UK NEQAS UNITED KINGDOM NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEMES FOR LEUCOCYTE IMMUNOPHENOTYPING

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BCR-ABL1 Quantitation: To IS or not to IS, that is the Question?

J R. Clark, A L. Jack, J L. Holden, D. Barnett and J T. Reilly

UK NEQAS for Leucocyte Immunophenotyping (UK NEQAS LI) , Sheffield, UK

INTRODUCTION

Reverse-transcription real-time quantitative polymerase chain reaction (RQ-PCR) measurement of *BCR-ABL1* oncogene expression is now a routine monitoring strategy for tyrosine kinase inhibitor (TKI) treated chronic myeloid leukaemia. A major molecular response of at least a 3-log reduction in *BCR-ABL1* expression is associated with prolonged disease free survival. Data from our UK NEQAS LI *BCR-ABL1* quantitation programme has shown, that from 389 results over a 4 year period, there is an average 105% inter-laboratory CV (Range 78%-130%). Recently, to overcome this problem, a new International Scale (IS) for *BCR-ABL1* measurement has been proposed. The aim of this study was to retrospectively analyse whether the use of the IS significantly reduced this variability and therefore improved inter-laboratory comparison.

METHODS

- UK NEQAS LI issued stable lyophilized cell line samples to over 100 laboratories worldwide for *BCR-ABL1* quantitation.
- The consensus median for %*BCR-ABL1*/control gene and the %*BCR-ABL1*/control gene (IS) were calculated from the returned results.
- The difference between each individual laboratory's result and the consensus median (delta) was determined for each pre- and post-conversion %*BCR-ABL1*/control gene result.
- This approach allowed us to determine whether IS conversion brought individual laboratory's results closer to, or further, from the median value.

RESULTS

- 122 paired results were analyzed from 8 different samples.
- Many laboratories were inappropriately converting to IS values when *BCR-ABL1*/control gene values were greater than 10% (note IS has not been validated for levels >10% due to the non-linearity of the *BCR-ABL1* and *ABL1* control gene relationship, Cross *et al* (2009)[1]). A fact that may relate to the failure to validate local conversion factors, as recommended by Branford *et al* (2008)[2].
- For samples with a consensus median ≤10%, the number of results that moved closer to the median, post conversion, was 57% (n=33), whilst unexpectedly 43% (n=25) moved away (Figure 1).
- The average pre conversion factor delta, minus post conversion factor delta was 0.047 for unconverted and 0.077 for IS. A paired t-test showed no significant difference (p=0.2095) (Figure 2)
- The average CV for all samples with a consensus median ≤ 10% was 72.1% (Range 48.5%-112.1%) for unconverted data and 99.7% (Range 34.5%-140.7%) for IS data

