



## Persistent polyclonal B lymphocytosis with multiple bcl-2/IgH rearrangements: a benign disorder

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### ABSTRACT

**The appearance of a chronic B lymphocytosis is usually associated with the existence of an underlying monoclonal malignant condition. However, a few cases of persistent polyclonal B cell lymphocytosis (PPBL), presented in young asymptomatic women with an uneventful course, have been reported in recent years. In these PPBL cases, since the lymphocytes usually display an anomalous morphology, a false diagnosis of a neoplastic chronic lymphoproliferative syndrome can be easily made. We report a typical case of PPBL that presents multiple bcl-2 rearrangements, the typical finding of follicular lymphomas. A review of different causes of benign non neoplastic lymphocytosis with special steadiness in changes in the lymphoid subsets will be made. ©1998, Ferrata Storti Foundation**

Key words: lymphoproliferative, polyclonal, Bcl-2, HLA-DR, lymphocytosis

The persistence of polyclonal B cell lymphocytosis (PPBL) is an unusual and presumably indolent syndrome, which was first reported by Gordon *et al.* in 1982.<sup>1</sup> It usually affects young or middle-aged women, who smoke and are generally asymptomatic. This syndrome associates a typical peripheral blood smear with binucleated lymphocytes and a polyclonal increase in serum IgM. Its etiology remains unclear and different factors have been involved: smoking habits, viral agents such as the Epstein-Barr virus (EBV)<sup>2,3</sup> and a genetic predisposition since an association with the DR-7 allele has been proposed. Most of the patients reported have followed an uneventful course, except for one patient who developed a malignant pulmonary blastoma 11 years after the onset of the hematological picture,<sup>4</sup> and another patient who presented a stage IV B large cell non Hodgkin lymphoma 19 years after the initial diagnosis of lymphocytosis.<sup>5</sup>

The correct diagnosis of this rare disorder is important, as it can be easily mistaken for chronic lymphocytic leukemia (CLL) or for a leukemic expression of non-Hodgkin lymphoma (NHL). Moreover, abnormal bcl-2 rearrangements have been described in some cases, a fact which increases the potential risk of misdiagnosing this disorder as a malignant disease.<sup>5</sup>

We report here a typical case of a young woman presenting PPBL in which we have documented the presence of multiple bcl-2/IgH rearrangements.

### Materials and Methods

#### Case report

The patient was a 24-year-old woman sent to our institution because of a persistent mononucleosis-like picture in peripheral blood during the previous 3 months. She had complained of symptoms of pharyngitis, but on admission to our hospital she was completely asymptomatic and she has remained well all the 19 months of follow-up. She lacked any past medical history, except that she failed to develop protective antibodies against the hepatitis B virus vaccine one year and a half before, and that she smoked about 20 cigarettes per day. On physical examination, neither adenopathy nor enlarged liver or spleen were discovered. The chest radiography was normal and an abdominal ultrasonography detected a mildly enlarged spleen of 14 centimeters.

#### Hematological findings

She presented normal hemoglobin concentration and platelet count. Her white cell count was about  $12 \times 10^9/L$  (55% lymphocytes). On peripheral blood, nearly 25% of the lymphocytes had an anomalous morphology, some of them mimicking those of the infectious mononucleosis and others showing either with two completely separated nuclei or a narrow chromatin bridge between two lobes of the nucleus (Figure 1). Bone marrow examination was not performed. It is worthy to note that the total leukocyte count was below  $11 \times 10^9$  in four out of seven determinations performed at different moments. The evolution of the hematological and biochemical parameters is detailed in Table 1.

Two siblings of the patient presented normal hematological counts and blood smears with normal morphology.

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### Biochemistry profile

The serum electrophoresis, immune electrophoresis and blood chemistry were normal. Serum IgM antibodies were increased, reaching 672 mg/dL, and serum IgG and IgA were slightly decreased to 364 mg/dL and 44 mg/dL, respectively.

### Immunophenotyping

An anomalous lymphoid subset reaching 70% of the lymphoid compartment was detected in peripheral blood. These lymphocytes were found to express the typical B cell markers CD19, CD20 showing a normal light expression of CD5, CD23 and CD11c. They were negative for CD10, CD25 and CD103. The ratio between the values of expression of  $\kappa$  and  $\lambda$  on the cell surface were within normal range. A normal T cell population with a normal CD4/CD8 ratio was present.

