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Epidemiology Study and Mutation Profile of Patients with Chronic Myeloid Leukemia (CML) in Indonesia

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Abstract

Aim: To assess CML patient's characteristic including demographic, clinical and hematological characteristic of patients with CML including quantitative BCR-ABL and BCR-ABL gene sequencing.

Methods: This study was an open-label, single arm, non-randomized, cross sectional study in patients with CML being treated with imatinib mesylate (IM) from 12 centers.

Result: A total of 100 patients were evaluated between January 1, 2009 and December 31, 2011. The median age was 34-35 years old (mean of age is 36 years old), and more patients in the productive age was found. ------- (?) were 80 of the 100 patients who had been examined for the BCR-ABL gene mutation with the sequencing method before consuming IM. Mutation in the P-loop was seen in 2,27% (1 out of 44 patients), this finding was beyond our expectation since 47,69% (31 out of 65 patients) of our patients did not achieved CHR at three months. On the other hand, 15,9% (7 out of 44 patients) of our patients had mutation outside the P-loop.

Conclusions: The characteristics of CML patients in Indonesia were not different from CML patients in Asia in general. Our finding concerning the high frequency mutation in the BCR-ABL gene outside the P-loop needs further study.

Keywords: Chronic myeloid leukemia; Imatinib mesylate (IM); Epidemiology

Introduction

Chronic Myeloid Leukemia (CML) is a myeloproliferative disorder characterized by the expansion of a clone of hematopoietic cells that carries the Philadelphia chromosome (Ph) [1]. The Ph chromosome results from a reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11) [2]. The molecular consequence of this translocation is a novel fusion gene, BCR-ABL, which encodes a constitutively active protein, tyrosine kinase [3-5].

CML has a slight male preponderance (male-to-female ratio is 1.6:1). Its annual incidence is about 1.5 cases per 100,000 individuals or about 15% of all adult leukemia case. This incidence has not changed over in the past few decades, and it increases with age. The median age at diagnosis is 55 to 60 years old, only 2.7% of CML cases are younger than 20 years old. The mean age of CML at the time of diagnosis is 50 years old, with 30% older than 60 (Jemal A) and

median survivals of 4 years if treated with the conventional chemotherapy and 6 years if treated with interferon.

Imatinib is the first line therapy of CML patients. Before imatinib therapy, the prevalence of CML was about 25,000 cases in the United States. Hochhaus et al.demonstrates that estimated rates of freedom from progression to accelerated phases and blastic phases and overall survival at 6 years of CML patients who received imatinib as initial therapy were 61% and 76%, respectively [1], and Druker et al. in IRIS study (International Randomized Study of Interferon and STI571) study demonstrates that the estimated overall of CML patients who received imatinib as initial therapy was 89% at 60 months [2]. Now that the annual mortality following imatinib therapy has been reduced to 2%, the prevalence of CML will continue to rise, reaching a plateau (in the next 20 years) at about 250,000 cases (when the annual incidence will equal the annual mortality). This will change CML from an uncommon disorder to a prevalent one.

Some of literature indicates a different in characteristic of CML among Caucasian and Asian races. Wing et al. mentioned in their journal that the median age of patients with CML in USA is 65 years old, where in China is 45-50 years, 36-38 years old in Thailand, in India is 38-40 years old, and in South Korea is 37 years old. Currently Indonesian's nationwide CML data, especially clinical and hematological characteristic of patients with CML is still not available. Until now, only a few data concerning CML in Indonesia has been published by Reksodiputro et al. CML local guideline will be developed so we need the clinical and hematological characteristic of patients with CML.

Study Objective

This study objective was to assess CML patient's characteristic including demographic (age, sex, education level, occupation, economic status, treatment before study), clinical and hematological characteristic of patients with CML including quantitative BCR-ABL and BCR-ABL gene sequencing.

Design and Methods

This study was an open-label, single arm, non-randomized, cross sectional study in patients with CML being treated with imatinib mesylate (IM) which was conducted during the period January 1th, 2009 to December 31th, 2011. The study population consisted of 100 patients with CML from several centers in Indonesia, such as: Medan, Padang, Palembang, Bandung, Jakarta, Semarang, Surabaya, Yogyakarta, Banjarmasin, Denpasar, Manado, and Malang.

Patients, who were selected as research subjects, are they who met the inclusion criteria. Criteria for inclusion in the study were male or female, 17-70 years old, patients were suspected of suffering from CML based on anamnesis, physical examination, and routine blood tests, and after an examination of molecular cytogenetic and RT-PCR showed the Philadelphia chromosome positive and/or BCR-ABL positive; and willing to engage (enroll?) in this study.

Patients who were suspected of CML from clinical and laboratory peripheral blood underwent a bone marrow aspiration for examination cytomorphology, cytogenetic, and RT-PCR BCR-ABL (BCR-ABL qualitative). Patients with the positive results, were informed consent to participate in this study and examined by quantitative BCR-ABL and BCR-ABL gene sequencing. The results were recorded in the Case Report Form in accordance with the variables listed in it.

Patients with the criteria for cytogenetic Philadelphia chromosome positive and/or BCR-ABL positive followed by Novartis Oncology Access program to acquire IM after their financial ability had been evaluated by independent surveyor. CML patients, who took (are taking?) IM, were recorded and monitored for their treatment responses and side effects during the period of 1 year, except the patients from outside Jakarta because of the difficulty (difficulties) in conducting follow-up during treatment.

