The coexistence of chronic myeloid leukemia (CML) and B-cell chronic lymphocytic leukemia (CLL) in the same patient is rare. A 71-year-old woman developed a B-lineage lymphoid blast crisis at 18 months after diagnosis of Ph-positive CML. At this time, a lymphoid cell population with morphologic and immunophenotypic features of CLL was demonstrated. The retrospective review of the tests performed at diagnosis and thereafter disclosed the presence of lymphoid nodules in the initial bone marrow biopsy in the absence of lymphocytosis. Subsequently, there was an appearance of moderate lymphocytosis in the following months. Therefore, diagnosis of CML and coexistent CLL was established. Although a transient remission of blast crisis was achieved, blast cells reappeared two months later and the patient died shortly afterwards. Molecular studies of the immunoglobulin heavy chain gene (JH) rearrangement pattern point to the origin of the diseases in two different cell clones. In addition, previously published cases of simultaneous CLL and CML are reviewed.

**Materials and Methods**

**Immunophenotypic analysis**

Double direct immunofluorescence phenotype was performed using a FACscan flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA) after incubation of the mononuclear cell fraction of peripheral blood (PB) and bone marrow (BM) samples with a panel of monoclonal antibodies labelled with fluorochromes against B, T-cell, and myeloid antigens (including Tdt, CD19, CD10, CD22, CD23, CD3, CD5, CD7, CD11b, CD13, CD33, CD14, HLA-DR, CD34 and surface M immunoglobulin heavy chain and \( \kappa \) and \( \lambda \) light chains, purchased from Becton-Dickinson, San José, CA, USA), followed by red cell lysis and analysis.

The expression of cytoplasmatic antigens was studied using the alkaline phosphatase anti-alkaline phosphatase technique. Additionally, immunohistochemical analysis using monoclonal antibodies CD20 and CD45RO was performed in the initial bone marrow biopsy in order to ascertain B-cell lineage of lymphoid nodules.
Molecular analysis

The clonality study of the lymphoid population was carried out by assessing the rearrangement of the heavy chain immunoglobulin gene (JH) pattern by Southern blot analysis and the polymerase chain reaction (PCR) technique. Southern blot analysis was performed after digestion of genomic DNA from peripheral blood and/or bone marrow samples with the EcoRI and HindIII restriction enzymes and hybridization with a P32-labelled JH probe with the random primer method. PCR analysis of the third complementary region (CDRIII) of the JH region was performed after 30 cycles of amplification using a primer based on a consensus sequence from the third framework portion (FR3) of the variable region (VH), and an antisense primer homologous to a conserved 3' sequence of the six joining (J) segments. The finding of a discrete band of an expected length of 80 to 120 base pairs after electrophoresis of the PCR product on a 4% agarose gel was indicative of the existence of a monoclonal population carrying the same rearrangement pattern in this region.

Case Report

A 71-year-old woman was referred to our center after the discovery of leukocytosis with immature myeloid cells in peripheral blood in a routine analysis. There was no previous history of exposure to radiation or myelotoxic drugs.

The physical examination was normal. The Hb was 129 g/L, WBC count was 12×109/L (45% neutrophils, 1% band cells, 4% eosinophils, 5% basophils, 22% lymphocytes, 8% monocytes, 1% metamyelocyte and 14% myelocytes), and the platelet count 172×109/L. The serum LDH level was 614 U/L (N:250-450), vitamin B12 was 1600 (N:200-1000 pg/mL), and the LAP score was 4 (N:30-60). A bone marrow aspirate was hypercellular with no fat cells and an increased proportion of the myeloid series in all maturative stages; the percentage of lymphocytes was 10%. A bone marrow aspirate disclosed 70% blast cells, whose immunophenotype corresponded to B-lymphoid lineage (CD34+, TdT+, CD10+, CD19+, and CD22+), and 30% lymphocytes with a mature appearance.

The immunophenotypic study of the latter population demonstrated expression of mature B cell markers (CD19, CD22) and coexpression of CD5 and CD23, weak IgM expression on the cell surface, a restricted pattern of the k light chain, and absence of immature markers such as TdT and CD10.

Southern blot for JH analysis of DNA from peripheral blood and bone marrow samples obtained at blast crisis showed the existence of a band which was differentiated from the germline band, indicating the existence of a monoclonal B-cell population. PCR amplification of CDRIII in the above samples yielded a double discrete band typical of a monoclonal rearrangement pattern of the JH region. Therefore, the diagnosis of B-cell lymphoid blast crisis of CML in association with B-CLL was made.

Retrospective analysis of initial bone marrow biopsy showed the B-cell nature of lymphoid nodules seen in the biopsy, as demonstrated by the CD20 expression of most lymphocytes in the nodule and the negativity of a pan-T cell marker, CD45RO. Additionally, DNA was extracted from the biopsy and a PCR of the CDRIII region was performed showing a unique band of around 100 bp, similar to the smaller band seen in the PCR product of blast crisis DNA. Doxorubicin (30 mg/m2 i.v./day × 2), vincristine (2 mg weekly × 4) and prednisone (60 mg/m2 during the first week and then gradually reducing the dose) were administered, which lead to the normalization of the peripheral blood hematological values showing no blast cells in either blood or bone marrow.

At that time, no blast cells in bone marrow were present, but the lymphoid population, with typical CLL morphology and phenotype, persisted. After having achieved a complete response, consolidation therapy with the same regimen as used to induce remission was given. However, a few weeks later, an increased proportion of blast cells in the bone marrow was observed, and a palliative treatment with 6-mercaptopurine was instituted. The patient died shortly afterwards, at five months after blast crisis diagnosis.

