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BCR-ABL TYROSINE KINASE DOMAIN MUTATIONS COMBINATIONS AFFECTS IMATINIB RESISTENCE IN CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA

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Abstract

Background: *Chronic myelogenous leukemia is a myeloproliferative disease, due to reciprocal translocations of chromosome 9 and 22, resulting to Bcr-Abl fusion gene. This gene has important role in chronic myelogenous leukemia development with activation of tyrosine kinase. In the era of tyrosine kinase inhibitor drugs, chronic myelogenous leukemia survival has improved greatly but unfortunately tyrosine kinase inhibitor resistence emerge as a treatment problem. It is important to determine the type, pattern and combination of Bcr-Abl kinase domain gene mutations that occur in chronic myelogenous leukemia patients with positive Bcr-Abl which had incomplete molecular response to imatinib, a first generation tirozin kinase inhibitor. Objective: To determine the point mutations C944T, T1052C, T932C in kinase domain of the Bcr-Abl gene and the influence of the number of point mutations to Bcr-Abl/G6PDH*

Methods: *Observational clinical and laboratories study of 40 Chronic Phase- chronic myelogenous leukemia patients Bcr-Abl positive, who received treatment for more than 18 months in. Venous blood sampling was done for point mutations C944T, T1052C, T932C and Bcr-Abl/G6PD ratio.*

Results: *90% of patients achieve complete Hematologic Response and majority of patients (60%) achieve Complete Molecular Response. Sixteen (40%) patients, who did not achieve chronic myelogenous leukemia , had point mutations in C944T, T 932C, and T1052C. Eight of the have 3 point mutations in C944T, T 932C, and T1052C, while 5 patients have T932 and T1052C, and 1 patient has mutation in T1052. The new finding in this study are the incidence of T932C mutations are quite high. The type of the Bcr-Abl transcripts will affect the increase of leukocytes and the number of mutations in the Bcr-Abl kinase domain affects blast number and the ratio of Bcr-Abl/G6PDH.*

Conclusion: *Most of the responses to treatment is first-generation tyrosine kinase inhibitors provides a complete hematologic response and most of the molecular response is undetectable.*

Keywords: *chronic myelogenous leukemia, Bcr-Abl, imatinib, tyrosine kinase Inhibitor*

Introduction

Chronic Myelogenous Leukemia is a hematologic malignancy included in the group of myeloproliferative diseases. This disease has clinical manifestations leukocytosis and spleen enlargement, and obtained a distinctive Philadelphia chromosome (Ph) which is the result of the proto-oncogene ABL translocation on chromosome 9 and the breakpoint cluster region protein (BCR) gene on chromosome 22 [*Leverrier Y et al., 1997; Deininger M et al., 2000; Jabbour E et al., 2008*].

Incidents of chronic myelogenous leukemia (CML) in the United States are 1.1 per 100,000 population and generally occurs at age 45-55 years, peak at the age of 53 years, and is rarely found in children. While in Asia seem even less. In China

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an estimated 0.39 -0.55 per 100,000 population, in Thailand about 0.50 per 100,000 population [*Au WY et al., 2009*]. In Indonesia there were 1109 patients [*Reksodiputro AH, 2015*].

Imatinib is a tyrosine kinase inhibitor class of agents, which since 2001 is used as a first-line CML treatment of all phases of CML. These agents are very effective and well tolerated by the body. Although most patients with CML showed hematologic response, but 3% of chronic phase CML, 20% of accelerated phase CML, and 50% blastik crisis phase CML does not respond at all to the Tyrosine Kinase Inhibitor [*Hughes TP et al., 2003*]. In fact, every year patients that response are still experiencing a recurrence of between 0.4 - 5.5% on. Resistance to agents is a challenge for the clinician to quickly determine the earliest possible signs of the development of drug resistance[*Corbin et al., 2003; Goldman and Melo, 2003; Beinortas et al., 2016*].