### Mirobiological assays

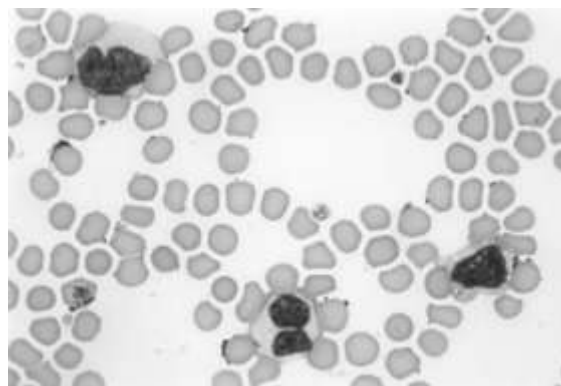
A PPD skin reaction was negative. The patient's serum was studied for the presence of antibodies against hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV), toxoplasma and *Coxiella burnetii* all of them being negative. The Rose Bengal plate agglutination test was negative. Complement-fixing antibodies against herpes simplex virus (HSV), varicella zoster virus (VZV) and cytomegalovirus (CMV) were found, all of them at a low titer and a rise on the titer was not detected.

With regard to EBV, heterophile antibodies (Paul Bunnell reaction) were not discovered. The presence of IgG (but not IgM) antibodies against the viral capsid antigen (VCA) of the EBV were measured by immunofluorescence. The same method was used to

**Table 1. Evolution of hematological and biochemical parameters. All the measures were made in peripheral blood. Bcl-2/IgH rearrangements were detected at April 30, 1997 and May 26, 1997.**

	3/25/ 1996	4/22/ 1996	5/21/ 1996	9/18/ 1996	4/1/ 1997	4/30/ 1997	10/7/ 1997
Leukocyte count ( $\times 10^9/L$ )	14.4	13.5	10	12.4	10.6	10.2	10.9
% Lymphocytes	52	42	57	49	59	57	55
% Atypical lymphocytes	32	26	20	Present	Present	Present	48
% CD19*	70	ND	ND	60	68	ND	ND
Ig M (mg/dL)	586	ND	561	586	672	654	634
Ig A (mg/dL)	60	ND	44	48	46	50	51
Ig G (mg/dL)	472	ND	428	377	364	367	403

ND: not determined; \*percentage of the lymphoid subset.



**Figure 1. Two mononucleosis-like cells beside one binucleated lymphocyte.**

test the serum against IgG antibodies to the early antigen (EA) of the EBV and IgG antibodies to the Epstein-Barr nuclear antigen (EBNA), both of them being positive. A genomic amplification by means of the polymerase chain reaction (PCR) was performed using probes against EBV, HSV, VZV, CMV and type VI herpes virus; none of them were discovered in serum. All of these findings argue on behalf of a past and non active history of EBV infection.

### HLA typing

HLA phenotype was found to be A2, A26, B60, B64, Cw3, Cw8, Bw6, DR7, DR8, DR53, DQ2, DQ4.

### Immunoglobulin heavy chain and BCL-2/IgH rearrangement assay

Blood samples were obtained for PCR analysis in two different moments after diagnosis. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque centrifugation and DNA was extracted by standard procedures.

PCR amplification of the Ig heavy chain was performed using V region family-specific and consensus J region oligonucleotides. Clonal rearrangement was not detected.

Amplifications at the major breakpoint region (MBR) and minor cluster region (MCR) of the bcl-2/IgH translocation were performed using nested oligonucleotides as previously described.<sup>6</sup> Three different rearrangements were observed, two within the MBR and one within the mcr.

### Other laboratory findings

The erythrocyte sedimentation rate, C3, C4, iso-hemagglutinins,  $\beta$ 2-microglobulin levels and antinuclear antibodies were measured, all of them being within normal values. Cytogenetic analysis in peripheral blood was performed, but no metaphases were visualized. The patient presented normal T lymphocyte function as she showed normal delayed hypersensitivity skin tests.

