Perspective

How to assess minimal residual disease in pediatric and adult acute myeloid leukemia?

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Complete remissions that are reached after therapy of acute leukemia patients are classically defined as <5% blast cells in the bone marrow as determined by morphology. Unfortunately, a high percentage of patients in remission ultimately relapse, usually within the first years (1). In order to improve prognostic value of blast counts, another approach has been embarked upon, namely the detection of minimal residual disease (MRD).

During the last decade, MRD has been shown in many studies to offer prognostic significance for clinical outcome, independent of other prognostic factors (2). In particular in pediatric acute lymphoblastic leukemia (ALL), MRD detection is facilitated by the presence of molecular (3), as well as immunophenotypic (4) aberrancies. In ALL current clinical decisions are made based on risk stratification by MRD levels (5). In pediatric acute myeloid leukemia (AML), MRD has only recently entered this stage (6). Although the relevance of MRD in adult AML has been extensively reported, it is currently not yet used for clinical decision making.

Early MRD studies in AML go back to the mid-nineties for both molecular MRD and MRD using flowcytometry (FCM-MRD), and the main obstacles for implementation in risk stratification still are: (I) the fact that only a minority of patients have molecular aberrancies suitable for MRD monitoring, and (II) the large heterogeneity of immunophenotypes (7). Besides PML-RAR for acute pro-myelocytic leukemia, molecular aberrancies used for molecular-MRD measurements include fusion transcripts of AML1-ETO, CBF-β-MYH11 and MLL, and more recently NPM1 mutations (1). In pediatric AML, the fusion transcripts make up around 20% of the patients, but NPM1 only about 5-10% (1). In contrast, in adult AML, NPM1 is mutated in about 35% of the patients, while the other aberrancies are less frequently present: 10-15% (1). Consequently, molecular MRD can be monitored using these molecular aberrancies only in a minority of pediatric and adult patients. In contrast, immunophenotype aberrancies [Leukemia Associated (Immunophenotypic) Aberrancies, LA(I)P] have been described for the majority (usually >85%) of pediatric (8) and adult (9) patients, making it potentially very suitable for MRD detection. However, the disadvantage of FCM-MRD is the large inter-patient as well as intra-patient heterogeneity, preventing an easy-to-use and easy-to-interpret application. In addition, an extensive antibody panel is required to define LA(I)Ps for each new patient. The different aberrancies used to determine FCM-MRD also have variable background expression in normal and regenerating bone marrow (different specificity), complicating the correct interpretation of LA(I)P in remission bone marrow. Hence, different LA(I)Ps allow different sensitivity levels. In general, 0.1% is considered as a cut off level that can be accurately assessed for most if not all LA(I)Ps. Lower levels (0.01-0.1% and <0.01%) are only possible for a subset of patients depending, not only on the specificity of the LA(I)P, but also on degree of LA(I)P coverage of blast population, and the numbers of bone marrow cells obtainable for analysis.

Considering these pros and cons of molecular- and FCM-MRD approaches, efforts to compare both approaches were warranted. In last year's October issue of *Journal of*

Clinical Oncology, Inaba and colleagues compared prognostic impact of blast count and morphological examination with MRD assessment using both molecular- and FCM-MRD approaches (10). As to the first two approaches, they found discrepancies between morphologic examination and FCM-MRD assessment with the latter being the best predictive for event free survival and without additional prognostic value by including blast counts. Although morphologic examination by pathologists offered strong prognostic value, this was unfortunately inconclusive in a high percentage of cases.

The general idea in literature is that FCM-MRD and molecular-MRD may be complementary. Because of higher sensitivities of PCR-based MRD, there may be some preference for molecular MRD in AML cases where both are possible. However, the Inaba study shows that AML1-ETO, CBF-β-MYH11 fusion transcripts do not offer reliable prognostic information whereas FCM-MRD assessment does. FCM-MRD could be performed on a large number of samples (over 1,500) in a large patient group (n=203), whereby molecular-MRD and FCM-MRD could be directly compared in 508 samples. Unfortunately, the total number of corresponding patients with sampling after two courses was not high: 55 and 66 after first and second induction course, respectively, for all transcripts together (AML1-ETO + CBF-β-MYH11 + MLL).