All data, that were collected, have been descriptive described by analysis from all patients who were received receiving IM at least one times visit after treatment. Adverse events were stated as numbers and percentages of all patients who had experienced with adverse event. Rate of overall survival that had been assessed in the study only for 2 centers (Cipto Mangunkusumo Hospital and Dharmais Cancer Center), indicated that the percentages of patients in this study who were still alive for a given period of time after diagnosis. We're using the analysis of survival with Kaplan-Meier method, according to the intention-to-treat (ITT) principle and also all data that were available. This was a part of survival analysis and an important things (directions?) for prognosis.

In a case of severe / serious adverse events (e.g severe neutropenia) which was required a study of a drug interruption or discontinuation as per physician's judgment, the treatment of this study were (will be) terminated. No further follow-up were required. Every patient had the right to discontinue this study participation at any time, and every patient might be discontinued from this study for any reason of beneficial to his/her wellbeing. All data that generated by the time of discontinuation from the study had been analyzed and the reason(s) for discontinuation had been recorded.

RQ-PCR and Sequencing Methods

RNA extraction

Total RNA was extracted from pellet of mononuclear cells using Trizol (Invitrogen) following the manufacturer's instructions with some modifications. After chloroform extraction and precipitation by centrifugation 13.000 rpm 1 hour with isopropanol, the pellet was washed twice by centrifugation with 75% ethanol. The RNA pellet was dried for about 60 minutes at room temperature and dissolved the RNA in diethylpyrocarbonate (DEPC)-treated water.

BCR-ABL nested RT-PCR and Sequencing

cDNA was synthesized using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas). The 20 ul reverse transcript (RT) reaction was performed from 2 µg of total RNA using random primers. A nested PCR for BCR/ABL fusion transcript was carried out using a KAPA HiFi Hot start Ready Mix (KAPA biosystems) and the following primers and conditions: forward primer B2A (5'-TTCAGAAGCTTCTCCCTGACAT-3') and the reverse primer A10R1 (5'-TGAGGCATCTCAGGCACGTC-3') for the 1st PCR round (35 cycles, annealing 62°C). And 1 µl of 1st PCR product (diluted 1:10) was used as template for the 2nd round PCR (32 cycles, annealing 62°C), with forward primer A4F (5'-CCAAAGCGCAACAAGCCCAC-3') and reverse primer A10R2 (5'-ACAGCCCCACGGACGCCTTG-3'). The resulting 905 bp of 2nd PCR product was purified from 2% agarose gel and then followed by sequencing.

Treatment

Glivec® 100 mg film-coated tablets contains 100 mg imatinib (as mesylate) which has very dark yellow to brownish orange film-coated tablets, round shape with "NVR" imprint on one side and "SA" and score on the other side. The recommended dose of Glivec® is 400 mg was once daily and was taken with food and a large glass of water. It was taken orally for the patients in chronic phase CML. Treatment was being continued as long as the patient continued to benefit. Dose was increased from 400 mg to 600 mg or 800 mg in patients with chronic phase disease, or from 600 mg to maximum of 800 mg daily in patients in accelerated phase or blast crisis might be considered in the absence of severe adverse drug reaction and severe non-leukemia-related neutropenia or thrombocytopenia in the following circumstances: disease progression (at any time); failure to achieve a satisfactory haematological response after at 3 months of treatment, failure to achieve a cytogenetic response (Philadelphia chromosome >65%) after 12 months of treatment, or loss of a previously achieved haematological and/or cytogenetic response.

Patients were being monitored closely following dose escalation given the potential for an increased incidence of adverse events at higher dosages.

Visits and assessments

Based on current medical practice, it was expected that the patient had at least 12 post baseline assessments in the first year. The frequency of clinical visits was determined by the physician and the health care needs of the patients. To contribute meaningful information it was possible to obtain information at 12 post baseline visits. Table below listed the expected assessments that had been reported periodically. Physical examination in first 3 months will be done in twice a month (Table 1).

Month/ Visit	Baseline	1	2	3	4	5	6	7	8	9	10	11	12
Anamnesis	X												
Demographic data	X												
Physical examination	X	X*	X*	X*	X	X	X	X	X	X	X	X	X
Laboratory													
BCR-ABL qualitative	X												
White-cell count	X	X	X	X	X	X	X	X	X	X	X	X	X
Platelet count	X	X	X	X	X	X	X	X	X	X	X	X	X
Hemoglobin	X	X	X	X	X	X	X	X	X	X	X	X	X
Ph+ chromosome cytogenetic	X												
BCR-ABL mutation (sequencing)	X												
BCR-ABL quantitative (RQ-PCR)	X												
Bone Marrow Puncture	X												
Adverse events			X	X	X	X	X	X	X	X	X	X	X

Table 1: Visits and assessments.

Visit 1/baseline including informed consent prior to any procedure planned by the protocol, verified inclusion/exclusion criteria, demography, anamnesis/medical history/background, physical examination, blood samples for laboratory evaluation (BCR-ABL qualitative, quantitative and mutation, white-cell count, platelet count, hemoglobin), bone marrow puncture and past and concomitant medications. Treatment follow-up period visits included query for adverse events and concomitant medications, performing symptom-directed physical examination as required, blood samples for laboratory evaluation, evaluating patient adherence to prescribed study medication (compliance) and prescribing study medication.