Resistance to tyrosine kinase inhibitor drugs can be divided into three, they are resistance in hematologic, cytogenetic and molecular. Resistance in hematologic is the lack of hematological response to tyrosine kinase inhibitors in CML chronic phase, failed to return into a chronic phase in the "blastic crisis" phase or partial response in blastic crisis phase CML. Cytogenetic resistance is defined as the loss of a major cytogenetic response or complete cytogenetic response. While the molecular resistance can be defined as the loss of a complete molecular response using RQ-PCR or lack of major molecular remission (ie, respectively, a decrease of Bcr-Abl transcripts of> 3 log or the ratio of Bcr-Abl/Abl <0.1%) [*Kantarjian H et al., 2007; Ramirez P, Di Persio J, 2008; Jabbour E, Kantarjian H, 2018*].

Imatinib resistance is divided into primary and secondary resistance. Primary resistance occurs when a drug is not effective from the start of administration. Secondary resistance is the loss of response to imatinib therapy in patients who had previously been the response to imatinib [*Kantarjian HM et al., 2007; Patel AB et al., 2017*]. Study reported that patients who fail to achieve a 1-log reduction in Bcr-Abl transcripts in three months or a decrease of> 2-log within 6 months, is unlikely provide a significant response and a high risk for progressive[*Branford S et al., 2003*]. Decrease transcript BcrAbl>2-log after getting treatment Tirozin kinaseinhibitor (TKI) for 3 months have the possibility of 100% for MMR in 24 months, whereas when a decrease of> 2-log transcripts of Bcr-Abl after 6 months then it is likely to MMR in 24 months only about 86 %.

The underlying mechanisme of Tyrosine Kinase Inhibitor resistance, are: 1. Depending on the Bcr-Abl (such as mutations in the kinase domain (KD) and the Abl gene amplification or duplication of Bcr-Abl), 2. Not relying Bcr-Abl (such as another kinase activation, changes influx of drugs or efflux protein (MDR1).

Mutation in the domain Abl kinase is the most frequent underlying mechanism of Tyrosine kinase inhibitor. Mutations in the domain Abl kinase of Bcr-Abl obtained in 42% - 90% of patients who are resistant to inhibitors Tyrosine kinase (imatinib). The effect of molecular which will result from mutations in domain kinase Bcr-Abl is a result of mutations would damage the hydrogen bonds between the drug and the protein Abl, could prevent the setting conformational required for binding of inhibitors Tyrosine kinase and can stabilize the conformation of kinase active [*O'Hare T et al., 2007*].

Until now, many mutations are discovered, and the most common is the kinase domain point mutations of Bcr-Abl. More than 90 mutations have been reported in four areas: arch binder ATP (P-loop), the contact area, a local-loop as well as the catalytic domain. Bcr-Abl mutations most frequently in the area P-loop (30-40% of all mutations) [*Corbin AS et al., 2003*].

MATERIAL AND METHODS

Observational prospective study of 40 patients with Chronic Phase CML in dr Soetomo Teaching Hospital Surabaya Indonesia. The CML diagnosis is based on history, physical examination, and laboratory tests such as complete blood count and examination of Bcr-Abl qualitative. The samples of patients are people with Bcr-Abl positive CML who received treatment of tyrosine kinase inhibitors more than 18 months.

Inclusion criteria c of the study are:

1. Karnofsky performance status > 60%.
2. Willing to follow research by giving approval to fill Informed consent letter.

The exclusion criteria of this study are:

1. Stop of treatment more than two weeks before evaluation of therapeutic results.
2. Patients experiencing severe infections

The steps of Research

Bcr Abl transcript detection

Blood samples were taken from CML patients who qualify the inclusion and exclusion criteria and had been receiving treatment for a minimum of 18 months. Peripheral blood samples were taken 20 cc in an Heparin preservative and stored in a refrigerator temperature of 2-8°C. Samples are then used to isolate RNA with the High Pure RNA Isolation Kit. After the isolation of mRNA from blood samples of patients, synthesis cDNA is performed. The results of the cDNA synthesis are used for examination RQ-PCR to detect *Bcr-Abl* fusion gene [*Sastre DA et al., 2007*].

