Combined use of reverse transcriptase polymerase chain reaction and flow cytometry to study minimal residual disease in Philadelphia positive acute lymphoblastic leukemia

Luz Muñoz, Olga López, Rodrigo Martino, Salut Brunet, Mar Bellido, Enriqueta Rubió, Jorge Sierra, Josep F. Nomdedéu
Hematology Division, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

ABSTRACT

Background and Objectives. The Philadelphia chromosome in acute lymphoblastic leukemia (Ph+ ALL) is associated with a poor prognosis given the high frequency of chemoresistance and leukemia relapse. Minimal residual disease (MRD) detection before cytogenetic and hematologic relapse could be useful in early therapy. The most suitable methods for detecting MRD in Ph+ ALL are flow cytometry (FC) and reverse transcriptase polymerase chain reaction (RT-PCR). However, since both techniques carry the risk of false-negative results the combined use of these two techniques could overcome this problem.

Design and Methods. We report our experience using this approach in 47 bone marrow samples obtained from 10 Ph+ ALL patients. Twenty-seven marrow aspirates were taken from patients in clinical remission (CR). The samples were considered positive for MRD by FC when two conditions were met: 1) detection of an abnormal B-cell differentiation pattern and 2) presence of more than 1x10^-3 cells coexpressing CD22/CD34/CD45 or CD66/CD34/CD10. After FC analysis, RNA was purified using standard methods.

Results. FC was positive in 23/27 samples in CR (sensitivity 85%). RT-PCR was successfully performed in 23 samples in CR. RT-PCR was positive in 18/23 samples (sensitivity 78%). There were 5 samples with discordant results. FC was positive in 3 samples with a negative RT-PCR and FC was negative in 2 samples with a positive RT. All the 10 patients relapsed and only 1 is currently alive after an allogeneic stem cell transplantation. The median (range) time from MRD detection to relapse in patients treated with chemotherapy was 42 (39-71) days.

Interpretation and Conclusions. These data suggest that RT-PCR may be negative despite the presence of neoplastic cells identified by their immunophenotypic traits. We conclude that immunologic and molecular techniques can be used in tandem for monitoring MRD in Ph+ ALL.

Keywords: acute lymphoblastic leukemia; bcr/abl; minimal residual disease; flow cytometry; RT-PCR

Correspondence: Josep F. Nomdedéu, M.D., Laboratori d’Hematologia, Hospital de la Santa Creu i Sant Pau, Avda Sant Antoni M. Claret 167, 08025 Barcelona, Spain.
teria. After marrow aspiration, only 1 sample was obtained, and flow cytometric analysis was always carried out before RNA extraction.

Immunologic methods

Sample preparation. The number of total cells was quantified by microscopy and adjusted to 2x10^6 in each tube. The immunophenotypic analysis was performed on lysed whole BM samples with direct conjugated monoclonal antibodies (MoAbs). To increase the possibility of detecting the maximum number of phenotypic aberrations present at diagnosis, antigen expression was analyzed using triple combinations of the following MoAbs conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCp) or phycoerythrin-cyanine 5 (PE/Cy 5) fluorochrome tandem: CD 66 (Kat4c-FITC), CD22 (4KB128 FITC), CD34 (8G12-FITC, PE), HLA-Dr (L243 PetCp), CD10 (W8E7 FITC), CD 20 (L138 PE), CD3 (SK7 PerCp), CD4 (Leu 3ª FITC), CD5 (Leu 1 FITC), CD8 (Leu 2ª PE) purchased from Becton Dickinson (BDIS), San José, Ca, USA; CD19 (SJ25-C1 PE/Cy 5) and MPO (H-43-5 FITC) from Caltag Laboratories, Burlingame, USA; CD36 (FAG-52 FITC) from Immunotech, Marseille, France and TdT (VNF-830 and VTF-510 FITC) from Harlam Sera Lab, Sussex, England. Direct immunofluorescence was performed incubating 2x10^6 cells with the specific MoAb for 15 min in the dark at room temperature. In all cases, isotype-matched immunoglobulins with no reactivity against BM cells and the CD4-FITC/CD8-PE/CD3-PerCP combinations were used as negative and positive controls, respectively. Cells were lysed (FACS lysis solution, BDIS) for 3 to 5 minutes and centrifuged at 250 g for 5 minutes. The cells were washed twice with phosphate buffered saline (PBS) before being resuspended in PBS and examined.

