Molecular Monitoring of *BCR-ABL* Fusion Transcripts in Patients with Chronic Myeloid Leukemia During Treatment Using the Endpoint Fluorescence Method

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Abbreviations: EPF, endpoint fluorescence; QPCR, quantitative real-time polymerase chain reaction; CML, chronic myeloid leukemia; TKI, tyrosine kinase inhibitors; CCyR, complete cytogenic response; MRD, minimum residual disease; FL, fluorescence; Ct, cycle threshold; RFU, relative fluorescence units; RT-PCR, reverse transcription polymerase chain reaction.

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ABSTRACT

Objective: The purpose of the study was to compare results and evaluate the agreement between the endpoint fluorescence (EPF) method and quantitative real-time polymerase chain reaction (QPCR) during molecular monitoring of patients with chronic myeloid leukemia (CML) receiving treatment.

Materials and Methods: The study was conducted at Molecular Lab of Riphah International University, Islamabad, Pakistan, from January 2017 to December 2018. A total of 150 blood specimens from 30 patients with CML were analyzed at regular intervals during therapy. The detection/quantification of transcript mRNA was done simultaneously using QPCR and the EPF method.

Results: Out of a total of 150 RNA specimens analyzed, 117 (78%) specimens were positive, whereas 33 (22%) were negative for the transcript using both methods at various stages of treatment. Strong linear negative correlations between the cycle threshold and relative fluorescence unit values were observed with $P < .0001$ at 0, 3, 6, 9, and 12 months of treatment. No significant difference ($P > .05$) between the means of the *BCR-ABL* percentage was observed in either method at all stages of treatment. The bias between the 2 methods was calculated as 0.069 ± 3.50, and 95% limits of agreement were 6.92% to –6.79%.

Conclusion: We found that EPF is s simple method to detect/quantify *BCR-ABL* mRNA expression during treatment with comparable results to QPCR.

Remission in patients with chronic myeloid leukemia (CML) during treatment with tyrosine kinase inhibitors (TKI) is usually assessed by blood counts and by cytogenetic techniques. In patients with complete cytogenetic response (CCyR), there can be as many as 10^7 leukemic cells still present.[1] After a patient achieves CCyR, it is only possible to follow the minimal residual disease (MRD) using molecular methods.[2] Molecular analysis of *BCR-ABL* transcripts shows a detectable level for many years, and gradual decline is seen in most patients.[3] Regular scrutiny of MRD helps identify the effectiveness of therapy and make decisions about alternative interventions in patients without optimum response.[3,4]

Because TKI therapy for CML reduces the disease burden below the threshold of hematologic and cytogenetic detection, molecular monitoring with quantitative real-time polymerase chain reaction (QPCR) is the best method to study therapy response.[3] It quantifies the disease with an increased sensitivity of up to 10^{-8} and has become the gold standard approach in the management of these patients.[4,5] However, QPCR presents some complexity because of its evolving and nonstandardized methodologies.[6] Efforts are being made for a better harmonization of the quantitative molecular monitoring of patients with CML.[7]

Currently, QPCR for drug monitoring is routinely not available in our region of the world. Major barriers to implementing routine QPCR in patients with CML in underresourced regions are its high cost and lack of technical expertise. The initial setup, including the instrumentation of QPCR, is quite expensive compared to the setup for conventional PCR.[8] The negative aspect of the QPCR machine is the expense of the instrument, which combines a thermocycler with an online fluorescence (FL) detector. Unavailability of this diagnostic facility provides a potential to investigate a method of post-PCR endpoint detection.

