

# Real-time PCR in Hematology.

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## Introduction

Molecular biological techniques have offered us a radical improvement in the evaluation of hematological disorders. Current treatment protocols for acute or chronic leukemia are based on prognostic factors, like aberrant fusion transcripts. But patient outcome cannot be reliably predicted solely on the basis of such parameters, underlining the importance of MRD testing. MRD or minimal residual disease information is important for prediction of relapse and early therapeutic intervention.

## Recognition

One of the most sensitive techniques for MRD detection is the PCR. One leukemic cell in roughly 100.000 normal cells can still be detected. Several PCR targets are available, like aberrant fusion transcripts (CML, AML and ALL) or fusion genes (lymphoma), rearranged immunoglobulin (Ig) and T-cell receptor (TCR) genes (in ALL, MM and lymphoma) or genes that are overexpressed in the leukemic cell (like WT1, PRV1 and CyclinD1).

## Quantification

Quantification of MRD can be performed by real-time PCR. The amount of target molecules in the sample can be calculated by use of the comparative method ( $\Delta\Delta C_t$  method) or by use of the standard curve method. A housekeeping gene or control gene should be amplified in the same sample to be able to correct for DNA/RNA quality and DNA/RNA input. The Abl, GUS and PBGD genes are often used as control genes in haematological disorders when a fusion transcript is quantified. For DNA markers the albumin gene is often used.

## Clinical applications

In CML patients careful monitoring of MRD by means of RQ-PCR of bcr-abl transcripts can help in therapeutic decisions like transplantation or not, when to give donor lymphocyte infusions (DLI's), prediction of relapse etc. A threshold level with prognostic significance has been established. Fusion transcripts can be quantified rather easily and with high sensitivity since they are unique for a given disorder and are normally not present in normal cells.

Quantification of rearranged Ig and TCR genes is a little bit harder since one should bear in mind that these genes are also present in normal B and T-cells. But since each rearrangement is unique for a B or T-cell and its progeny, a specific feature of this rearranged gene like the length or the sequence can be used to recognize the leukemic clone. For real-time PCR an allele specific oligo (ASO) is designed for each patient based on the sequence of the monoclonal Ig and/or TCR genes. After extensive optimization this ASO is then used as a primer together with consensus primers and probes to quantify follow-up samples of this patient. The tailor-made approach makes this technique very expensive but often Ig and TCR genes are the only markers that can be used in follow-up since fusion transcripts are only present in 10-15% of acute leukemia's. The sensitivity depends on the variability in the rearranged gene and the normal distribution of certain rearrangements. Usually, sensitivity lies between  $10^{-4}$  and  $10^{-5}$ . The usefulness of this qASO-PCR has been shown in MM and ALL patients.

Over expression of certain genes can also be quantified. The WT1 gene is normally expressed in haematopoietic precursor cells but can be upregulated in AML, CML, MDS and ALL. WT1 can be considered as a panleukemic marker but PCR sensitivity is not that good since normal expression levels, especially in the bone marrow, can mask the presence of low numbers of leukemic cells. Our policy is to use WT1 when no other marker is available.

## Standardisation

RQ-PCR is already in use in a lot of hematological laboratories but standardisation remains a big issue. Also communication of data towards the clinicians is not at all regulated. Further discussions with all the experts in the field and quality control rounds should solve the main problems in the near future.