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ARTICLE

Measurable residual disease (MRD) testing for acute leukemia in EBMT transplant centers: a survey on behalf of the ALWP of the EBMT

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Abstract

Detectable measurable residual disease (MRD) is a key prognostic factor in both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) patients. Thus, we conducted a survey in EBMT transplant centers focusing on pre- and post-allo-HCT MRD. One hundred and six centers from 29 countries responded. One hundred had a formal strategy for routine MRD assessment, 91 for both ALL and AML. For ALL ($n = 95$), assessing MRD has been routine practice starting from 2010 (range, 1990–2019). Techniques used for MRD assessment consisted of PCR techniques alone ($n = 27$), multiparameter flow cytometry (MFC, $n = 16$), both techniques ($n = 43$), next-generation sequencing (NGS) + PCR ($n = 2$), or PCR + MFC + NGS ($n = 7$). The majority of centers assessed MRD every 2–3 months for 2 (range, 1-until relapse) years. For AML, assessing MRD was routine in 92 centers starting in 2010 (range 1990–2019). Assessment of MRD was by PCR ($n = 23$), MFC ($n = 13$), both PCR and MFC ($n = 39$), both PCR and NGS ($n = 3$), and by all three techniques ($n = 14$). The majority assesses MRD for AML every 2–3 months for 2 (range, 1-until relapse) years. This survey is the first step in the aim to include MRD status as a routine registry capture parameter in acute leukemia.

Introduction

Over the last decade, persistence of detectable measurable residual disease (MRD) after intensive chemotherapy has proven to be a key prognostic factor in adult patients with acute leukemia. Specifically, in the setting of acute lymphoblastic leukemia (ALL), MRD monitoring has become an important parameter to guide therapeutic strategy including indication or not of performing an allogeneic hematopoietic stem cell transplantation (allo-HCT) in first complete remission [1, 2]. Indeed, in a recent meta-analysis achievement of MRD negativity was strongly associated with better overall survival (OS) [3]. Similarly, in the setting of acute myeloid leukemia (AML), persistence of detectable MRD after induction and/or consolidation chemotherapy has been identified as a strong prognostic factor in patients with NPM1-wt standard-risk AML patients [4] as well as in NPM1-mutated patients [5, 6]. Based on these observations, recent trials have included MRD data to allocate patients or not to allo-HCT [7], while the 2017 EuropeanLeukemiaNet (ELN) recommendations introduced the concept of CR without MRD as a response criteria for AML [8].

Despite allo-HCT remaining one of the most potent anti-leukemic strategy in patients with acute leukemia [9, 10], several recent studies have demonstrated that persistent detectable MRD at transplantation remains a key prognostic factor in both ALL and AML patients [11]. Specifically, in children with ALL, pretransplant MRD $\geq 10^{-3}$ (assessed with quantitative polymerase chain reaction (qPCR)

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detecting leukemic clone-specific T-cell-receptor (TCR)/Immunoglobulin (Ig) gene rearrangements) was associated with a high risk of relapse leading to worse OS [12]. In the AML setting, pretransplant MRD (assessed by multiparameter flow cytometry (MFC)) ≥0.01% was associated with higher risk of relapse and lower OS even after adjusting with disease status at transplantation and ELN risk group [13]. Interestingly, the negative impact of persistent MRD (at least when assessed by MFC) was comparable in patients with MRD levels >1% and those with MRD levels <0.01%, suggesting that any level of persistent MRD was of poor prognosis [14]. Confirming these findings, a recent meta-analysis including data from 19 articles reported between 2005 and 2016 demonstrated that detectable MRD (regardless of MRD methodology, MRD threshold, and conditioning regimen intensity) at transplantation predicted for higher relapse incidence and worse OS in allo-HCT patients [15]. Interestingly, detectable MRD has remained predictive of allo-HCT outcome in patients with monosomal karyotype (which is associated with a particularly poor prognosis by itself [16]) [17]. Thus, incorporating pretransplant MRD as an additional parameter in large transplant registry databases, though challenging, is of special importance. Accordingly, several reports from the ALWP of the EBMT have evidenced higher risk of relapse and worse transplantation outcomes in patients with detectable MRD at transplantation in various transplantation setting [18–23].