## Discussion

As far as we know, a few less than 50 cases of PPBL have been reported since 1982, when Gordon *et al.*<sup>1</sup> communicated the finding of a persistent lymphocytosis in 3 women. This syndrome has so far been reported almost always in women, most of them smokers, and it is characterized by a mild lymphocytosis with binucleated lymphocytes on peripheral blood smears, a polyclonal increase in the amount of serum IgM, and in some cases lymphadenopathies and/or splenomegaly are observed. However, Delanoy *et al.*<sup>7</sup> reported one case with a normal lymphocyte count, thus pointing out the feasibility of failing to diagnose this disorder. In fact, our patient presented a white blood cell count below  $11 \times 10^9/L$  in four out of seven determinations while being asymptomatic and showing the same increment in serum IgM and the presence of atypical lymphocytes. Thus, the actual prevalence of this syndrome may be higher than expected so far and it can be overlooked unless careful observation of peripheral blood smears is made. Patients usually present either no symptoms or complaints of mild weariness, and in one case, recurrent erythema nodosum/multiforme<sup>8</sup> was referred. The lymphocyte population was found to be polyclonal by immunophenotyping light chains on lymphocyte surface or by searching for a rearrangement of the heavy chain immunoglobulin (Ig) gene. However, the presence of a monoclonal population has been concluded by analysis of the heavy chain Ig gene with an uneventful follow-up in at least three cases. Therefore, it seems that different clones could appear in these patients without an aggressive clinical behavior. Thus, 21 out of 23 cases in which the evolution was communicated had an indolent course with a follow-up that ranged between 1 and 17 years. In the other two cases, a pulmonary blastoma and a non-Hodgkin lymphoma developed 11 and 19 years after the presentation of the hematological disorder, respectively.<sup>4,5</sup>

The pathogenesis of this syndrome is unsolved. Most of the patients are heavy smokers and in some instances, disappearance of the lymphocytosis has been observed after ceasing smoking.<sup>9</sup> A great association with the female sex, the presence of the allele DR7 of the HLA system and the presentation of this rare disorder in some members of the same family, including cases in monozygotic twins, argues in behalf of a genetic susceptibility.<sup>10,11</sup> We have studied two patient's siblings, a male and a female, both smokers and they presented normal peripheral blood smears. However, a HLA typing was not performed in either one of them. Persons who harbor the DR7 allele are supposed to present some type of hyporesponsiveness to viral infection, like the inability of developing protective antibodies against the Australia antigen of the hepatitis B virus (HBV)<sup>12</sup> as seen in our patient, and a susceptibility to chronic infection by HBV.<sup>13</sup> DR7 positivity has also been involved in an increased

risk of developing CMV retinitis in AIDS patients<sup>14</sup> and CMV disease in renal transplant recipients.

The presence of atypical lymphocytes has suggested a possible role of viruses in the pathogeny and the interest has focused on EBV. Most of the reported patients show serologic assays compatible with past, but neither acute nor persistent EBV infection. Thus, although Gordon *et al.*<sup>1</sup> could not discover viruses inside the cells by electron microscopical examination, Chow *et al.*<sup>2</sup> have demonstrated the presence of EBV DNA in the peripheral blood lymphocytes by PCR and *in situ* hybridization, and Larcher *et al.*<sup>8</sup> established a stable cell line that represented the atypical lymphocytes seen in a patient with PPBL, and that harbored the EBV genome. Interestingly, this cell line was positive for the bcl-2 protein, but no rearrangement of the bcl-2/IgH fusion product was found by PCR. Larcher *et al.* have also discovered a patient with PPBL who carry a mutation in the LMP1 protein. This peptide is known to be one of the EBV encoded proteins expressed during viral latency, and it has the ability to enhance the expression of bcl-2,<sup>15</sup> therefore protecting EBV infected cells from apoptosis. We have used PCR to analyze the presence of EBV genome in the serum, but not in the lymphocytes of our patient, and we have found it to be negative.

The bcl-2 oncogene was first discovered because of its involvement in the t(14;18) chromosomal translocation found in 80% of follicular lymphomas. Bcl-2 rearrangements have also been described in about 20% of diffuse large cell lymphomas and in some cases of CLL. In this patient, we detected three different rearrangements, two localized in the MBR and one in the mcr region. Delage *et al.* have described multiple bcl-2/IgH rearrangements in 5 patients with PPBL.<sup>5</sup> Bcl-2/IgH rearrangements in MBR may be detected in approximately 50% of normal individuals,<sup>16</sup> increasing the frequency of circulating translocation t(14;18) positive cells with age and heavy smoking.<sup>17</sup>

When a clinical hematologist has to manage a patient with a persistent lymphocytosis, the first steps to take are the immunophenotyping study and the establishment of the clonal or non clonal nature of this proliferation. In B cells, the latter is usually made by studying the expression of  $\kappa$  and  $\lambda$  light chains on the lymphocyte surface by flow cytometry,<sup>18</sup> or analyzing the rearrangement of the heavy chain. With regard to T cells, the clonality can be proved by examination of the rearrangement of the T cell receptor (TCR)  $\alpha$  and  $\gamma$  chains. However, it is more difficult to assess the clonal character of NK cells, as they do not rearrange any genes, and it can be demonstrated by X-linked DNA analysis<sup>19</sup> in female patients or by clonal cytogenetic abnormalities.

