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MINIMAL RESIDUAL DISEASE IN AML: WHY HAS IT LAGGED BEHIND PEDIATRIC ALL?

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Abstract

Although the concept of minimal residual disease (MRD) as an indicator for the quality of treatment response is the same in acute myeloid (AML) and acute lymphoid leukemia (ALL), the practice of measuring MRD levels for monitoring response and guiding post-induction therapy has been implemented much more rapidly in ALL, particularly pediatric ALL, than in AML. This perspective will look at the facts and discuss why ALL appears to be more amenable to MRD-shaped risk-allocation and a revised definition of complete remission.

Keywords

Response definition; acute leukemias; MRD

INTRODUCTION

The prognostic outlook of individual patients with acute leukemia currently is based predominantly on cytogenetic and molecular aberrations that are found in the leukemic blast cells at the time of diagnosis. However, pre-therapeutic prognostic factors alone yield incomplete information; this is suggested by the uncontested observation that long-term treatment outcomes within upfront-defined risk classes vary widely. Although 85%–90% of acute leukemia patients achieve a morphologic remission when intensive induction chemotherapy is used, 40–60% of remitting older ALL and AML patients will eventually relapse and succumb to their disease. The accepted hypothesis remains that the predominant root of relapses is the persistence of minimal residual disease, or MRD, that remains after treatment at levels too low to be detected under the microscope. It is a work in progress to determine whether the aberrant hematopoietic cells which escape treatment represent a therapy-resistant minor blast clone, leukemic stem cells, the elusive pre-leukemic stem cell or residual cells from the original leukemic bulk as the result of insufficient therapy. Given that most standard MRD assays rely on the recognition of the original phenotypic or genotypic features of leukemic bulk cells and, despite of this limitation, produce data with

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prognostic relevance, it seems fair to state that monitoring the leukemic bulk, though incomplete, provides valuable clinical information, both in AML and ALL.

The level of MRD after induction chemotherapy reflects the quality of the response and, therefore, serves as a post-therapy prognosticator. Within each conventional risk-category, MRD status adds independent prognostic information. In other words, patients with favorable standard risk will do much more poorly if they remain MRD positive after therapy than favorable-risk patients without detectable post-therapy disease (e.g., in Core-Binding Factor AML¹ or TEL/AML1 ALL²), while patients with intermediate-risk who become MRD-negative with induction therapy may do as well as MRD-negative favorable-risk patients (e.g., FLT3-mutated normal karyotype AML³). Even poor-risk patients, such as those with BCR/ABL positive ALL,⁴ have a better outcome if they become MRD negative with tyrosine kinase inhibitors and chemotherapy than patients who remain MRD positive. The integration of pre-therapeutic prognostic features, e.g., expression of CD25 in AML³ or presence of *IKAROS* gene alterations in ALL,⁵ with MRD status may optimize our ability to predict relapse, particularly in patients with intermediate risk based on the standard classification and patients with slow rate of blast clearance during induction.

Given the extent of our understanding that MRD has a decisive role in treatment stratification, one cannot help but ask what the reasons are that post-treatment MRD status is not yet used routinely to dictate post-remission therapy in all leukemia subtypes. Indeed, in ALL, measurement of MRD is increasingly used as a tool for adjusting therapy after initial treatment response and for stratifying patients into MRD-risk classes.^{6,7} The limited success of MRD-based clinical interventions in ALL patients determined high-risk based on unremitting MRD during MRD-guided treatment intensification,^{8–10} however, suggests that novel treatment strategies are needed to overcome the chemoresistance to current therapies as reflected by a persistent positive MRD status. One promising innovative modality is MRD-targeted therapy with the CD19/CD3-bispecific antibody blinatumomab in B-lineage ALL.¹¹ With respect to MRD-directed therapy in AML, robust clinical data are lacking. The pediatric AML02 trial¹² failed to reduce MRD levels by intensifying induction therapy but demonstrated an MRD-lowering effect of gemtuzumab ozogamicin, the anti-CD33 antibody, when given after the first course of induction. Strikingly, however, a high level of MRD after induction 1 was the only significant adverse prognostic factor for outcome. Similar to the above mentioned data from the ALL trials, allogeneic stem cell transplantation has not proven to be a panacea for MRD-high risk AML patients. Importantly, AML02 confirmed data from retrospective analyses¹³ which suggested that the threshold of prognostically significant MRD was at least 10-times higher in AML than ALL. Furthermore, this study demonstrated that patients with low MRD levels after initial treatment did as well long-term as those with undetectable MRD irrespective of subsequent treatment allocation, a finding quite different from what is seen in ALL, where higher levels of MRD are associated with a proportional increase in risk of relapse.^{14–16} Remarkably, the effect of MRD pre-transplantation is also quite different in ALL from AML;¹⁷ while in ALL survival probability post transplant was tightly linked to pre-transplant MRD levels, the same was not the case in AML.^{17,18} In other words, in ALL but not AML, increasing MRD levels prior to transplantation were associated with increased risk of relapse or death after

