Revista Brasileira de Hematologia e Hemoterapia
Brazilian Journal of Hematology and Hemotherapy

www.rbhh.org

Special article

Proposal for the standardization of flow cytometry protocols to detect minimal residual disease in acute lymphoblastic leukemia

Maura Rosane Valério Ikoma[a,*], *Miriam Perlingeiro Beltrame*[b], *Silvia Inês Alejandra Cordoba Pires Ferreira*[c], *Elizabeth Xisto Souto*[d], *Mariester Malvezzi*[b], *Mihoko Yamamoto*[e], *on behalf of Minimal Residual Disease Working Group of the Brazilian Society of Bone Marrow Transplantation (SBTMO)*[1]

[a] *Fundação Amaral Carvalho (FAC), Jaú, SP, Brazil*
[b] *Universidade Federal do Paraná (UFPR), Curitiba, PR, Brazil*
[c] *Centro de Hematologia e Hemoterapia de Santa Catarina (HEMOSC), Florianópolis, SC, Brazil*
[d] *Diagnósticos da América (DASA), São Paulo, SP, Brazil*
[e] *Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil*

ARTICLE INFO

Article history:
Received 3 May 2015
Accepted 27 July 2015
Available online 28 September 2015

Keywords:
Minimal residual disease
MRD acute lymphoblastic leukemia
Flow cytometry
Immunophenotyping

ABSTRACT

Minimal residual disease is the most powerful predictor of outcome in acute leukemia and is useful in therapeutic stratification for acute lymphoblastic leukemia protocols. Nowadays, the most reliable methods for studying minimal residual disease in acute lymphoblastic leukemia are multiparametric flow cytometry and polymerase chain reaction. Both provide similar results at a minimal residual disease level of 0.01% of normal cells, that is, detection of one leukemic cell in up to 10,000 normal nucleated cells. Currently, therapeutic protocols establish the minimal residual disease threshold value at the most informative time points according to the appropriate methodology employed. The expertise of the laboratory in a cancer center or a cooperative group could be the most important factor in determining which method should be used. In Brazil, multiparametric flow cytometry laboratories are available in most leukemia treatment centers, but multiparametric flow cytometry processes must be standardized for minimal residual disease investigations in order to offer reliable and reproducible results that ensure quality in the clinical application of the method. The Minimal Residual Disease Working Group of the Brazilian Society of Bone Marrow Transplantation (SBTMO) was created with that aim. This paper presents recommendations for the detection of minimal residual disease in acute lymphoblastic leukemia based on the literature and expertise of the laboratories who participated in this consensus, including pre-analytical and analytical methods. This paper also recommends that both

* *Corresponding author at*: Fundação Amaral Carvalho (FAC), Rua das Palmeiras, 106, 17210-120 Jau, SP, Brazil.
E-mail address: msikoma@uol.com.br (M.R.V. Ikoma).
[1] Members of MRD Working Group of SBTMO are listed in Appendix.
http://dx.doi.org/10.1016/j.bjhh.2015.07.012
1516-8484/© 2015 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. All rights reserved.

multiparametric flow cytometry and polymerase chain reaction are complementary methods, and so more laboratories with expertise in *immunoglobulin/T cell receptor (Ig/TCR)* gene assays are necessary in Brazil.

© 2015 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. All rights reserved.

Introduction

Minimal residual disease (MRD) is today considered the most powerful predictor of outcome in acute leukemias, including acute lymphoblastic leukemia (ALL). Although classical factors such as age, cytogenetic and molecular features, and leukocyte count are taken into account to establish the initial risk groups for therapeutic purposes, the evaluation of treatment response by MRD detection allows clinicians to identify relapse risk categories for ALL and stratify the chemotherapy according to well-established adult or pediatric therapeutic protocols.[1–7] Results of MRD studies can also be used to select treatment intensity and duration, and estimate the optimal timing for hematopoietic stem cell transplantation (HSCT) in childhood ALL.[8]

Both multiparametric flow cytometry (MFC) and the amplification of *immunoglobulin/T cell receptor* (*Ig/TCR*) genes by polymerase chain reaction (PCR) have similar results in MRD detection with a level of 10^{-4} cells. However the best time points for detection are different between the two techniques.