Importantly, latest studies have unraveled that posttransplant MRD re-appearance in patients with acute leukemia was associated with grim outcomes [24]. Specifically, in the ALL-BFM-SCT 2003 Trial, MRD load $>10^{-4}$ leukemic cells after all-HCT predicted for high relapse incidence at any time point after transplantation. However, interestingly, the predictive value of MRD positivity was lower on day 30 after transplantation than thereafter [25]. Similarly, Wethmar et al. observed that posttransplant residual/relapsing MRD (assessed by qPCR targeting disease-specific genetic rearrangements and/or lineage-sorted donor cell chimerism) in ALL patients predicted for a high relapse incidence and poor OS [26].

In the AML setting, Pozzi et al. demonstrated that WT1 overexpression (defined >100 messenger copies/10^4 ABL1) after allo-HCT was the strongest predictor of relapse in AML patients [27]. Interestingly, among patients with posttransplant WT1 overexpression OS was improved in patients who received preemptive donor lymphocyte infusions. In the study from the Fred Hutchinson Cancer Center, pretransplant MRD (assessed by MFC) was associated with grim outcomes regardless of whether or not MRD was cleared on day 28 after allo-HCT [24]. However, two patients MRD negative at allo-HCT who were MRD positive on day 28 died of AML relapse before day 28 [24]. Thus, capturing posttransplant MRD data at key time points is also of major interest for transplantation registries.

Several MFC and molecular biology approaches have been developed to assess MRD in patients with acute leukemia [28]. Their sensitivity ranges from 10^{-3} to 10^{-6}. In ALL, qPCR detecting leukemic clone-specific TCR/Ig gene rearrangements can be used in ~90% of the patients [1]. MCF can also be used in >90% of the patients [1]. In AML while qPCR methods can only be used to quantify molecular markers and gene fusions such as BCR/ABL, NPM1, CBFB-MYH11, RUNX1-RUNX1T1, PML-RARA or KMT2A-AFF4, MCF and next-generation sequencing (NGS) methods are considered as more universal [29, 30]. However, they generally have a lower sensitivity. Given its even lower sensitivity, WT1 expression is currently considered to be a suboptimal MRD marker that should be used only if other techniques are not available [29].

As a first step aimed at including pre- and posttransplant MRD status as routine registry capture parameters [31], we conducted a survey among European Blood and Marrow Transplantation Society (EBMT)-affiliated centers focused on MRD assessment pre- and post-allo-HCT for patients with either AML or ALL.

Methods

A total of 325 EBMT-affiliated centers were contacted by e-mail to invite them to complete an online survey assessing how MRD was assessed in their center. Specifically, the questionnaire surveyed the location of MRD analysis (in-house, outsourced, or both), the methodology (PCR, FCM, NGS, or combination of these techniques), cut-off points for MRD negativity, and timing as well as duration of MRD monitoring after transplantation for both AML and ALL. The detailed survey is provided in Supplementary Table 1.

The board of the acute leukemia working party of the EBMT approved this survey.

Results

One hundred and six centers out of 325 (33%) contacted centers responded to the survey (Fig. 1). Among the 219 centers that did not answer to the survey, 189 (88%) and 170 (78%) centers reported data on pretransplant MRD for AML or ALL patients transplanted in 2018, respectively. Among the 106 centers that answered to the survey, the figures were 95 (91%) and 92 (87%), respectively. Responding centers were from 29 countries, including Italy ($n = 23$), Germany ($n = 12$), France ($n = 11$), Spain ($n = 8$), and Belgium ($n = 5$). Responding centers were of various sizes reporting a median of 1–97 allogeneic or autologous

