



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

[www.rbhh.org](http://www.rbhh.org)



### Scientific Comment

# Qualitative polymerase chain reaction versus quantitative polymerase chain reaction for the detection of minimal residual disease in children with acute lymphoblastic leukemia<sup>☆</sup>



Carlos Alberto Scrideli\*, Luiz Gonzaga Tone

Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil

In acute lymphoblastic leukemia (ALL), remission is classically defined as the reestablishment of normal hematopoiesis and the presence of less than 5% of the nucleated blast cell population found by conventional microscopy; this is used in older protocols to assess treatment response. Morphological analysis, although useful and applicable at any center, has proven to be of limited sensitivity, subjective and imprecise to study early response to treatment and this technique does not appear to be sufficient to identify patients at true risk of relapse who might benefit from the intensification of treatment.<sup>1,2</sup> For this reason, cytomorphological analysis has been replaced by minimal residual disease (MRD) monitoring in several treatment protocols and new definitions of remission and relapse in childhood ALL have been proposed.<sup>3</sup>

The analysis of MRD has proved to be the strongest independent prognostic factor in all studies analyzing large series of patients with B-lineage and T-cell ALL, and specific molecular subgroups such as patients with the BCR-ABL fusion gene and ALL patients with MLL gene rearrangements. This analysis allows more accurate risk group assignment and tailoring the intensity of treatment, permitting reduction or intensification at the different treatment time points according to the MRD level.<sup>4–9</sup> MRD monitoring can also guide treatment decisions in relapsed patients and those who are candidates for bone marrow transplantation.<sup>4,5,10,11</sup>

Sequential monitoring of MRD using more sensitive and specific techniques, such as quantitative real-time polymerase chain reaction (RQ-PCR) for immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements and flow cytometry analysis, with a detection power of one blast cell in 10<sup>4</sup>–10<sup>6</sup> normal cells, has substantially refined the assessment of early response to treatment. Unfortunately these methods are not only expensive, but technically complex and require considerable technology and highly-specialized laboratories to be routinely used in risk stratification protocols for ALL; they are therefore inaccessible to most treatment centers, especially in developing countries.<sup>4,12</sup> The development of simplified MRD technologies is essential to allow the potential benefits of MRD monitoring to be extended to all children with leukemia including those treated in low-budget countries. Table 1 shows some characteristics of the main methodologies used to detect MRD in ALL.

A clinically useful simplified MRD technique should be economically viable, widely applicable, specific and sensitive enough to predict the course of the disease. The detection of clonal Ig and TCR rearrangements by PCR and homoduplex analysis has proved to be a rapid and much simpler and cheaper method than the use of clone-specific probes or flow cytometry. In a multicenter retrospective study, this was the strongest independent prognostic factor

DOI of original article: <http://dx.doi.org/10.1016/j.bjhh.2015.08.003>.

<sup>☆</sup> See paper by Paula et al. on pages 373–80.

\* Corresponding author at: Departamento de Puericultura e Pediatria, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (USP), Avenida Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brazil.

E-mail address: [scrideli@fmrp.usp.br](mailto:scrideli@fmrp.usp.br) (C.A. Scrideli).

<http://dx.doi.org/10.1016/j.bjhh.2015.08.010>

1516-8484/© 2015 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. All rights reserved.

**Table 1 – Characteristics of the methodologies used for minimal residual disease detection in acute lymphoblastic leukemia (ALL)<sup>a</sup>.**