Detection of intracellular antigens (cytoplasmic and nuclear) by FC. The cells were incubated for fixation and permeabilization with Fix & Perm solution (CALTAG, Burlingame, CA, USA). They were then washed twice with PBS and incubated for 15 min with 20 µL of antibody. After incubation, the cells were washed again with PBS before resuspension and examination. Immunologic criteria for lineage involvement. Lineage affiliation was performed according to the EGIL recommendations. The diagnosis of B-cell lineage acute leukemia was established when cytoplasmic CD79a (cyCD79a) was positive regardless of the pattern of surface markers. Acute myelogenous leukemia (AML) was excluded from this series by the lack of cytoplasmic myeloperoxidase expression. Cytoplasmic CD3 was tested in every case.

Data acquisition and analysis. A FACScan flow cytometer was used for analysis.

Table 1. Clinical and biological characteristics of Ph+ ALL patients.

<table>
<thead>
<tr>
<th>Pts.</th>
<th>Sex</th>
<th>Age</th>
<th>FAB</th>
<th>WBC count (x 10^9/L)</th>
<th>BCR/ABL transcript</th>
<th>Induction therapy</th>
<th>Salvage therapy</th>
<th>SCT</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>79</td>
<td>L1</td>
<td>7</td>
<td>b2a2</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Dead</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>50</td>
<td>L1</td>
<td>11</td>
<td>e2a2</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Dead</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>15</td>
<td>L1</td>
<td>20</td>
<td>e2a2</td>
<td>SHOP-94</td>
<td>Yes</td>
<td>All</td>
<td>TRM</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>21</td>
<td>L1</td>
<td>18</td>
<td>e2a2</td>
<td>PETHEMA</td>
<td>RelAL</td>
<td>All</td>
<td>TRM</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>45</td>
<td>L2</td>
<td>69</td>
<td>e2a2</td>
<td>PETHEMA</td>
<td>RelAL</td>
<td>No</td>
<td>REL</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>42</td>
<td>L2</td>
<td>15</td>
<td>e2a2</td>
<td>PETHEMA</td>
<td>RelAL</td>
<td>All</td>
<td>REL</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>28</td>
<td>L2</td>
<td>84</td>
<td>e2a2</td>
<td>PETHEMA</td>
<td>RelAL</td>
<td>All</td>
<td>REL</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>47</td>
<td>L2</td>
<td>7.5</td>
<td>b2a2</td>
<td>PETHEMA</td>
<td>RelAL</td>
<td>No</td>
<td>REL</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>66</td>
<td>L2</td>
<td>10.3</td>
<td>e2a2</td>
<td>PETHEMA</td>
<td>No</td>
<td>No</td>
<td>REL</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>61</td>
<td>L2</td>
<td>41</td>
<td>e2a2 (b2a2)*</td>
<td>PETHEMA</td>
<td>RelAL</td>
<td>All</td>
<td>Alive in CR</td>
</tr>
</tbody>
</table>

*Predominant e2a: expression with a small amount of b2a: induction therapy: Pethema ALL; salvage therapy: RelAL; REL: relapse; TRM: transplant-related mortality.

Table 2. Immunophenotype of Ph+ ALL patients.