Mutation analysis with Allele-specific oligonucleotide (ASO) PCR method [*Iqbal Z et al., 2004*].

To search for mutation type that may occur in patients who do not give a complete molecular response, then performed mutation analysis using PCR ASO with 3 primers to detect point mutations in T932C, C944T and T1052C.

Results

General overview of the characteristics of respondents consists are gender, age and complaints. More details can be studied as shown in table 1.

Most respondents were in the age group 30-50 years is 60%. The lowest age of respondents was 15 years old while the oldest is 69 years old, with mean 39,75

Treatment response

Hematologic Response of Bcr-Abl positive CML chronic phase patients who received therapy first-generation tyrosine kinase inhibitors were determined by clinical examination and laboratory in the form of the number of leukocytes, platelet count after 3 months of treatment. These are the distribution of hematological responses to tyrosine kinase inhibitor therapy are shown in table 1 below.

Molecular response evaluation performed after a minimum of tyrosine kinase inhibitor treatment after 18 months of treatment. Molecular evaluation is done in several stages. After the cDNA synthesis from mRNA (reverse transcription process), a process realtime PCR using the LightCycler machine II. Furthermore, to evaluate the response of molecular therapy, the results obtained from the RQ-PCR compared to G6PD, a house keeping gene.

Table 1.

Distribution characteristicsof respondents by sex, age and to treatment tyrosine kinase inhibitor

	Frequency	%
Sex		
Male	21	52.5
Female	19	47.5
Sum	40	100.0
Age (year)		
0 – 10	0	0
11 – 20	3	7.5
21 – 30	5	12.5
31 – 40	15	37.5
41 - 50	9	22.5
51 – 60	6	15.0
61 - 70	2	5.0
>71	0	0
Sum	40	100.0
Mean	39.75	
Response to treatment tyrosine kinase inhibitor		
Hematologic response	36	90
Non hematologic response	4	47.5
Sum	40	100.0

These are the values of the ratio of *Bcr-Abl* compared with G6PDH after administration of tyrosine kinase inhibitors were examined by using RQ-PCR sre shown in table 2.

From Table 4, it can be seen that the molecular response is mostly CMR (undetectable) by 60%, while giving major molecular response (MMR) in 3 (7.5%) patients, and no response in 13 (32.5%) patients.

Abl Kinase Domain Gene Mutations

There are 16 patients who didn't achieve complete molecular response. Detection of mutations were performed in these patientsThe detection method used is ASO PCR using three primer pairs to detect in T932C, C944T, T 1052C. Of the 16 patients who did not achieve a complete molecular response, one type of

Table 2.

Distribution Ratio of Bcr-Abl compared with G6PDH (molecular response)

BCR-ABL/G6PD ratio	Frekuensi	%
Bcr Abl undetectable	24	60.00
Bcr-Abl/G6PDH< 0.001 (MMR)	3	7.50
Bcr-Abl/G6PDH> 0.001	13	32.50

TABLE 3.
Mutation distribution C944T, T1052C, T932C

No	Mutation	Yes	No
1	C944T	9 (56,25%)	7 (43.75%)
2	T1052C	16 (100%)	0
3	T932C	13 (81.25%)	3(18.75%)

mutation was found in 2 patients, 2 a point mutation in 6 patients, and 3 mutations in 8 patients.

The results of the analysis of mutation C944T, T1052C, T932C are shown in table 3. Based on the results of the mutation to all patients who showed mutations, these results can be grouped into four groups of variations, which are, the first variation, the 8 patients who experienced three types of mutations simultaneously (T 1052 C, T C 932, C 944 T); group second variation, 5 patients with two types of mutations simultaneously (T 1052 C, T 932 C); The third group, one patient who experienced the same two types of mutations (T 1052 C, C 944 T); variation group 4, which are 2 patients with only one type of mutation (T 1052 C).