With the endpoint fluorescence (EPF) method, target fluorescence can be measured after performing PCR in an ordinary thermocycler with a fluorescent labeled probe. Fluorometry is often selected because

of its sensitivity and high specificity.[9] It was first used for qualitative testing, but its quantitative results correlate very well with QPCR results.[10] Other advantages of this analytical technique are its low cost and simplicity of handling. It is used in medical diagnostics, DNA sequencing, forensics, genetic analysis, and biotechnology applications and has proved to be a helpful tool for both quantitative and qualitative analysis.[11]

The implementation of a cost-effective and simple strategy for monitoring therapeutic response in patients with CML is indispensable in underresourced populations. This study evaluated patients' test results obtained using QPCR and EPF to determine the specificity, sensitivity, and mean difference between transcript percentages. The results of QPCR and EPF were correlated, and agreement between the 2 methods was also established using blood specimens from patients with CML at different stages of treatment.

Materials and Methods

A total of 150 peripheral blood specimens from newly diagnosed patients with CML in the chronic phase were studied. Thirty patients were consecutively enrolled from tertiary care hospitals in Rawalpindi, Pakistan from January 2017 to December 2017 and were followed until December 2018. Molecular analysis was carried out in the laboratory of Riphah International University. Informed consent was taken from every patient, and the study was approved by the ethical review committee of Islamic International Medical College, Riphah International University, (reference number Riphah/IIMC/ERC/16/0115).

The diagnosis of CML was made according to clinical presentation and morphologic criteria of peripheral blood film and bone marrow aspirate. Only those patients who started treatment with TKI after their diagnosis were included in the study regardless of age and sex. Patients with atypical fusion transcript (other than e13a2 and e14a2) were not included in the study.

Peripheral blood specimens were obtained before starting therapy for baseline record and then after every 3 months during the first 12 months of treatment. Peripheral blood specimens were rapidly transported at 2° to 8°C to the laboratory for extraction of total RNA using the TRIzol reagent (TRI Reagent L.S.).

The concentration of RNA specimens obtained was determined using the Nanondrop spectrophotometer (Thermo Fisher Scientific) by measuring the absorbance at 260 nm. The presence of protein and contamination in RNA specimens was measured at A260/280 and A260/230. If these ratios were <2, then RNA extraction was repeated. The RNA specimens were normalized to a concentration of approximately 500 ng for cDNA synthesis for real-time amplification and EPF detection (EPF) using a fluorometer.

The reverse transcription and amplification for the fusion transcript at baseline and 3, 6, and 12 months was performed using Taqman probe based on real-time PCR using an already described method in previous studies with little modification[12,13] on the Sa Cycler 96 (Sacace Biotechnologies). The cDNA synthesis and DNA amplification were conducted in a 15 μL reaction mixture containing 8 μL of PCR Mix (Invitrogen PCR Super Mix, with buffer, Mg++, dNTPs, and recombinant *Taq*DNA polymerase), 1 μL of Primer mix (forward, reverse primer and probe) (Primer mix ABL gene for internal control or Primer mix BCR-ABL fusion gene for each specimen), 0.25 μL of enzyme RT (SuperScript III Reverse Transcriptase) (Invitrogen), and 6 μL of RNA template.

Various primer combinations of *ABL-2* with *M-BCR* and *BCR-ABL* and *ABL* probes were used.[14,15] Primer and probe sequences for QPCR are given in the next paragraph. All primers and probes were obtained from Integrated DNA Technologies in lyophilized form and stored at −20°C. The primer sequences were verified using the National Center for Biotechnology Information nucleotide Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/). Two forward primers, e-13 and e-14, were used with a common reverse primer, *ABL-2*. A common *BCR-ABL* TaqMan probe complementary to the *ABL* gene was used. A fragment of the *ABL* gene was amplified as an internal control and for normalization using a separate pair of *ABL* primers and probe.