<table>
<thead>
<tr>
<th>Pts.</th>
<th>CD10</th>
<th>CD20</th>
<th>CD19</th>
<th>CD33</th>
<th>CD117</th>
<th>CD66</th>
<th>CD34</th>
<th>CD45</th>
<th>HLA-DR</th>
<th>MPO</th>
<th>CD79a</th>
<th>CD3</th>
<th>Tdt</th>
<th>µ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+100%</td>
<td>+50%</td>
<td>+100%</td>
<td>+76%</td>
<td>+37%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+100%</td>
<td>-</td>
<td>+100%</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>+100%</td>
<td>-</td>
<td>+100%</td>
<td>+21%</td>
<td>+56%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+100%</td>
<td>-</td>
<td>+100%</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>+100%</td>
<td>+50%</td>
<td>+100%</td>
<td>+47%</td>
<td>+62%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+100%</td>
<td>-</td>
<td>+100%</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>+100%</td>
<td>+45%</td>
<td>+100%</td>
<td>+58%</td>
<td>+30%</td>
<td>-</td>
<td>+79%</td>
<td>+73%</td>
<td>+100%</td>
<td>+62%</td>
<td>-</td>
<td>+100%</td>
<td>+100%</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>+100%</td>
<td>+62%</td>
<td>+100%</td>
<td>+42%</td>
<td>+25%</td>
<td>-</td>
<td>+50%</td>
<td>+100%</td>
<td>+44%</td>
<td>+100%</td>
<td>-</td>
<td>+100%</td>
<td>+100%</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>+100%</td>
<td>-</td>
<td>+100%</td>
<td>+43%</td>
<td>+35%</td>
<td>-</td>
<td>+49%</td>
<td>+86%</td>
<td>+100%</td>
<td>+60%</td>
<td>+70%</td>
<td>-</td>
<td>+100%</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+100%</td>
<td>+42%</td>
<td>+100%</td>
<td>+40%</td>
<td>+50%</td>
<td>-</td>
<td>+92%</td>
<td>+90%</td>
<td>+100%</td>
<td>+62%</td>
<td>+70%</td>
<td>-</td>
<td>+100%</td>
<td>+100%</td>
</tr>
<tr>
<td>8</td>
<td>+100%</td>
<td>+22%</td>
<td>+100%</td>
<td>+35%</td>
<td>+85%</td>
<td>+31%</td>
<td>+82%</td>
<td>+100%</td>
<td>+100%</td>
<td>+25%</td>
<td>+90%</td>
<td>-</td>
<td>+100%</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>+100%</td>
<td>+74%</td>
<td>+100%</td>
<td>+32%</td>
<td>-</td>
<td>-</td>
<td>+80%</td>
<td>+100%</td>
<td>+100%</td>
<td>+26%</td>
<td>+52%</td>
<td>+100%</td>
<td>+100%</td>
<td>+100%</td>
</tr>
</tbody>
</table>

*Cytoplasmic; ND: not done.
During all reactions to decrease the possibility of PCR tips and included non-nucleic acid containing blanks described by Saglio

ed-PCR to quantify bcr/abl cDNA were those samples produced any bands after PCR amplification.

Without adding reverse transcriptase. None of these cDNA synthesis was carried out for some samples.

Activity in the second step of the nested PCR. Repeat the experiments for all the samples showing PCR positivity in the second step of the nested PCR. Repeat the experiments for all the samples showing PCR positivity in the second step of the nested PCR.

For MMRD detection included two criteria based on previously published results: 1) an abnormal B-cell pattern as revealed by the percentages in the cell population identified using the CD10/CD20/CD19 tube and ii) detection exceeding 1x10−3 cells coexpressing an aberrant phenotype. For MMRD studies three triple-antigen combinations were employed: CD10/CD20/CD19, CD22/CD34/CD45 and CD66/CD34/CD10 or CD66/CD34/CD45 (Table 3). Evaluation of cells displaying leukemia-associated phenotypes was performed using a two-step acquisition procedure according to a previously defined method.

Cytogenetic analysis

Cytogenetic analysis was performed on bone marrow cells after short-term culture without stimulation.

RT-PCR analysis

Total RNA was prepared either by the guanidinium thiocyanate-phenol chloroform method (Ultraspec, Biotecx laboratories, Inc., Houston, Texas, USA) (27 samples) or by using a RNeasy Kit (Qiagen GmbH, Hilden, Germany) (17 samples), following the manufacturers’ recommendations. The RNA was dissolved in RNase-free water and 7 µL were withdrawn for quantification on a GeneQuant RNA/DNA (Pharmacia Biotech, Sollentuna, Sweden) and stored until use at −80ºC.