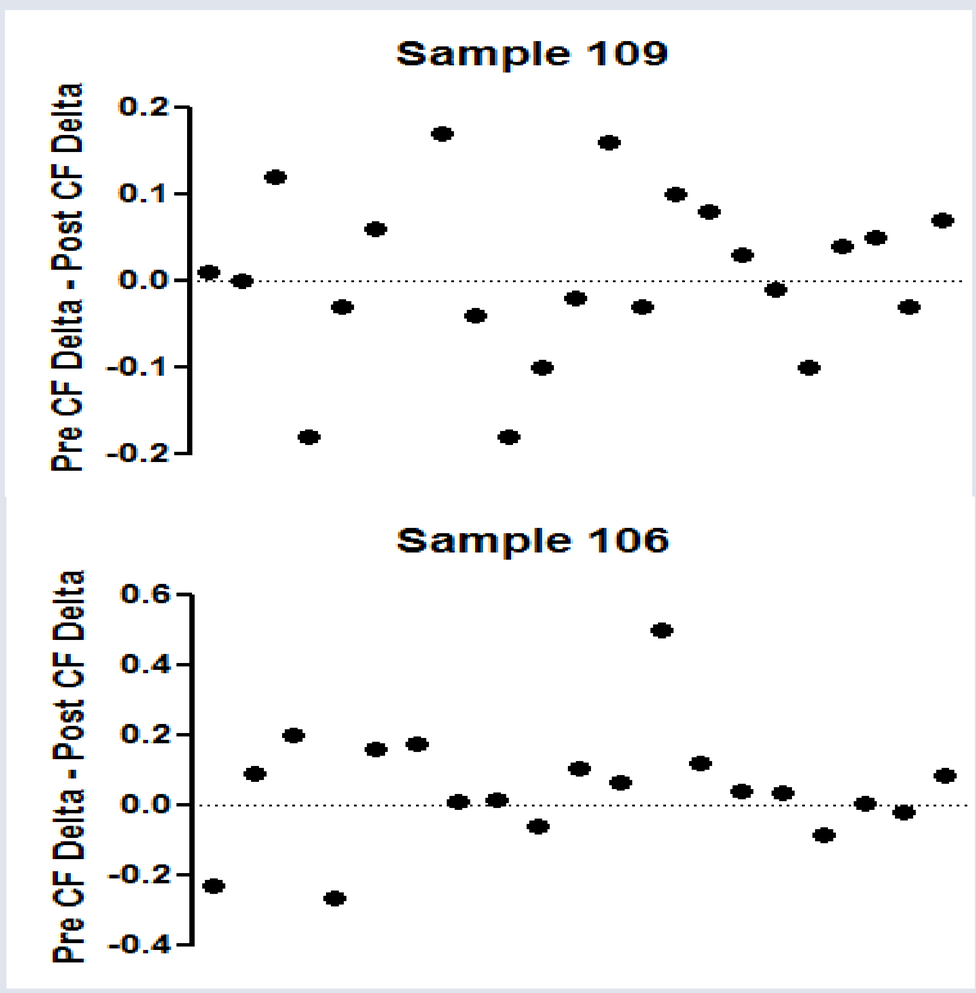

Figure 1 Graph showing the pre conversion factor (CF) delta minus post conversion factor (CF) delta. Positive numbers equate to results closer to the median on IS. Whilst negative numbers equate to pre converted results being closer to the median

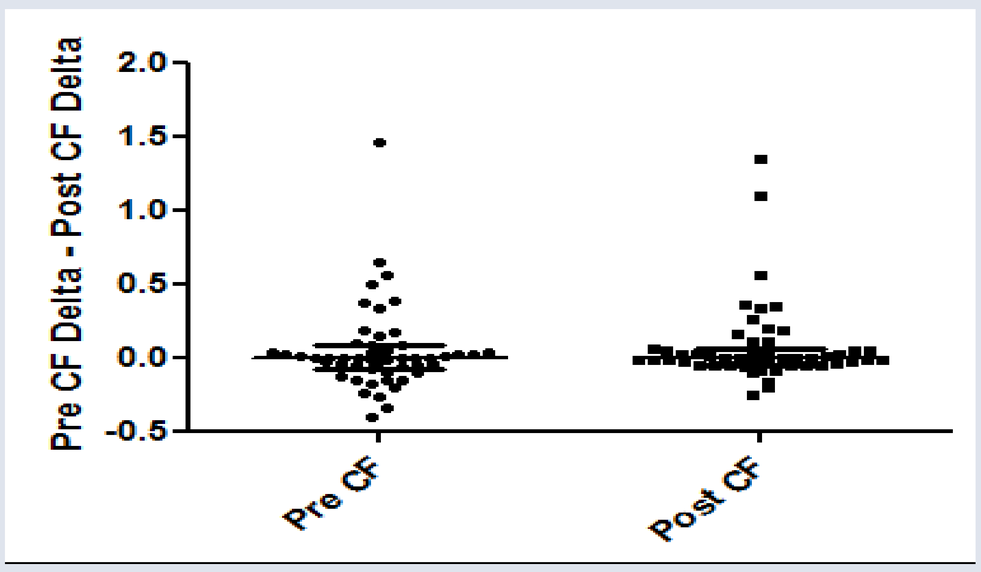

Figure 2 Graph showing the pre conversion factor (CF) delta minus the post conversion factor (CF) delta grouped for all samples with a consensus median ≤ 10%

CONCLUSION

- This study has shown that the use of the IS has not reduced inter-laboratory variability.
- Our findings show that inter-laboratory variation is a topic that still needs to be addressed.
- Future development of reference reagents should help and hopefully lead to better internal quality control systems and facilitate comparative inter-laboratory studies.
- This study has highlighted the need for further education and guidance on conversion factor determination/usage and IS application.

References

1. N. Cross *et al*. Standardisation of molecular monitoring for chronic myeloid leukaemia. *Best Pract Res Clin Haematol* 2009;22:355-365
2. S. Branford *et al*. Desirable performance characteristics for *BCR-ABL* measurement on an international reporting scale to allow consistent interpretation of individual patients response and comparison of response rates between clinical trials. Blood. 2008; 112: 3330-3338.