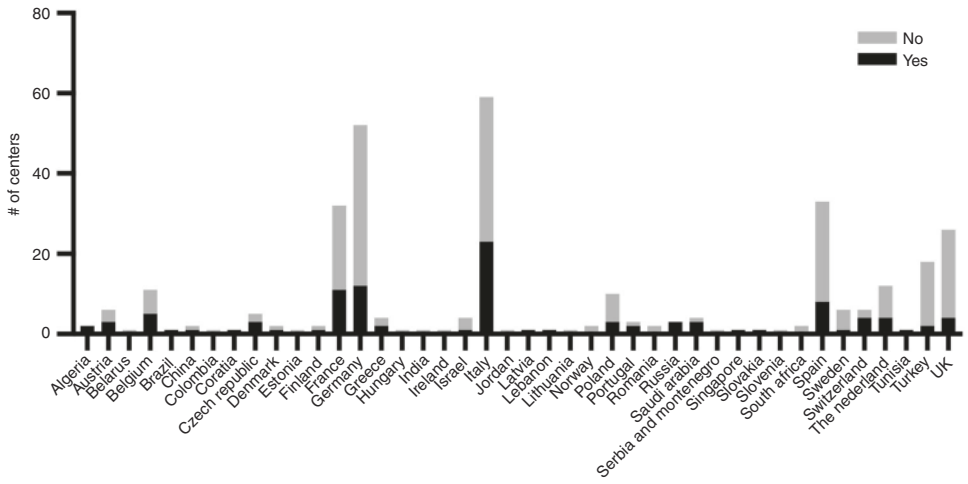

Fig. 1 Number of responding centers per country. Number of centers responding to the MRD survey (black bars) according to the number of centers reporting allo-HCT (gray bars) to the EBMT registry per country.

transplantation for acute leukemia per year from 2014 to 2018. Out of the 106 centers that answered the survey, 48 performed <100 transplants and 58 more than 100 transplants from January 2014 to December 2018. Among centers that performed 100 or more transplants, 55% and 78% of the centers performed the MRD testing only in their hospital for ALL and AML, respectively. Among smaller size centers, the figures were 45% and 68%, respectively. There was no significant difference between the methods used according to center size (data not shown). One hundred centers had a formal strategy for routine MRD assessment for both AML and ALL ($n = 91$), ALL only ($n = 4$), and AML only ($n = 1$). Four centers performed routine MRD assessments but failed to answer the remaining survey questions. In contrast, six centers reported not having a formal strategy for routine MRD assessment, including two centers from Algeria, two from Germany, one from Brazil, and one from Saudi Arabia.

Acute lymphoblastic leukemia

Ninety-five centers indicated that, for ALL, assessing MRD has been routine practice starting from 1990 to 2019 (median, 2010). Of these, 48 (51%) do it in-house, 20 (21%) outsource, and 27 (28%) use both options. Twenty-seven centers assess MRD by PCR, 16 by MFC, 43 use both, 2 use PCR and NGS, and 7 use all three techniques (Fig. 2a). By PCR ($n = 79$), MRD negativity is regarded as 10^{-5} in 40, 10^{-4} in 18 and 10^{-6} in 7, and 10^{-3} in 5 centers (Fig. 2b). The data were missing for six centers, and three centers answered that the threshold depended on which marker was used. For those assessing MRD by MFC ($n = 66$), MRD negativity threshold is 10^{-4} in 37, 10^{-5} in 13, 10^{-3} in 8, 10^{-6} in 4, and 10^{-2} in 1 centers. The data were missing for two centers and one center answered that the threshold depended on which marker was used. For those centers assessing MRD by NGS ($n = 9$),

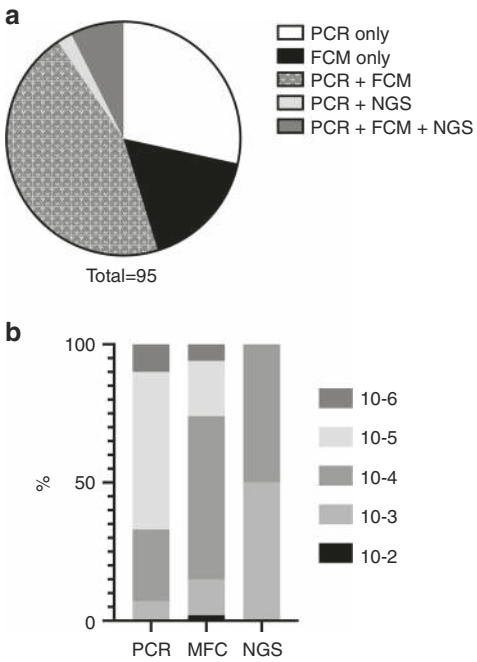

Fig. 2 MRD assessment in ALL. a Methods used for MRD assessment in ALL. **b** Cut-off point regarding as negative MRD according to the technique used.

MRD negativity is regarded as 10^{-3} in 2 and 10^{-4} in 2 centers. The data were missing for three centers and two centers answered that the threshold depended on which marker was used.

