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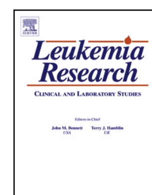

Research paper

Cepheid xpert monitor platform for the confirmation of *BCR-ABL1* IS conversion factors for the molecular monitoring of chronic myeloid leukaemia

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ABSTRACT

Molecular monitoring of *BCR-ABL1* expression in chronic myeloid leukaemia (CML) is well established. As the International Scale (IS) normalised *BCR-ABL1/ABL1* ratio at 3 months post-treatment is now an important milestone in patients' treatment schedule, the reliable and reproducible measurement of *BCR-ABL1* levels is therefore paramount. IS conversion factors (CF) are established via sample exchange and yearly ratification with an external reference laboratory. Since any change to an established IS CF could lead to discontinuity in longitudinal results, we wished to add an internal verification step as an additional safeguard. We used the Cepheid GeneXpert qPCR and IS calibrated Xpert BCR-ABL Monitor cartridge system, parallel to our in-house pipeline on 50 CML samples, over the period of one week to verify the CF for those samples and compare it to the externally provided CF. The median non-IS in-house *BCR-ABL1/ABL1* values were significantly different than that from the IS GeneXpert, but they became non-significant when adjusted to CF provided by the CXM and by the current external CF, validating it. These metrics can help decide to accept or reject an updated CF value, especially where a significant change in CF might lead to a discontinuity in ongoing patient monitoring.

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1. Introduction

Chronic myeloid leukaemia (CML) is viewed as a model malignancy, since only a single genomic event, the t(9;22)(q34;q11) translocation and resultant *BCR-ABL1* fusion gene, is considered necessary for leukemogenesis [1]. Likewise, the use of molecular monitoring using real-time quantitative polymerase chain reaction (RT-qPCR) was pioneered in CML, initially as an early warning of relapse following allogeneic stem cell transplantation (ASCT), but subsequently as the model system for measuring minimal residual disease (MRD). There are numerous platforms available for the molecular monitoring of *BCR-ABL1* by RT-qPCR, with the majority based on the use of dual-labelled hydrolysis probes (TaqMan; Life Technologies, Carlsabad, USA) and primers flanking the junctional region of the first-strand synthesis converted cDNA product of total extracted RNA. However, the individual workflows, conversion kits, hardware and informatics software vary greatly between monitoring centres. Because of this inherent heterogeneity, much effort has been made to standardise the workflow and data interpretation between centres; both nationally and internationally [2,3]. One aspect of this inter-centre standardisation is the concept of the International Scale (IS), based on a set of control samples used for calibration during the IRIS trial, and the application of conversion factors (CF) derived for each centre to allow them to report on the IS [4]. These CFs are administered by designated reference laboratories and must be re-validated by means of sample exchange every one or two years. Because this methodology represents a 'snap-shot' of the multiple factors involved and therefore may be prone to temporal error effects, we wished to use an internal control to corroborate any change in CF, especially since accurate reporting of the 3 month molecular results is critical because treatment change may be enacted by a greater than 10% *BCR-ABL1/ABL1* IS at this juncture [5]. We investigated the potential to use the Cepheid

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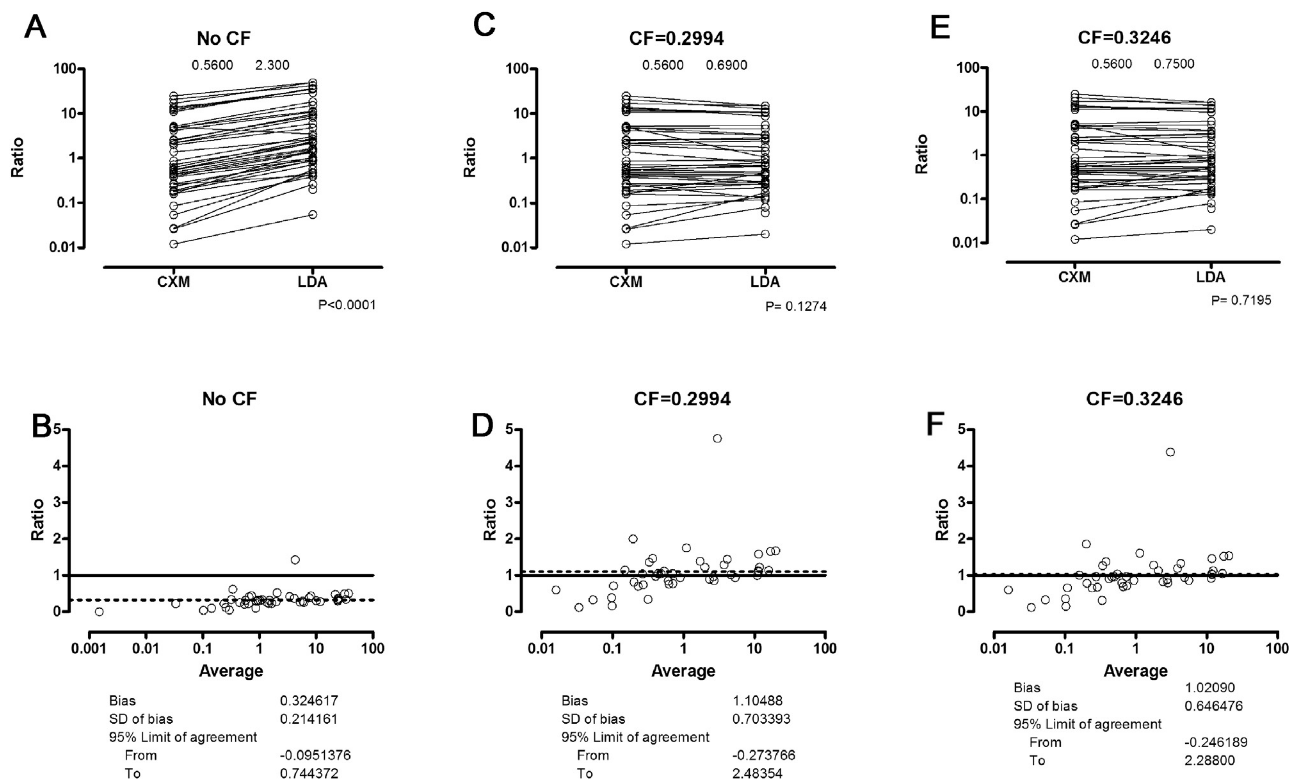