Procedure Flow

Suspected CML patients who met inclusion criteria and did not have exclusion criteria were recorded in every variable in case report form (CRF). Anamnesis, physical examination, laboratory examination (peripheral blood test), morphology of bone marrow puncture (BMP), and BCR-ABL test were recorded in CRF as well. The planned treatment: imatinib mesylate 400 mg daily orally. The sequence and duration period were 1 month for baseline and 1 year for period. The post study treatment was at physician's description, but imatinib was highly recommended till disease progression.

Investigator/co-investigator/investigator staff entered the completed information into CRF. Before the collected data were being analyzed, we had validated the extensiveness and veracity of data (data cleaning), coding, tabulation, and entered the input data into computer (Figure 1).

Adverse Events

Safety assessments consisted of monitoring and recording (serious) adverse events. An adverse event (AE) is any untoward medical occurrence in patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a casual

relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

A serious adverse event (SAE) is any untoward medical occurrence that results in death, is life threatening, required inpatient hospitalization or prolonged hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly or a birth defect. Other significant medical events that were not immediately life-threatening or results in death or hospitalization but jeopardized the subject and required intervention to prevent one of the outcomes listed above was considered serious.

Participating physicians were requested to record all adverse events at the follow-up visits or when they notified of the occurrence of adverse events. Serious adverse events reports and pregnancy notifications were forwarded by fax to the local medical/IMS officer within 24 hours. These were confirmed by ticking a special check-box on the CRF.

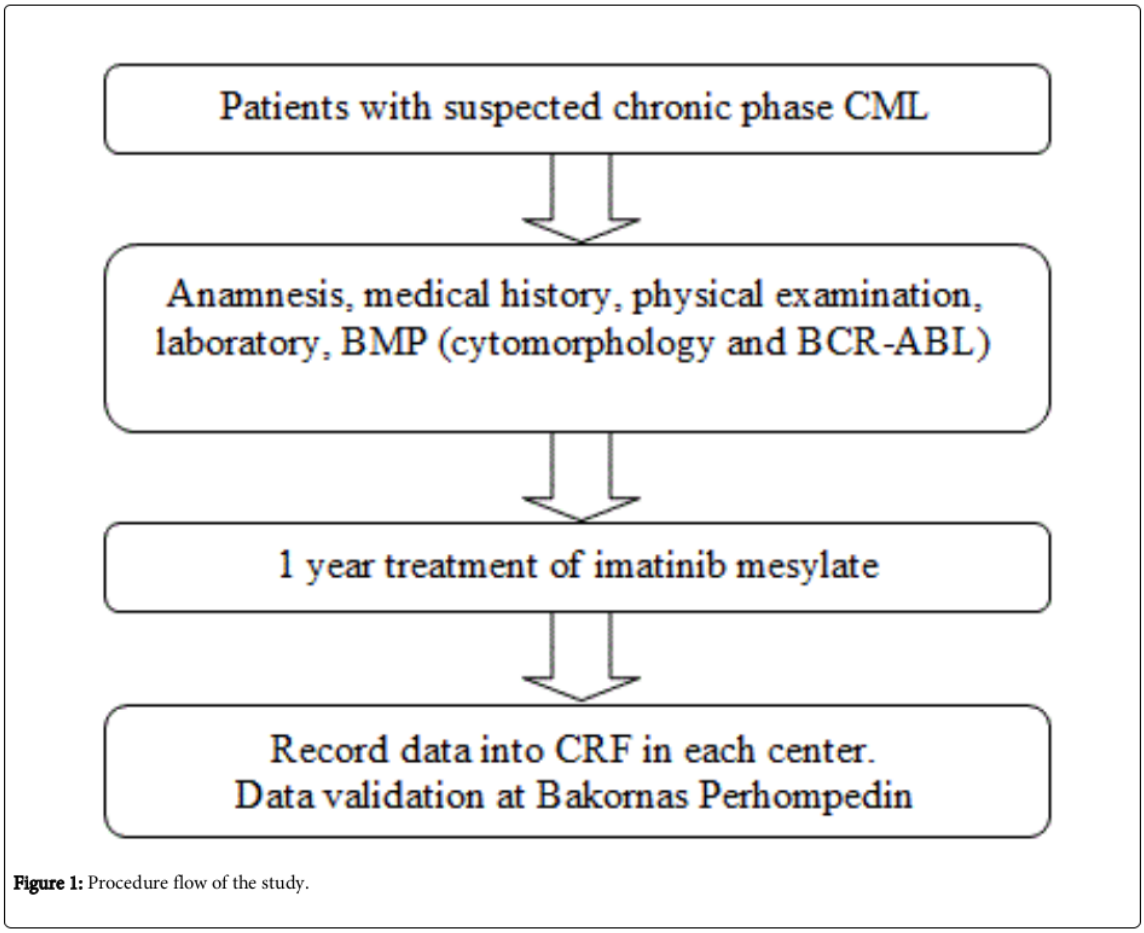

Figure 1: Procedure flow of the study.

Pregnancies

Any pregnancy that occurred during study participation was reported using the Pregnancy Form. The pregnancy was being followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence any birth defects, congenital abnormalities or maternal and newborn complications.

