



Use of Quantitative PCR in the Monitoring of Patients with Chronic Myelogenous Leukemia

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The *BCR-ABL1* fusion, resulting from the reciprocal translocation $t(9;22)(q34;q11)$, produces a constitutively active tyrosine kinase. In the right hematologic context, the finding of a *BCR-ABL1* fusion is diagnostic of chronic myelogenous leukemia (CML). The use of imatinib and later-generation tyrosine kinase inhibitors (TKIs) in recent years has revolutionized the treatment of patients with CML. The majority of chronic phase CML patients treated with TKIs will achieve a complete cytogenetic response (zero chromosomes with a *BCR-ABL1* fusion detected in bone marrow metaphases); therefore, more sensitive polymerase chain reaction (PCR) based methods are often used for monitoring subsequent low levels of disease.

Real-time quantitative reverse transcription PCR (RQ-PCR) is used at many centers as an adjunct to hematologic and cytogenetic monitoring. Although laboratories use a variety of methods and reagents, the underlying principles are generally the same. RNA is extracted from peripheral blood or bone marrow and reverse transcribed into cDNA. Real-time PCR with fluorescent probes is then used to quantify *BCR-ABL1* and a control gene. The results are expressed as a ratio of *BCR-ABL1* to the control gene.

RQ-PCR assays are technically challenging to perform accurately and precisely, and it is necessary to understand these limitations to use them optimally. Changes in *BCR-ABL1* levels between serial specimens may represent variation related to specimen collection, transport, or testing, rather than changes in disease burden. These seemingly minor variations in collection and testing may result in changes of *BCR-ABL1* levels of up to 0.5 log (about three-fold) or greater. Consequently, changes of less than 0.5 log are not considered significantly different in most laboratories; and for changes greater than 0.5 log, it is advisable to repeat RQ-PCR testing or confirm the results with other methods prior to changing clinical management. Furthermore, due to the variety of methods, reagents, and control genes used, results from different laboratories are generally not directly comparable; therefore, it is recommended that the same laboratory test a patient's serial specimens. Finally, some patients have significantly different levels of *BCR-ABL1* in the peripheral blood and bone marrow, so it is necessary to compare results from the same compartment.

Peripheral blood is quite representative of the disease burden and is routinely used for disease monitoring.

Multiple publications have discussed how to integrate molecular studies in the monitoring of patients with CML^{1,2,3,4}. At diagnosis, RQ-PCR studies establish a pre-treatment baseline. Subsequent serial monitoring provides a sensitive method to follow low levels of disease. Rising *BCR-ABL 1* levels confirmed by repeat RQ-PCR testing, may prompt closer monitoring, further testing (bone marrow examination with cytogenetics or *ABL 1* mutation analysis), or reevaluation of therapy. Finally, RQ-PCR data may provide prognostic information. Patients treated with TKIs that achieved a major molecular response ($\geq 1,000$ -fold or 3.0 log reduction in *BCR-ABL 1*) and a complete cytogenetic response have a very low likelihood of disease progression⁵. How to optimally integrate RQ-PCR monitoring for patients with CML is an actively studied area, and this provides a unique opportunity for collaboration between the treating physician and the clinical laboratorian.

References

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