transplant. In addition to these potential biologic disparities between MRD in ALL and AML, profound differences exist in available targets for MRD detection and the methodologies to monitor them which may explain why the clinical application of MRD to AML treatment is lagging behind that in ALL.

DISCUSSION

A search in PubMed¹⁹ in October 2014 yielded >2,000 studies of MRD in ALL and not quite 400 in AML. Though only a rough estimate, these numbers clearly demonstrate the fact that much more work is done on MRD in ALL than AML and this is also reflected in the disparate numbers of retrospective clinical trial analyses or prospective MRD-directed interventions. In my opinion, before we engage into a discussion of biologic differences of MRD between the two diseases, the more mundane issue of MRD measurement should be discussed.

When antibodies to hematopoietic antigens, became available, Bradstock et al²⁰ in 1981 demonstrated that several patients in morphologic remission after treatment for ALL had 'minimal residual disease' in their bone marrows, based on unique phenotypic aberrations which allowed the distinction of leukemic from normal immature cells. Because the first monoclonal antibodies were directed against lymphoid antigens, both the immunophenotypic characterization of ALL and the detection of residual lymphoid blasts after treatment from the beginning were far ahead of similar efforts in AML. While all methodology for MRD detection relies on the recognition of phenotypic or genotypic differences between normal and leukemia hematopoietic cells, there are obvious advantages to measuring MRD in ALL compared with AML which are summarized in Table 1. The fact that the majority of ALL cases have suitable somatic gene rearrangements is probably the most important difference given that AML lacks comparative immunogenotypes. Multiparameter flow cytometry, the quickest and cheapest way to determine MRD, is much more straight forward in ALL than AML, mostly because AML patients have a tendency to present with multiple immunophenotypic clones, everyone of which could contribute to MRD and thus requires monitoring. This statement applies whether investigators prefer using Leukemia-Associated-Immunophenotypes or the Different-From-Normal model. The reader is referred to the vast literature on these flow cytometric approaches to MRD evaluation. The main difficulty in B-lineage ALL is the occurrence of normal B-lymphoid precursor cells, hematogones,^{21,22} which can be easily misinterpreted as MRD. Changes in antigen profiles with treatment are found in both diseases though they rarely interfere with MRD detection in ALL. Suitable fusion genes as the result of chromosomal rearrangements are much more frequent in ALL than AML, while gene mutations are increasingly being used for MRD detection in AML. We have spent the last years improving the technologies of MRD detection in a great effort to detect increasingly lower levels of residual disease; and with the advent of Next-Generation Sequencing (NGS), we are succeeding.²³⁻²⁸ We have to remember though that NGS can detect significantly lower MRD levels only if specific genes are targeted, which have previously been characterized in a given patient's leukemic bulk.