The conditions for reverse transcription and nested-PCR to quantify bcr/abl cDNA were those described by Saglio et al. with minor modifications. Briefly, 5 µg of RNA were reverse-transcribed into cDNA using a specific antisense primer in a final reaction volume of 50 µL with 10 µL of buffer 5x (Gibco BRL), 5 µL of DTT 0.1 M (Gibco BRL), 200 U of M-MV reverse transcriptase (Gibco BRL), 3 µL of each dNTPs (1.5 mM each in final concentration) (Promega, Madison, USA) and 40 U of RNAsin (Promega). The reaction mixture was incubated at 37ºC for 1 h 25'.

To rule out contamination by DNA, we repeated the experiments for all the samples showing PCR positivity in the second step of the nested PCR. Repeat cDNA synthesis was carried out for some samples without adding reverse transcriptase. None of these samples produced any bands after PCR amplification.

We used specially dedicated pipettes and filtered tips and included non-nucleic acid containing blanks during all reactions to decrease the possibility of PCR product carry over and cross-contamination. Quality control tests were carried out with 10 µL of cDNA in a one-step PCR amplification of normal ABL and E2A sequences.

The remaining cDNA from each sample was then divided into 2x100 μL PCR tests for the first-step amplification of a given fusion gene transcript followed by a second-step (nested) amplification of 6 µL of first-step products. Separate amplifications of p210 and p190 BCR-ABL transcripts were performed under the same conditions: each PCR mixture contained 10 µL of buffer (KCl 50 mM, Tris-HCl pH 10 mM, MgCl2 25 mM, dNTPs 0.2 mM each, BSA 0.2 mg/mL final concentration), 0.3 µL of Taq polymerase (Perkin Elmer 5 U/µL, Branchburg, New Jersey, USA) and two specific primers for p210 and p190 amplification. Nested-PCR amplification of the bcr/abl gene was performed sequentially with two internal specific primers for p210 and p190 detection. Amplification reactions were performed on a temperature controller (Perkin Elmer GeneAmp PCR System 9700 Thermocycler). Amplified PCR products from PCR-1 and PCR-2 were separated by electrophoresis on a 2% agarose gel.

In order to assess the sensitivity of this technique, CML-T1 (b2a2), EM-2 (b2a2) and TQM-1 (e1a2) cells were diluted with another cell line (non-Ph+ cells). These preparations represented dilutions of 1/10, 1/10², 1/10³, 1/10⁴, 1/10⁵, 1/10⁶ and 0 Ph cells in 10⁶ non-Ph cells. Each aliquot was processed as described above.

MRD analysis with RT-PCR

RNA isolation from MMRD samples and the conditions and specific primer sequences used in reverse transcription/PCR amplification were as described above.

Results

Immunophenotypic characteristics

All the cases were CD10, CD19, CD34, HLA-DR, CD79a and TdT positive. In our series there was a high incidence of myeloid antigen expression: CD66 in 6 out of 6 cases tested, CD33 in 9 out of 10 cases, CD13 in 8 out of 10 cases, and CD117 in 1 out of 10 cases. Another interesting feature was the expression of the CD45 antigen, which was negative in 2 patients and showed a dim expression in 3 cases (Table 2).

