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Research

Not all Imatinib treated Chronic Myeloid Leukemia Patients with BCR-ABL1 more than 10% at 3 Months have Unsatisfactory Outcome

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Abstract

Background

Outcomes are heterogeneous among chronic myeloid leukemia chronic phase (CML CP) patients with BCR-ABL1 transcript percent>10% at 3 months and some have satisfactory outcomes. NCCN guidelines include a change of CML therapy if BCR-ABL1 >10% at 3 months, whereas the ELN recommends additional testing and a therapy change for patients who are still >10% after 6 months treatment.

Aim of the work

To compare relative ratio at 3 months (RR3) combined to BCR-ABL1 transcript percent at 3 months versus BCR-ABL1 transcript percent at 6 months on the long-term outcome of CML patients treated with frontline imatinib.

Materials and methods

The present study included 77 Egyptian CML CP patients treated with imatinib 400 mg daily during the first 6 months. RR3 was calculated to all patients. It was defined as BCR-ABL1 transcript percent at 0 month - BCR-ABL1 transcript percent at 3 month / BCR-ABL1 transcript percent at 0 month. Peripheral blood BCR-ABL1 transcript percent was measured by quantitative PCR prior to commencing imatinib (baseline, M0), after 3 months (M3) and 6 months (M6) for all patients and after 1 year (M12) for responders only. Response assessment was done according to ELN criteria.

Results

Range of BCR-ABL1 transcript percent at initial diagnosis was 13–100%. Fifty five patients (71.43%)showed BCR-ABL1 transcript percent>10% and 22 patients (28.57%) showed BCR-ABL1 transcript percent<10% at 3 months. At 6-month, 13 out of 55 patients (23.64%) showed BCR-ABL1 transcript percent <1%. Ten out of 13 patients had RR3 >64.5 while 3 patients had RR3<64.5. Those 10 patients were of low Sokal score while the other 3 were intermediate Sokal score.

Conclusion

low Sokal score CML CP with RR3>64.5 and BCR-ABL1 transcript percent>10% at 3 months may define a group of patients who need BCR-ABL reassessment thoroughly as these patients may achieve BCR-ABL1< 1% at 6 months. Further studies on a larger scale of patients are still required to confirm this finding.

Keywords: Relative ratio at 3 months; BCR-ABL1 transcript percent; chronic myeloid leukemia

Introduction

The standard frontline therapy for chronic myeloid leukemia patients in chronic phase (CML-CP) is the tyrosine kinase inhibitor (TKI) imatinib. The IRIS trial (International Randomized Study of Interferon Versus STI571)suggested the use of second-generation (2G) TKIs dasatinib and nilotinib as front-line treatment [1] because of their better cytogenetic and molecular responses than imatinib [2]. However, imatinib has the following advantages: its side-effect profile is well understood and is likely to be more affordable in the near future with the expiry of its patency in many countries. that is why there is an increasing interest in developing strategies to identify patients who will not respond optimally to imatinib, as early as possible, so that they can be offered an alternative TKI [2].

Early molecular assessment had superior value over serial cytogenetic studies. Perhaps, this reflects the unreliability of extrapolating cytogenetic results from a small number of analyzable metaphases. Furthermore, the dividing Philadephia-positive cells in the bone marrow are poorly representative of the specialized, more primitive leukemia cells population resistant to eradication by TKI. This hypothetical population might be better characterized by their capacity to express more BCR-ABL1transcripts [3].

The range of BCR-ABL transcript levels at diagnosis is very wide, and no particular cutoff point at that time could be identified to predict

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overall survival or progression-free survival [4]. Achievement of major molecular remission in the first year of imatinib treatment indicates long-lasting CCyR. However,waiting for 12 months is not appropriate. The ELN and the NCCN showed better outcome if patients achieved10% or less BCR/ABL1 transcript by IS at 3-month time point [5].

NCCN guidelines include a change of therapy if BCR-ABL1 is >10% at 3 months, whereas the ELN recommends additional testing and a therapy change for patients who are still >10% after 6 months of treatment [6]. The prognostic impact of 3-month response landmarks could be driven by individual differences in tumor load at diagnosis i.e. BCR-ABL transcript levels, or by the individual reduction of transcript levels within the first 3 months of treatment [7]. The use of transcript cutoffs assessments at 6 and 12 months contributed little (if anything) more to identify patients with a high risk of progression, any additional deaths or cytogenetic failures [3].

Aim of the work

The objective of this study was to compare relative ratio at 3 months (RR3) combined to BCR-ABL1transcript percent at 3 months versus BCR-ABL1 transcript percent at 6 months on the long-term outcome of Egyptian CML patients treated with frontline imatinib.