The majority of centers assess MRD every 2–3 months post-allo-HCT ($n = 63$) or once every 3 months post-allo-HCT ($n = 9$). The duration of post-allo-HCT MRD assessment was 1 year in 19 centers, 2 years in 36 centers, 5 years in 10 centers, or until relapse in 20 centers. Less frequent answers included depending on patient ($n = 2$), other ($n = 2$), for 3 year ($n = 1$), according to chimerism ($n = 1$), ALL

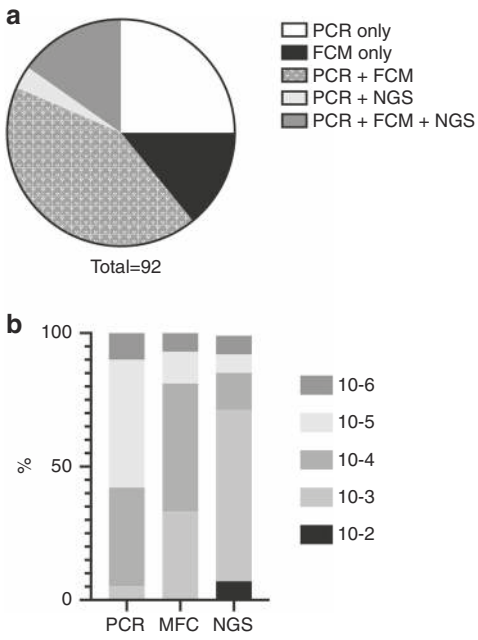

Fig. 3 MRD assessment in AML. a Methods used for MRD assessment in AML. **b** Cut-off point regarding as negative MRD according to the technique used.

study regulations ($n = 1$), not routinely done after transplantation ($n = 1$), on demand ($n = 1$), and missing ($n = 1$).

Acute myeloblastic leukemia

For AML, assessing MRD was routine in 92 centers starting from 1990 to 2019 (median, 2010). It has been done in-house in 67 (74%), outsourced in 9 (10%), and by both in 15 (16%) of centers. The information was missing for one patient. Assessment of MRD was performed by PCR only in 23 centers, by MFC only in 13, by both PCR and MFC in 39, by PCR and NGS in 3, and by all three techniques in 14 centers (Fig. 3a). Using PCR ($n = 79$), MRD negativity is regarded as 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} in 3, 22, 28, and 6 centers, respectively. The data were missing for 8 centers and 12 centers answered that the threshold depended on which marker was used (Fig. 3b). By MFC ($n = 66$), MRD negativity is regarded as 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} in 20, 29, 7, and 4 centers, respectively. The data were missing for 1 center and 5 centers answered that the threshold depended on which marker was used. Finally, for those centers assessing MRD by NGS ($n = 17$), MRD negativity is regarded as 10^{-2}, 10^{-3} 10^{-4}, 10^{-5}, and 10^{-6} in 1, 9, 2, 1, and 1 centers, respectively. The data were missing for 1 center and 2 centers answered that the threshold depended on which marker was used. The majority assess MRD for AML every 2–3 months posttransplant ($n = 38$) or once every 3 months posttransplant ($n = 25$). The duration of post-allo-HCT MRD assessment was 1 year in 15 centers, 2 years in 37 centers, 5 years in 9 centers, until relapse in 22 centers or depending on patients in 4 centers. Less frequent answers included 3 months ($n = 1$), according to chimerism ($n = 1$), not routinely done except for acute promyelocytic leukemia ($n = 1$), on demand ($n = 1$), or missing ($n = 1$).

Discussion

As mentioned in the introduction, several techniques have been used to assess pre- and posttransplant MRD after transplantation. In ALL, qPCR techniques targeting antigen-receptor gene rearrangements can be used in >90% of the patients, with most often a sensitivity of 10^{-5} [1]. Molecular detection of fusion transcripts (such as BCR/ABL) can also be used for MRD detection with a high sensitivity (10^{-5}) but can be biased by the fact that the number of RNA transcripts per leukemic cell varies among different leukemic cells. MCF can also be used in >90% of the patients and can reach a sensitivity of 10^{-3} to 10^{-5} [1]. The main advantage of MCF (in comparison to qPCR targeting antigen-receptor gene rearrangements) is that this technique can provide results in a few hours but the samples need to be processed relatively quickly after collection (i.e., within 3 days at the most to avoid cell death). In ALL setting, MFC can be affected by posttransplant regeneration of normal lymphoid cells co-expressing some ALL-type antigens. Recently, the use of high-throughput methods (next-generation flow cytometry) allowed processing large amounts of cells (>10^7 stained cells) in parallel, substantially increasing the sensitivity of the technique to 10^{-5} with a very high applicability (>98%) [32]. Further, recent studies have suggested a complementary role of combining qPCR MRD assessment with lineage-specific chimerism evaluations [26].