The primer sequences for the *BCR-ABL* fusion gene were as follows:
BCR e13-F GCA TTC CGC TGA CCA TCA ATA A
BCR e14- F CAG CCA CTG GAT TTA AGC AGA GT
ABL-2–R TCC AAC GAG CGG ATT CAC T
BCR-ABL probe:
6-FAM/AAG CCC TTC /ZEN/AGC GGC CAG TAG CAT CT/3 IABkFQ
Primer sequences for *ABL* gene:
ABL-2F GCT GGG TCC CAA GCA ACT AC
ABL-2–R ACA CAG GCC CAT GGT ACC A
ABL probe:
5HEX/TCA CGC CAG/ZEN/TCA ACA GTC TGG AGA AAC A/3IABkFQ
Cycling conditions for QPCR:
Reverse transcription × 1 cycle at 42°C for 15 minutes
Initial denaturation × 1 cycle at 95°C for 10 minutes
PCR cycling × 40 cycles at 95°C for 15 seconds
60°C for 60 seconds

The results were expressed in a cycle threshold (Ct) value in each specimen. The percentage of the *BCR-ABL* transcript was extrapolated from the standard curve made from the dilution of the known positive control (the value of the positive specimen was ascertained using the GeneXpert BCR-ABL; Cepheid) and was expressed as a normalized ratio of the *BCR-ABL* transcript to the control *ABL* gene transcript. In each QPCR batch, the known positive and negative RNA for *BCR-ABL* was included.

At the end of each QPCR, every PCR product in the same 0.2 mL PCR tube was transferred to a fluorometer (GTI PCR Reader, Genetic Technology Instrumentation). Reaction vials of all positive and negative specimens were read in the fluorometer. The amount of green fluorescence (FAM dye) was recorded in the computer software, and the results were expressed in relative fluorescence units (RFU) after subtracting the background fluorescence of a known negative specimen. The same PCR template, primers, probe, and thermocycling conditions were used as described earlier. The estimated expenditures for RNA extraction, reverse transcription PCR (RT-PCR) on conventional thermocycler, and technician time were also calculated as per the local market price and converted into US$ to observe the cost-effectiveness of the method.

Molecular detection and quantification of the *BCR-ABL* fusion gene were performed using QPCR and the EPF method on all specimens. The amount of target DNA was determined by the FL emitted by the FAM green dye. In QPCR, FL measurements were carried out at the end of each thermal cycle, and in EPF, offline FL measurements were carried out using a PCR reader after completion of the PCR.

To approximately calculate the target DNA in unknown specimens using the EPF method, a calibration graph was constructed. The RFU of serial dilutions of known *BCR-ABL* positive RNA were plotted with the value of each dilution.

The sensitivity of the 2 assays for the detection of the fusion transcript were compared by generating a 10-fold serial dilution of known RNA concentration (1/10, 1/100, 1/1000, 1/10,000, 1/100,000). These 5 dilutions and 1 known negative specimen were run using the RT-PCR protocol on both instruments in identical conditions in 5 separate reaction tubes. Repeated measures of specimen dilutions were performed and compared with known negative specimens. Each specimen of a corresponding dilution point was tested in duplicate (n = 10), and the assay response (detected or not detected) was noted.

All statistical calculations were done using SPSS 22 software for descriptive and inferential statistics. Frequency and percentages were calculated for the presence or absence of the transcript. Sensitivity and specificity were calculated using PCR as the gold standard. Results of EPF and QPCR were compared using the Fisher's exact test. Descriptive data were expressed as mean ± standard deviation. Pearson correlations between Ct and RFU values were calculated. The paired *t*-test was used to observe the difference between the mean percentages of the transcript in both methods. A *P* value <.05 was taken as a level of significance. Using the method comparison procedure of Bland and Altman, the bias and 95% limits of agreement between the 2 methods were determined.

Results

One hundred fifty specimens from 30 newly diagnosed patients with CML (18 male and 12 female) in the chronic phase were studied at different stages of treatment (0, 3, 6, 9, and 12 months). The mean age of the patients was 41.2 ± 8.61 years, with an age range of 11 to 70 years.

