Natančnost in hitrost poročanja o molekularnem odzivu pri KML bolnikih z in vitro diagnostičnim testom Xpert BCR-ABL

Accuracy and speed of molecular response reporting with Xpert BCR-ABL Ultra in vitro diagnostic test in CML patients

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Izvleček

Namen: *Cilj študije je bil uvesti diagnostični test BCR-ABL, ki temelji na GeneXpert Ultra sistemu, za rutinsko molekularno spremljanje minimalne preostale bolezni (MRD) pri bolnikih s kronično mieloidno levkemijo (CML), ki so na zaviralcih tirozin kinaze (TKI).* *Metode: Kvantifikacija transkriptov BCR-ABL1 je bila izvedena v 100 vzorcih periferne krvi 33 bolnikov s CML v različnih fazah bolezni z avtomatiziranim GeneXpert BCR-ABL Ultra testom (Cepheid, ZDA) in s standardiziranim laboratorijskim protokolom (SP).*

Rezultati: *Rezultati obeh analiz so bili izraženi kot % na mednarodni le-*

Abstract

Purpose: *The objective of this study was to introduce the GeneXpert Ultra cartridge-based BCR-ABL diagnostic test into routine molecular monitoring of minimal residual disease for chronic myeloid leukemia (CML) patients who are on tyrosine kinase inhibitors (TKIs).*

Methods: *BCR-ABL1 transcript quantification was performed on 100 peripheral blood samples from 33 CML patients at different stages of the disease using automated GeneXpert BCR-ABL Ultra assay and following a standardized laboratory protocol (SP).*

Results: *Analysis results were expressed as % on the International Scale (IS) for*

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stvici (IS) in so bili ocenjeni z molekularnim odzivom (MR) v skladu z laboratorijskimi priporočili za oceno globokih molekulskih odzivov po zdravljenju kronične mieloične levkemije. Njihova primerjava je pokazala, da ni bilo statistično pomembne razlike v rezultatih, izraženih kot % na IS, p = 0,098. Vzorce glede na ocenjeno MR smo razdelili v 6 skupin (MR[1], MR[2], MR[3], MR[4], MR[4,5] in MR[5]). 74 od 100 vzorcev je imelo z obema metodama popolnoma enak MR. 12 vzorcev je imelo višji MR, pridobljen z GeneXpertom, medtem ko je imelo 14 vzorcev nižji MR v primerjavi s SP. Pri 3 od 74 vzorcev se je GeneXpert razlikoval od SP za dva MR razreda, medtem ko se je pri ostalih razlikoval za en MR razred. Analiza glavnega molekularnega odziva (MMR) je pokazala, da smo dosegli 82 % ujemanje. V osmih vzorcih so bile z GeneXpertom zaznane minimalne količine kopij BCR-ABL1, medtem ko jih SP ni zaznal. Ključno je, da pri nobenem izmed bolnikov rezultat, pridobljen z GeneXpertom, ni vplival na drugačno klinično odločitev o zdravljenju s TKI kot rezultat, ki je bil pridobljen po SP.

Zaključek: *Glede na ugotovitve lahko Xpert BCR-ABL Ultra test štejemo kot koristno in zelo hitro klinično orodje za molekularno spremljanje bolnikov na TKI.*

BCR-ABL1 real-time PCR quantification and estimated using molecular response (MR) according to the laboratory recommendations for scoring deep MRs following CML treatment. A comparison of the two methods showed that there was no statistically significant difference in the results expressed as % on the IS (p = 0.098). Samples were divided into six groups (MR[1], MR[2], MR[3], MR[4], MR[4.5], and MR[5]) according to the estimated MR. A total of 74/100 samples had a completely identical MR with both methods, 12/100 samples had a higher MR obtained with GeneXpert, and 14/100 samples had a lower MR compared to the SP. In addition, 2/74 samples had a different MR for two classes obtained with GeneXpert compared to the SP, while the others 72/74 samples differed in one MR class. Analysis of the major molecular response resulted in 82% agreement. Minimal amounts of BCR-ABL1 copies were detected with GeneExpert in eight samples while the SP did not detect them. None of the GeneXpert results had any effect on the clinical decision for treatment with TKIs obtained using the SP.

