ORIGINAL ARTICLE

Frequency of the minor BCR-ABL (e1;a2) transcript oncogene in a Mexican population with adult acute lymphoblastic leukaemia

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KEYWORDS
Acute lymphoblastic leukaemia;
Philadelphia chromosome;
BCR-ABL oncogene

Abstract

\textbf{Background}: The minor BCR-ABL (e1;a2) transcript oncogene is the most common genetic alteration in adults with acute lymphoblastic leukaemia (ALL). It is associated with a poor prognosis.

\textbf{Aim}: To determine the frequency of minor BCR-ABL (e1;a2) transcript oncogene expression in ALL patients in Mexico.

\textbf{Material and methods}: A cohort of 411 patients with \textit{de novo} ALL were tested for the oncogene using reverse transcription polymerase chain reaction (RT-PCR).

\textbf{Results}: The oncogene was found in 14\% (n = 57) of the study population. Mean age was 29 years, and 53\% were male. Median leucocyte count was $53 \times 10^3$ \text{\textmu}l.

\textbf{Conclusion}: Prevalence of BCR-ABL expression by RT-PCR has not previously been reported in Mexico. Our laboratory found a higher prevalence than that reported in Latin-American series, but lower than that reported for the European population.

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Introduction

Acute lymphoblastic leukaemia (ALL) is one of the most common types of cancer found in Mexico, with an average incidence of 5 cases per 100,000 inhabitants.1 On average, 70 new cases of ALL are admitted to the Haematology Department of the General Hospital of Mexico each year. Several cytogenetic abnormalities are involved in the development of this type of cancer. The t(9;22) (q34;q11) translocation, known as the Philadelphia chromosome or Ph, gives rise to the BCR-ABL fusion transcript. This transcript, together with abnormalities such as t(4;11), is associated with an adverse prognosis.2 Incidence of this gene varies; reports suggest it to be 5% in the paediatric population,3-5 and 25-50% in adults.6-8 The minor BCR-ABL transcript codes for a chimeric protein (190 kDa) with tyrosine kinase activity, which is implicated in both the activation of various cell signalling pathways (RAS-GTP) and cell apoptosis (PI3K).9-13 The BCR-ABL transcript has been associated with an adverse prognosis in most international studies.14 The introduction of therapies that act on specific molecular targets, such as BCR-ABL tyrosine kinase (TK) inhibitors (Glivec®), Novartis) has improved overall survival rates when compared to traditional chemotherapy. There are various methods for isolating the BCR-ABL transcript, the most common being conventional karyotyping, fluorescent in situ hybridization (FISH), and polymerase chain reaction (PCR). In Mexico, the Philadelphia chromosome is found in around 3.8% of the paediatric population15 and 16.7% of adults,16 isolated by reverse transcription polymerase chain reaction (RT-PCR) and conventional cytogenetics, respectively. In our laboratory, we amplify the BCR-ABL fusion transcript by means of RT-PCR, and perform around 60 tests on ALL patients each year. In this study, we describe the frequency of minor BCR-ABL expression in ALL patients compared with the international literature.

Materials and methods

An experimental, prospective, longitudinal study conducted from February 2000 to January 2010 in the molecular biology laboratory of the Haematology Department. The study was approved by the institution’s independent ethics committees. Male and female patients with de novo diagnosis of ALL that agreed to give peripheral blood samples after having signed the informed consent form were included in the study. ALL was diagnosed in accordance with the French–American–British (FAB) classification systems, with the help of immunophenotyping and cytochemistry assays. Clinical data were sourced from the patient’s medical records (Table 1).

Methodology

Leukaemia cells
Bone marrow samples were collected from ALL patients that had signed the informed consent form. Samples were collected in heparinized tubes containing Lymphoprep (Nycomed Pharma AS, Oslo, Norway) and centrifuged to obtain mononuclear cells.

