Perspective

Minimal residual disease detection in pediatric acute myeloid leukemia: does flow cytometry score a point over molecular biology?

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Submitted Sep 27, 2012. Accepted for publication Oct 24, 2012.
doi: 10.3978/j.issn.2224-4336.2012.10.01
View this article at: http://www.thetp.org/article/view/1158/1831

In acute myeloid leukemia (AML), achievement of morphologic complete remission (RC) still remains the "gold standard" to make a judgment on treatment response and yet hematologists wonder about its utility in the era of minimal residual disease (MRD) detection. In fact, whatever the techniques used, a number of studies has convincingly demonstrated that the persistence in the bone marrow of leukemic cells below the threshold of conventional morphology - MRD - identifies patients at a significantly higher risk of relapse (1). Owing to the superior potential for sensitivity, flow cytometry and polymerase chain reaction (PCR) are the most favored methods to track residual leukemic cells that survived cytotoxic chemotherapy. Although using different time-points and quantitative thresholds, several studies from different worldwide groups came to the converging conclusion that MRD detection by flow cytometry does have a prognostic role in AML, especially in term of relapse prediction (2-4). On the other hand, more controversial data have been generated about the role of PCR. Qualitative reverse trascriptase-PCR (RT-PCR) in core binding factor positive AML (AML carrying *RUNX1-RUNX1T1* formerly *AML1-ETO* or *CBFB-MYH11* transcript) has a limited clinical applicability since a persistent positivity has been observed in long survivors even after allogeneic stem cell transplantation. Using quantitative RT-PCR, Corbacioglu *et al.* (5) identified clinically relevant time-points at which persistence of *CBFB-MYH11* transcript positivity was associated with a significantly increased risk of relapse, suggesting that this technique should be preferred over qualitative RT-PCR. In a recent publication of the National Cancer Research Institute (formerly United Kingdom Medical Research Council) (6), the role of quantitative RT-PCR in MRD monitoring was confirmed not only for *CBFB-MYH11* positive AML but also for cases expressing the *RUNX1-RUNX1T1* fusion gene. The role of WT1 over-expression as a universal marker for MRD detection in AML still await a conclusive validation. On the behalf of LeukemiaNet, Cilloni *et al.* (7) have made available a common standard protocol to measure WT1 copy number with MRD purpose. However, they reported that only in 46% and 13% of peripheral blood and bone marrow samples, respectively, were the levels of WT1 sufficiently over-expressed, compared with normal samples, to allow MRD determination and risk-assessment. Therefore, concerns still remain about the power of the present PCR approaches to distinguish between the physiologic background of WT1 and its "*bona fide*" over-expression in leukemic blasts. Whereas the role of FLT3 mutations in MRD monitoring is still unproven as a consequence of its instability at diagnosis and relapse (8), Schnittger *et al.* (9) have demonstrated that mutations of NPM1 are very stable at relapse and that the persistence of a mutated signature at different time-points after achievement of morphologic CR significantly predicts disease recurrence. One of the key-point in the MRD scenario is that nothing is really known of the relation between the two main opponents: flow versus PCR. What is the relation between MRD results obtained by flow and those by PCR? Answering this question is central to further developments of our skills in MRD surveillance. In their manuscript, Inaba *et al.* (10) tried to address exactly this issue by examining the relation

between morphology, flow cytometry and PCR in response monitoring of bone marrow follow-up samples from 203 children and adolescents with newly diagnosed AML. MRD studies by flow cytometry were performed using leukemia associated phenotypes whereas MRD by PCR was evaluated in patients whose leukemic blasts harbored *AML1-ETO*, *CBFB-MYH11*, *RBM15-MKL1* and *MLL* fusion transcripts. Overall, they found a poor correlation between the three methods, with morphology showing the major limitations in terms of sensitivity. Of much more interest was the comparison between flow cytometry and PCR. In 308 of 311 (99%) samples, MRD by PCR and flow cytometry were concurrently negative. On the other hand, only 19 (10%) of 197 PCR-positive samples were flow cytometry positive, with analyses of *AML1-ETO* and *CBFB-MYH11* accounting for most discrepancies. In 8 of 13 *MLL*-positive samples, MRD was detected both by flow cytometry and PCR. The authors point out that whereas detection of MRD by PCR did not improve risk-assessment and outcome prediction, MRD by flow cytometry after induction 1 or 2 predicted shorter event-free survival, higher relapse rate ($P<0.001$) and was an independent prognostic factor in a multivariable analysis. The authors reason that in samples *AML1-ETO* or *CBFB-MYH11* positive, the clinico-biologic variance between flow cytometry and quantitative RT-PCR might be explained based on persistence of very low (below flow cytometry sensitivity) but clinically irrelevant levels of MRD. Alternatively, quantitative RT-PCR might have captured pre-leukemic or partially differentiated cells that have lost both aberrant phenotype and clonogenic potential. Based on this, the authors recommend that targeting *AML1-ETO* and *CBFB-MYH11* transcripts for MRD investigation should be undertaken cautiously or indeed abandoned, especially when robust flow cytometry data can be obtained. Although there was a more consistent relation between results of flow cytometry and detection of *MLL* transcripts as compared to *AML1-ETO* and *CBFB-MYH11* transcripts, the authors point out that studies of MRD targeting *MLL* fusion genes are at their very beginning. Therefore, a more extensive background is needed for a proper positioning of this approach. Finally, the authors point out that, although being demonstrated highly qualified targets for MRD detection in adult AML, NPM1 mutations are expressed in less than 10% of pediatric AML (11). Based on this body of evidence, Inaba and co-workers delineate a general picture of MRD strategy in pediatric AML where flow cytometry retains a central role, providing strong prognostic information. To our knowledge, this is the first formal demonstration of the superiority of flow cytometry over PCR in MRD monitoring of pediatric AML. Although in the Inaba experience flow cytometry scores a point over PCR, the game is not over. The more we known the genetic signature of AML the higher the chances to discover suitable targets for MRD determination and the way forward will hopefully witness an integration of the two techniques. A close cooperation between cytometrists and molecular biologists sounds critical in a time when the MRD biomarker is firmly rising to the attention of the experts and authorities as a potential clinical trial end-point for acute leukemias.

Acknowledgements

None.

Footnote

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

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Cite this article as: Venditti A, Del Principe MI, Maurillo L, Buccisano F, Amadori S. Minimal residual disease detection in pediatric acute myeloid leukemia: does flow cytometry score a point over molecular biology? Transl Pediatr 2013;2(1):43-45. doi: 10.3978/j.issn.2224-4336.2012.10.01