Mutation combination affects Bcr-Abl/G6PD ratio

In this research data analysis technique used is Partial Least Square. According to model analysis C944T mutation, T1052C mutation, T932C mutation affects Bcr-Abl/G6PD ratio (coefficient more than 1,96) as shown in figure 1. The biggest coefficient is in T1052C mutation (8,134) and C944T (6,297) mutation.

DISCUSSION

There were 40 patients met the inclusion and exclusion criteria with age range 15 to 63 years, with an average age 39 years. The comparison between men and women was 1.1: 1, which is not much different from the Japanese study with a ratio of male: female = 1.05: 1. However, different from events in Thailand ratio of male to female was 1.7: 1. Likewise in China, the ratio of male and female was 1.5: 1[*Au et al., 2009*].

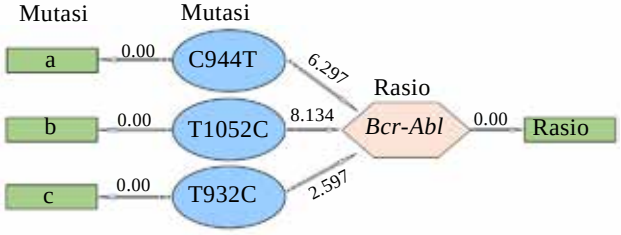

FIGURE 1. *Model analysis C944T mutation, T1052C mutation, T932C mutation and Bcr-Abl/G6PD ratio*

In recent years, Tyrosine Kinase Inhibitor first generation has shown encouraging responses in patients with *Bcr-Abl* positive CML chronic phase [*Garside R, 2002*]. *Bcr-Abl* plays an important role in the pathogenesis of CML, and the ABL tyrosine kinase activity of the treatment targets. Tyrosine Kinase Inhibitor was able to induce apoptosis selectively Bcr-Abl, and successfully treatin CML patients. In patients with chronic phase CML, tyrosine kinase inhibitor capable of producing a full cytogenetic responses in 80% of patients.

Monitoring response to therapy and the detection of early onset of relapse is very important in the management of CML. There are three criterias to evaluate the results of treatment response in CML therapy, namely: Hematologic Response, Cytogenetics Responses, and Molecular Response. Hematologic responses can be evaluated after four weeks of treatment with an evaluation complete blood and blood smears. Complete hematologic response defined as number of leukocytes < 10x10 9/L with counts of leucocytes normal platelet count <450,000, myelosit and metamyelosit less than 5%, basophils less than 20% and not promyelosit obtained young cells and peripheral blood, and without symptoms and signs of disease. Cytogenetic response is a standard method used to detect the Philadelphia chromosome. While the molecular response is a measure to evaluate the response to treatment by measuring molecular residue levels of *Bcr-Abl* with Real-time (quantitative) RQ-PCR. Molecular testing is the most sensitive monitoring techniques, thus can detect signs of early drug resistance.

In this study, Hematologic response were performed monthly by means of a clinical examination and a complete blood count, while the molecular response to therapy was examined after minimal treatment was 18 months. Of the 40 patients who received Imatinib the vast majority (90%) patients are on CHR. This result is slightly lower than in developed countries, namely O'Brien, 2003, in the IRIS study Trial reported experiencing CHR 95% of the 553 patients who received first-generation tyrosine kinase inhibitors, as well as in, 98% of 488 patients; achieve CHR. Arora 2008 in India gained 96%, of the 79 patients [*Au WY et al., 2009*].

Eventhough most patients with CML showed hematologic response, but 3% of patients with chronic phase, 20% of accelerated phase, and 50% of pa-

tients from the crisis blastic phase did not respond at all. In fact, each year recurrence between 0.4 - 5.5% will loss molecular response. In this study of 40 patients, 16 (40%) patients. Did not achieve complete molecular response by 18 months of treatment.