Conclusion: *The Xpert BCR-ABL Ultra assay can be considered a useful and very fast clinical tool for molecular follow-up of patients on TKIs.*

INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal malignant disease of pluripotent stem cells directed towards granulopoiesis. According to the World Health Organization (WHO), it is classified as chronic myeloproliferative disease with an annual incidence of 1.6/100,000, where one out of every 62,500 men and women will be diagnosed with CML during their lifetime (1). The average age at diagnosis is 50–60 years, although the disease can occur during all stages of life, even in childhood. Exposure to ionizing radiation is the only known risk factor for the occurrence of the disease. CML accounts for 20–25% of all leukemia cases. It is

the first malignant disease in which a change leading to its development was recognized at a molecular level (2). It is caused by the formation of the Philadelphia chromosome, a reciprocal translocation of the chromosomal parts between chromosomes 9 (9q34.1) and 22 (22q11.2), resulting in the formation of the BCR-ABL1 fusion gene. The resulting fusion oncogene carries the BCR-ABL1 (tyrosine kinase) protein that is responsible for the development of CML because it maintains the activity of the signaling pathway in the cell, thereby accelerating the proliferation of stem cells, interfering with maturation, and preventing apoptosis. Genetically modified cells overwhelm the bone marrow. Genetic instability of the cell clone may cause resistance to the treatment and progression of the disease to secondary acute leukemia. Therefore, BCR-ABL1 can be employed as a specific leukemic marker for minimal residual disease (MRD) follow-up (3). After treatment with tyrosine kinase inhibitors (TKIs), BCR-ABL1 quantification may predict the outcome in CML patients, especially in those who achieve a complete cytogenetic response (4).

Monitoring of BCR-ABL transcript levels with real-time quantitative polymerase chain reaction (qRT-PCR) in patients on TKI therapy is the standard of care in the management of CML. Furthermore, it is very important to achieve sensitivity of 4.5 logs below baseline in patients who are considered for TKI discontinuation (5). In order to assess the treatment response, it is essential to ensure that variability in testing methodologies is tightly controlled. This was the cause of BCR-ABL molecular measurement standardization across laboratories, which introduced the International Scale (IS) defined by WHO (5).

The GeneXpert system utilizes microfluidics to directly process peripheral blood samples in a cartridge for RNA extraction, reverse transcriptase PCR (RT-PCR), and fluorescence detection in an "all-in-one" self-contained instrument (6). The GeneXpert BCR-ABL system uses ABL1 as its control gene, which is recommended for molecular response (MR) follow-up. It calculates the BCR-ABL1/ABL1 ratio using the delta-crossing threshold (Ct) comparative-quantitative method (7). Jobbagy et al. and Winn-Deen et al. performed an early comparison of the GeneXpert assay with standard qRT-PCR assay and found that the system had the potential to translate into clinical practice despite the differences in quantitative methods (8, 9). However, the system did not report BCR-ABL1 values on the IS and result interpretation and inter-laboratory comparison were hindered. Enjeti et al. developed a conversion factor specific to GeneXpert assay and used it to adjust the BCR-ABL1/ABL1 ratio to the IS. A batch-specific standard curve and an efficiency value were also introduced to ensure cartridge lot-specific calibration (10). This very important quality control assay improvement enables inter-laboratory comparison. It also achieved an accurate measurement of 0.01% of BCR-ABL1 on the IS, a level sufficient to detect MR4 (molecular response at 4-log reduction) (11, 12).

In our study, we introduced the GeneXpert Ultra cartridge-based BCR-ABL diagnostic test into routine molecular monitoring of MRD for CML patients who are on TKIs.

MATERIALS AND METHODS

Study sample

Peripheral blood samples were obtained from CML patients on TKI therapy referred to our laboratory of Medical Genetics from the Department of Haematology and Haematological Oncology, Clinic for Internal Medicine, University Medical Centre Maribor, Maribor, Slovenia for monitoring of BCR-ABL transcript levels. All samples were from individuals of Caucasian descent who were residents of different geographical areas of east Slovenia. The study was approved by the ethical committee (No. UKC-MB-KME-24-08/17). Informed consent was obtained from all participating individuals.

Assay design

An automated GeneXpert BCR–ABL Ultra cartridge System (Cepheid Europe S.A.S., Vira-Solelh, Maurens-Scopont, France) consists of an instrument and a personal computer with software for running tests and viewing results. It simplifies generation of very accurate and reproducible results using integration of nucleic acid extraction, target amplification, and quantification of the BCR-ABL e13a2/b2a2 and e14a2/b3a2 transcripts relative to ABL in peripheral blood samples. Disposable GeneXpert cartridges

contain the RT-PCR and nested PCR reagents and host the RT-PCR and nested PCR processes. Cross-contamination concerns are minimized because the cartridges are self-contained.