In AML, qPCR can only be used for gene fusions such as BCR/ABL, NPM1, CBFB-MYH11, RUNX1-RUNX1T1, PML-RARA, or KMT2A-AFF4. In contrast, MFC has a large applicability (>90% of AML patients) with a good sensitivity (10^{-3}–10^{-5}) [28]. Two different approaches are used for assessing MRD with MCF: (1) the leukemia-associated aberrant immunophenotypes (LAIP) approach, which identifies LAIP at diagnosis and tracks these in following samples; and (2) the "different-from-normal" approach, which is based on the identification of aberrant differentiation/maturation profiles even without a diagnostic phenotype for reference. Since the LAIP approach can be affected by phenotypic shift of the leukemic cells, ELN experts recommend that both approaches are combined to follow MRD in AML by FCM [29]. NGS is also becoming an important tool for MRD monitoring in AML. This technique is applicable to virtually all AML patients but the interpretation of the results requires complex bioinformatics

approaches. The sensitivity of this approach is generally lower than what can be achieved with qPCR or MFC but this technique also allows the identification of leukemic sub clones [33].

As a first step aimed at including pre- and posttransplant MRD status as routine registry capture parameters, we conducted a survey among EBMT-affiliated centers focused on MRD assessment pre- and post-allo-HCT for patients with either AML or ALL. This survey provided useful information on how MRD is currently assessed in acute leukemia patients undergoing an allo-HCT.

An initial observation was that among the 106 transplant centers who responded to the survey, 95 (90%) and 92 (87%) had a formal strategy for routine MRD assessment in ALL and/or AML, respectively. This indicates the increasing routine use of MRD data in the management of transplant patients. However, although centers from 29 different countries took part in the survey, it is likely that there was some participation bias in favor of centers assessing MRD, these probably being more likely to answer the survey than centers not assessing MRD.

In ALL patients, MRD was most frequently assessed by PCR techniques (74% of centers) either alone (28% of centers) or in combination with flow cytometry (45% of centers). In contrast, MCF was the sole method of MRD assessment in 16% of centers.

Similarly to that observed in ALL patients, among AML patients, MRD was most frequently assessed by PCR techniques (67% of centers) either alone (25% of centers) or in combination with flow cytometry (42% of centers). In contrast, MCF was the sole method of MRD assessment in 16% of centers.

In concordance with data from the literature, the median sensitivity reported by the centers was a log higher for PCR techniques (10^{-5}) than for MFC methods (10^{-4}). Importantly, there was a considerable variation from center to center in the cut-off point for MRD negativity ranging from 10^{-2} to 10^{-6}. This suggests that future collection of MRD registry data should ideally not be restricted to dichotomous responses such as MRD positivity or not, and that questionnaires should allow the recording of the level of MRD positivity or the cut-off for MRD negativity (in the case of MRD negative patients). This will likely increase the predictive value of pretransplant MRD status on relapse risk and OS above what has been observed with MRD positivity as currently ascertained in the EBMT registry database. Further, having these data in the registry will also benefit to study assessing the impact of prophylactic intervention such as posttransplant immunosuppression discontinuation, donor lymphocyte infusion, or targeted low-intensity therapies on leukemia progression [34–39].

In summary, these data indicate that assessing MRD for both ALL and AML has become routine practice in close to 100 EBMT transplant centers, with 50–74% of them establishing the assay in-house. The assessment technique and threshold values varied between centers. In these centers, MRD status is assessed every 2–3 months for a median of 2 years after transplantation or until relapse. Based on these consideration, one could propose that AL transplant registries could collect MRD data pretransplant on day 40, 100, 180, and 365 after transplantation. These data might consist of capturing the MRD level, the technique used, the sensitivity of the technique, and the cell type assessed (bone marrow, peripheral blood. or lineage-specific cells).

Author contributions AN wrote the manuscript, designed the study, and interpreted the data; FB wrote the manuscript and interpreted the data; ML designed the study, analyzed and interpreted the data, and edited the manuscript; EP designed the study, analyzed and interpreted the data, and edited the manuscript; MM designed the study, interpreted the data, and edited the manuscript; all remaining authors helped in the design of the study, interpreted the data, and edited the manuscript. All authors approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest FB has received travel grants from Celgene, Abbvie, Novartis, and Sanofi as well as honoraria from Merck and Abbvie. The remaining authors declare that they have no relevant conflict of interest.

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