Maximal benefit of minimal residual disease monitoring in pediatric acute myeloid leukemia

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In this issue of *Haematologica*, Maurer-Granofszky *et al.*[1] report on the role of genomic breakpoint-specific monitoring of minimal/measurable residual disease (MRD) in pediatric non-standard-risk acute myeloid leukemia (AML).

The event-free survival for pediatric AML reaches 65% with an overall survival of 80% with current treatment protocols. Relapse remains the main risk of treatment failure and cause of death. Optimal treatment stratification and early detection of relapse may improve the outcome and MRD monitoring may be the most relevant avenues to explore in achieving this goal. Indeed, MRD has become an important tool in the management of pediatric AML. The two methods currently applied in clinical practice are flow cytometry (FCM) and reverse transcription quantitative polymerase chain reaction (RT-qPCR). Both technologies have been applied in many series but their comparative role, optimal timepoints of application, and the use of peripheral blood *versus* bone marrow are less clear.

The study by Maurer-Granofszky *et al.* provides a welcome comparison of the two methods of PCR- and FCM-based MRD monitoring, which is sparse in the literature. The authors describe MRD quantification using genomic breakpoint-specific sequences via quantitative polymerase chain reaction (gDNA-PCR), which allows residual disease assessment representative of absolute leukemic cell quantities as opposed to fusion transcripts detected by RT-qPCR. gDNA-PCR MRD was performed in 49 children with non-standard-risk AML and the results compared to those obtained with FCM MRD in 183 paired samples.

The overall concordance was high (90%) considering a cutoff threshold of 0.1% and both methodologies were superior to morphological evaluation. Both PCR- and FCM-based methodologies showed much higher specificity than morphology, which may challenge the traditional definition of complete response based upon less than 5% leukemic blasts detected by morphology.[2]

PCR-based methods may overestimate MRD compared with FCM during the early phase of therapy since PCR is also able to detect mature cells with the genetic fusion.[3,4] In contrast FCM-MRD identifies cells with an immature/blast immunophenotype and immunophenotypic aberrancies, which are often lacking in already mature cells.

The technology of gDNA-PCR is complex and depends on identification of patient-specific markers through genomic breakpoint characterization but the turnaround time of the assays is 5-7 weeks after diagnosis allowing the implementation of gDNA-MRD for the combined assessment of end-of-induction response. Markers were identified in more than 90% of the selected patients with a sensitivity of at least 10^{-4}.

FCM-based MRD detection during and at the end of induction has contributed to significant improvements in risk stratification and optimal post-remission therapy in AML.[5-7] Whether or not MRD is sufficient for risk stratification, neutralizing the independent prognostic impact of genetic risk groups, is the focus of ongoing studies by the Nordic Society for Pediatric Hematology Oncology (NOPHO) study group. Most pediatric AML study groups have a number of genetic aberrations defining high risk, regardless of response assessment. The role of PCR-based MRD during induction is limited and larger studies are needed using this more sensitive technology to determine whether risk stratification can be further improved.

It would have been of interest to analyze the concordance between peripheral blood and bone marrow findings. However, the study by Maurer-Granofszky *et al.* included very few peripheral blood samples and no data derived from peripheral blood in the early phase of treatment. FCM of peripheral blood may be of clinical relevance during the first weeks after initiating therapy[8] but not sufficiently sensitive later during or after therapy. For PCR-based follow-up after the end of therapy peripheral blood is at least as sensitive as and more specific than bone marrow.[9]

Table 1. Advantages and limitations of measurable/minimal residual disease assessments by flow cytometry and polymerase chain reaction in childhood acute myeloid leukemia.

	Advantages	Disadvantages
FCM	Widely applicable (>90% of patients) Rapid turn-around time Useful for early response assessment and risk-stratification Persistence of MRD highly predictive of relapse	Limited sensitivity (0.1%) Only applicable in BM Sample quality variation (risk of PB dilution) Leukemia-specific immunophenotype may be non-informative or unstable over time Considerable operator- and expertise dependence
PCR	Highly sensitive (up to 0.001%) Applicable in both BM and PB Standardized through established and validated protocols Useful tool for post-therapy disease monitoring in PB	Applicable in only 40% (RT-qPCR) to 70% of patients (gDNA-PCR)* Prolonged turn-around time, particularly for individualized gDNA-PCR assays Delayed response during early assessment Persistence of MRD in BM common despite continuous CR

*Applicable targets: *RUNX1::RUNX1T1*, *CBFB::MYH11*, *KMT2A::MLLT3* and mutated *NPM1* (RT-qPCR); all fusion transcripts (gDNA-PCR). BM: bone marrow; PB: peripheral blood; FCM: flow cytometry; MRD: measurable/minimal residual disease; PCR: polymerase chain reaction: RT-qPCR: reverse transcription quantitative PCR; gDNA-PCR: genomic DNA-based PCR; CR: complete remission.

Some samples showed persistent positivity as assessed by gDNA-PCR but were negative by FCM, suggesting the persistence of gene fusions in maturing or terminally differentiated AML cells. While the study by Maurer-Granofszky *et al.* focused on the feasibility and performance of two MRD technologies, future investigations should explore the complementary prognostic impact of gDNA-PCR MRD in FCM-negative patients, of whom approximately one third are still destined to relapse.[6,7]

Persistence of stable, low-level, RT-qPCR-detectable MRD in bone marrow of patients with CBF-AML subtypes during consolidation or after therapy completion does not have a negative impact on outcome. However, sustained MRD positivity in peripheral blood (rather than bone marrow) or increasing levels above 10^{-4} during or after consolidation indicates impending relapse. Since persistent, low-level MRD in bone marrow is common and not predictive of relapse,[10] routine bone marrow sampling after the end of therapy is not recommended. In contrast, peripheral blood samples are easier to collect and sustained MRD positivity in peripheral blood is strongly predictive of impending relapse.[9] The PCR-based monitoring of peripheral blood may allow early detection of relapse, which may suggest the need for preemptive therapy alleviating toxicity before hematopoietic stem cell transplantation.

One limitation of the PCR-based technology is the lack of markers for some patients, which was the case for one-third of the patients in the study by Maurer-Granofszky *et al.* Only a handful of targets are currently used for MRD assessment by RT-qPCR and most of these aberrations are found in standard-risk patients. Even though the study by Maurer-Granofszky *et al.*[1] increases the number of patients with useful markers, a significant number of children have no marker for PCR-based MRD monitoring. In contrast, multicolor FCM is applicable in more than 90% of pediatric AML patients and is therefore currently the method of choice for response assessment in most clinicals trials (Table 1).

Newer methods using whole exome sequencing or droplet digital PCR with a patient-tailored approach for molecular MRD monitoring in peripheral blood may ensure sensitive markers for almost all AML patients and enable response assessment and close monitoring in peripheral blood for early detection of AML relapse.[11]

Disclosures

No conflicts of interest to disclose.

Contributions

Both authors contributed equally to the writing of this editorial comment.

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