The qualitative results of QPCR and EPF (transcript status: positive or undetectable) in patients with CML analyzed 5 times at regular treatment intervals are summarized in **TABLE 1**. Analysis of all the specimens taken at the time of diagnosis and after 3 months of treatment showed 100% positivity for the *BCR-ABL* transcript. However, after 6 months of therapy, transcripts disappeared in 3 patients (10%). The *BCR-ABL* transcripts could not be detected in 10 (33%) and 20 (66%) patients after 9 and 12 months of treatment, respectively (**TABLE 1**).

Every specimen with a detectable Ct value using QPCR was considered as positive. For the EPF method, specimens with >20 RFU were considered positive.

TABLE 2 indicates that of the 150 RNA specimens that were analyzed from 30 patients at different treatment intervals, 117 (78%) specimens were found positive, whereas 33 (22%) were found negative for the transcript using both the methods at various stages of treatment.

TABLE 1. QPCR and EPF Results for Detection of *BCR-ABL* Transcript at Different Stages of Treatment

Duration of Treatment	Qualitative			
	QPCR		EPF	
	Positive	Negative	Positive	Negative
Baseline	30 (100%)	0	30 (100%)	0
3 mo	30 (100%)	0	30 (100%)	0
6 mo	27 (90%)	3 (10%)	27 (90%)	3 (10%)
9 mo	20 (67%)	10 (33%)	20 (67%)	10 (33%)
12 mo	10 (33%)	20 (67%)	10 (33%)	20 (67%)

EPF, endpoint fluorescence; QPCR, quantitative real-time polymerase chain reaction.

TABLE 2. QPCR vs EPF in Detecting *BCR-ABL* Transcripts in Patients with CML Undergoing Treatment

EPF	Q PCR		Total
	BCR-ABL (Positive)	BCR-ABL (Negative)	
Positive	117 (TP) a	0 (FP) b	117
Negative	0 (FN) c	33 (TN) d	33
Total	117	33	150

CML, chronic myeloid leukemia; EPF, endpoint fluorescence; FN, false negative; FP, false positive; QPCR, quantitative real-time polymerase chain reaction; TN, true negative; TP, true positive.
Sensitivity = a/a + c × 100% = 117/117 + 0 × 100 = 100%.
Specificity = d/b + d = 33/0 + 33 × 100 = 100%.
P *value = .000.*

A contingency table for the calculation of the sensitivity and specificity of the EPF method, considering QPCR as the gold standard is shown in **TABLE 2**. The sensitivity and specificity for EPF were both 100%. The Fisher's exact test was applied to determine the statistical significance, which was found to be high (*P* value of .000).

In determining the analytical sensitivity, we found that the dilutions that were tested showed that as few as 1 cell in a background of 10^4 normal cells could be detected using both methods repeatedly. The 1/100,000 dilution was consistently negative for the fusion transcript using EPF and QPCR. A dilution of 1/10,000 was consistently positive using both the methods, indicating a limit of detection of 10^{-4} (95% confidence interval).

The quantitative results of QPCR and the EPF method in patients with CML are summarized in **TABLE 3**. The mean Ct value of QPCR varied with the duration of treatment in patients with CML, ranging from 24.56 to 37.2 cycles (see **TABLE 3**). As the duration of treatment increased, the mean Ct increased. The mean Ct was 24.5 at the time of diagnosis and gradually increased to 30.27, 34.25, 36.11, and 37.2 at 3, 6, 9, and 12 months of treatment, respectively (a Ct value is inversely proportional to the amount of target DNA present in a specimen). The QPCR results showed a strong positive correlation between the duration of treatment and the mean Ct values ($r = 0.956$).

The mean RFU value of EPF also altered with the duration of treatment, ranging from 199.06 at the beginning of treatment to 26.50 at the end of 1 year (**TABLE 3**; the RFU value is directly proportional to the amount of target DNA present in a specimen). The EPF results indicated a strong negative correlation with the duration of treatment and the mean RFU values ($r = -0.889$).