Results

Demography of patients and clinical characteristic

A total of 100 patients were evaluated between January 1, 2009 and December 31, 2011. The median age was 34-35 years old (mean of age is 36 years old), and more patients in the productive age was found (Table 2). IRIS study (Figure 2) showed that CML disease was more common in the elderly age group and very different from the age group of CML patients in Indonesia. Although the description looked

different from Caucasian, age of CML patients in Indonesia appear to be similar with the data in Asia, where the median age of patients with CML in China was 45-50 years, in Thailand was 36-38 years, in India was 38-40 years, and in South Korea was 37 years. Males were slightly more frequent than females with a ratio of 1.5:1 (Table 2). In China and Hong Kong, CML disease was also more common in men than women with the same ratio (1.5:1), whereas in Thailand and USA the ratio was 1.7:1. According to the description of sex and age, the characteristics of CML patients in Indonesia were not different from CML patients in Asia in general.

Twenty six percent of 100 patients (?) were employees. More than half of 100 patients were not exposed by carcinogenic agent. Nevertheless, 17% of 100 patients were smokers [6,7] (Table 3).

No.	Characteristic	Median	SD	95% CI	Frequency	%
1	**Sex**					
	Male				61	61
	Female				39	39
2	Age	34.67	11.765	33.57-38.26		
3	**Educational Background**					
	None				4	4.3
	Elementary School				5	5.4
	Junior High School				10	10.8
	Senior High School				44	47.3
	College/ university degree				30	32.3
4	**Occupational types**					
	None				3	3.1
	Farmer				2	2.1
	Gardener				1	1
	Fisherman				1	1
	Employee				26	26.8
	Labor				5	5.2
	Civil servant				15	15.5
	TNI/ POLRI				1	1
	House wife				20	20.6
	Student				7	7.2
	Others				16	16.5
5	**Income**					
	None				5	6.6
	<$50				5	6.6
	$50- $100				8	10.5
	$100- $150				11	14.5
	$150- $200				19	25
	>$200				28	36.8
6	**Exposure types**					
	None				63	63

	Radiation				4	4
	Pesticide				3	3
	Latex industry				0	0
	Petroleum industry				4	4
	Leather industry/Shoes					
	manufacture				2	2
	Hair colouring				3	3
	Air pollution				3	3
	Smoking				17	17

Table 2: Demography of CML patients (n=100).

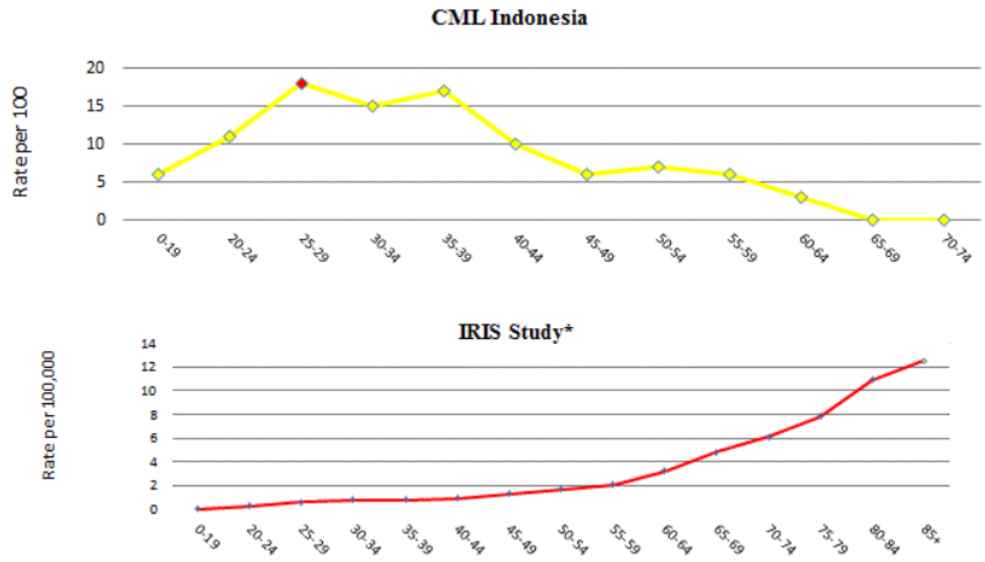

Figure 2: Age distribution.

No.	Characteristic	Chronic Phase (n=79)		Accelerated Phase (n= 15)		Blast Crisis Phase (n=6)		Total	
		Frequency	%	Frequency	%	Frequency	%	Frequency	%
1	**Duration of illness before Imatinib therapy (in months)**								
	<6 months	52	65.8	11	73.3	5	83.3	68	68
	6-12 months	8	10.1	0	0	1	0	9	9
	13-18 months	2	2.5	1	6.7	0	16.7	3	3

	19-24 months	1	1.3	0	0	0	0	1	1
	>24 months	9	11.4	2	13.3	0	0	11	11
	Unknown	7	8.9	1	6.7	0	0	8	8
	Total	79	100	15	100	6	100	100	100
2	**Duration of illness before Imatinib therapy (in year)**								
	<1 year	60	75.9	11	73.3	6	100	77	77
	≥1 year	12	17.1	3	20	0	0	15	15
	Unknown	7	7	1	6.7	0	0	8	8
	Total	79	100	15	100	6	100	100	100
3	**Previous treatment**								
	HU	54	68.4	10	66.7	3	50	67	67
	IM+HU	3	3.8	0	0	0	0	3	3
	Others	1	1.3	0	0	1	16.7	2	2
	Unknown	21	26.6	5	33.3	2	33.3	28	28
	Total	79	100	15	100	6	100	100	100
4	**Duration of Hidroxyurea therapy before Imatinib started**								
	≤6 months	38	70.4	5	50	2	66.7	45	67.2
	>6 months	16	29.6	5	50	1	33.3	22	32.8
	Total	54	100	10	100	3	100	67	100
5	**Splenomegaly**								
	<10 cm	34	43	5	33.3	2	33.3	41	41
	≥10 cm	45	57	10	66.7	4	66.7	59	59
	Total	79	100	15	100	6	100	100	100
6	**Blast cells (in%)**								
	<5	61	77.2	3	20	0	0	64	64
	5-10	18	22.8	6	40	0	0	24	24
	>10	0	0	6	40	6	100	12	12
	Total	79	100	15	100	6	100	100	100
7	**Basophils (in%)**								
	<5	40	50.6	8	53.3	6	100	54	54
	≥5	20	25.3	6	40	0	0	26	26
	Unknown	19	24.1	1	6.7	0	0	20	20
	Total	79	100	15	100	6	100	100	100
8	**White blood count (x/uL)**								
	<4,500	7	8.9	0	0	0	0	7	7
	4,500-11,000	10	12.7	0	0	0	0	10	10

