

Profile of BCR-ABL Transcript Levels Based on Sokal Prognostic Score in Chronic Myeloid Leukemia Patients Treated with Imatinib

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ABSTRAK

Tujuan: mengevaluasi pola dari respons molekular yang dinilai dengan reduksi kadar transkrip BCR-ABL berdasarkan skor prognosis sokal pada pasien leukemia mieloid kronis (LMK) fase kronis yang mendapat terapi Imatinib. **Metode:** penelitian dengan desain potong lintang dilakukan di Instalasi Rawat Jalan Hematologi, RSU Dr. Soetomo Surabaya, pada semua pasien LMK fase kronis, sejak Juni 2008 hingga Juni 2012. Skoring Sokal ditentukan saat sebelum terapi. Data yang dikumpulkan meliputi karakteristik subjek (usia, jenis kelamin), kadar hemoglobin, trombosit, leukosit, sel muda mieloblas, dan ukuran limpa. Pemeriksaan real-time kuantitatif PCR (RT-qPCR) digunakan untuk mendeteksi residual molekular respons dari BCR-ABL. Hasil RT-qPCR yang dicatat merupakan rasio dari BCR-ABL dan gen acuan (G6PDH) sebagai standar internal. Perbedaan proporsi respons molekular lengkap pada kelompok risiko Sokal rendah dan tinggi dianalisis dengan uji kai kuadrat, sementara perbedaan kadar transkrip BCR-ABL di antara kelompok risiko Sokal dianalisis dengan uji Kruskal-Wallis. **Hasil:** 40 subjek menyelesaikan penelitian. Setelah 18 bulan terapi Imatinib, kadar transkrip BCR-ABL tidak terdeteksi (molekular respons lengkap) pada 7(70%), 8(66,7%), dan 9(50%) berturut-turut pada kelompok subjek risiko Sokal rendah-, sedang-, dan tinggi ($p=0,417$). Respons molekular lengkap pada kelompok risiko Sokal rendah didapatkan lebih tinggi dibanding risiko Sokal tinggi (70% vs 50%), secara statistik tidak berbeda bermakna ($p=0,557$). Analisis statistik menggunakan Kruskal-Wallis menunjukkan tidak ada perbedaan secara bermakna distribusi kadar transkrip BCR-ABL di antara subkelompok skor prognostik Sokal ($p=0,734$). **Kesimpulan:** tidak ada perbedaan kadar transkrip BCR-ABL antara subkelompok skor prognostik sokal pada pasien LMK fase kronik yang diterapi dengan imatinib.

Kata kunci: LMK, imatinib, kadar transkrip BCR-ABL, skor sokal.

ABSTRACT

Aim: to elucidate the pattern of molecular response assessed by logarithmic reduction in BCR-ABL transcription levels based on Sokal prognostic score in chronic phase chronic myeloid leukemia (CML) patients receiving Imatinib treatment. **Methods:** cross-sectional study was conducted in the Hematologic Outpatient Clinic, Dr. Soetomo Hospital Surabaya in all chronic phase CML patients from June 2008 to June 2012. Data on subject characteristics (age and sex), complete blood count with differential and spleen size were collected. Patients were stratified according to Sokal score at diagnosis. Real-time quantitative PCR (RT-qPCR) were used to monitor BCR-ABL levels in patients who fulfilled study. Proportion difference of complete molecular response (MR) was analyzed by chi-square test, while differences of BCR-ABL transcript level among Sokal

prognostic score subgroups was analyzed by Kruskal-Wallis test. **Results:** 40 subjects finished the study. After 18 months of Imatinib treatment, the undetected BCR-ABL transcript level (complete MR) were 7(70%), 8(66.7%), and 9(50%) in low-, intermediate-, and high risk group patients, respectively ($p=0.417$). Although proportion of subjects with complete MR is higher in sokal low risk group compared to in sokal high risk groups (70% v.s. 50%), but this difference is not statistically significant ($p=0.557$). Kruskal-Wallis test showed that there was no significant difference of BCR-ABL transcript level among Sokal prognostic score subgroup ($p=0.734$). **Conclusion:** there was no difference of BCR-ABL transcript level among sokal prognostic score risk groups in chronic phase CML patients treated with Imatinib.

Key words: CML, Imatinib, Sokal prognostic score, BCR-ABL/G6PDH transcript.