Reverse transcription polymerase chain reaction (RT-PCR)
Total-cell RNA was isolated with Trizol (Life Technologies, Paisley, UK), and 1 µg of RNA was used for cDNA synthesis by means of MMLV (Life Technologies, Paisley, UK). The CMLB primers 5’ ATCTCCACTGCCCACAAATACATACA 3’. ALLA 5’ AGATCTGGGGCCACGATGCGAGGAC 3’ were used for PCR amplification. Results were validated by sequencing two positive samples (ABI PRISM 3100, Applied Biosystem, San Francisco, USA). Each cDNA was tested by PCR using primers specific for the constituent β2 microglobulin gene.
PCR cycles of 1 min 94 °C, 1 min 55 °C, 1 min 72 °C were repeated 35 times. The PCR products were stained with ethidium bromide and visualized in a 1.5% agarose gel.

### Results

#### Patient characteristics

A total of 411 patients with a mean age of 29 years (range 16–62) were studied. By morphology, most (n = 98%) presented acute lymphoblastic leukaemia (ALL-L2), with 81.6% corresponding to the B-cell immunophenotype. Only 2% showed central nervous system infiltration at diagnosis.

#### Expression of the minor BCR-ABL oncogene

All 411 de novo ALL cases were studied for BCR-ABL oncogene expression. RNA quality was evaluated by amplification of the constituent β2 microglobulin gene, which amplifies a fragment of 397 bp by RT-PCR. BCR-ABL was isolated in 57 patients, amplifying a fragment of 196 bp. This represents 13.8% of the study population.

Mean age of the 57 BCR-ABL-positive patients was 25 years (range 18–56); 53% (n = 30) were men, and 47% (n = 27) were women. Mean leucocyte count at diagnosis was 54 x 10^3/μl (range 1.2–207 x 10^3/μl). All (100%) patients were of the B-cell immunophenotype, and none showed central nervous system involvement (Fig. 1).

#### Discussion

In this study, we evaluated the prevalence of the BCR-ABL transcript fusion (e1;α2) in a population of ALL patients in Mexico using reverse transcription polymerase chain reaction (RT-PCR). This technique has been used since the 1990s by various international groups in both the diagnosis and follow-up of ALL Ph+. The first studies in RT-PCR reported a prevalence of the minor BCR-ABL transcript of 50%, with no difference in either prognosis or clinical presentation. Researchers in the GIMEMA 0496 trial reported a prevalence of minor vs. major breakpoint of 58.5% and 41.5%, respectively. Prevalence continues to vary across Latin America, ranging from 5.7% in adults and between 2.3% and 2.7% in the paediatric population. Prevalence in ALL patients in the US is estimated at 19%, and from 25% to 39% in Asia (Table 2). Very few studies in BCR-ABL prevalence in children have been conducted in Mexico, and none in adults. In our laboratory, we found prevalence to be greater than that reported for Latin America, and lower than that reported for the American and European population. These discrepancies could be due to the genetic diversity of the Latin American population. Nowadays, it is particularly important to isolate the BCR-ABL transcript in ALL patients due to the potential benefits of tyrosine kinase (TK) inhibitors, such as imatinib, nilotinib, or Dasatinib.

Research suggests that the combination of BCR-ABL and TK inhibitor therapy reverses the disease by providing a specific molecular target. In contrast to previous interpretations, this marker is now thought to indicate a good prognosis. In conclusion, ALL is one of the most common malignancies seen in the Haematology Department. Isolation of BCR-ABL in ALL patients is of primordial importance, particularly in view of the potential action of tyrosine kinase inhibitors. Advances in molecular biology, such as real-time PCR, will allow clinicians to monitor BCR-ABL transcript levels more closely. An understanding of the prevalence of this fusion gene in the Mexican population will give greater insight into ALL, improve management and monitoring of the disease, and introduce more specific TK-based therapy.