	>11,000	62	78.5	14	93.3	6	100	82	82
	Unknown	0	0	1	6.7	0	0	1	1
	Total	79	100	15	100	100	100	100	100
9	**Platelets ($1x10^3$/uL)**								
	<150	4	5.1	0	0	2	33.3	6	6
	150-400	23	29.1	3	20	3	50	29	29
	>400	52	65.8	8	53.3	1	16.7	61	61
	Unknown	0	0	4	26.7	0	0	4	4
	Total	79	100	15	100	6	100	100	100
10	**Sokal's risk**								
	Low	26	32.9	2	13.3	0	0	28	28
	Intermediate	21	26.6	1	6.7	0	0	22	22
	High	32	40.5	12	80	6	100	50	50
	Total	79	100	15	100	6	100	100	100
11	**Survival**								
	Live	74	93.7	13	86.7	2	33.3	89	89
	Dead	5	6.3	2	13.3	4	66.7	11	11
	Total	79	100	15	100	6	100	100	100

Table 3: Clinical characteristics of CML patients (n=100).

Eleven of 100 patients with CML had duration of illness more than 24 months before imatinib mesylate (IM) was started. Among these 11 patients, 9 were in chronic phase. These 9 patients were 11.4% of 79 patients who were in chronic phase. The rest 2 patients that were in accelerated phase constituted 13.3% of all the 15 patients who were in accelerated phase. None of these 11 patients were in blast crisis phase. On the other hand, 5 of 6 patients (83.3%) in blast crisis phase had duration of illness less than 6 months before IM was started. Before IM was started, 67 of 100 patients had been treated with hydroxyurea (HU). HU was consumed by twenty two of these 67 patients (32.8%) for more than 6 months.

Our evaluation of these 100 patients showed those who had consumed HU for more than 6 months before IM were more difficult to achieved 3 months complete hematologic response (CHR) than those who had consumed HU for less than 6 months.

Seventy nine of 100 CML patients were in chronic phase at baseline, 62 of these 79 patients (78.5%) had more than 11,000 /uL of WBC. Most of the patients in chronic phase had more than 400,000 /uL of platelet, less than 5% of blast cells in the peripheral blood and a spleen with a diameter of more than 10 cm. Fifty of these 100 patients were in high Sokal's risk. Nevertheless, 90% of the whole population studied were still alive at the moment.

There were 5 of 79 patients (6.3%) in chronic phase who died during this study because of blast crisis. Two of these patients had intermediate Sokal's risk and 3 patients had high Sokal's risk at diagnosis. Two of these 5 patients had consumed IM for 3 months and the others for 15 months, 18 months and 19 months, respectively. Four patients never achieved CHR within 3 months and only 1 patient achieved it but lost the 3 months' CHR at 10th month. One of these 5 patients had HU therapy for more than 6 months and 4 patients had it for less than 6 months before IM therapy.

Two patients (13.3%) from 15 patients in accelerated phase died because of blast crisis. These patients had high Sokal's risk at baseline and one patient had consumed HU for less than 6 months where the other patient had not consumed HU. One patient only consumed IM for 1 month before progressed to blast crisis. Another patient consumed IM for 18 months and stayed in accelerated phase before progressed to blast crisis. Thirteen patients in accelerated phase are still alive during this study. From these 13 patients, 5 patients turned into chronic phase after consuming IM only for one month, 3 patients needed 3 months, 1 patient needed 5 months, 1 patient lost to follow up and 3 patients from outside Jakarta could not be followed up. Three of 9 patients, who were recorded, achieved CHR within 3 months while 3 patients were achieved it at 4^{th} months, 2 patients at 5^{th} months and 1 patient at 11^{th} months. Two of 6 patients (33.3%) who were in blast crisis were still alive during this study. These two patients with blast crisis were achieved chronic phase and entered CHR within 3 and 5 months subsequently. One patient had consumed HU for one month only and other patient had not consumed any tyrosine kinase inhibitors before IM started. According to survival graph (Figure 3), there were 89% patients still alive until the end of study. Eleven patients died during the study because of blast crisis (6 patients) and others (5 patients).