Assay procedure

Peripheral venous blood samples were collected in standard vacutainer tubes containing ethylenediamine tetraacetic acid (EDTA) anticoagulant. A total of 4 mL of whole peripheral blood was mixed with 100 µL of proteinase K (Cepheid Europe S.A.S.). After 1 min of incubation at room temperature, 2.5 mL of lysis reagent (Cepheid Europe S.A.S.) was added to inactivate nucleases in order to release nucleic acids from the cells, followed by vortexing and two incubation periods of 5 min at room temperature. After the second incubation, 1 mL of lysate was transferred to a new tube and mixed with 1.5 mL of lysis reagent. After 10 min of incubation at room temperature, 2 mL of pure ethanol (Merck KgaA, Darmstadt, Germany) was added to the sample and 4.5 mL of mixture was transferred to the test cartridge. Washing reagent (Cepheid Europe S.A.S.) was then added to the designated port in the test cartridge, the lid was closed, and the cartridge was loaded into the GeneXpert System. The GeneXpert System isolates the total RNA and runs a nested RT-PCR. Total assay time was approximately 2 h and 20 min.

Assay quality control

Each GeneXpert BCR-ABL Ultra kit lot is standardized using in-house secondary standards calibrated according to the WHO (Figure 1).

A lot-specific conversation factor is applied to each lot (Figure 2).

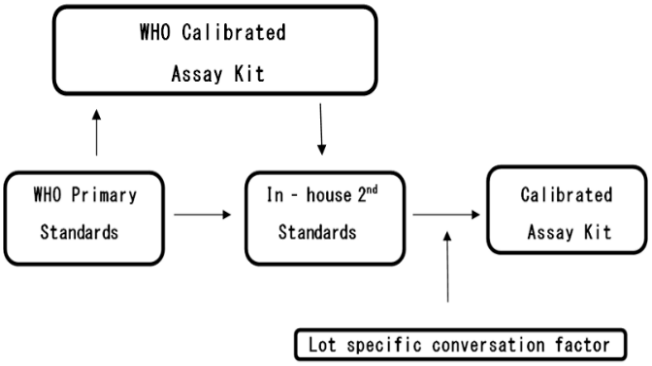

Figure 2. *Calibration to WHO IS.*

Statistical Analysis

MRs were compared using the t-test, where $p \leq 0.05$ was considered statistically significant.

RESULTS

BCR-ABL1 transcript quantification was performed on 100 peripheral blood samples from 33 CML patients at different stages of the disease in two different laboratories. The first method used the automated GeneXpert BCR-ABL Ultra assay (Cepheid Europe S.A.S.), while the second used a standardized laboratory protocol (SP). The blood samples were collected over three years in the period from 2017 to 2020.

The negative control group included 14 blood samples obtained from patients with hematological disorders

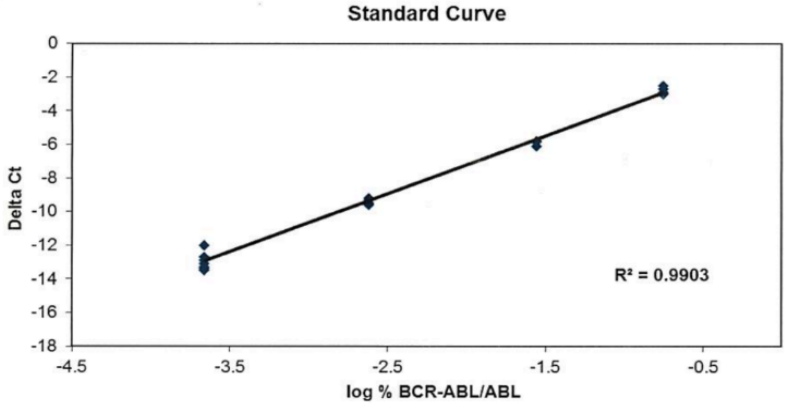

Figure 1. *Sample of the BCR-ABL ΔCt standard curve. EΔCt=1.96 (lot-specific efficiency value) is derived from the slope of the standard curve; IS-SF = 1.26 (lot-specific scaling factor) corrects the quantitative output of the assay to the international scale established based on WHO International Genetic Reference Panel.*

unrelated to BCR-ABL gene fusion. The negative predictive value was 100%. A total of 74 of 100 (74%) samples had a completely identical MR using both methods (Table 1). Furthermore, 12 of 100 samples had a higher MR obtained with GeneXpert, while 14 of 100 samples had a lower MR compared to the SP. Analysis by intervals did not show any significant difference in concordance (p = 0.098).