Fig. 1. Statistical analysis between two RT-qPCR platforms: CXM = Cepheid Xpert Monitor and LDA = Local Diagnostic Assay.
Graphs A, C and E show the median, interquartile ranges and results of Wilcoxon tests between the median%*BCR-ABL1/ABL1* ratios with different International Scale (IS) conversion factors (CF) applied: A = No CF; C = extant CF (0.2994); E = No CF ratio bias (CF = 0.3246). Graphs B, D and E show Bland-Altman analyses with associated ratio biases: B = No CF; D = CF 0.2994; E = CF 0.3246; the solid line represents the ratio of equivalence (=1) and the broken line the ratio bias. The 95% limits of agreement are given numerically below the graphs.

GeneXpert qPCR and Xpert BCR-ABL Monitor cartridge system as an internal control, by virtue of its low material input requirements and because it, by default, reports on the IS without the need for further external calibration [6,7].

2. Methods

EDTA peripheral blood (PB) samples from 50 patients with chronic-phase CML were analysed in parallel, using the 4-module GeneXpert platform and Xpert BCR-ABL Monitor (V1i) system (Cepheid, Sunnyvale, CA, USA) and an in-house workflow in a nationally accredited clinical diagnostic laboratory with an externally calibrated IS CF (Scientific Laboratory, Faculty of Medicine, University of Heidelberg, Pettenkoferstr 22, 68169, Mannheim, Germany). The samples were selected based on prior molecular monitoring results as 'intermediate' level positive, i.e., with target *BCR-ABL1/ABL1* levels between 0.1% and 10% (which represents the validated dynamic range of the GeneXpert platform). Each sample was processed in parallel: 500 μl PB aliquoted for the Cepheid Xpert Monitor (CXM) and the remainder for the local diagnostic assay (LDA).

The CXM workflow was performed according to the manufacturer's instructions: 200 μl PB was placed in lysis buffer, vortexed and incubated at room temperature for 10 min. 1 ml of 100% ethanol was added to precipitate the nucleic acids and this mix was then placed into the CXM cartridge, which was inserted into the CXM qPCR platform and qPCR was performed using pre-set cycling conditions. Cq calling was by the on-board software and data was generated as%*BCR-ABL1/ABL1* IS via an internal batch-controlled CF.

The LDA was based on the Europe Against Cancer (EAC) *BCR-ABL1* workflow, modified to allow the use of duplexing via differentially labelled TaqMan MGB probes (6-FAM for *BCR-ABL1* and VIC for *ABL1*) [8,9]. In brief, PB samples were incubated twice at 4 °C with pre-cooled red cell lysis buffer and once with PBS at RT; between each step the samples were centrifuged at 1800 RPM for 7mins. The tubes were then inverted on paper towels to remove all supernatant and the white cell pellets disrupted in RLT chaotropic lysis buffer (Qiagen RNeasy Mini Kit; Qiagen, Venlo, Netherlands). After overnight storage at −20 °C, RNA was extracted using 350 μl of the lysates with a 60 μl elution volume, in an automated workflow (RNeasy Mini Kit and QIAcube robotic platform; Qiagen). The total volume of RNA was taken through to first-strand synthesis of cDNA, using MMLV reverse transcriptase and random hexamer primers (both Invitrogen, Carlsbad, CA, USA), with a final volume of 100 μl.

RT-qPCR was performed on the ABI 7900HT platform, using 3 μl of cDNA in 20 μl reactions, with modified fast-mode cycling conditions, as previously described [8]. Cq calling was by the onboard SDS 4.4 software, with a cycling threshold of 1.2. Quality control was according to the UK guidelines and included no template control (NTC), buffer blank, and high and low *BCR-ABL1* cell line controls. Post-run quality controls included slope, intercept and replicate monitoring [3].

Statistical analysis (Wilcoxon matched pairs test, and Bland-Altman) was performed using Graphpad Prism 4.2. The Bland-Altman ratio bias between the CXM and LDA was used to generate a confirmatory CF for the LDA, with the CXM as reference. The 95% limits of agreement (95% LoA) were used to assess CF concordance, with overlap with the ratio of equivalence (RoE; = 1) denoting a non-significant difference.

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