|                       | Flow cytometry immunophenotyping                                                                                                                                                                                                                                                                                           | RQ-PCR analysis of fusion gene transcripts                                                                                          | RQ-PCR analysis of Ig/TCR genes                                                                                                                                                                                                                                           | Conventional PCR of Ig/TCR genes and homo/heteroduplex analysis                  |
|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Estimated sensibility | 3–4 colors: $10^{-3}$ – $10^{-4}$<br>6–8 colors: $10^{-4}$                                                                                                                                                                                                                                                                 | $10^{-4}$ – $10^{-6}$                                                                                                               | $10^{-4}$ – $10^{-5}$                                                                                                                                                                                                                                                     | $10^{-2}$ – $10^{-3}$                                                            |
| Applicability         | B-ALL: >90%<br>T-ALL: >90%                                                                                                                                                                                                                                                                                                 | B-ALL: 25–40%<br>T-ALL: 10–15%                                                                                                      | B-ALL: 95%<br>T-ALL: 90–95%                                                                                                                                                                                                                                               | B-ALL: >90%<br>T-ALL: >90%                                                       |
| Advantages            | Fast                                                                                                                                                                                                                                                                                                                       | Relatively easy                                                                                                                     | Applicable to the great majority of B-ALL and T-ALL cases                                                                                                                                                                                                                 | Cheaper                                                                          |
| Disadvantages         | Information about the whole sample cellularity<br>Applicable to the great majority of patients                                                                                                                                                                                                                             | Sensitive                                                                                                                           | Sensitive                                                                                                                                                                                                                                                                 | Relatively easy                                                                  |
|                       | Variable sensitivity due to similarities between normal regenerating cells and leukemic cells<br>Ideal at least two aberrant immunophenotypes per patient (chance of immunophenotypic shifts)<br>Drug-induced modulation of the immunophenotype might influence the levels of antigenic expression<br>Relatively expensive | Applicable to specific leukemia subgroups (BCR-ABL, MLL-AF4, etc.)<br>Limited standardization                                       | Well standardized<br>Expensive                                                                                                                                                                                                                                            | Applicable to the great majority of patients<br>Not quantitative                 |
|                       |                                                                                                                                                                                                                                                                                                                            | Limited applicability in ALL (absence of targets in >50% of cases)<br>Risk of contamination                                         | Requires extensive experience and knowledge<br>Time consuming at diagnosis: identification of the junctional regions and sensitivity testing<br>Need for (preferably) two sensitive PCR targets per patient ( $\geq 10^{-4}$ ), because of the chance of clonal evolution | Low sensitivity—patients with MRD levels $<10^{-2}$ – $10^{-3}$ are not detected |
|                       | Limited standardization                                                                                                                                                                                                                                                                                                    | Differences in fusion transcript expression levels between the patients<br>Stability of fusion gene transcripts decreases over time |                                                                                                                                                                                                                                                                           |                                                                                  |

PCR: polymerase chain reaction; RQ-PCR: quantitative real time polymerase chain reaction; Ig: immunoglobulin gene; TCR: T-cell receptor gene; B-ALL: B-lineage ALL; T-All: T-cell ALL.

<sup>a</sup> Based on van Dongen et al.<sup>4</sup>, Schrappe et al.<sup>12</sup> and Conter et al.<sup>13</sup>

in patients treated according to the Grupo Brasileiro de Tratamento da Leucemia Infantil-leucemia linfóide aguda protocol 1999 (GBTLI-99).<sup>13</sup> This method represents a good predictive criterion of unfavorable course in children with ALL as it is able to identify patients with a high risk of relapse. This method, however, was not truly quantitative and, due to its lower sensitivity, it should be employed only to identify patients with a high residual tumor load.

Actually in the GBTLI-2009 protocols, MRD analysis at Days 14 and 35 of the induction phase has been used to stratify patients as good and poor responders, guiding treatment decisions in all pediatric ALL subtypes.<sup>14</sup> Due to the cost and technical complexity, MRD analysis using patient specific probes by RQ-PCR has been routinely used in very few treatment centers in Brazil and no comparison of this method with simplified MRD strategies to detect Ig or TCR clonal rearrangements by conventional PCR and homo-heteroduplex analysis has been published until now.

In this issue of the Revista Brasileira de Hematologia e Hemoterapia, Paula et al.<sup>15</sup> compared MRD monitoring using Ig and TCR gene rearrangements by conventional PCR followed by homo-heteroduplex analysis with clone-specific probes to RQ-PCR at the end of induction in 44 children with ALL. According to RQ-PCR MRD cut-off points established by the GBTLI-2009 protocol, the agreement between the two methods was 40% for B lineage ALL and 100% for T-cell ALL. MDR detection by the simplified method was a significant prognostic factor for 3.5-year leukemia free survival. Surprisingly, the same was not observed using the clone-specific RQ-PCR method. Despite the deficiencies associated with the study design, especially the relatively small number of patients analyzed, the short follow up and the different protocols used – which are well recognized by the authors – the results are interesting and can be useful to aid the validation of alternative and cost effective methods to detect MRD in centers with lower technological resources. Analysis of a larger series of patients with

ALL using the same protocol is essential to define the real utility of this simplified strategy in the treatment stratification.