INTRODUCTION

Chronic myeloid leukemia (CML) is the first malignancy associated with a specific chromosome abnormality with an annual incidence of 1 to 2 cases per 100.000 per year and a median onset in the fifth or sixth decade of life.^{1,2} The Philadelphia (Ph) chromosome is a shortened chromosome 22 that results from a reciprocal translocation between the long arms of chromosomes 9 and 22. The consequence of this translocation is the fusion of the c-abl oncogene from chromosome 9 with bcr gene from chromosome 22, giving rise to a fused bcr-abl gene. The different fusion proteins encoded by BCR-ABL vary in size depending on the breakpoint in the BCR gene and share a high tyrosine kinase activity, in part responsible for the leukemogenesis. The Philadelphia (Ph) chromosome is seen in 95% of patients with CML.^{2,3} Tyrosine kinases are enzymes that transfer phosphate from ATP to tyrosine residues on substrate proteins that in turn regulate cellular processes such as proliferation, differentiation, and survival. Therefore, it is not surprising that deregulated tyrosine kinase activity has a central role in malignant transformation. Until the last decade before targeted therapy, the prospect for patients diagnosed with CML had been relatively unfavorable. Imatinib mesylate (STI571/Gleevec) is the first therapy to target tyrosine kinase activity. The introduction of imatinib has led not only to more favorable outcomes, but has driven the development of advances in monitoring response to therapy at molecular level. The total number of leukemia cells in the body is reduced very substantially in CML patients with BCR-ABL-positive

responding to imatinib. This reduction is seen first as restoration of Ph negativity in blood and marrow and there after as decreasing BCR-ABL transcript levels assayed by quantitative polymerase chain reaction. Most patients with chronic-phase CML who receive imatinib achieve complete cytogenetic response (CCyR) and low levels of BCR-ABL transcripts, a status that seems to predict for relatively long survival compared with previous treatments.⁴

Cytogenetic remission with monitoring of the percentage of Philadelphia chromosome-positive cells is the best validated system for the assessment of response to interferon- α and tyrosine kinase inhibitors, since the cytogenetic response is the best surrogate marker of survival. For patients who achieve a CCyR to interferon- α , the 10-year survival is about 75%. For patients who achieve a CCyR to imatinib, the 5-year survival rate is close to 100%. The response is conventionally determined by chromosome banding analysis of marrow cell metaphases. If there are fewer than 20 metaphases, the cytogenetic response can be validated by determining the level of BCR-ABL transcripts with quantitative techniques PCR.⁴

More recently, real-time quantitative PCR (RT-qPCR) has been developed. Results of RT-qPCR usually report the ratio of BCR-ABL transcript level to a reference gene (recommended genes include ABL, BCR, and G6PDH). The level of molecular response (MR) at 12-18 months was confirmed to be predictive of long-term clinical outcomes.⁴ Patients treated with 400 mg daily who achieved a reduction in BCR-ABL transcript numbers equal or greater than 3 logs compared with a baseline value have

a significantly better progression-free survival than those who achieved lesser degrees of response.⁴

Two scoring systems (Sokal and the Hasford score), are available for prognostic risk evaluation of CML. Both scores have confirmed their predictive value. The Sokal scoring system has been applied to stratify the patient by risk in all the largest clinical Imatinib trials in CML. The cytogenetic response monitoring require bone marrow aspirates and the accuracy of this test depend on metaphase number, while the Sokal score only require clinical data and peripheral blood as the source of residual cells. Moreover, as far as we know, this is the first study on Sokal prognostic score that was ever conducted in Surabaya. Based on these facts, the purpose of this study is to investigate residual molecular response assessed by logarithmic reduction in BCR-ABL transcription levels through peripheral blood related with prognostic risk score of Sokal in CML chronic phase treated with Imatinib.^{3,5-14}

METHODS

A cross-sectional study was conducted at the Hematology Outpatient Clinic, Dr. Soetomo General Hospital Surabaya, between June 2008 and June 2012. The study population included all patients who fulfilled the inclusion criteria: diagnosed as chronic phase CML according WHO criteria and will be treated with Imatinib more than 18 months, had Karnofsky's performance status >60%, and agreed to be included in this study. The exclusion criteria were patients who stopped treatment >2 weeks before evaluation, and had severe infection. Data collected include subject characteristics (age, sex). Before beginning treatment, patient's history, physical examination, complete blood count with differential, blood chemistry and bone marrow evaluation for morphology and cytogenesis were performed at baseline. Patients were stratified according to Sokal score at diagnosis. Complete blood counts were measured at baseline; at weeks 1, 2, and 4 monthly until month 6; and every 3 months thereafter until the end of the study. Molecular studies: total RNA was extracted from 106-107