Using the EPF method, fluorescence was detected by the PCR reader in every specimen. Keeping in mind the mean EPF results in all the negative specimens (mean, 6 RFU; range, 1–16) and in the highest dilution of the positive DNA specimens (weakest positive specimens; mean, 31 RFU; range, 21–37), the cutoff limit for positive specimens was arbitrarily identified at 20 RFU. This finding was also validated in another study using the same instrument.[10] In all QPCR negative specimens, the EPF method had a clear negative result (<20 RFU).The EPF results in the QPCR positive specimens ranged from 21 to 332 RFU (**TABLE 3**).

The mean *BCR-ABL* percentages at regular treatment intervals in patients with CML were also calculated using both methods (**TABLE 3**). A paired *t*-test was applied on the mean percentages of *BCR-ABL*, and no significant difference was observed between 2 readings using QPCR and the EPF method in all stages of treatment (**TABLE 3**).

TABLE 3. QPCR and EPF Results for Quantification of Detectable *BCR-ABL* Transcripts at Regular Treatment Intervals

Treatment Duration	Mean Ct (QPCR)	Mean RFU (EPF)	Correlation Coefficient (*r* Value)	Mean % of *BCR-ABL*		*P* Value
				QPCR	EPF	
Baseline	24.56 ± 2.36 (20–29.16)	199.063 ± 55.38 (87–332)	–0.96 (*P* < .0001)	81.93 ± 15.7 (45%–100%)	81.19 ± 15.25 (40%–100%)	.366
3 months	30.27 ± 2.01 (26.4–34.1)	79.76 ± 28.87 (35–149)	–0.97 (*P* < .0001)	30.53 ± 15.87 (8%–65%)	30.13 ± 15.16 (5%–60%)	.571
6 months	34.25 ± 2.29 (29.5–38.2)	45.5 ± 19.9 (21–97)	–0.96 (*P* < .0001)	9.73 ± 9.4 (1%–35%)	10.19 ± 10.7 (1%–40%)	.356
9 months	36.11 ± 1.73 (32.4–38.2)	32.8 ± 11.55 (21–59)	–0.98 (*P* < .0001)	4.1 ± 3.7 (1%–15%)	4.89 ± 5.2 (1%–20%)	.09
12 months	37.22 ± 0.92 (35.8–38.2)	26.50 ± 5.31 (21–34)	–0.97 (*P* < .0001)	1.9 ± 0.99 (1%–3%)	1.6 ± 1.49 (0%–5%)	.188

Ct, cycle threshold, EPF, endpoint fluorescence; QPCR, quantitative real-time polymerase chain reaction; RFU, relative fluorescence unit.

FIGURE 1. Correlation of Ct and RFU values of QPCR with EPF in patients with CML at different stages of treatment. CML, chronic myeloid leukemia; Ct, cycle threshold; EPF, endpoint fluorescence; QPCR, quantitative real-time polymerase chain reaction; RFU, relative fluorescence units.