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**Table 1** General clinical characteristics of patients with acute lymphoblastic leukaemia.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>BCR-ABL Negative N (%)</th>
<th>BCR-ABL Positive N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total patients = 411</strong></td>
<td>354 (86.13)</td>
<td>57 (13.86)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>194 (54.8)</td>
<td>30 (53)</td>
</tr>
<tr>
<td>Women</td>
<td>160 (45.2)</td>
<td>27 (47)</td>
</tr>
<tr>
<td><strong>Median age (years)</strong></td>
<td>29 (16–62)</td>
<td>25 (18–56)</td>
</tr>
<tr>
<td><strong>Laboratory tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline leucocyte count (×10^3/μl)</td>
<td>55.9 (0.7–789)</td>
<td>54 (1.2–207)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>7.18 (4–10.9)</td>
<td>7.05 (5.4–11.5)</td>
</tr>
<tr>
<td>Platelets (×10^3/μl)</td>
<td>53 (0.88–388)</td>
<td>45 (2–432)</td>
</tr>
<tr>
<td><strong>FAB classification</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>7 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>L2</td>
<td>347 (98)</td>
<td>57 (100)</td>
</tr>
<tr>
<td><strong>Immunophenotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-cell</td>
<td>111 (81.6)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>T-cell</td>
<td>25 (18.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Nervous system infiltration</strong></td>
<td>7 (2)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Table 2  Prevalence of the BCR-ABL Ph+ oncogene worldwide, by cyogenetic and RT-PCR testing.

<table>
<thead>
<tr>
<th>Region</th>
<th>Patients</th>
<th>N</th>
<th>Prevalence (%)</th>
<th>Testing technique</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td></td>
<td></td>
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<tr>
<td>China</td>
<td>Adults</td>
<td>389</td>
<td>28.3</td>
<td>RT-PCR</td>
<td>Li et al.27</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>137</td>
<td>37</td>
<td>RT-PCR</td>
<td>Bao et al.28</td>
</tr>
<tr>
<td>Malaysia</td>
<td>Children</td>
<td>299</td>
<td>7.8</td>
<td>RT-PCR</td>
<td>Ariffin et al.29</td>
</tr>
<tr>
<td>Japan</td>
<td>Adults</td>
<td>285</td>
<td>22</td>
<td>Cytogenetic</td>
<td>Takeuchi et al.34</td>
</tr>
<tr>
<td>India</td>
<td>Adults and children</td>
<td>33</td>
<td>24 children19 adults</td>
<td>RT-PCR</td>
<td>Gurbuxani et al.30</td>
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<tr>
<td>US</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Canada</td>
<td>Adults</td>
<td>53</td>
<td>24</td>
<td>RT-PCR</td>
<td>Brandwein et al.35</td>
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<tr>
<td>Mexico</td>
<td>Children</td>
<td>59</td>
<td>2.7</td>
<td>FISH</td>
<td>Pérez-Vera et al.17</td>
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<td>Mexico</td>
<td>Children</td>
<td>2</td>
<td>3.8</td>
<td>RT-PCR</td>
<td>Jiménez-Morales et al.18</td>
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<tr>
<td>Chile</td>
<td>Adults</td>
<td>35</td>
<td>5.7</td>
<td>Cytogenetic</td>
<td>Arteaga-Ortíz et al.19</td>
</tr>
<tr>
<td>Chile</td>
<td>Children</td>
<td>44</td>
<td>2.3</td>
<td>Cytogenetic</td>
<td>Legües et al.31</td>
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<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>USA–UK</td>
<td>Adults</td>
<td>1521</td>
<td>19</td>
<td>RT-PCR</td>
<td>Rowe et al.14</td>
</tr>
<tr>
<td>France</td>
<td>Adolescents</td>
<td>100</td>
<td>6</td>
<td>Cytogenetic</td>
<td>Boissel et al.36</td>
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<tr>
<td>Italy</td>
<td>Adults</td>
<td>216</td>
<td>19</td>
<td>Cytogenetic</td>
<td>Anino et al.37</td>
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</table>

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Conflict of interest

The authors declare that they have no conflict of interests.

References

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