An important observation was that in 311 molecular MRD negative samples, almost all [308] samples were also negative (<0.1%) by FCM-MRD, whereas all (n=57) samples with detectable but very low (<0.01%) levels of transcripts were negative by FCM-MRD (<0.1%). In addition, only 7/61 samples with relatively low levels of transcripts (0.01-0.1%), were positive (≥0.1%) by FCM-MRD. Altogether, for PCR with no or low transcript levels (<0.1%) there is a discrepancy with FCM-MRD in only 10/429 samples. A large discrepancy was, however, found for PCR positive (n=197) samples (≥0.01%). The vast majority (86.3%) of samples positive with molecular MRD, were negative (<0.1%) by FCM MRD. As the authors state, a fair comparison between the two approaches is to use 0.1% cut-off for both methods; this revealed that molecular MRD positive samples (≥0.1%) were negative by FCM-MRD in about 85% (67/79) of the cases. Theoretically, negativity by FCM-MRD in PCR positive patients may imply that the aberrant immunophenotype(s) used has disappeared, which indeed is one of the major drawbacks of FCM-MRD. Consequently, this would lead to false-negative FCM-MRD, but with possible persistence of the molecular aberration. Inversely, due to background expression of several markers on normal cells, some LA(I)Ps may give rise to over-estimation of MRD by immunophenotyping, although this may not be a major problem in this study, seen the large congruency between molecular MRD and FCM-MRD in the large group of PCR negative patients.

To determine which MRD approach would best reflect the actual situation, the authors related both approaches to clinical outcome using event free survival (EFS) analysis. FCM-MRD turned out to be a strong predictor of relapse, but molecular MRD was not. However, it has to be emphasized that the PCR results are shown only for AML1-ETO, CBF-β-MYH11 and MLL fusion transcripts (in total n=55 after first course and n=66 after second course), which reflect in majority good prognosis patients, while FCM-MRD has been performed on (almost) all available patients (203 after first course and 194 after second course). This latter patient group not only includes the good prognostic patients, but also intermediate and poor prognosis AML. In fact the honest comparison should contain the results of prognosis for patients assessable with both molecular MRD and FCM-MRD (theoretically 55 and 66, after course 1 and course 2, respectively). It might be asked how the analysis shown in the 6th figure of the article would turn out for all PCR-MRD patients, at cut-off levels of 0.1%, and moreover, to study how FCM-MRD would contribute to prognosis in these groups. A further drawback of the small group of patients for whom molecular MRD was available is that the group is too small for further stratification on other known risk factors, such as relevant mutations such as FLT3-ITD and NPM1.

The main message of the study is that FCM-MRD best predicts outcome and molecular MRD did not add to the prognostic value of MRD in FCM-MRD negative patients. The argument against PCR as the method of choice for MRD assessment seems mainly based on persistence of PCR positivity in patients who nevertheless remain in remission and, moreover, are negative with FCM-MRD. The clue for the discrepancies seen between PCR and FCM may be found in author's Supplementary in the figure of S5: for both AML1-ETO and CBFβ-MYH11 AML the kinetics of reduction of transcript is very capricious with ups and downs up to a few log differences, while crossing the important 0.1% point on different occasions. These can reflect real changes, but also may reflect variations in the assays itself, which at one time point may lead to false negativity, and on another time point to false positivity. It would therefore be highly informative to compare the molecular MRD kinetics with kinetics

of FCM-MRD in those patients in which FCM-MRD is sensitive enough (also in the 0.01-0.1% range), in order to assess whether the latter shows more stable kinetics. Lastly, if persistent PCR positivity in remission patients represents true MRD, which does not lead to relapses and can be considered as an obscuring factor, pleading against molecular MRD as the method of choice, it is still intriguing why in such large number of samples (311/508) MRD by PCR is completely undetectable.

Although these data suggest that FCM-MRD is the optimal method of choice, the numbers of patients in the molecular MRD group is also in this study still limited and should be extended with additional types of mutations applicable for PCR. The authors alluded to FLT3-ITD as a potential target for molecular MRD in pediatric AML, although the aberrancy has a lower frequency than in adult AML (25% versus 15%). However, FLT3-ITDs are quite unstable during/after treatment with frequent losses, gains or losses with gains of new mutations (11-13). Such new mutations may be present in small sub-populations present at diagnosis (14), and which outgrowth may be paralleled by appearance of new immunophenotypes and disappearance of original LA(I)P (14). The latter may contribute to false-negativity seen for FCM-MRD.

Altogether, the authors show once more that MRD as measured by FCM-MRD is a powerful tool to define new risk groups and to use FCM-MRD as a guide for therapeutic intervention as they showed in their previous paper (6). It should be realized, however, that FCM-MRD still needs large experience in recognizing aberrant immunophenotypes both at diagnosis and in remission bone marrow. For that reason, further optimization of the techniques and targets available for the assessment of MRD by both FCM-MRD and molecular-MRD approaches is still warranted.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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