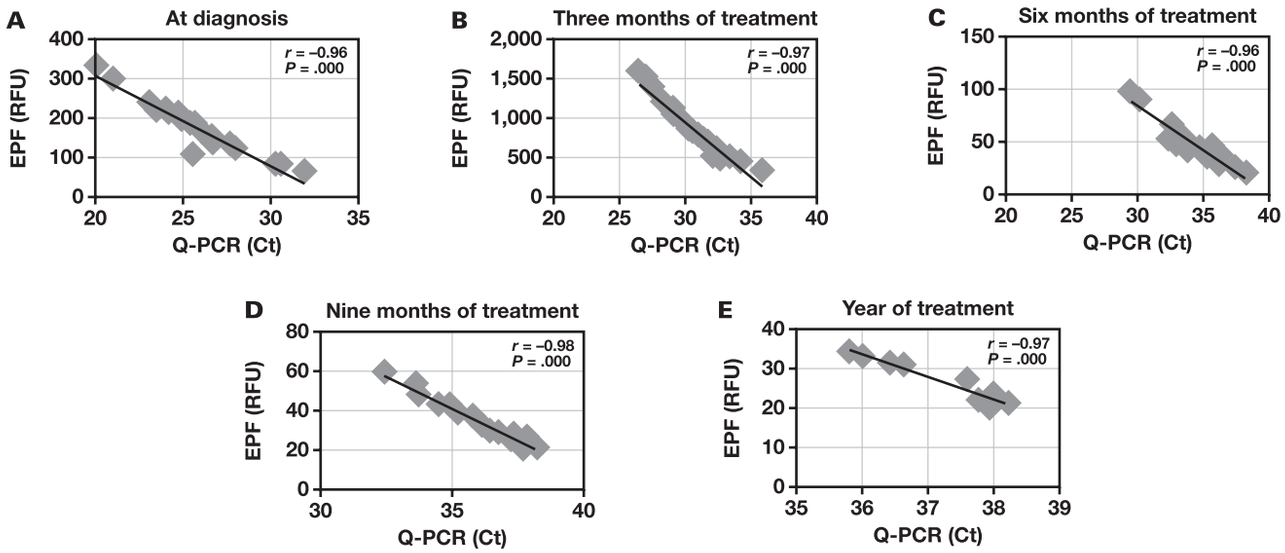
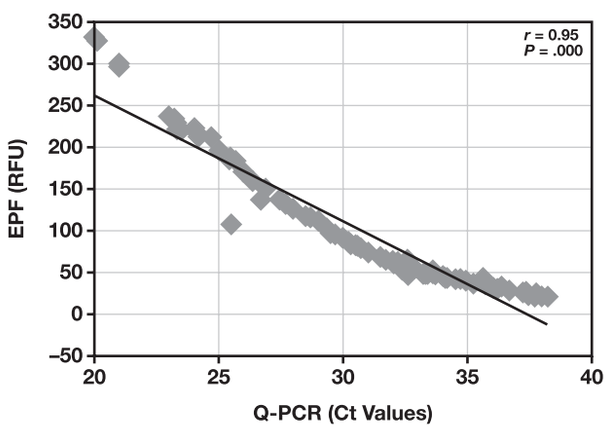


FIGURE 2. Correlation of Ct and RFU values of QPCR with EPF in patients with CML in aggregate. CML, chronic myeloid leukemia; Ct, cycle threshold; EPF, endpoint fluorescence; QPCR, quantitative real-time polymerase chain reaction; RFU, relative fluorescence units.



The Ct values of specimens that were detected as *BCR-ABL* positive using QPCR were correlated with their corresponding RFU values. The correlation curve revealed a strong linear negative association with a correlation coefficient (*r*) of –0.96, –0.97, –0.96, –0.98, and –0.97, respectively, at different stages of treatment (**FIGURE 1**). **FIGURE 2** represents the correlation between Ct values and their corresponding RFU in aggregate.

The values of RFU using EPF were compared directly to the Ct values using RT-PCR. The Ct value was less when the quantity of target DNA was more, and vice versa. The direct comparison of the fluorescence units of the PCR reader with the Ct values of RT-PCR showed an inverse association of signal intensities (the lower the Ct values, the higher the fluorescence; **FIGURE 3**). **FIGURE 3** presents the CT value for a similar amount of target DNA in a specimen and its corresponding RFU value using EPF, which provides a clearer idea about the relations of both displayed units.

A scatterplot was obtained by plotting the difference between the test results of the EPF method and QPCR on the *y* axis and the mean of the percentage transcript using both methods on the *x* axis. Graphing test laboratory values on a Bland-Altman plot showed good agreement

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FIGURE 3. Direct comparison of fluorescence units with Ct values of QPCR (amount of target DNA). Ct, cycle threshold; QPCR, quantitative real-time polymerase chain reaction.

