Table 1, patients with chronic myeloid leukemia who underwent the tyrosine kinase inhibitor therapy experienced an increase in hemoglobin levels. On the other hand, the leukocyte counts and platelet counts decreased significantly after the therapy. Similar results were also found by Jbireal et al and Bhutani et al who also found different hematological profiles before and after 12 months of tyrosine kinase inhibitor therapy (Bhutani et al., 2020; Jbireal et al., 2019).

There were 17 patients who experienced anemia (hemoglobin<9.0 g/dl). This condition can cause cognitive disorders such as dizziness and malaise because due to fewer amount of oxygen transported which can lead to hypoxia thereby affecting the quality of life of CML patients (Efficace et al., 2013; Le, 2016). Previous studies have explained that the pathogenesis of anemia in chronic myeloid leukemia occurs due to excessive granulocyte cell proliferation which inhibits the erythropoietic hematopoietic activity (Park et al., 2017). Patients with chronic myeloid leukemia have a significant increase in the number of leukocytes following the fusion of the BCR-ABL gene that produces a protein tyrosine kinase. The tyrosine kinase activity of BCR-ABL can also take over the phosphate group from ATP without stimulation (autophosphorylation). Phosphate groups are further transferred to tyrosine residues of various substrates within the cell, thereby activating various intracellular signaling pathways. Activation of these signals leads to increased proliferation, decreased apoptosis and differentiation of hematopoietic stem cells, and decreased myeloid cell adhesion to the bone marrow stroma, leading to chronic myeloid leukemia (Jabbour & Kantarjian, 2020). In this study, 6 CML patients had platelet counts exceeding 700,000/mm3. This could trigger the risk of thrombocytosis and even bleeding due to platelet dysfunction. The risk is even higher if the platelet count is >700,000/mm3 (Dia Rofinda, 2012).

Based on Table 3, after 12 months of tyrosine kinase inhibitor therapy, 54.7% of CML patients showed optimal molecular response (BCR-ABL <0.1%). Meanwhile, 45.3% of CML patients did not show optimal molecular response. Likewise Rahem, et.al also reported that more than 50% of CML patients undergoing kinase inhibitor therapy achieved optimal molecular response (Rahem et al., 2016). The optimal molecular response predicts that CML patients can live longer (Rajabto et al., 2022). Non-optimal molecular response is considered a "warning" that CML patients require more intensive monitoring (Press, 2010).

On the other hand, different results were found by Nasser et al who conducted their study in Tanzania. They found that only 23.4% of CML patients achieved an optimal molecular response and 76.6% of CML patients did not have optimal molecular response after the therapy. They assumed that this condition might be associated with inadequate optimal molecular response after 12 months of therapy are the level of therapeutic toxicity and decreased drug adherence (Nasser et al., 2021). Imatinib can cause side effects including weight gain, fatigue (Jabbour & Kantarjian, 2020), bone and muscle pain, as well as increased creatinine. A study conducted by Francis et al reported that 68% of CML patients treated with Imatinib experienced various side effects, especially hematological toxicity-thrombocytopenia as much as 21% (Francis et al., 2015).

Alves et al and Okouango et al reported that the level of drug adherence in CML patients ranged from 20-50% (Alves et al., 2016; Okouango et al., 2017). Medication adherence may decrease due to prolonged drug consumption, asymptomatic feeling, and normal blood count. As patients find their conditions better, they often miss several doses of medication (dos Reis et al., 2013; Gater et al., 2012; Marin et al., 2010).

This study limitedly analyzed the evaluation in the hematological and molecular profiles of BCR-ABL in patients with chronic myeloid leukemia who underwent the tyrosine kinase inhibitor Imatinib mesylate. Further researchers are recommended to evaluate the type of tyrosine kinase inhibitor therapy between Imatinib and Nilotinib on the hematological and molecular profiles of BCR-ABL in patients with chronic myeloid leukemia with a larger sample count.

CONCLUSIONS

Tyrosine kinase inhibitor therapy improves the hematological profiles including increased hemoglobin levels, decreased leukocyte and platelet counts in patients with chronic myeloid leukemia. The therapy also decreases the percentage of BCR-ABL molecular. For patients with chronic myeloid leukemia, it is recommended to perform a quantitative BCR-ABL examination as a therapeutic evaluation and follow the treatment as directed by the clinicians.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper

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