<table>
<thead>
<tr>
<th>Aberrant phenotypes</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD22FITC/CD34PE/CD19PerCP</td>
<td>8/10</td>
</tr>
<tr>
<td>CD66FITC/CD34PE/CD45PerCP</td>
<td>6/6</td>
</tr>
<tr>
<td>CD10FITC/CD20PE/CD19PerCP</td>
<td>10/10</td>
</tr>
</tbody>
</table>

Table 3. MoAbs combinations used to detect MDR and their distribution at diagnosis in Ph+ ALL patients.
MRD analysis by FC

We analyzed 27 BM samples in CR with FC. The criteria used to define MRD have been described previously. MRD was detected in 23 out of 27 samples. Both criteria, an abnormal B-cell differentiation pattern and a population with CD22/CD34/CD45 or CD66/CD34/CD10 >10⁻³ cells were positive in 20 samples. In one sample MRD was only detected by an abnormal B-cell differentiation pattern and in another sample it was only associated with >1x10⁻³ cells coexpressing CD66/CD34/CD10. The median (range) of residual leukemic cells detected was 4x10⁻³ (0.6-40x10⁻³) of total nucleated cells. The median (range) time between MRD detection and relapse in patients treated with chemotherapy was 42 (39-71) days. After MRD detection two patients treated with allogeneic stem cell transplantation relapsed 2.5 and 4 months later. The sensitivity of FC in aberrant phenotype detection using cell dilutions was 1x10⁻⁴. The sensitivity for detecting B-cell differentiation pathway abnormalities has not been established.

Molecular characteristics

Ten Ph+ ALL patients were tested by qualitative bcr/abl RT-PCR. Eight patients expressed p190 transcript (e1a2) and two showed p210 (b2a2).

MRD analysis by RT-PCR

All samples tested at diagnosis were positive for some type of bcr/abl transcript. Eighteen patients in CR were bcr/abl positive.

We found a sensitivity of 10⁶ for bcr/abl p210 and 10⁵ for bcr/abl p190.

MRD detection combining both methods

The presence of MRD was simultaneously assessed by FC and RT-PCR in 23 samples from patients in CR. FC was performed on 27 CR samples. FC was positive in 23 of these 27 samples. RT-PCR was successfully performed in 23 samples from patients in CR. RT-PCR was positive in 18/23 samples. The results of the two methods showed a good correlation. However, 5 samples showed discordant findings; FC was positive in 3 samples with a negative RT-PCR and FC was negative in 2 samples with a positive RT-PCR (Table 4, Figure 1). The clinical outcome suggests that these discordant results were false-negatives. These disparate results always preceded hematologic relapse. FC and RT-PCR were negative in only two samples taken consecutively from the same patient who was allografted and suffered from acute graft-versus-host disease (GvHD). All the patients in this series relapsed and only one is currently alive after allogeneic stem cell transplantation.

Table 4. Discordant findings between FC and RT-PCR results of MRD detection in our series.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Timing</th>
<th>Flow cytometry</th>
<th># of aberrant cells detected by FC</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>After AlloSCT</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>After AlloSCT</td>
<td>+</td>
<td>1x10⁻³</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>After salvage chemotherapy</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>After salvage chemotherapy</td>
<td>+</td>
<td>2x10⁻³</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>After induction chemotherapy</td>
<td>+</td>
<td>2x10⁻³</td>
<td>-</td>
</tr>
</tbody>
</table>

* of the total nucleated cells.

Figure 1. Left: flow cytometric detection of abnormal B-cell maturation (red: lymphoblasts, green: normal B-lymphocytes). B-cell gate was established on the basis of CD19 expression and low right angle light scatter (SSC) and additional analysis of B-cell differentiation pathways. We acquired 10⁵ of total events and CD19⁺ cells represent 2% of the total nucleated cells. Right: RT-PCR analysis in Ph+ ALL. Lane 1: Molecular weight marker. Positive controls b2a2, b3a2, b2a2 and e1a2, lanes 2, 3, 4 and 7, respectively. Lane 5: Negative control. Lane 6: RT-PCR negativity in one case with MRD detected by FC (dot plots A). Lane 8: RT-PCR positivity in one case with normal immunophenotype (dot plots B).
Discussion

MRD studies are currently performed to estimate the total burden of leukemic cells during clinical remission. A number of techniques have been recently developed to improve the morphologic methods in assessing response to therapy. These include immunophenotype analysis and molecular methods, which are the most sensitive and reproducible techniques to date. However, both methods require technical expertise and are not problem free.5,17-19

