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Received: August 22, 2023.
Accepted: August 29, 2023.


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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 (non-SR) acute myeloid leukemia (AML).

The event-free survival for pediatric AML reaches 65\% with 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.

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 PCR (RT-qPCR). Both technologies have been applied in many series but their comparative role and the optimal time points and the use of peripheral blood (PB) vs bone marrow (BM) are less clarified.

The present study provides a welcome comparison of the two methods of PCR and FCM based MRD, which is sparse in the literature. The authors describe MRD quantification using genomic breakpoint specific sequences via qPCR (gDNA-PCR) which allow 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-SR AML and compared to those obtained with FCM MRD results in 183 paired samples.

The overall concordance is high (90\%) considering a cut-off threshold of 0.1\% and both methodologies were superior to morphologic 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 by morphology\(^2\).

PCR based methods may overestimate MRD compared with FCM during the early phase of therapy since PCR is able to detect also mature cells with the genetic fusion\(^3,4\). In contrast FCM-MRD describes cells with immature/blast immunophenotype and immunophenotypic aberrancies, which are often lacking in already matured 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 during and at the end of induction has contributed to significant improvement in the risk stratification and the optimal post remission therapy in AML\(^5,6,7\). Whether, MRD is sufficient as risk stratification neutralizing the independent prognostic impact of genetic risk groups
is the focus of the ongoing studies from the Nordic Society for Pediatric Hematology Oncology (NOPHO) study group. Most pediatric AML study groups have a number of genetic aberrations as high-risk defining 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 study whether risk stratification can be further improved.

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

Some samples showed persistent positivity as assessed by gDNA-PCR but negative by FCM, suggesting the persistence of gene fusions in maturing or terminally differentiated AML cells. While the present study focus on 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. Persistence of stable low-level RT-qPCR MRD in CBF-AML subtypes BM during consolidation or after therapy completion has no negative impact on outcome. However, sustained MRD positivity in PB (rather than BM) or increasing levels above 10−4 during or after consolidation indicates impending relapse. Since persistent MRD at low level in BM is common and not predictive of relapse routine BM sampling after end of therapy is not recommended. In contrast PB samples are easier to collect and sustained MRD positivity in PB is strongly predictive of impending relapse. The PCR based monitoring of PB may allow early detection of relapse which may opt for preemptive therapy alleviating toxicity before HSCT.

One limitation of the PCR based technology is the lack of markers for some of the patients, in the present study one third of the patients. Only a handful of targets are currently used for MRD assessment by RT-qPCR and most of these aberrations are found in SR patients. Even though the study by Maurer-Granofszky et al increases the number of patients with useful markers a significant number of children has 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. Newer methods using whole exome sequencing or droplet digital PCR with a patient-tailored approach for molecular MRD monitoring in PB may ensure sensitive markers for almost all AML patients and allow for response assessment and close monitoring in PB for early detection of AML relapse.
References

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<th>Advantages</th>
<th>Disadvantages</th>
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<td><strong>FCM</strong></td>
<td>• Widely applicable (&gt;90% of patients)</td>
<td>• Limited sensitivity (0.1%)</td>
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<td></td>
<td>• Rapid turn-around time</td>
<td>• Only applicable in BM</td>
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<td></td>
<td>• Useful for early response assessment and risk-stratification</td>
<td>• Sample quality variation (risk of PB dilution)</td>
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<td>• Persistence of MRD highly predictive of relapse</td>
<td>• Leukemia-specific immunophenotype may be non-informative or instable over time</td>
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<td></td>
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<td>• Considerable operator- and expertise dependence</td>
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<tr>
<td><strong>PCR</strong></td>
<td>• Highly sensitive (up to 0.001%)</td>
<td>• Applicable in only 40% (RT-qPCR) to 70% of patients (gDNA-PCR)*</td>
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<td>• Applicable in both BM and PB</td>
<td>• Prolonged turn-around time, particularly in individualized gDNA-PCR assays</td>
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<td>• Standardized through established and validated protocols</td>
<td>• Delayed response during early assessment</td>
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<td>• Useful tool for post-therapy disease monitoring in PB</td>
<td>• Persistence of MRD in BM common despite continuous CR</td>
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Table I: Advantages and limitations of measurable/minimal residual disease (MRD) assessments by flow cytometry (FCM) and polymerase chain reaction (PCR) in childhood AML

Abbreviations: BM, bone marrow; CR, complete remission; gDNA-PCR, genomic DNA PCR; PB, peripheral blood; RT-qPCR, reverse transcription quantitative PCR

* Applicable targets: *RUNXI::RUNXIT1*, *CBFB::MYH11*, *KMT2A::MLLT3* and mutated *NPM1* (RT-qPCR); all fusion transcripts (gDNA-PCR)