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.

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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.[1] 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,[2] 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.[3] The GIMEMA CML Working Party adopted *B2M* as the control gene in studies evaluating minimal residual disease until 2004,[4-6] when, according to the current recommendation,[3] 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.[7]

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 $1.04x10^{-8}$ to 0.80 and *BCR-ABL/ABL* ratios ranged from $9.44x10^{-7}$ to 45.18. 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=0.121+57.74**BCR-ABL/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

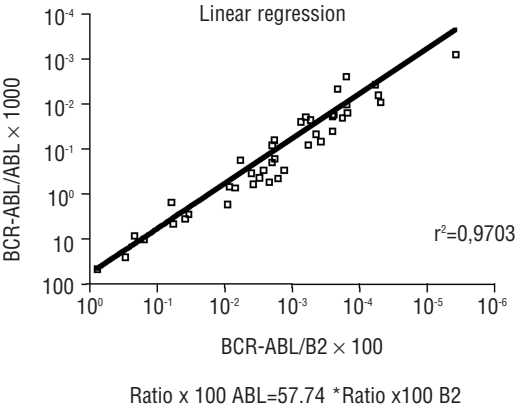

Figure 1. Derivation of estimated *BCR-ABL/ABL* ratios. *BCR-ABL/B2M* ratios obtained by RQ-PCR analysis were plotted against *BCR-ABL/ABL* ratios also obtained by RQ-PCR analysis. The slope of the linear regression equation was used to derivate the estimated *BCR-ABL/ABL* ratios, applying the formula: *BCR-ABL/ABL* ratio=57.74**BCR-ABL/B2M*.

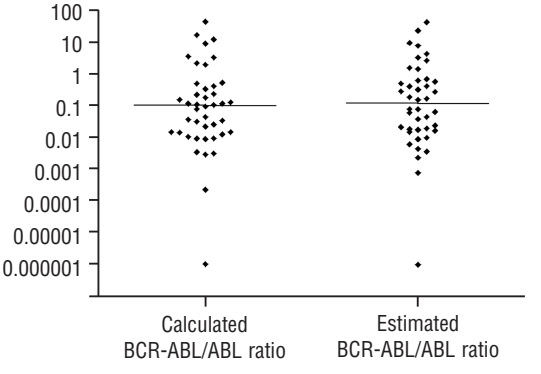

Figure 2. Comparison of BCR-ABL/ABL results before and after conversion. The median values for the two groups were statistically the same (0.102 versus 0.118, p=0.9441) and the ranges overlap.

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,[8] 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,

p=0.9441) and 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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