peripheral blood cells of patients using the acid guanidinium thiocyanate and phenolchloroform method and a commercially available extraction kit. cDNA synthesis was performed according to the manufacturer's instructions using random hexamer priming and AMV reverse transcriptase (First Strand cDNA Synthesis Kit for RT-PCR, Roche Diagnostic, Mannheim, Germany). cDNA was amplified using a LightCycler (Roche Diagnostics). Primers and probes were included in the kit and they designed to detect b3a2, b2a2 and e1a2 fusion transcripts, covering 95% of the described t(9;22) translocations. Results of real-time RT-PCR usually report the ratio of BCR-ABL transcripts level to a reference gene (recommended genes include ABL, BCR, and G6PDH).

All of the patients were in early chronic phase of CML and had received 400 mg/day Imatinib orally. Hematological response was evaluated after four weeks after commencement of therapy. Molecular response was assessed by RT-qPCR at baseline, then every 3 months and on 18 months of treatment.

Measurement Methods

The calculation of Sokal prognostic score is $0.0116 \times (\text{age in years} - 43.4) + 0.0345 \times (\text{spleen} - 7.51) + 0.188 \times [(\text{platelet count} \div 700)^2 - 0.563] + 0.0887 \times (\text{blast cells} - 2.10)$; and risk by calculation are low score (<0.8), intermediate (0.8-1.2), high (>1.2).⁸

Complete hematological response (CHR) was defined as normalization of the bone marrow (blast cells less than or equal to 5%) for at least four weeks and the peripheral leucocyte count <10 x 10⁹/L and platelets <450 x 10⁹/L, (without peripheral blasts, promyelocytes and myelocytes), in addition to the disappearance of all signs and symptoms of CML (including palpable splenomegaly).⁸

Cytogenetic analysis of bone marrow metaphases was not evaluated in our study. RT-qPCR for ratio BCR-ABL/G6PDH transcript level; an international reporting scale (IS) was proposed to represent major molecular response (MMR) less than 0.1% (≥ 3 log reduction in BCR-ABL transcripts level). A value of 1.0% (<3 log reduction in BCR-ABL transcripts level) is approximately equivalent to the achievement

of a complete cytogenetic response (CCyR). And, a complete molecular response (CMR), or undetected BCR-ABL transcript, is still somewhat controversial (ratio BCR-ABL/G6PDH transcripts = <0.001%).^{5,9}

Statistical Analysis

Data were analyzed using SPSS version 13.0. Numerical data is presented as mean (with its standard deviation, SD) or median (with range), while categorical data is presented as proportion (%). The proportion difference of complete MR between low- and high-risk Sokal Prognostic Score was analyzed by chi-square test. Statistical comparisons between patient subgroup were performed using chi-square test, and Kruskal-Wallis test for nonparametric proportions. A *p* value below 0.05 was considered as statistically significant.

RESULTS

Overall, 40 patients with chronic myeloid leukemia were included into analysis. There were 21 (53%) male and 19 (47%) female patients. Mean age at diagnosis of CML was 39.7±12.9 years. At the time of diagnosis, the

characteristic of subject in this study was based on Sokal prognostic risk classification can be seen in **Table 1**. After 3 months of treatment, 90% patients had complete hematologic remission. After 18 months of imatinib treatment, ≥3 log reduction in BCR-ABL transcript level (major MR) was seen in 3 (7.5%) patients, <3 log reduction in 13 (32.5%) patients and undetected (complete MR) in 24 (60%) patients (**Table 2**). The rates of major MR were 1 (10% of low risk group), 1 (8.3%), and 1 (5.5%) in low-, intermediate-, and high-risk group patients, respectively (**Table 3**). The rates of complete MR were 7 (70%), 8 (66.7%), and 9 (50%) in low-, intermediate-, and high-risk group patients, respectively. Chi-square test showed that there was no significant difference between risk subgroups in terms of complete MR (*p*=0.417).

Although proportion of patients with complete MR in low Sokal risk group is higher compared to high Sokal risk group (70% versus 50%) but this difference is not statistically significant (*p*=0.557) (**Table 2**). Kruskal-Wallis test showed there was no statistically significant difference of BCR-ABL transcript level among subgroup of Sokal prognostic score (**Figure 1**) (*p*=0.734).

