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RELEVANCE OF MINIMAL RESIDUAL DISEASE FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA: AN OVERVIEW

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Running title: IMPORTANCE OF CD34+ FOR HEMATOPOIETIC

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ABSTRACT

Minimal residual or measurable disease (MRD) can predict relapse in AML patients. Depending on patients` risk stratification, MRD status may indicate that the patient will benefit from autoSCT and alloSCT in first clinical remission. In case of persistent MRD positivity, there is no consensus on whether there are benefits to perform additional consolidation treatments to eradicate MRD before alloSCT. Anyway, the persistence of pre-transplant MRD does not contraindicate alloSCT, but indicates an urgent need for transplantation, in addition to being considered a strong independent predictor of posttransplant outcomes in AML. Currently two approaches can be used to detect MRD in clinical practice, multiparameter flow cytometry (MFC) and real time PCR (RT-qPCR), although more sensitive new technologies are emerging, such as digital droplet PCR and next generation sequencing (NGS). Despite the differences of each distinct methodology available, MRD monitoring is currently part of the standard of care for AML patients.

Key Words: Minimal Residual Disease, Acute Myeloid Leukemia, Stem Cell Transplantation

Minimal residual or measurable disease (MRD) can predict relapse in AML patients, regardless of the variability of results among the currently available methodologies. However, some data are conflicting between different studies, which can be explained by differences both in the evaluation of pre- and post-transplant approaches and in the methods for MRD detection.

MRD persistence in the postinduction phase can predict poor outcomes in AML standard risk patients, indicating that they may benefit from allo-SCT in first clinical remission (CR1).[1]

In intermediate-risk patients, MRD status may indicate consolidation with autoSCT if MRD negative (MRDneg), or allo-SCT in patients with positive MRD (MRD+). In patients with MRD+, alloSCT was able to prolong OS and increase the duration of DFS to the level of favorable risk patients[2-3], suggesting that the transplantation can reverse the adverse prognosis of positive MRD.[2,4]

On the other hand, a study observed that both patients with MRD+ in morphological remission and those with active disease at the time of allo-SCT had similar worse outcomes.[5] However, there is a bias in

this study, which includes both pediatric and adult patients, without differentiating between different molecular risk groups. Currently there is no consensus on whether there are benefits to perform additional consolidation treatments to eradicate MRD before alloSCT.[1,6-7] Furthermore, the ability of alloSCT to overcome MRD positivity, the impact of the conditioning regimen intensity on MRD clearance and the selection of the ideal donor are not fully established.[1] A recent study showed that pre-alloSCT MRD negative (MRDneg) patients, OS and RFS at three years were longer for those who received myeloablative conditioning (MAC) compared to reduced intensity (RIC) and non-myeloablative (NMA) regimens, suggesting that MAC should still be considered for patients with MRDneg AML, if tolerated.[8] It must be taken into account that many patients who are apparently MRDneg, may in fact have occult disease or have pre-leukemic clones responsible for post-transplant relapse. In addition, patients <50 years in CR1 and MRD+ pre-transplant should preferably receive MAC allo-HCT, according to a retrospective study from EBMT.[9]

Anyway, the persistence of pre-transplant MRD does not contraindicate alloSCT1 but in fact indicates an urgent need for transplantation10, in addition to being considered a strong independent predictor of posttransplant outcomes in AML.[11-13] A metanalysis showed a robust association between MRD status, post-SCT relapse and mortality, regardless the method of detection, patients age, conditioning intensity, adverse cytogenetics[13] and donor-recipient HLA-matching.[4]

However, the conversion from MRD positivity pre-transplant to MRD negativity after myeloablative conditioning alloSCT does not substantially improve the relapse rate or overall survival (OS). [11-12]

Pre-SCT MRD is not associated with a significantly increased risk of non-relapse mortality (NRM). The association between pre-SCT MRD and OS in entirely accounted by disease relapse without significant contribution from SCT toxicity.[13]

Some studies have shown that early detection of MRD post-alloSCT (<D + 100) can predict AML patients' progression (relapse or death) over an average of 13 to 94 days from the detection of MRD.[14-16] On the other hand, post-alloSCT MRD status has not been independently associated with OS, RFS and RR in other series.[13,15]

The prevention of disease relapse, mainly for high-risk patients with AML, including those with high-risk cytogenetics or molecular markers, persistent MRD, and those who responded poorly to prior therapy, is the aim of future trials to determine most effective strategies for these patients.[10]

Therefore, post-transplant MRD results allow assessments of the effectiveness of preemptive therapeutic approaches to prevent relapses, such as discontinuation of immunosuppression and donor lymphocyte infusion, as well as the role of post-remission maintenance therapies and potential new drugs under investigation to mitigate the risk of AML recurrence after aloHCT.[17]

Methods for AML MRD detection: Currently two approaches can be used to detect MRD in clinical practice, multiparameter flow cytometry (MFC) and real time PCR (RT-qPCR). Each methodology differs in the applicability and sensitivity to detect MRD.[3,11,18] More sensitive and promising new technologies are emerging, such as digital droplet PCR and next generation sequencing (NGS), which can reach sensitivity superior to RT-qPCR and MFC[18-19], but are not ready for routine application outside of clinical trials.[11,18]