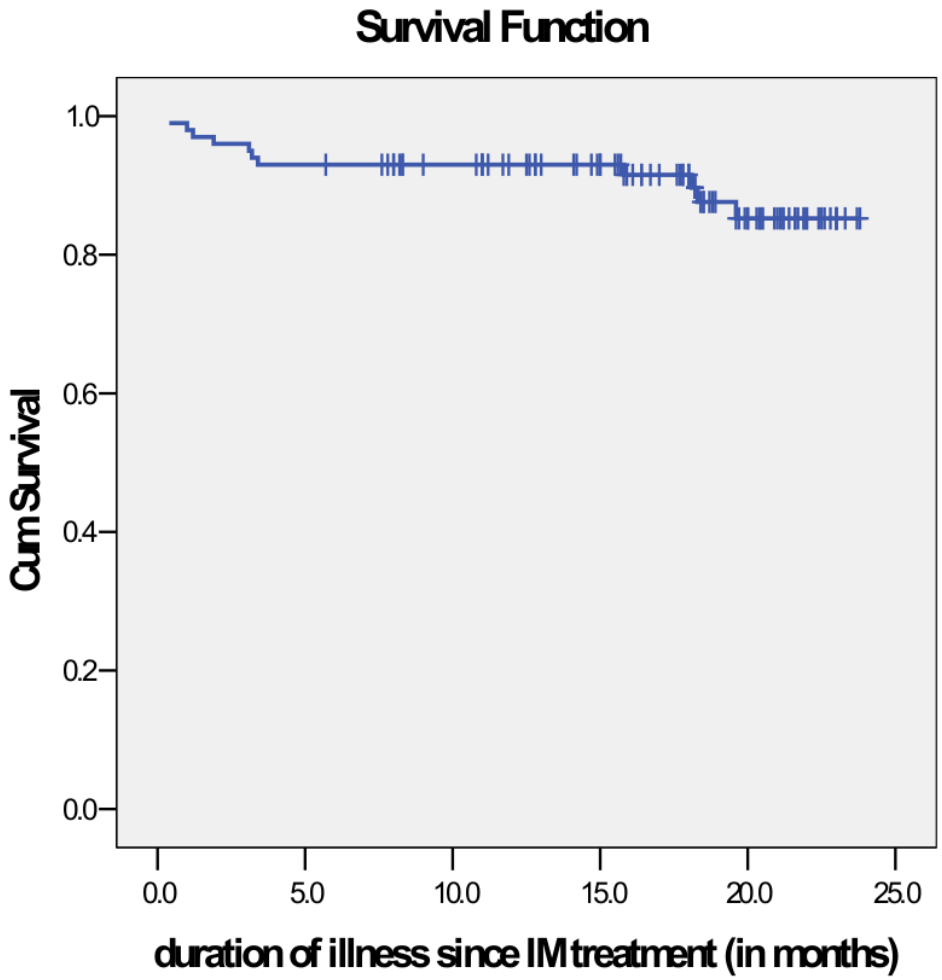

Figure 3: Survival since IM consumption to end of study.

No.	Hematologic Response	Chronic Phase (n=61)		Accelerated Phase (n= 13)		Blast Crisis Phase (n= 6)		Total	
		Frequency	%	Frequency	%	Frequency	%	Frequency	%
1	CHR in 3 months								
	Yes	38	62.3	3	23.1	2	33.3	43	53.8
	No	18	29.5	8	61.5	4	66.7	30	36.3

	ND*	5	8.2	2	15.4	0	0	7	10
	Total	61	100	13	100	6	100	80	100
2	**Group of CHR**								
	1-3 months	38	62.3	3	23.1	2	33.3	43	53.8
	4-6 months	7	11.5	4	30.8	0	0	11	13.8
	7-9 months	1	1.6	0	0	0	0	1	1.3
	10-12 months	0	0	2	15.4	0	0	2	2.5
	>12 months	1	1.6	0	0	0	0	1	1.3
	No CHR**	9	14.8	2	15.4	4	66.7	15	18.8
	ND*	5	8.2	2	15.4	0	0	7	8.8
	Total	61	100	13	100	6	100	80	100

*ND: not determined

**No CHR: 4 patients in chronic phase died before CHR was achieved and 5 patients still not achieve CHR yet; 1 patient in accelerated phase died before CHR was achieved and 1 patient still in accelerated phase; 4 patients in blast crisis died.

Table 4: Hematologic Response in 1 year of treatment.

Forty three of 80 patients (53.8%) achieved complete hematologic response within 3 months (Table 4). Among those in chronic phase, 62.3% achieved CHR within 3 months while 7 patients (11.5%) achieved it in 4 to 6 months. There were 3 of 13 patients (23.1%) in accelerated phase who achieved CHR within 3 months while 4 patients (30.8%) achieved it in 4 to 6 months. Two of 6 patients (33.3%) in blast crisis succeeded to achieve CHR within 3 months.

There was no serious adverse effect attributed to imatinib observed among 100 patients during the past one year. The three most frequent adverse effects were nausea (44%), fatigue (42%), and myalgia (39%), followed by muscle cramps (36%), superficial edema (36%), hypopigmentation (29%), diarrhea (26%), headache (25%), weight gain (21%), and alopecia (19%). IRIS study reported, the most frequent adverse effects were superficial edema, nausea, and muscle cramps.

RQ-PCR

RT-PCR is used to detect BCR/ABL RNA transcripts. White blood cells (WBCs) are separated from the peripheral blood sample by red blood cell lysis and subsequent washing. Total RNA is extracted from the WBCs using the TRIzol® method. Then reverse transcription was performed using primer random hexamer and MMLV enzyme for cDNA synthesis. Presence or absence of the amplified sequence is visualized using agarose gel electrophoresis and using G6PDH as a control.