We used two FC criteria to study MRD: detection of an abnormal B-cell maturation pattern and the presence of a cell population >1x10^-3 with an aberrant phenotype. We chose these criteria because both are reproducible and can be applied to all ALL samples.11,12,20 We found that CD66 is a good marker in ALL and that it could be used to detect residual leukemic cells in a large number of cases of B-cell lineage ALL.21

The main reasons for false-negative results are: 1) uneven distribution of leukemic cells during clinical remission leading to sampling variability, and 2) clonal evolution, during and after treatment which may lead to the disappearance of one or more markers detected at diagnosis. Sample variability could be resolved by taking samples from multiple sites, whereas immunophenotypic shifts could be avoided by increasing the number of marker combinations.22

However, major phenotypic changes did not seem to be common in Ph+ ALL.

The possibilities and limitations of molecular methods such as RT-PCR assay for detecting minimal residual bcr/abl depend not only on their specificity, sensitivity and reproducibility but also on their simplicity and speed.

In our study, all patients were monitored for both the p210 and p190 transcripts. Two patients had the p210 transcript (both b2a2) and seven had the p190 transcript (e1a2). We observed that one case presented two bcr/abl transcripts simultaneously with a predominant e1a2 expression and a small amount of b2a2 expression. A false negative bcr/abl RT-PCR analysis could result from an inadequate template, poor RNA isolation or failed reverse transcription or PCR amplification.23,24

Although RT-PCR has been shown to be a highly sensitive assay for bcr/abl detection, we encountered a number of false-negative samples. We attributed this discordance to our limited sample volume and technical errors, in part because in our study RNA was obtained after FC analysis. RT-PCR could have been improved by obtaining a separate BM sample. However, the high sensitivity described for RT-PCR is not always achieved routinely and variations in each sample could arise.25,26

Both methods were good at predicting relapse. In our study, positive MRD almost invariably persisted in...
all chemotherapy-treated patients. These results in conjunction with prior studies27,28 suggest that chemotherapy cannot eradicate the neoplastic clone in Ph+ ALL. AlloSCT is the only curative treatment although there is a high frequency of relapses.29,30 In our series of patients treated with alloSCT, we detected M RD in all the samples before this procedure. After alloSCT, all but two samples were M RD positive. Of these patients two relapsed at 3 and 4 months after the transplantation and two patients died with M RD and CR. Only one patient yielded two consecutive negative samples, at 7 and 8 weeks after transplantation. This patient suffered from acute GvHD. Residual leukemic cells were detected by FC a month later and the patient relapsed and died 9 months after alloSCT. The other 4 patients treated with alloSCT did not suffer from GvHD. The negative M RD findings in this patient with acute GvHD could have been due to a graft-versus-leukemia effect23 which could have reduced the neoplastic clone below the detection sensitivity threshold. We detected only a small aberrant cell population in all the immunophenotypic samples but we were not able to detect the slope of increase before the hematologic relapse. This could be explained by the high division rate of the leukemic cells in Ph+.30,31 New methods which allow quantification of the tumor population such as quantitative PCR,32-34 and the shortening of the interval between samples could provide insights into disease evolution.

In conclusion, our findings suggest that M RD detection in Ph+ ALL is associated with a high probability of hematologic relapse within a short time. Our results show a correlation between FC and RT-PCR but 5 samples were positive using only one of the two techniques. Bearing this in mind, we propose the use of both methods to study MRD in Ph+ ALL, thereby minimizing the risk of false-negative results. Their use in tandem may reduce false results. The most suitable methods for detecting MRD in these patients are flow cytometry and RT-PCR. However, both assays carry the risk of false negative results. Their use in tandem may reduce false results.

The potential implications for clinical practice are as follows:

- Chemotherapy cannot eradicate the neoplastic clone in Ph+ ALL patients.
- The most suitable methods for detecting M RD in these patients are flow cytometry and RT-PCR. However, both assays carry the risk of false negative results. Their use in tandem may reduce false results.
- M RD detection in Ph+ ALL is associated with a high probability of hematologic relapse within a short time.

References

13. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-