This brings us to the question, though, whether very low levels of MRD, detectable only by NGS or similar methods, are clinically relevant. Do MRD levels $<0.001\%$, which is >10 -times lower than the accepted threshold for MRD positivity in ALL and >100 times lower than the one in AML, really matter for outcome? In pediatric B-lineage ALL, there is evidence that such low levels of MRD after induction do indeed increase the probability of relapse.¹⁶ In AML, on the other hand, patients with slow blast cell clearance whose MRD levels at the end of induction therapy are above 0.1% but less than 1% , long-term do as well as those patients with MRD levels $<0.1\%$ post induction.^{29–31} A strict association between presence of quantifiable MRD, even at ultra-low levels, and outcome has also been reported for ALL patients prior to transplant,^{9,17} whereas this absolute need for MRD reduction prior to transplant may not exist in AML.^{17,18} Aside from important clinical consequences, such as benefits to the implementation of interim therapy in MRD positive pre-transplant ALL but not AML patients, these observations also suggest that MRD assays with sensitivities higher than those currently available will be advantageous predominantly in ALL but not in AML patients.

Potential reasons for the disparate threshold levels which define clinically meaningful MRD in ALL vs AML remain under discussion without definitive answers. A potential explanation for differences in prognostic pre-transplant MRD levels between ALL and AML, is higher susceptibility of AML blasts to graft-versus-leukemia effects.³² Buccisano et al¹³ hypothesized that less effective chemotherapy was the reason that the threshold for the definition of MRD positivity post induction chemotherapy was higher in AML than in ALL. In support of this concept, these investigators observed that increased intensity of chemotherapy in AML lowered the prognostic MRD threshold. Undoubtedly, with any novel, improved therapy, the MRD level with clinical efficacy will change. In other words, for every new drug or therapeutic strategy, the clinically relevant MRD level will first need to be established retrospectively, a requirement that complicates the introduction of MRD-guided interventions.

Whatever the methodology and disease subtype, it is important to remember that accurate MRD data depend on sample quality. In particular, in AML and B-lineage ALL, MRD levels have been found to be drastically lower in blood than bone marrow.³³ In an elegant, though little noticed study, Helgestad and co-workers³⁴ demonstrated that the technique of marrow aspiration dramatically influenced the level of MRD in the aspirate. Even a second pull from the same aspiration site reduced the percentage of leukemic cells by almost 50% due to dilution with blood. Furthermore, when a large amount of marrow was aspirated in one single pull (e.g., 10ml), the percentage of leukemic cells was significantly lower than when only a small aspirate was obtained (e.g., 2.5ml). These findings are of crucial importance for multi-institutional trials with central MRD evaluation given the common practice to send the first aspirate to the local pathology laboratory and to continue aspirating from the same puncture site for additional samples to be sent off to the central place. Several National Cancer Trial Network groups who have active leukemia protocols which involve MRD determinations have, therefore, adopted the policy to request marrow aspirates from a separate puncture site to be submitted to the central laboratory by redirecting the needle after the first pull. But how does this problem translate into the ALL vs AML disparity? Between the Children's Oncology Group (COG) and St. Jude's Children Hospital, thousands of

children with ALL have been tested for MRD and its association with outcome. COG investigators were the first to introduce the “first pull” requirement for MRD samples in their leukemia treatment protocols, while trials performed at a single institution, like at St. Jude’s, have the advantage of being under tight performance control regarding aspiration practices. In adult groups, there is still a learning process underway and we occasionally meet with unexpected resistance. In general, marrow aspirates in ALL, particularly in younger patients, are of better quality than those in AML, especially in the cohort >50 years old, given that many older AML patients have a history of myelodysplasia or other antecedent hematologic disorder.

One more notable difference between MRD in AML and ALL relates to potentially measurable MRD targets. In ALL, it is common practice to define MRD as the detection of lymphoid cells with aberrant pheno- or genotype, based on the findings in the leukemic bulk at presentation. To date, there has not been any apparent need to monitor leukemic stem cells in B- or T-lineage ALL, though the frequency of CD34^{POS}CD38^{NEG} stem cells at diagnosis of B-ALL, but likely not T-ALL, predicted for high MRD levels.³⁵ This is in stark contrast to efforts in AML MRD monitoring which aim at detecting leukemic stem cells (LSC),^{36–39} and even the elusive pre-leukemic stem cell.⁴⁰ Fortunately, in AML, the phenotype of LSCs is well-characterized,^{36–42} much better than in ALL.⁴³ However, the potential need for accurate measurement of leukemic progenitors, in addition to or in lieu of measuring residual cells from the presenting leukemic bulk, adds an additional complex layer to routine MRD detection in AML. At the same time, not measuring LICs in AML may result in underestimating MRD positivity and explain why a substantial fraction of AML patients, negative for MRD by flow cytometry or molecular studies, experience relapse.