Quantification of this target using Light Cycler TagMan Master and designed primer for BCR ABL/ ABL targeted to the major breakpoint cluster region (M-BCR or p210) region of chromosome 22 (exon 2 or exon 3) and the ABL endogenous control. Amplification and quantification using Light Cycler 1.5 of Roche. Quantitation of BCR/ABL is determined by a ratio of BCR/ABL to ABL.

Standards (Plasmids) and patient samples are run in duplicate and shows good reproducible results. The standard error shows range 0.014–0.05 in both BCR-ABL and ABL. Although the standard error must be less than 0.01, there are still found standard error values (up to 0.05) and the standard efficiency appears in the range 1.821–2.031(must be approaching the value of 2).

No	Initial	Age at dx	Phase	Sokal's risk	Mutation at diagnosis		Response
					P-loop	Outside P-loop	
1	PP	28	Chronic	Low	NM	NM	CHR within 3 months
2	N	24	Chronic	Low	NM	NM	CHR within 3 months
3	AL	38	Chronic	High	NM	NM	CHR within 3 months
4	JR	61	Chronic	High	NM	NM	CHR within 3 months
5	F	24	Chronic	High	NM	NM	CHR within 3 months
6	SR	45	Chronic	Low	NM	NM	CHR within 3 months
7	M	36	Chronic	High	NM	n.1152 C→T (silent mutation)	Drop out

8	KKK (†)	47	Chronic	Intermediate	NM	V506S	No response
9	UBW (†)	54	Chronic	High	NM	N231Q	No response
10	A (†)	45	Chronic	High	NM	c.661_662insG and c. 1555_1557insG	No response
11	I	59	Accelerated	Low	NM	NM	CHR in 4 to 6 months
12	AP	20	Accelerated	High	NM	NM	CHR in 10 to 12 months
13	AH (†)	50	Accelerated	High	NM	E509G	No response
14	H (†)	41	Blast crisis	High	NM	NM	No response
15	M (†)	51	Chronic	High	NM	NM	No response
16	TS	56	Chronic	Intermediate	NM	NM	CHR within 3 months
17	S	32	Chronic	High	NM	NM	CHR in 7 to 9 months
18	ME	32	Chronic	Intermediate	NM	NM	CHR within 3 months
19	MFA (†)	39	Chronic	Intermediate	NM	NM	CHR within 3 months
20	ES	34	Accelerated	High	NM	NM	CHR within 3 months
21	PR	39	Accelerated	High	NM	NM	CHR in 4 to 6 months
22	S (†)	21	Blast crisis	High	NM	NM	No response
23	TF	26	Blast crisis	High	NM	NM	CHR within 3 months
24	MA	34	Chronic	High	NM	NM	CHR within 3 months
25	YNK	40	Chronic	Intermediate	NM	NM	Not determined
26	WG		Chronic	Low	NM	NM	CHR within 3 months
27	HY		Chronic	High	NM	M351T	Died of blastic crisis
28	Was		Chronic	Intermediate	NM	NM	CHR within 3 months
29	WF		Chronic	Intermediate	NM	NM	Died of blastic crisis
30	UK		Chronic	High	NM	NM	Died of blastic crisis
31	TF		Blast crisis	High	NM	NM	Not MMR
32	TA		Blast crisis	High	NM	NM	CHR within 3 months
33	Syaf		Chronic	Intermediate	NM	NM	CHR within 3 months
34	Sur		Chronic	Low	NM	NM	CHR within 3 months
35	Sol		Chronic	High	NM	NM	Died of blastic crisis
36	SA		Chronic	High	NM	NM	Died of blastic crisis
37	RP		Chronic	Intermediate	NM	NM	CHR within 3 months
38	RSu		Accelerated	High	NM	NM	CHR within 3 months
39	R So		Chronic	Low	NM	NM	CHR within 3 months
40	RY		Chronic	High	NM	NM	CHR within 3 months
41	PR		Accelerated	High	NM	NM	CHR within 3 months
42	NM		Accelated	High	NM	NM	No MMR, but still alive
43	NS		Chronic	High	NM	NM	CHR within 3 months

44	TS		Chronic	Intermediate	Y253H	NM	Died during 15 mo's IM treatment
*NM: no mutation							

Table 5: Mutations profile of CML patients.

Mutations profile of CML patients

There were 44 of the 100 patients who had been examined for the BCR-ABL gene mutation with the sequencing method before consuming Imatinib as shown in Table 5, only 44 of the patients had a successfully performed sequencing test.

Mutation in the P-loop was seen in 2.3% (1 out of 44 patients). On the other hand, 15.9% (7 out of 44 patients) of our patients had mutations outside the P-loop. Further evaluation concerning these findings will be reported in another paper.

From 44 patients we concluded 8 patients who were mutated by BCR-Abl gene, after we calculated with Chi-square method, we found that there were correlation with MMR (Table 6).

Mutations before IM	No MMR n (%)	MMR n (%)	p	OR
Yes	8 (34.7%)	1 (4.7%)		10.7
No	15 (65.3%)	20 (95.3%)	0,01	1
Total	23 (100%)	21 (100%)		

Table 6: Relationship between BCR-Abl gene mutation with MMR at CML patients.