A review of previously reported cases in literature concerning coexistent myeloproliferative and lymphoproliferative disorders was performed through a MedLine® search (Table 1).
Table 1. Patients previously reported with coexistence of CLL and CML.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Age/sex</th>
<th>Interval CLL-CML (months)</th>
<th>Treatment for CML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcassonne et al. (1968)</td>
<td>59/F</td>
<td>36 mos.</td>
<td>CLB + PDN</td>
</tr>
<tr>
<td>Whang-Peng et al. (1974)</td>
<td>62/M</td>
<td>36 mos.</td>
<td>TBI</td>
</tr>
<tr>
<td>Whang-Peng et al. (1974)</td>
<td>74/M</td>
<td>24 mos.</td>
<td>None</td>
</tr>
<tr>
<td>Vilpo et al. (1980)</td>
<td>58/M</td>
<td>Simultaneous</td>
<td>CLB</td>
</tr>
<tr>
<td>Khojasteh et al. (1981)</td>
<td>55/M</td>
<td>61 mos.</td>
<td>CLB</td>
</tr>
<tr>
<td>Schreiber et al. (1984)</td>
<td>55/M</td>
<td>87 mos.</td>
<td>CLB</td>
</tr>
<tr>
<td>Teichmann et al. (1986)</td>
<td>47/F</td>
<td>72 mos.</td>
<td>CLB + PDN, Vincristine + Bleo COP</td>
</tr>
<tr>
<td>Hashimi et al. (1986)</td>
<td>82/F</td>
<td>62 mos.</td>
<td>None</td>
</tr>
<tr>
<td>Leoni et al. (1987)</td>
<td>55/M</td>
<td>Simultaneous</td>
<td>CLB</td>
</tr>
<tr>
<td>Browett et al. (1988)</td>
<td>69/M</td>
<td>Simultaneous</td>
<td>CLB</td>
</tr>
<tr>
<td>Zollino et al. (1991)</td>
<td>66/M</td>
<td>72 mos.</td>
<td>CLB</td>
</tr>
<tr>
<td>Maher et al. (1993)</td>
<td>68/M</td>
<td>Simultaneous</td>
<td>CLB</td>
</tr>
<tr>
<td>Nanjangud et al. (1996)</td>
<td>43/M</td>
<td>72 mos.</td>
<td>TBI</td>
</tr>
</tbody>
</table>

CLB: chlorambucil; PDN: prednisone; Bleo: bleomycin; TBI: total body irradiation. COP: cyclophosphamide + vincristine + prednisone

Discussion

The coexistence of myeloproliferative and lymphoproliferative disorders in the same patient is uncommon. However, different lymphoproliferative disorders, including Waldenström's macroglobulinemia,14 Hodgkin's disease,15 non-Hodgkin's lymphoma,16 multiple myeloma17 and especially CLL have been observed in association with myeloproliferative disorders like CML,1-12 but can also be associated with idiopathic myelofibrosis18 and polycythemia vera.19,20 In most cases, the diagnosis of the myeloproliferative disorder followed that of the lymphoid disorder,2,4-11 whereas in other cases, both diagnoses were made simultaneously.5,9,11 In the cases previously reported, the interval between the diagnoses of CLL and CML ranged from 24 to 87 months (see Table 1).

In the attempt of explaining the association between CLL and CML, several mechanisms have been suggested. Thus, the leukemogenic effect of the treatment employed for CLL might be related to the appearance of CML in those cases supervening after treatment of the lymphoproliferative disorder. In this sense, the mutagenic effect of chlorambucil (the cytolytic agent most commonly employed in such patients) and radiotherapy is well-known.12,21-24 However, the latter mechanism would not apply in those patients in whom the diagnoses of the myeloid and lymphoid disorders are simultaneously established. The impaired immune surveillance associated with CLL might also contribute to the increased risk of second neoplasias registered in these patients.25 In addition, a coincidental occurrence of CML and CLL in the same patient can not be ruled out. Finally, an alternative explanation for the association above could be that both diseases originate from a unique stem cell capable of differentiating into two different cell lines; however, molecular studies aimed at ascertaining such a possibility are scarce.9,17 Zollino et al.9 analyzed the expression of molecular markers specific of both proliferations (bcr/abl rearrangement for CML and the monoclonal pattern of JH rearrangement for CLL) in differentially cell-enriched blood fractions from a patient with CML following CLL, detecting bcr/abl rearrangement in the granulocytic but not in the mononuclear cell fraction, probably composed mainly of CLL cells; on the contrary, they observed a characteristic band corresponding to a monoclonal lymphoid population after JH rearrangement analysis in the lymphoid-enriched but not in the myeloid blood fraction. Therefore, these authors could determine the different origin of CLL and CML in their patient. Likewise, Maher et al. and Browett et al. reported similar findings in two additional cases of simultaneous CML and CLL.9,11 The patient reported herein had CML and CLL in an early stage at the time of presentation; she further developed B-lineage lymphoid blast crisis, which prompted us to study the JH rearrangement pattern as a specific molecular marker of the clone. PCR of the CDRIII region in a DNA sample extracted from the initial bone marrow biopsy showed a unique discrete band, probably corresponding to the CLL population. When this analysis was repeated in mononuclear cell fractions obtained at blast cell crisis, a double pattern band was clearly observed. There were also the appearance of a larger-sized band, which was not seen after amplification of chronic phase DNA, and suggested the existence of a different monoclonal population, probably corresponding to the blast crisis clone. Thus, the analysis of JH rearrangement pattern of sequential samples supports the origin of both disorders in two different cell clones.

References

Coexistence of B-CLL and CML