

# Comparative Study of Quantitative Real-Time Monitoring Qrt-Pcr For Bcr-Abl Gene In Chronic Myelogenous Leukemia (Cml) Between Blood and Bone Marrow Samples

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**Abstract**

Quantitative RT-PCR technique present remarkable diagnostic information for molecular surveillance of the BCR-ABL duplicate in Chronic Myelogenous Leukemia (CML) using in both peripheral blood (PB) and bone marrow (BM) samples, prospective analysis has been conducted a by quantitative real-time RT-PCR (QRT-PCR) for both PB and BM specimens, from 25Re patients with untreated CML. QRT-PCR investigation was carried out previous and during treatment with Imatinib for untreated CML. Statistical examinations showed useful agreement of PB and BM pre- treatment specimens. Nevertheless, using the SPSS statistical method that estimates the agreement between PB and BM data, results showed low correspond of BCR-ABL measurements in PB and BM for specimens acquired through treatment. PB values tended to be lower than the conformable BM values [average difference = -0.37 ( $p < 0.001$ ) in 36 coupled samples] and the 95% limits of agreement ranged from -1.23 to 0.48. Nevertheless, the present study showed that BM and PB QRT-PCR values followed a similar direction during treatment (Spearman correlation coefficient, 0.83; 95% CI, 0.70, 0.96). Our findings imply that PB and BM measures of BCR-ABL are frequently quantitatively different. The most accurate way to determine whether there is minimal residual disease is through BM sampling because BM results tend to be greater than PB values (MRD). Based on these findings, we advise avoiding switching BM and PB sampling for MRD monitoring during CML treatment because doing so could result in incorrect interpretation of treatment outcomes.

**Keywords:** Imatinib, CML, Cytogenetic response, QRT-PCR.

**Introduction**

At some point of the past decade, molecular diagnostic strategies, which includes Southern blotting and RT-PCR, were beneficial to clinicians for prognostication and assessment of treatment efficacy for patients with Chronic Myelogenous Leukemia (CML [-4]. We and others have stated previously that Southern blot tracking of the BCR-ABL fusion gene rearrangements in peripheral blood (PB) samples correlated with cytogenetic monitoring of bone marrow (BM) aspirate samples, as a result supplying a much less invasive method for the assessment of therapeutic reaction all through remedy of CML (1-4) [5-8].

Quantitative real-time RT-PCR (QRT-PCR) affords a rapid, automated and especially sensitive manner of accurately quantifying BCR-ABL transcripts as an alternative sign of disorder that appears to have unbiased prognostic significance for sufferer's present process curative remedy for CML with allogeneic stem

cells implanted [9-12]. Recently, molecular monitoring data from the international Randomized observe of Interferon and STI571 (IRIS) phase III trial of patients with previously untreated CML recommend that quantitation of MRD at some phase in early treatment time-factors is an essential sign of response and prolonging lifetime [10]. Seeing that frequent tracking the usage of BM sampling is inconvenient and expensive, the potential to locate and quantify minimum remaining disease in PB samples provides several wonderful advantages. To determine whether or not QRT-PCR tracking of PB is similar to BM tracking in patients receiving intensive combination chemo-remedy, the BCR-ABL levels the usage of QRT-PCR of coupled PB and BM samples of CML patients were initiated in 1998, previous to the advent of imatinib that examined the combination of homoharringtonine and cytarabine for newly diagnosed chronic phase CML.

## Patients and methods

Twenty-five adults with a confirmed identification of untreated CML in chronic phase were treated by intravenously Single daily dose of Imatinib (450 mg/day) for 7 days. Process have been reiterated every 28 days. Patients were Planned to receive as many as nine month-to-month processes of treatment; Patients who carried out a chief cytogenetic reaction (>77% Everyday metaphases) after nine months have been eligible to keep remedy of the twenty-five patients who acquired treatment, the median number of treatment processes acquired turned into nine. Most effective 6 patients showed a major cytogenetic response after 10 processes of therapy. The clinical and cytogenetic impacts of this trial have been shown elsewhere. All patients additionally enrolled on this experiment, for the molecular comparison of BCR-ABL transcripts by utilized of QRT-PCR of coupled PB and BM samples after three and nine months of initiation of remedy.