Material and Methods

The present study included 77 Egyptian CML patients in chronic phase. They were on imatinib 400 mg daily during the first 6 months. They were followed up in the Hematology Outpatient Clinic of Alexandria Hospital between February 2013 and March 2015.Patients gave written informed consent for their data to be used in this analysis. The study was approved by local ethics committees in accordance with the Declaration of Helsinki.

Peripheral blood BCR-ABL1 transcript percent was evaluated by quantitative PCR (Qiagen) prior to commencing imatinib (baseline, M0), after 3 months (M3) and 6 months (M6) for all patients and after 1 year (M12)for responders only.

Measurement of BCR- ABL1 Transcripts

Total RNA was extracted from bone marrow or peripheral blood mononuclear cells using RNeasy Midi Kit (Qiagen) and was synthesized into cDNA according to standard procedures in the ipsogen RT kit (Qiagen). RQ-PCR was done on Rotor-gene Q instrument using ipsogen BCR-ABL1 kit (Qiagen). The absolute quantities of BCR-ABL and ABL transcripts in patient specimens were determined by reference to standard curves. RQ-PCR results were reported as a ratio of BCR-ABL/ABL (%) [8].

Response assessment was done according to ELN criteria [9]. There is wide variation in BCR-ABL1 transcript percent at initial diagnosis with a range of 13–100%. Using BCR-ABL1 transcript percent cut off of 10% at 3 months and 1% at 6 months. Fifty five patients (71.43%) showed BCR-ABL1 transcript percent >10% and 22 patients (28.57%) showed BCR-ABL1 transcript percent <10% at 3 months (responders at 3 months). At 6-month,13 out of 55 patients (23.64%) BCR-ABL1 transcript percent dropped to <1% (responders at 6 months).

Relative ratio at 3 months (RR3) defined as BCR-ABL1 transcript percent at 0 month - BCR-ABL1transcript percent at 3 month / BCR-ABL1 transcript percent at 0 month was calculated to all patients. High relative ratio means rapid rate of transcript reduction. At 3 months, the optimum value for prediction of progression free survival at 3 years was RR3 of 64.5%. [10].

Statistical Analysis

The statistical data were analyzed using Statistical Package for the Social Sciences (SPSS) (version 21.0; SPSS Inc., Chicago, IL, USA). Qualitative data were compared using Pearson's Chi-square and Fisher's exact while quantitative data were compared using ANOVA test (F test) to compare means of more than two groups. Least significant difference (LSD) was used when F-value is significant to detect the presence of significance between each 2 groups. A cut off P-value of 0.05 was adopted for all the statistical analyses.

Results

Patients' characteristics at diagnosis are summarized in table 1. BCR-ABL transcript percent in the studied patients at diagnosis varies from 13–100% with a median of 80%. At 3 months, the mean value of BCR-ABL transcript percent was statistically significantly different between the three groups. At 6 months, the mean value of BCR-ABL transcript percent was statistically significantly differ between non responders and both responders at 3 and 6 months while non-statistically significant difference was present between responders at 3 and 6 months. Mean values of BCR-ABL1 transcript percent at diagnosis, 3 months and at 6 months in non-responders, responders at 3 months and responders at 6 months are shown in figure 1.

Table 2 shows number of patients according to BCR-ABL1 transcript percent at 3 months,6 months and 1 year (in responders) after imatinib treatment. Forty two patients (54.55%) had BCR-ABL1 transcript percent >10% at 3 and 6 months, 13 patients (16.88%) had BCR-ABL1 transcript percent >10% at 3 months and <1% at 6 months and 22 patients (28.57%) had BCR-ABL1 transcript percent <10% at 3 patients and <1% at 6 months. RR3>64.5 was present in all responders at 3 months and in 10 out of 13 responders at 6 months. These three patients with RR3<64.5 have intermediate Sokal score. None of responders at 3 months and responders at 6 months had high Sokal score.

Discussion

A single measurement of *BCR-ABL1* transcripts percent at 3 months is the most informative way to identify poor responders CML-CP patients to imatinib treatment thus allowing early clinical intervention [3]. At 3 months, however, predictive cut off did emerge. A 3-month reduction ratio of 0.35-fold was found to be the best cut off point, which corresponded to a 0.46-log reduction in BCR-ABL transcript levels. Early decline in the transcript level response at 3-month measurement may reflect decline of mature precursors [2].