The fact that up to 30% of acute leukemia patients in first morphologic remission who are MRD negative (by whatever methodology and threshold level) will relapse while many patients who are MRD positive patients at this time point will not, prompted the definition of “false MRD negativity” or “false MRD positivity” (R. Gale, personal communication). As discussed in the prior paragraph, in AML false MRD negativity can result from merely monitoring the leukemic bulk, which may not be the culprit for relapse. Oligoclonality, the outgrowth of a minor leukemic subclone that was undetectable or missed at presentation, represents another possible cause of falsely defined MRD negativity.^{44,45} MRD monitoring by sequencing eliminates the risk of false negative MRD results due to clonal evolution of a specific gene during the course of disease by detecting alternate mutations of target gene(s).^{23–28} Potential pitfalls leading to false MRD negativity by flow cytometry include therapy-induced alterations in the leukemia phenotype, such as in response to steroids⁴⁶ or due to loss of the crucial gating antigen in response to antibody treatment, e.g., CD19 in the case of blinatumomab.⁴⁷ Most detrimental is a frequent lack of sufficient training of pathologists in the correct analysis and interpretation of MRD given that flow cytometric MRD detection is done in many routine laboratories. This can lead to basic errors such as the flow cytometric acquisition of insufficient white blood cells to yield the required sensitivity of one target cell in 10,000 – 100,000 normal cells and the choice of aberrant immune profiles with low sensitivity (or specificity).⁴⁸ Lack of experience leads to misinterpretation of hematogones as residual B-lymphoblasts or of normal immature

myeloid cells as residual leukemic myeloblasts in a recovering marrow following chemotherapy, as well as a failure to recognize an immunophenotypic shift. Finally, it is important to remember that MRD is by far not the sole factor predicting relapse and that other parameters provide independent outcome information, as exemplified by data from pediatric ALL.^{49,50}

On the other hand, false MRD positivity has been reported both in AML and ALL due to the detection of a clonal molecular marker in differentiating leukemic cells destined for cell death,^{23,51} a phenomenon of particular clinical relevance in acute promyelocytic leukemia treated with all-trans retinoic acid where PML/RAR α levels post induction do not represent reliable MRD values.⁵² Furthermore, the persistence and detection of leukemia fusion transcripts in non-leukemic stem cells, as proposed for *RUNX1/RUNX1T1* (*AML1/ETO*) AML,⁵⁰ may not be an indication for relapse.

Given the increased complexity associated with MRD measurements and their potential significance in AML, it is surprising that all but one⁵³ of the studies published to date have found a powerful predictive value of MRD status for outcome. This observation suggests that the prognostic value of MRD in AML, just as in ALL, is robust and not easily swayed by technical aspects. However, the uncertainty surrounding the most proper MRD target, the most trustworthy methodology, and the clinically most relevant MRD level at various time-points during treatment continue to hamper the implementation of MRD in the design of AML trials, more so than in ALL. Despite all the methodological aspects discussed in this perspective, I would suggest, though, that true biologic disparities exist in the significance of MRD between AML and ALL.

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Table 1

MRD Methods in ALL vs AML

ALL	AML
Somatic Receptor Gene Rearrangements (~90%)	No Immunogenotype
Simple Immunophenotypes (Hematogones in B-ALL)	Multiple Immunophenotypic Clones (lower sensitivities)
Changes in Antigen Profile with Therapy <u>Not Significant</u>	Changes in Antigen Profile with Therapy <u>Common and Significant</u>
Fusion Genes (up to 75%)	Fusion Genes (~30%)
Gene Mutations (Ikaros, JAK)	Gene Mutations : FLT3, NPM1, IDH, DNMT3A (<u>oligoclonality</u>)