## RNA preparation and cDNA synthesis

Overall RNA become extracted from BM and PB mononuclear cells. General RNA (1-15µg) changed into synthesized into cDNA according to traditional Processes in the pre-amplification device (Promega, USA).

## Quantitative Real-Time PCR (QRT-PCR)

The QRT-PCR Analyses had been performed within molecular lab of Al-Khdumya Teaching Hospital. QRT-PCR of patient samples, serial dilutions, and the negative controls were surveyed in duplicate utilizing a Promega instrument (USA). BCR-ABL transcripts were transcripts in 50µl mixture containing 0.1µl

of cDNA; 4mM MgCl<sub>2</sub>; 10mM Tris-HCl, pH 8.4; 50 mM of KCl; 0.2mM of each dNTP; 5µg BSA; 1.25U AmpliTaq GDNA polymerase; 500nM of each primer; and 250nM of the probe. Also, ABL transcripts were amplified to indemnify for differences in RNA probity and cDNA synthesis efficiency. The final concentrations of primer and probe were 500nM and 250nM, respectively. The following BCR-ABL p210 transcripts were amplified using in this study: (p190 5') GCAGATCTGGCCCAACGAT, (p190 3') TCAGACCCTGA-GGCTCAAAGTC, and (p190 probe)6FAM-CATGGAGACGCAGAAGCCCTTCAGC-TAMRA; (ABL 5') AAAATGACCCCAACCTTTTCG, (ABL 3') CCATTCCCATTGTGATTATAGC, and (ABL probe) 6FAMTCTAAGCATAACTAAAGGTGAAAAGCTCCGGGTCTT-TAMRA. Standard TaqMan PCR parameters (ABI PRISM 7700 SDS) were applied to all BCR-ABL and ABL amplifications. BCR-ABL [15-17].

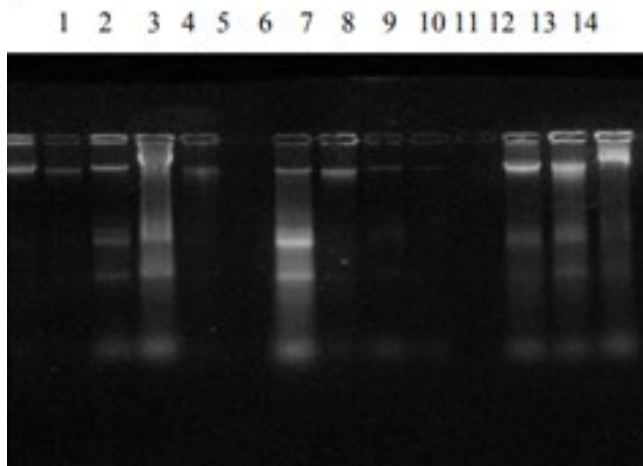
## Quantification and Normalization of BCR-ABL

The affected person's exact BCR-ABL and ABL transcript levels the selection of specimens was made using conventional curves as a guide. All preferred curves were created using 5-fold serial dilutions of cDNA from the CML cell line (from 80 pg to 250 ng), which included the appropriate BCR-ABL transcript. As a ratio or normalized quotient (NQ) of BCR-ABL/ABL, real-time RT-PCR effects have been observed. NQ levels 0.0001 are below the assay's level of detection and could be regarded as a "molecular remission." Every assay's sensitivity altered into the p190 transcript, between 10-6 and 10-7, and between 10-5 and 10-6 for both p210 transcripts.