PCR assays have high sensitivity (10^{-5} – 10^{-6}) but a limited applicability to ~40% of AML patients that harbor 1 or more gene abnormalities.[11] It is considered the gold standard method for patients with NPM1 mutations, with fusion genes RUNX1-RUNX1T1, CBFB-MYH11, and PML-RARA. [11,18] Mutations not recommended by European Leukemia Net (ELN) for MRD are FLT3-ITD, FLT3-TKD, NRAS, KRAS, IDH1, IDH2, MLL-PTD, EV1 and WT1 expression, because of frequent losses or gains after treatment and at relapse.[11,18] The persistence of mutations related to clonal hematopoiesis indeterminate potential (CHIP), such as DNMT3A, TET2 and ASXL1, which are detected by NGS, also have no prognostic implications.[11] They may require the acquisition of new mutations to induce relapse, a process that may take longer.[20]

As an advantage, MFC does not require the availability of a previously determined leukemia-associated immunophenotype and is applicable to approximately 90% of AML patients[12,18], but has a limited sensitivity compared with PCR-based methods[12] due to the heterogeneity of approaches and interpretation of tests among the laboratories.[13,18] Two MFC approaches are used to assess MRD: 1) the detection of the leukemia associated immunophenotype (LAIP), which defines LAIPs at diagnosis and tracks these in subsequent samples; and 2) the different-from-normal (DFN) approach, which is based on the identification of aberrant differentiation/maturation profiles at follow up. [11,18-19, 21] Both approaches should be combined to best define MFC MRD burden.[11]

A promising MFC approach is the identification of Leukemic Stem Cells (LSC). The frequency of LCS in patients in remission is an independent prognostic factor for patient outcome[22], including in a post-transplant setting.[14] LSC and conventional MFC or PCR MRD double positivity predicts a very poor outcome in AML patients.[22] Despite this promising result, this approach is still investigational.

An important issue for AML MFC MRD is that immunophenotypic shift may occur and losses and gains of antigens expressions are frequent.[23] In the same way, clonal evolution is a potential obstacle when using somatic mutations as basis for MRD analysis.[23] NGS MRD strategies may overcome these issues as it potentially has more MRD targets than MFC and PCR. [23]

NGS allows millions of DNA fragments to be sequenced simultaneously and can detect mutations with a sensitivity that is superior to RT-qPCR and MFC in some implementations.[19] Despite promising results, measurement of MRD using NGS techniques are under development but are not ready for routine application outside of clinical trials.[11]

Samples for AML MRD detection: MFC MRD should be assessed from bone marrow (BM). ELN recommends 5-10mL BM and to use the first pull for MRD assessment to avoid hemodilution. [11] Molecular MRD should be assessed preferentially from peripheral blood (PB) and requires at least 20mL PB or more, if WBC count below 1000 cells/µL, to assure sensitivity of MRD detection.[11] For patients with a previous PB MRD negative result, subsequent MRD assessments should be from BM.[11]

Threshold for MRD level: The ELN MRD Working Party suggests a threshold of 0.1% to distinguish between MRD positivity and negativity.[11] However, even patients with MRD below this threshold may have significant residual leukemia and are still at risk of relapse.[19] MRD levels below 0.1% showed prognostic significance and some studies. Cutoff levels below 0.1% (eg,,0.01%) may define patients with particularly good outcome.[11] Some studies that measured MRD by MFC used lower detection thresholds, for example, from 0.01% to 1.0%, but the thresholds depend on the presence of informative LAIPs in the study.[24]

To reach the minimum sensitivity level of 0.1%, at least 500,000 to 1 million cells must be analyzed in a flow cytometry setting, with recommended panels of ≥ 8 colors, laboratories must have complete pre, post and standardization and analytical processes, and the MRD assessment must be performed by experienced analysts.[11]

MRD timepoints: For patients undergoing allo-SCT, MRD should be assessed not earlier than 4 weeks before conditioning treatment. [11] The exact time points for post-alloSCT MRD assessments are not well established. According the recommendations of ELN, MRD should be assessed every 3 months in bone marrow during the first two years after the end of treatment.[11] Alternatively, RTqPCR MRD can be assessed in peripheral blood every 4 to 6 weeks.[11]Monitoring beyond this period of follow up should be based on the relapse risk of the patient and decided individually. [11] The definition of molecular progression is the increase ≥ 1 log10 in MRD copy numbers between two positive samples.[11] A positive MRD molecular result should be confirmed after 4 weeks.[11]

CONCLUSIONS

Despite the differences of each distinct methodology available, MRD monitoring is currently part of the standard of care for AML patients.[10-11]

For molecular MRD this is limited to APL, CBF AML, and NPM1-mutated AML. For other AML patients, MRD should be assessed using MFC. [11]

The ELN 2017 recommendations for diagnosis and treatment of AML highlight that MRD testing should be performed in experienced, centralized diagnostic laboratories.[11]

Pre alloSCT MRD in AML is an irrefutable predictor of post-transplant relapse. [2-4,11-13]

In the post alloSCT setting, regular MRD assessments can be effective tools to identify patients at increased risk of relapse and assist with therapeutic decisions.[10,17]

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