There are several mechanisms that underlie resistance to tyrosine kinase inhibitors, namely: 1. kinase domain point mutation (KD) Abl, 2. Bcr-Abl gene amplification, 3.Drug influx changes 4. efflux proteins. But until now the most common and the most understandable are mutations in the ABL kinase domain is the underlying mechanism of resistance Tyrosine Kinase Inhibitor [*Corbin AS et al., 2003*].

Of the 16 patients who did not achieve complete molecular response, all (100%) were point mutations. Different to the study reported by research conducted of the 297 patients with CML who are resistant to tyrosine kinase inhibitors, only 127 (43%) patients who had mutations [*Soverini S et al., 2006*].

In this study T1052C mutation was found in all patients who do not complete response, C944T mutation occurs in 9 among 16 patients who did not achieve CMR, T932C mutation was found in 13 patients among 16 patients who did not achieve CMR. The discovery of these mutations have been reported in case reports by [*Iqbal Z et al., 2004*] but only get two types of domain ABL gene mutation, ie C944T and T1052C in a patient resistant to Tyrosine Kinase Inhibitor first generation [*Iqbal Z et al., 2004*].

Based on data analysis interaction Tirozin kinase inhibitor to the wild-type ABL gene there are four hydrogens interaction with Tirozin kinase inhibitor ligand in amino acid residues Glu286, Thr315, Met318, Asn381. But the Abl gene mutant cause disruption of hydrogen interaction with ligands of Tirozin kinase inhibitor.

The C944T mutation, changes cytosine into thymine at nucleotide position 944 Abl gene that causes the amino acid substitutions threonin into isoleucine at codon 315 in onco protein Bcr-Abl bound to ATP (T315I).

Based on the crystal structure of the Abl kinase domain that the T315I mutation, when isoleucin replaced threonin as a result of the C944T mutation, this substance does not provide oxygen atom for Tirozin kinase inhibitor binding. In addition, this substance contains an extra hydrocarbon group in the side chain that produces steric hindrans to first generation Tyrosine Tirozin kinase inhibitor, so that the bond is weak and lead to drug resistance. Mutation of threonine 315, now referred to as "gatekeeper" position, is the most frequently reported mutation resistant to first-generation Tirozin kinase inhibitor (imatinib), carrying 12% of all mutations in the database. Replacement of threonine by isoleucine disrupt hydrogen bonds to imatinib and prevent Tirozin kinase inhibitor binding to amino acid residues. T315I is insensitive to imatinib and second-generation tyrosine kinase inhibitor nilotinib and dasatinib.

In T1052C mutation, the nucleotide sequence number 1052 where the ATG codon encoding amino acid Methionin at codon 351 amino acids will be changed to codons that encode the amino acid TCC Threonin. These mutations would weaken the partial bond Tyrosine Kinase Inhibitor first generation on the target and cause resistance to the drug partially. Mutations have also been reported outside the kinase domain but with a lower frequency. In vitro mutagenesis predicted that mutations can be found in the cap, SH2 SH3. As the T932C mutation, the nucleotide sequence number 932 where TTC codons that encode amino acid Phenilalanin at codon 311 amino acids will be changed to TCC codon encoding the amino acid serine. This mutation is rarely found, with the number of cases and incidence of only 1.8% .

Kinase domain mutation analysis in patient resistant to first generation Tirozin kinase inhibitor is still based on single point mutation. We should consider multiple mutations that lead patients to drug resistance. In this study of 16 patients who did not achieve a complete molecular response, all have Bcr-Abl kinase domain point mutation, three point mutations simultaneously obtained in 8 patients, 2 a point mutation in 6 patients, 1 point mutation in 2 patients. New finding of this study is the is role of mutations combinations with Bcr-Abl/G6PD ratio. The more point mutation occur there will be higher probability of resistency of first line Tirozin kinase inhibitor.

Conclusion

Most of the responses to treatment is first-generation tyrosine kinase inhibitors provides a complete hematologic response and most of the molecular response is undetectable.

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