**Table 1: Normalized quotient of BCR-ABL/ABL during treatment with Imatinib**

Patient No	pre-treatment		3- month		9- month		Past 9- months	
	BM	PB	BM	PB	BM	PB	BM	PB
1	1.33	1.28	0.61	0.228	1.22	0.62		
2	1.84	1.31	0.88	0.338	1.300	1.150		
3	1.42	1.20	0.84	0.671	1.850	0.887	0.066	0.081
4	1.60	1.26	0.76	0.347	0.035	0.068		
5	1.55	1.53	0.79	0.256	0.730	0.178		
6	1.23	1.11	0.85	0.761	1.78	1.200	0.044	0.027
8	0.59	0.48	0.12	0.117				
9	0.87	0.36	0.55	0.287				
10	3.21	2.44	0.89	1.002				
11	0.88	0.510	NA	NA				
12	1.62	0.63	0.64	0.044				
13	1.25	1.32	1.21	0.740				
14	1.82	1.12	0.57	0.582				
15	3.23	1.98	0.98	0.85				
16	2.44	1.77	1.66	1.22	1.450	1.220		
17	1.37	0.77	0.58	0.52	0.580	0.477		
18	2.66	1.66	1.58	0.54	0.574	0.421		
19	1.52	1.28	1.33	0.87	0.320	0.650		

20	1.78	1.21	0.84	0.66	NA	NA	0.140	
21	1.66	0.68	0.278	0.078	0.510	0.180		
22	2.24	1.82	0.62	1.24	1.80	1.00		NA
23	1.32	0.87	0.32	NA				NA
24	1.47	0.965	0.98	NA				NA
25	2.38	1.44	1.25	NA				NA
Patients who achieved a major cytogenetic response.NA, sample was not available for examination.								



**Figure 1:** Total RNA separated from PB CML Iraqi patients'

### Result and Discussion

Fifty paired untreated PB and BM specimens were assessed using QRT-PCR transcript, 10 patients expressed b3a2 BCR-ABL. The median value (range) for untreated BM NQs was 1.5 (0.32-11.2) compared to the median value in PB of 1.3 (0.11- 4.8). After 3 months of therapy, the median values for BM and PB were 0.5 (undetectable 24.0) and 0.4 (undetectable 3.0), respectively. After 9 months of therapy, average BM and PB NQs were 1.2 (0.08-44.0) and 1.0 (0.02-4.23), respectively. Three patients (No 4, 7, and 20) achieved a temporary major cytogenetic response at 9, 10, and 11 months of treatment, respectively. All three had observable MRD in both PB and BM at the time of their most reasonable cytogenetic response. One patient (No. 24) had a transient complete cytogenetic response following 3 months of therapy but had continued MRD in a PB sample at that time. Only 1 patient (No. 11) had Corresponded PB and BM specimens that were transiently negative (undetectable) for MRD following 3 months of treatment [5-7]. The cytogenetic examination was not conducted at this time point. According to this protocol of therapy for CML has changed to imatinib therapy, accurate assessed of MRD forms is becoming an important surrogate for response and prediction [12].

Many recent studies recommend that the degree of transformation in the NQ value using QRT-PCR during the first months of therapy with imatinib is predictive of succeeding cytogenetic response and also appears to associate with clinical results for patients with both

chronic phase and advanced CML. The successes of imatinib as a single agent to eliminate MRD in the majority of CML patients, led to apply it as a tyrosine kinase inhibitor and of imatinib-based combination therapies [17]. While shown to induce significant cytogenetic and molecular responses to this experiment, imatinib has great activity in CML this agent can assess its ability to further decrease or eliminate MRD [15-16]. Study shows that PB and BM quantification of BCR-ABL transcripts using QRT-PCR exhibit only slightly agreement with one another in complete terms. During treatment, BM values were greater than those in PB. Regardless, no cases were found in which BM had detectable no local study dealt both blood and marrow BCR-ABL transcript.

Spearman correlation coefficient has used the calculates the association of the coupled samples but doesn't calculate the actual agreement between PB and BM values [5-12]. Coupled samples have a 10-fold difference in absolute copy number. Also, the standard Pearson correlation coefficient calculates the potency of the relationship between two variables, not their absolute agreement [11]. Actual agreement between BM and PB BCR-ABL. Based on our results, we recommend that QRT-PCR analysis of BM supplies more accurate data about true MRD status in patients receiving therapy for CML. Although our data indicate that similar trends occurred in matched PB and BM values allowing clinicians to measure molecular results employing PB sampling, BM BCR-ABL levels during therapy were always higher than those in PB. Periodic BM tests

During CML therapy also stay necessary for assessment of other disease parameters, including morphology and cytogenetic.

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