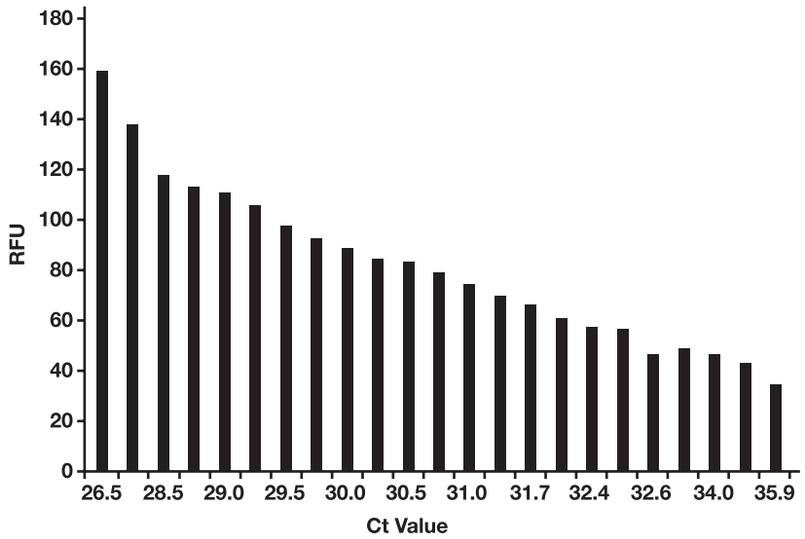
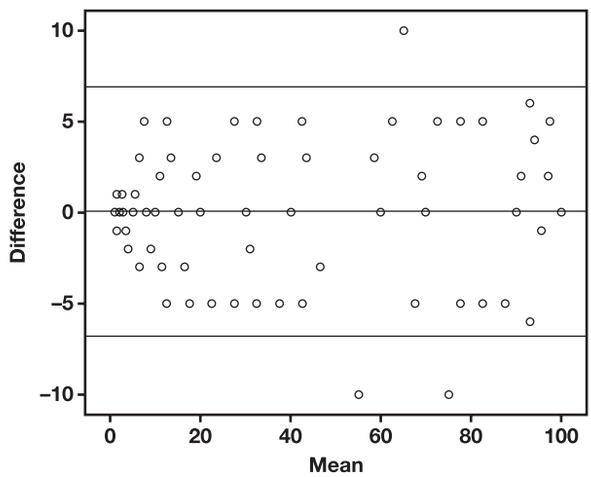


FIGURE 4. Bland-Altman scatterplot of the distribution of the percentage of the *BCR-ABL* transcript in patients with CML measured using QPCR and EPF. The *y* axis shows the mean differences in the percentage of the transcript and the *x* axis shows the average of the percentage of the transcript. The lines represent the mean difference and the upper and lower limits of agreement. CML, chronic myeloid leukemia; EPF, endpoint fluorescence; QPCR, quantitative real-time polymerase chain reaction.



between the methods. The constant bias was 0.069%, the standard deviation was 3.50, and 95% limits of agreement values were found to be from 6.92% to –6.79% (**FIGURE 4**). The cost estimated for RNA extraction, the thermocycler, the RT-PCR, and technician time was estimated to be approximately $10 to $12 per test.

Discussion

The constitutively active oncogenic feature of the *BCR-ABL* gene is targeted by TKI. It is an effective and standard therapeutic option for patients with CML.[16] For the best optimization and outcome of the therapy, it is essential to monitor these patients with treatment.[17]

Therapy progressively decreases the disease burden; therefore, the sensitivity of the technique to measure the residual disease must be increased accordingly.[18] Testing of MRD at the molecular level is performed using QPCR, which is very useful for monitoring different types of hematological neoplasms; however, this method is not easy to use and is confined to specialized laboratories. The PCR technique is much easier to use for qualitative analysis but becomes a complicated procedure when quantification is required.[19] It is also afflicted with interlaboratory variability, resulting in difficulty in the interpretation and comparison of data. To address this problem, international standardization has been proposed but is offered in only few specialized laboratories.[20]