### Conflicts of interest

The authors declare no conflict of interest.

### REFERENCES

1. Cazzaniga G, Biondi A. Molecular monitoring of childhood acute lymphoblastic leukemia using antigen receptor gene rearrangements and quantitative polymerase chain reaction technology. *Haematologica*. 2005;90(3):382-90.
2. Stanulla M, Schrauder A. Bridging the gap between the north and south of the world: the case of treatment response in childhood acute lymphoblastic leukemia. *Haematologica*. 2009;94(6):748-52.
3. Schnittger S. Minimal residual disease monitoring: a new era for childhood ALL. *Lancet Oncol*. 2015;16(4):362-4.
4. van Dongen JJ, van der Velden VH, Brüggemann M, Orfao A. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. *Blood*. 2015;125(26):3996-4009.
5. Vora A, Goulden N, Mitchell C, Hancock J, Hough R, Rowntree C, et al. Augmented post-remission therapy for a minimal residual disease-defined high-risk subgroup of children and young people with clinical standard-risk and intermediate-risk acute lymphoblastic leukaemia (UKALL 2003): a randomised controlled trial. *Lancet Oncol*. 2014;15(8):809-18.
6. Pui CH, Pei D, Coustan-Smith E, Jeha S, Cheng C, Bowman WP, et al. Clinical utility of sequential minimal residual disease measurements in the context of risk-based therapy in childhood acute lymphoblastic leukaemia: a prospective study. *Lancet Oncol*. 2015;16(4):465-74.
7. Borowitz MJ, Wood BL, Devidas M, Loh ML, Raetz EA, Salzer WL, et al. Prognostic significance of minimal residual disease in high risk B-ALL: a report from Children's Oncology Group study AALL0232. *Blood*. 2015;126(8):964-71.
8. Schrappe M, Valsecchi MG, Bartram CR, Schrauder A, Panzer-Grümayer R, Möricke A, et al. Late MRD response determines relapse risk overall and in subsets of childhood T-cell ALL: results of the AIEOP-BFM-ALL 2000 study. *Blood*. 2011;118(8):2077-84.
9. Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grümayer R, Möricke A, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. *Blood*. 2010;115(16):3206-14.
10. Eckert C, Hagedorn N, Sramkova L, Mann G, Panzer-Grümayer R, Peters C, et al. Monitoring minimal residual disease in children with high-risk relapses of acute lymphoblastic leukemia: prognostic relevance of early and late assessment. *Leukemia*. 2015;29(8):1648-55.
11. Conter V, Valsecchi MG, Parasole R, Putti MC, Locatelli F, Barisone E, et al. Childhood high-risk acute lymphoblastic leukemia in first remission: results after chemotherapy or transplant from the AIEOP ALL 2000 study. *Blood*. 2014;123(10):1470-8.
12. Szczepański T. Why and how to quantify minimal residual disease in acute lymphoblastic leukemia? *Leukemia*. 2007;21(4):622-6.
13. Scrideli CA, Assumpção JG, Ganazza MA, Araújo M, Toledo SR, Lee ML, et al. A simplified minimal residual disease polymerase chain reaction method at early treatment points can stratify children with acute lymphoblastic leukemia into good and poor outcome groups. *Haematologica*. 2009;94(6):781-9.
14. GBTLI (Grupo Brasileiro para o Tratamento de Leucemia Infantil). Protocolo de tratamento da leucemia linfóide aguda em crianças. *Soc Bras Oncol Pediatr*. 2009.
15. Paula FD, Santos SM, Xavier SG, Ganazza MA, Jotta P, Yunes JE, et al. Comparison between qualitative and real-time polymerase chain reaction to evaluate minimal residual disease in children with acute lymphoblastic leukemia. *Rev Bras Hematol Hemoter*. 2015;37(6):373-80.