Table 1. *Concordance between standardized laboratory protocol (SP) and GeneXpert Ultra assay results at different logarithmic intervals and overall.*

MR (%IS)	Number of samples at the interval		Concordance between methods All samples
	SP	GeneXpert Ultra	
MR[1] (>1)	7	5	5/7 (71 %)
MR[2] (≤1)	5 (50.0)	34 (18.7)	6/6 (100 %)
MR[3] (≤0.1)	0 (0.0)	22 (12.1)	13/16 (81 %)
MR[4] (≤0.01)	0 (0.0)	13 (7.1)	5/7 (71 %)
MR[4.5] (≤0.0032)	10 (100.0)	182 (100.0)	4/6 (67 %)
MR[5] (≤0.001)	0 (0.0)	13 (7.1)	41/50(82 %)
Not detected	10 (100.0)	182 (100.0)	0/8 (0 %)
Total	0 (0.0)	13 (7.1)	74/100 (74 %)

Furthermore, only two of 74 (2.7%) samples had a different MR for two classes obtained with GeneXpert compared to the SP. The others 72 samples differed in one MR class. Analysis of the major molecular response (MMR) resulted in 82% agreement (Table 1). Minimal amounts of BCR-ABL1 copies were detected in eight samples that were not detected by the SP (Table 1). None of the GeneXpert results had any effect on the clinical decision for treatment with TKIs obtained using the SP.

DISCUSSION

A major advantage of the GeneXpert platform is its simplified work flow, which takes approximately 2 h and 20 min. The system thus provides a faster turnaround, reduces hands-on time, and requires less technical expertise compared to the standard BCR-ABL1 qRT-PCR assay. The GeneXpert automates both the pre-analytical and analytical phases. The risk of contamination with the equipment or with other samples is insignificant because the assay is performed inside a closed cartridge. All of these factors support the choice of GeneXpert for BCR-ABL1 quantification and CML monitoring. Cepheid has also introduced the GeneXpert BCR-ABL Ultra, a high-sensitivity assay, which demonstrates a limit of detection reaching MR4.5 and below (13). BCR-ABL assays that are sensitive in their measurement of deep-level response may aid in the identification of potential candidates for treatment discontinuation. GeneXpert BCR-ABL Ultra detects the most common BCR-ABL transcripts below MR4.5 or 0.0032%, which is widely accepted as the clinical threshold that defines candidates who can safely discontinue TKI therapy (13).

This important GeneXpert BCR-ABL1 assay improvement has led our laboratory to introduce the system into routine molecular monitoring of MRD for CML patients who are on TKIs, because the ability to consistently detect low levels of BCR-ABL transcripts in CML patients is important for the assessment of treatment outcomes in patients on TKI therapy. For that reason, GeneXpert BCR-ABL Ultra results in the present study were compared to the SP results. The concordance analysis showed that 74% of samples agreed at the same logarithmic interval and had a similar distribution of GeneXpert disagreement in values above and below those obtained using the SP. Analysis by intervals did not show any significant difference in concordance (p = 0.098). Analysis of MMR showed an 82% agreement. MR was demonstrated to be properly determined using GeneXpert when compared to the SP. Our results also highlight the fact that GeneXpert was able to detect a low number of copies in cases that turned out to be negative when determined using the SP. This indicated a higher sensitivity as a result of the fact that GeneXpert provides a more sensitive

assay (nested PCR) that is able to detect reduction of up to 5 log in BCR-ABL1 transcript concentrations. The negative control group also had a high specificity (100%) using GeneXpert.

It can therefore be concluded that the GeneXpert BCR-ABL Ultra assay is a reproducible and robust alternative to laboratory-developed assays. Its ease of use may allow some laboratories to offer BCR-ABL testing to the patients they serve. It is also important that the short assay time enables same-day results for treating clinicians. Such fast sample turnaround can reduce patient anxiety and allow a faster treatment adjustment if there is an increase in BCR-ABL concentration.

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