The 6- and 12-month assessments has a little in identifying patients

Table 1: Clinical and laboratory data of the studied patients

Parameter	Non responders	Responders at 6 m	Responders at 3 m	Test of significance	p value
BCR-ABL transcript%	> 10% at 3m > 10% 6 m	> 10% at 3 m < 1% at 6m	< 10% at 3 < 1% 6 m		
Case no.	42 (54.55%)	13 (16.88%)	22 (28.57%)		
Age (yrs)	46.71±9.69	51.46 ±14.32	50.55±10.43	1.46	0.239
Sex Male Female	 22 20	 5 8	 6 16	5.03	0.081
Sokal score Low Intermediate High	 19 20 3	 10 3 0	 18 4 0	10.52	0.33
BCR-ABL% 0 m 3 m 6 m	 79.71 ±18.566 52.31±24.66 79.71 ±18.566	 73.31±26.69 21.31±15.18 0.01± 0.010	 70.27±27.23 4.92±3.19 0.00±0.00	 1.349 46.097 317.58	 0.266 0.00 0.00
RR3 <64.5 >64.5	 35 7	 3 10	 0 22	44.43	00

m.: month; p is significant if ≤ 0.05

Table 2: Number of patients according to RR3 and BCR-ABL% at 3, 6 and 12 months

No. of Patients (%)	BCR-ABL transcript %			RR3	
	3 m	6 m	12 m	<64.5	>64.5
22 (28.57%)	< 10	<1	<0.1	0	22
-	< 10	>1	-	-	-
13 (16.88%)	>10	<1	-	3	10
42 (54.55%)	>10	>1	-	35	7

at a high risk of progression. At 6-month, other factors, such as adherence to therapy and the need to adjust treatment for toxicity influence the response and interfere with the prognostic power of transcript measurement [3].

Not all imatinib-treated patients with BCR-ABL1 transcript percent>10% at 3 months have unsatisfactory outcomes, particularly if the sample is collected at the earliest extreme of the 3-month assessment window [6].

Around 54.55% of our patients (55 patients) achieved BCR-ABL1 transcript percent>10% at 3 months. However, 13 out of 55 patients (23.64%) achieved BCR-ABL1 transcript percent <1% at 6 months and <0.1 at 1 year. In other study, 2% of CML patients had high transcript levels at 3 months but low levels at 6 months. These patients had a significantly worse outcome than those with lower transcripts at both time points [6]. So, there is a need for other early additive assessment method for this group of patients.

The prognostic value of the 3-month assessment was independent of temporarily reduced or discontinued imatinib dose because of adverse effects [3]. Measurements classified as 3 months could span a wide collection window from the TKI starting day. A shift in the day of the 3-month sample collection could theoretically change the response category from warning or failure to optimal and vice versa [6].

False low BCR-ABL/ABL transcript ratios may be obtained at diagnosis. The international scale is only applicable for ratios up to 10%, a range in which BCR-ABL/ABL is considered to reflect BCR-ABL ratios in an almost linear way [7]. The dynamics of early molecular response may be more important than the molecular

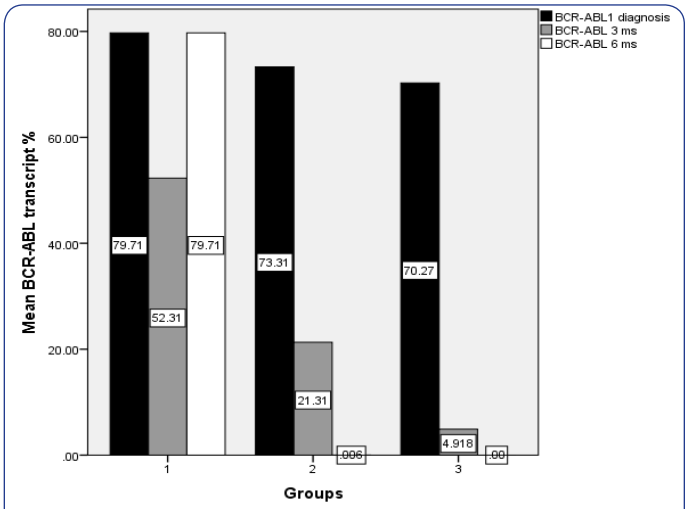

Figure 1: Mean value of BCR-ABL transcript percent at diagnosis, at 3 months and at 6 months in the three studied groups

response at 3 months. This requires an RT-Q-PCR every month and a different housekeeping, control gene, like beta glucuronidase. The evaluation of the dynamics of early molecular response is not yet recommended in practice [10].

RR3>64.5 was present in all our responders and in 10 out of 13 patients with BCR-ABL transcript percent> 10% at 3 months and <1% at 6 months. These 10 patients had low Sokal score. Three patients who have RR3<64.5 in responders at 6 months have intermediate Sokal score. High relative ratio denotes more rapid rate of transcript reduction. There is no high Sokal score among those who are responders at 3 months and responders at 6 months. Close attention should be paid to those in the intermediate/high risk scores for whom the risk of development of primary resistance is higher while patients with low Sokal appear to benefit from imatinib [5].

Conclusion

RR3>64.5 in the presence of BCR-ABL transcript percent>10% at 3 months in low Sokal score CML CP may define a group of patients who need BCR-ABL1 reassessment thoroughly as those may achieve BCR-ABL< 1% later at 6 months. Further studies on a larger scale of patients are still required to confirm this finding which if proved may have a greater impact in early modification of therapy.

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