Table 1. Characteristic based on Sokal risk group classification

Characteristic	Sokal low risk group (n=10)	Sokal intermediate risk group (n=12)	Sokal high risk group (n=18)
Age (year)			
- Mean	41.40	41.83	37.4
- SD	10.679	12.03	14.585
Hemoglobin (g/dl)			
- Median	10.150	11.650	10.100
- Range (Min-Max)	7.70-14,00	8.60-13.30	7.60-13.90
WBC (x109/L)			
- Median	7.8	191.5	196
- Range (Min-Max)	4-589	5.17-345	12-484
Platelet number (x109/L)			
- Median	298	534.5	613.5
- Range (Min-Max)	59-646	192-1044	163-2454
Blast cell number (%)			
- Mean	0.557	1.962	3.911
- SD	0.752	3.416	3.831
Spleen (cm)			
- Median	2	15	17
- Range (Min-Max)	1-8	2-20	2-28

Table 2. Distribution of BCR-ABL transcript level/GAPDH after Imatinib treatment

Ratio BCR-ABL/G6PDH (%)	Frequency (%)
BCR-ABL undetectable (<0.001)	24 (60)
BCR-ABL/G6PDH <0.1- 0.001 (≥3 log reduction)	3 (7.5)
BCR-ABL/G6PDH >0.1 (<3 log reduction)	13 (32.5)

DISCUSSION

In chronic myeloid leukemia (CML) patients with targeted therapy; monitoring is essential for treatment optimization and for a cost-effective outcome. At the beginning, and during the first 3 months, clinical, biochemical and haematological monitoring is recommended every 2 weeks, to ensure the CHR of the patient. Further reduction in the burden of disease results in cytogenetic remission, defined by the degree of reduction of Ph-positive cells in marrow or blood testing. The standard method of determining cytogenetic response is bone marrow cytogenetics (by the National Comprehensive Cancer Network guidelines). Once CHR has been documented, monitoring continues with karyotyping of at least 20 bone marrow metaphases, which is currently recommended at 6, 12, and 18 months, or until CCyR had been achieved. Once CCyR had been documented, quantitative PCR (qPCR) should be performed at three-month intervals for testing of minimal residual disease (MRD) at the molecular level.^{1,10,11} Low levels of minimal residual disease is measured by real-time quantitative PCR (RT-qPCR). The result of detection of RT-qPCR represents the scientific paradigm for successful molecular diagnostic monitoring of targeted cancer therapy. Complete MR predicts a very low risk of relapse, but it is not equivalent to disease eradication.^{4,11,12}

The superiority of Imatinib mesylate over interferon and other traditional treatment has been shown in a variety of prospective randomized international studies. MMR after 18 months of Imatinib therapy lead optimal results to continuous remission and better prognosis. Although Imatinib has been used in the treatment of the CML patients since 2001, the number of studies including molecular response (MR) to Imatinib treatment from different countries is still low.^{4,5} A higher rate of complete MR (undetectable BCR-ABL) has been reported in patients receiving prolonged Imatinib therapy.^{4,13}

In our study, the response of treatment is evaluated hematologically every two weeks, and molecularly after the treatment has reached minimal amount of time of 18 months. From 40 patients that received Imatinib, complete hematological response (CHR) and major MR were 90% and 32.5%, respectively. These results are still lower than a study conducted by Bixby (2009) in 204 subjects were reported 98.5% CHR, and 50.1% MMR at 1 year treatment.¹⁴ Study by Ozatli et al. (2010) in 73 subjects, were seen complete hematologic response (CHR) and major MR rates at 12 months Imatinib treatment were over 90% and 40-62%, respectively.⁵ In other multicenter-retrospective study, the molecular response at 12 months of Imatinib treatment was evaluated for the first time in Turkish CML patients. Sixty-seven (87%) patients were in hematologic remission at 12 months of treatment. Major MR to treatment was seen in 45 (58.4%) patients. Of these 45 patients, 25 (32.5%) patients were in complete MR. The percentage of patients having hematologic response, major and complete MR at 12th months treatment are comparable to others studies. We supposed that according to different racial groups of the patients, response

Table 3. The molecular response to the treatment of patients according to Sokal risk classification