The lack of diagnostic capabilities is an enormous challenge in treating patients with this targeted therapy. The cost of monitoring by QPCR in Pakistan rupee (PKR) is estimated to be Rs 10,000 to Rs 16,000 (approximately $60–$110), depending upon whether a manual or an automated system is used. This high cost becomes more relevant when frequent testing is required with lifelong therapy.[20] The recommended protocol for patients with CML during therapy is quantification of the *BCR-ABL* transcript every 3 months.[21] This schedule becomes quite difficult to follow in our geographic region, owing to the affordability issue for most of our patients. The prices of RT-PCR instruments range from $20,000 to $90,000, depending on the manufacturer, and are therefore not easily affordable for the majority of laboratories. The cost of reagents, supplies, and trained technical staff is also very high. The approximate cost of *BCR-ABL* detection using QPCR, including the cost of the QPCR

instrument, RNA extraction, RT-PCR, and technician time, was found to be $60 in our center.

For EPF, the cost of the PCR reader is approximately $1000. Studies have shown that EPF reading invites no extra expenditure or specialized technical expertise. The cost of the entire procedure, along with the cost of the PCR reader (fluorometer), thermal cycler, RNA extraction, RT-PCR, and technician time, was calculated in US$ as per the local market price and was estimated to be $10 to $12 per test in our center. Meanwhile, *BCR-ABL* monitoring with an automated cartridge-based detection system (GeneXpert; Cepheid) in our country ranges between $100 and $110. The EPF method has a clear edge over QPCR in terms of cost and ease of reporting and can well serve the purpose of molecular monitoring in underresourced countries.

Keeping in mind the above scenario, this study tried to compare the test results of a simple and cost-effective methodology to determine whether it could be a used as an alternative to a QPCR assay for monitoring the MRD of patients with CML. This methodology is based on an approach of performing RT-PCR on any conventional thermocycler (countering the high cost and unavailability issues of real-time instruments) followed by *BCR-ABL* transcript detection and quantification using a fluorometer (PCR reader). Although real-time QPCR provides a direct measurement of the PCR product during the amplification process and also provides an accurate number of targets present,[22] it requires international standardization for the reliable clinical management of patients with CML,[23] which is a logistical and fiscal challenge for many laboratories in our region. The EPF technique uses the same primer/probe combination as in QPCR, but fluorescence is measured at the end of the PCR reaction. We found that EPF was a sensitive and specific method for the detection of the BCR-ABL fusion transcript.

Both methods displayed a strong linear negative correlation ($r = -0.9$) between the Ct and RFU values. The standard curves were later generated to calculate the *BCR-ABL* transcript percentage at different treatment intervals and for direct comparison of the means between the 2 assays. These results established that EPF can reliably quantify the *BCR-ABL* percentage compared with that obtained by QPCR assay. It can quantify *BCR-ABL* concentrations almost as low as 1 copy in 10,000 reference copies, a magnitude much more sensitive than the clinical cutoff values of 1 in 1000 for MRD in patients with CML.

This system may prove beneficial over currently used QPCR or other commercially used *BCR-ABL* fusion detection methods by providing low-cost results, requiring fewer technical skills and offering ease of reporting. Although this assay may not be as ideal and sophisticated as an automated cartridge-based detection system (eg, GeneXpert; Cepheid) and cannot match sensitivity at extremely low copy numbers, it may prove to be accessible and sustainable in the MRD assessment of the majority of patients with CML in low-resource countries.

Conclusion

Molecular methods were used to assess the kinetics of the BCR-ABL transcript in patients with CML undergoing treatment. We found that EPF is a less-complicated and cost-effective method with the same sensitivity and specificity as QPCR in patients with CML. The quantification of the mean transcript percentages at different stages of treatment exhibited no significant difference between QPCR and EPF results. Good agreement was also evident between the 2 methods with a relatively constant mean difference. Therefore, the EPF method may be considered in patients with CML undergoing treatment for the detection and quantification of transcripts.

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