Discussion

Cytogenetic studies established CML as the first human neoplasm which is consistently associated with a particular chromosome abnormality known as the Philadelphia chromosome. The Philadelphia translocation juxstaposes to c-ABL proto oncogene sequence on chromosome 9 with the BCR gene on chromosome 22. This result in a BCR-ABL fusion protein which has a tyrosine specific activity that is indispensable for its capacity to transform cells. This BCR-ABL protein which has an elevated tyrosine kinase activity relative to c-ABL appears to be able to induce various hematopoietic malignancies in transgenic mice.

The BCR-ABL gene products result in an active protein tyrosine kinase enzyme protein p210, which is critical in the pathogenesis of CML. In normal physiological conditions the ABL tyrosine kinase enzyme regulate and modulate a number of downstream target molecules, including c-Myc, Akt and Jun which are essential for the proliferation and survival on normal cells. In contrast the aberrant BCR-ABL tyrosine kinase provides survival advantage to malignant cells by promoting uncontrolled proliferation and survival and altering the addition properties of these malignant cells.

In the advance stage the chronic phase CML enters the intermediate accelerated phase and the blast crisis end-stage. The accelerated phase usually last around 3 to 18 months while the blast crisis phase usually last around 3 to 6 months. The pathogenesis of the transformation from chronic to accelerated and to blast crisis is not yet well understood until now. Nevertheless it seems that the acquisition of other gene mutation by genomically unstable BCR-ABL cell play important roles.

BCR-ABL gene is the oncogene which plays the most important role in the development of CML. The BCR–ABL gene expression is a constitutively active BCR-ABL tyrosine kinase and suspected to play an initiating role in the pathogenesis of CML. Therefore study of the mutation this gene becomes more important in the management of CML, since the standard treatment for the BCR ABL positive CML is a group of agent from a class of drugs called Tyrosine kinase protein inhibitor. The BCR-ABL gene code is a protein in the cytoplasm of the cell that functions as a tyrosine kinase enzyme. This constitutively BCR-ABL tyrosine kinase is the cause of CML.

In 2009, Muller and colleagues [8] analyzed three researches of phase two/ three clinical trials on the response to Dasatinib in patients with chronic CML with or without BCR-ABL mutation who have been treated with Imatinib. Of 1,043 patients who underwent BCR-ABL assessment only 39% had BCR-ABL mutation prior to Dasatinib treatment. Of 850 patients who were Imatinib resistant or responded poorly to Imatinib, 48% had BCR-ABL gene mutation. Of the patients who had BCR-ABL gene mutation, 69 different mutations have been discovered, with G250, M351, M244 and F359 most frequently found.

BCR-ABL mutation confer resistant to imatinib through a variety mechanism but mostly effect conformation of the protein tyrosine kinase inhibitor binding site on the tyrosine kinase phosphate binding loop (P-loop) or the ATP binding site. The ABL tyrosine kinase moves between a catalytically active open conformation and an inactive closed one. The inhibitory activity of imatinib comes from its ability to bind the inactive non-ATP binding conformation and by this its make the enzyme not active. If the non-ATP binding conformation is nonactive, the A-loop occludes the catalytic site of and prevents activity. Our study found BCR-ABL mutation in the P-loop only in 2.3%. Our finding concerning the high frequency mutation in the BCR-ABL gene outside the P-loop need further study.

In 2006, Jabbour and colleagues study BCR-ABL mutations in 171 patients that fail protein tyrosine kinase inhibitor therapy and found that 36% of these patients had BCR-ABL mutations and 66 types of mutations were identified. Factors that were associated with mutation were older age, prior Interferon therapy, and accelerated or blast crisis phase at the beginning of tyrosine kinase inhibitor therapy.

Although certain mutations are associated with protein kinase inhibitor resistant, the role of many (other ?) mutations are unknown. The presence of a mutation do not always result in resistance (Korashad in 2006, Willis in2005). Resistance to imatinib could be because of BCR-ABL dependent which are BCR-ABL kinase domain mutation (>50%) where mutations have been reported at various region and covey variant degrees (activation loop<phosphorilation loop< kinase binding pocket) and amplification of the BCR-ABL gene (7-10%) which is associated with imatinib–related relapse; and BCR-ABL independent which are cellular pharmacology (drug import/ export), over expression of BCR-ABL, Ph+ clonal evolution and others (such as Lyn (other Src kinases)).

Resistant kinase domain mutation is not only seen in CML patients, but also has been reported in other cancers which are treated with kinase domain inhibitors, such gastro intestinal stromal tumors (Tamborini in 2004) and lung cancer (Pao W; Kobayashi).

Only 43 of 80 patients (53.8%) among our patients have achieved CHR within 3 months. Thirty patients have not achieved CHR within 3 months. Nevertheless, these 30 of 80 patients have reached CHR in 6 months (11 patients), in 9 months (1 patients), in 12 months (2 patients), more than 12 months (1 patients), and have not achieved CHR in 18 months (15 patients).The relation between BCR-ABL mutation will be reported in another research.

Conclusion

We reported the median age was 34-35 years old (mean of age is 36 years old), and more patients in the productive age was found. The characteristics of CML patients in Indonesia were not different from CML patients in Asia in general.

Mutation in the P-loop was seen in 2.3% (1 out of 44 patients), this finding was beyond our expectation since 47.69% (31 out of 65 patients) of our patients did not achieved CHR at three months. On the other hand, 15.9% (7 out of 44 patients) of our patients had mutation outside the P-loop. Further evaluation concerning these findings will be reported in another paper. Our finding concerning the high frequency mutation in the BCR-ABL gene outside the p-loop needs further study.

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