Sokal classification	Reduction In BCR-ABL transcript level			Total
	≥3 log (Major MR)	<3 log	Undetected	
Low Risk Group	1 (10.0%)	2 (20.0%)	7 (70.0%)	10 (100.0%)
Intermediate Risk Group	1 (8.30%)	3 (25.0%)	8 (66.7%)	12 (100.0%)
High Risk Group	1 (5.50%)	8 (44.5%)	9 (50.0%)	18 (100.0%)

rates can be variable due to gene polymorphisms in different countries.^{5,15}

The prognostic score of Sokal has been reported to predict the response to treatment and overall survival. In the IRIS study, rates of major MR at 12 months among cases with complete cytogenetic responses were 66%, 45% and 38% in low-, intermediate-, and high-risk patients, respectively ($p=0.007$).⁵ Another study in 73 subjects, rates of major MR at 12 months were 44%, 63%, and 53.6% in low-, intermediate-, and high-risk patients, respectively ($p>0.05$).^{5,15} In our study, the rates of major MR at 18 months were 1 (10%), 1 (8.3%), and 1 (5.5%) in low, intermediate-, and high-risk group patients, respectively (**Table 3**). We did not find any statistically significant difference between risk subgroups in Sokal of major MR rates ($p=0.823$). This contrast finding may be due to the low number of cases of the present study.

Our study also found undetected BCR-ABL transcript level (complete MR) were 7 (70%), 8 (66.7%), and 9 (50%) in low-, intermediate-, and high-risk group patients, respectively (**Table 3**). Analysis showed no statistically significant difference between risk subgroups in terms of complete MR ($p=0.417$). Although, Sokal low risk group were higher than Sokal high risk groups in terms of complete MR (70%

versus 50%), in analysis, this difference is not statistically significant ($p=0.557$). Similar results were found in study by Goldman, which showed that in Sokal low risk group CCyR and of Major MR are higher than Sokal high risk group.^{16,18,19} But, a study by Ozatli et al. in 73 subjects, showed that the Sokal low risk group had complete MR lower (11%), than Sokal high risk group (17.8%) ($p>0.05$).¹ However, there was no significant difference of BCR-ABL transcript level/G6PDH among prognostic score of Sokal in our study ($p=0.734$). These results might be due to a small sample size. In the future, we hope that we will have more samples, so results will be more accurate in representing the population.

The outcome of Imatinib treatment in CML patients has been improved. Until now, monitoring outcome of Imatinib treatment is still challenging. We did not do cytogenetic response monitoring because it required bone marrow aspirates and the accuracy of the test depends on metaphase the number. It is much more convenient for the patient to have their peripheral blood as the source of residual cells to be evaluated by real time quantitative PCR and use the prognostic score of Sokal as treatment monitoring first.

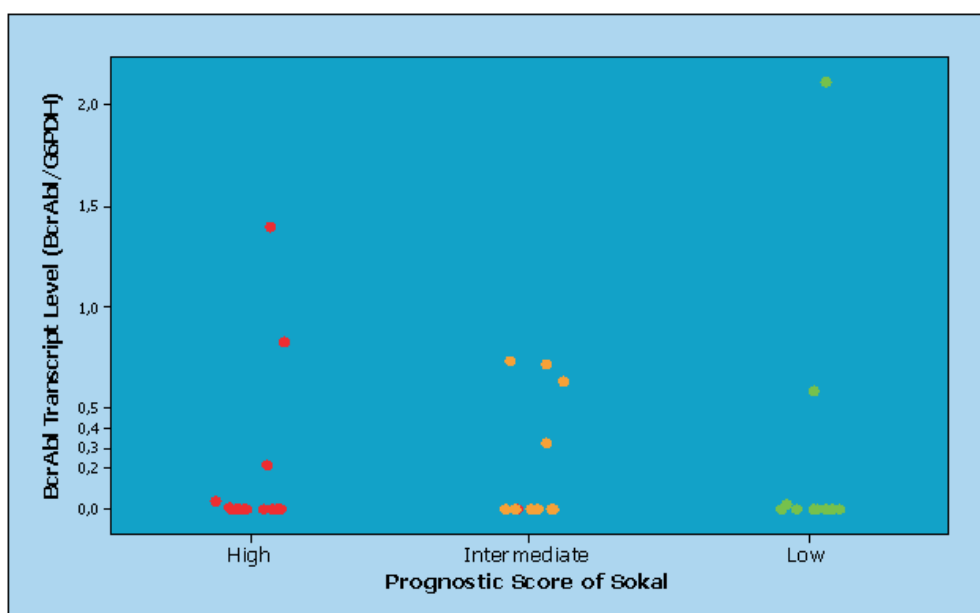


Figure 1. Distribution (scatter) of BCR-ABL transcript level and prognostic score of Sokal

CONCLUSION

Proportion of patients with complete MR are higher in the Sokal low risk group compared to Sokal high risk group, although this difference is not statistically significant, and there is no difference in BCR-ABL transcript level/G6PDH among Sokal risk groups.

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