Monitoring BCR-ABL transcript levels by real-time quantitative polymerase chain reaction: a linear regression equation to convert from BCR-ABL/B2M ratio to estimated BCR-ABL/ABL ratio

In order to overcome the problem of different control genes for BCR-ABL normalization, we used a linear regression equation to compare our results previously obtained using B2M as the control gene with those calculated using the ABL gene and validated the slope as a factor to convert from B2M to ABL results.

Real-time quantitative polymerase chain reaction (RQ-PCR) of BCR-ABL hybrid transcripts is now the standard method for monitoring the response to treatment in patients with chronic myeloid leukemia (CML) who have been induced into complete cytogenetic remission. Recently, various methods based on RQ-PCR have been adopted to monitor residual disease in clinical studies. An attempt to standardize this methodology was made by the comprehensive work of a European network of experienced laboratories, resulting in the suggestion to use three different control genes, glucuronidase (GUS), Abelson (ABL) and β-2 microglobulin (B2M), to correct quantitative analyses for RNA quality and quantity variations. A suitable control gene should (i) have an expression level broadly similar to that of BCR-ABL at diagnosis of CML; (ii) have a stability similar to that of BCR-ABL; (iii) not have any pseudogenes, in order to avoid any genomic amplification; and (iv) have the same expression in bone marrow and peripheral blood. The GIMEMA CML Working Party adopted B2M as the control gene in studies evaluating minimal residual disease until 2004, when, according to the current recommendation, it was replaced by ABL.

In order to compare results previously obtained using the B2M gene and those obtained using the ABL gene, we performed RQ-PCR on 50 peripheral blood or bone marrow fresh and stored leukemic samples in duplicate with both ABL and B2M as the control genes. The main aim was to find a conversion factor potentially useful for meta-analysis purposes to convert values obtained with B2M to the same scale as those using ABL as the control gene. We choose samples both at diagnosis and during treatment with minimal residual disease to have BCR-ABL levels that covered at least a 3-log range, particularly at low levels. Molecular analysis was performed using a standardized RQ-PCR method that was established in the framework of the UE Concerted Action.

All samples processed were evaluable. The Ct values of B2M ranged from 16.31 to 23.62, while those of ABL ranged from 24.02 to 29.52. BCR-ABL/B2M ratios ranged from 1.04×10^-7 to 9.44×10^-2 and BCR-ABL/ABL ratios ranged from 24.02 to 29.52. In order to find the model that best fitted the data, BCR-ABL/B2M and BCR-ABL/ABL ratio results were plotted against each other in a scatter plot graph, then basic descriptive analysis was performed (median, range, standard deviation) and the linear regression equation was generated: BCR-ABL/ABL ratio=57.74×Ratio x 100 B2M ratio (Figure 1), with a highly significant r²=0.9703 (F=1307, p<0.001). The line was forced to 0 to null the intercept as the BCR-ABL/B2M ratio never assumes the value of 0, and the same happens for BCR-ABL/ABL ratios, even if these were samples with undetectable levels of BCR-ABL transcript (under the limit of sensitivity). Thus, while maintaining its mathematical importance in the calculation of the regression line, the use of the intercept in the estimate of the ABL ratio fails to best describe the biological phenomenon under study.

We could use just the angular coefficient of the regression line to predict BCR-ABL/ABL ratio values from BCR-ABL/B2M ratios by applying the formula: BCR-ABL/ABL ratio=57.74×BCR-ABL/B2M ratio.

Since mRNA degradation can occur after the shipment of samples, resulting in altered stability or altered expression of transcripts, we analyzed both stored and fresh samples processed immediately after sampling. It is important to note that we found the same estimated conversion factor between BCR-ABL/ABL and BCR-ABL/B2M comparing both fresh samples and stored samples.

To validate our model we matched BCR-ABL/ABL ratios obtained from the RQ-PCR analysis with those estimated by the regression line. The medians of the two groups are statistically the same (0.102 versus 0.118,
The ranges of the two groups overlap, as shown in Figure 2. To check the validation of our model we analyzed, calculated and estimated *BCR-ABL/ABL* ratios in relation to molecular response. This is very important to ensure that the conversion by our model does not change the clinical assessment of patients. If a major molecular response was considered as a *BCR-ABL/ABL* ratio less than 0.10, there were 21 samples with such a response according to both the calculated and estimated ratios. Furthermore, samples with a *BCR-ABL/ABL* ratio less than 0.001 also maintained their values with the estimated ratio. These data are very encouraging, since a way to transform data previously calculated using *B2M* as the control gene was needed. Our model allows us: (i) to determine whether *BCR-ABL* transcript levels decrease or increase with respect to previous quantifications using *B2M*, even if we now use a different control gene, and (ii) to evaluate long term molecular response.

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Key words: *BCR-ABL* ratio, RT-PCR.

Funding: Supported by European LeukemiaNet, COFIN 2003 (M. Baccarani), AIRC, Fondazione Del Monte di Bologna e Ravenna, FIRB 2001 and Ateneo 60% grants.

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