Clinical significance of minimal residual disease levels

The goals of MRD studies for clinical purposes are to establish: (i) the levels of MRD that are relevant to the therapeutic decision; (ii) the most informative time points during treatment; and (iii) the clinical relevance of information that each method provides at the different time points.

The cut-off value to define ALL MRD positivity is 0.01% or 10^{-4} cells, because this represents the limit of detection by immunophenotyping and molecular assays, although it is possible to achieve a higher sensitivity (better than 0.01%) by PCR techniques. Moreover, with the recent improvements in technology, this threshold can now be achieved by flow cytometry.[8,9] Currently, therapeutic protocols establish a cut-off point at the most informative time to predict danger of relapse according to the appropriate methodology employed for MRD detection (Table 1).

The identification of the disease relapse risk allows therapeutic stratification and better clinical management, including recognition of patients who require less intensive therapy and those eligible for HSCT at first remission.[5,10]

The level of MRD in pediatric patients prior to conditioning for allogeneic HSCT has a significant impact on post-transplant outcomes and it is the most important predictor of relapse after HSCT. Patients with high-level MRD at the time of transplant ($>10^{-3}$ or 0.1% malignant cells) have significantly poorer outcomes than those who entered the transplantation with negative MRD ($<10^{-3}$ cells).[11] The Acute Lymphoblastic Leukemia Berlin-Frankfurt-Münster Stem Cell Transplantation Group (ALL-BFM-SCT) 2003 trial assessed MRD in the bone marrow (BM) at Days 30, 60, 90, 180, and 365 after HSCT and each time point with a MRD $\geq 10^{-4}$ leukemic cells was consistently correlated with shorter event free survival.[12]

Two techniques are available for post-transplant monitoring of disease remission: MRD detection and the characterization of post-transplant chimerism. The MRD detection techniques search for the malignant clone, while assessments of chimerism characterize the origin of post-transplant hematopoiesis.[11] The sensitivity of investigations of chimerism vary greatly depending on the method used.[13]

Patients with a low MRD level after HSCT ($<10^{-3}$), can convert mixed chimerism to complete chimerism by pre-emptive immunotherapy,[11,14,15] which demonstrates the importance of MRD monitoring after HSCT. Although there is not a well-established management schedule for these cases, MRD status provides a real perspective of rational therapeutic intervention after HSCT to prevent recurrence of the disease.[14]

Methods of minimal residual disease detection

The most reliable methods of evaluating MRD are MFC analysis with the identification of leukemia-associated immunophenotypes (LAIPs) and amplifying antigen-receptor (*Ig/TCR*) gene rearrangements and fusion transcripts by PCR. Both MFC and amplification of *Ig/TCR* genes by PCR provide similar results at a MRD level of 0.01%,[5,16] and both MFC and PCR have advantages and disadvantages. MFC is a rapid method, useful in >95% of ALL cases and is more informative than PCR during the first phase of induction therapy, while PCR is preferable for studies after HSCT or at the end of therapy because of its high sensitivity in those moments.[10,17] During the first 2–3 weeks of remission-induction therapy, BM specimens do not contain lymphoid progenitors, and so the detection of immature B-cells by MFC can be an indication of residual disease.[17]

The most important causes of discrepancy between MFC and PCR assays are: (i) samples containing a limited cell number for MFC assays; (ii) phenotype variations of regenerating precursor B-cells (PBC) in BM during therapy and related to age; (iii) drug induced antigenic modulation; (iv) quality of PCR clonal markers; (v) amplification of nonspecific DNA from dead cells; and (vi) oligoclonality and clonal evolution.[6,18–20]

The main disadvantages of *Ig/TCR* rearrangement investigations are: (i) they are labor intensive and time consuming; (ii) require extensive experience and knowledge concerning the different types of *Ig/TCR* gene rearrangements; (iii) real-time PCR technology is demanding because of the design and sensitivity of testing using specific probe-prime sets for individual

Download English Version:

https://daneshyari.com/en/article/3332939

Download Persian Version:

https://daneshyari.com/article/3332939

Daneshyari.com