Benign lymphocytosis may be found in a wide variety of diseases, which are detailed in Table 2. Most of these causes are transient and related to infectious or drug reactions.<sup>20</sup> However, although persistent lymphocytosis is more likely to be associated with a

malignant lymphoproliferative condition, some other causes have been identified.

Lymphocytosis is rare during bacterial infections except for pertussis. Pertussis is a well known cause of lymphocytosis in children. The absolute lymphocyte count (ALC) is usually above  $12 \times 10^9/L$ . It is predominantly composed of CD4<sup>21</sup> and seems to be due to the lymphocytosis promoting factor (LPF) that inhibits the migration of the lymphocytes from the blood to the lymph nodes. Most of these lymphocytes appear as normal small mature cells, but there is an increase in small lymphocytes with convolutes and cleaved nuclei. Although the common perception among physicians is that pertussis is solely a disease of children, there is recent evidence that pertussis could be a common cause of persistent cough in adults who usually lack the typical lymphocytosis.<sup>22</sup> Other bacterial infections, such as rickettsiosis, syphilis, shigellosis or brucellosis have been found in association with a relative or absolute increase in the number of lymphocytes. Thus, an expansion of  $\gamma\delta$  T cells with an ALC reaching to  $18 \times 10^9/L$  has been seen in human erlichiosis.<sup>23</sup>

It is more common to observe an increase in ALC during most of viral diseases. Perhaps the most frequently suspected is the *infectious mononucleosis* caused

by the infection of the B lymphocyte by EBV,<sup>24</sup> and the following expansion of T lymphocytes that usually appears as atypical lymphocytes. Mononucleosis-like pictures are also seen during cytomegalovirus (CMV) infection (typical heterophile antibodies negative),<sup>25</sup> measles, mumps, viral hepatitis, type VI herpes virus,<sup>26</sup> and some parasitic diseases like babesiosis<sup>27</sup> and toxoplasmosis.<sup>28</sup>

*Acute infectious lymphocytosis* is a rare self-limiting disease that mostly affects children under the age of 10 years. The main feature of this syndrome is the elevation of the ALC with an average of  $20$  to  $30 \times 10^9/L$  normal appearing small lymphocytes. In most of the cases this increase is due to CD4 T cells,<sup>29</sup> although Saulsbury reported one child with elevation of B cells.<sup>30</sup> This disease has an incubation period of 12 to 21 days. Some viruses such as adenovirus, Coxsackie A, Coxsackie B6 or Echo 7 have been involved in the pathogenesis of this syndrome that still remains obscure. Most of the affected individuals are asymptomatic and fever and/or diarrhea are experienced by a few of the subjects.

With regard to *HIV infection*, it is known that it may cause heterophile negative mononucleosis like pictures<sup>31,32</sup> during the seroconversion. Initial lymphopenia is followed by CD8 lymphocytosis and inversion of the CD4/CD8 ratio. During the follow up, the CD8 count gradually returns to normal. Apart from this initial acute event, a persistent mild and normal appearing CD8CD29 lymphocytosis may be developed in less than 1% of HIV infected patients who suffer what is called the *diffuse infiltrative lymphocytosis syndrome* (DILS).<sup>33</sup> It affects mainly black male patients, usually with a slower progression to AIDS. Almost all patients have bilateral parotid gland enlargement and the majority have sicca symptoms. Typical extraglandular sites involved are lung (lymphocytic interstitial pneumonitis), liver and muscle. DILS development seems to be related to certain HLA types, so the lymphocytosis represents an MHC restricted antigen driven oligoclonal selection<sup>34</sup> that suppresses HIV replication in some organs. A salivary gland biopsy and/or scintigraphy with <sup>67</sup>Ga may be useful in the diagnosis. Frequently, patients respond well to steroids or anti-retroviral therapy.

The term *tropical splenomegaly* (TS) usually refers to chronic forms of malaria.<sup>35,36</sup> It is characterized by a B cell lymphocytosis, an increase in serum IgM and big splenomegaly. TS usually responds to proguanil therapy. Bates *et al.* have shown the presence of IgH rearrangements in some of these TS.<sup>37</sup> Atypical lymphocytes have also been found in acute malaria.<sup>38</sup>

*Hypersensitivity reactions to drugs* are a well known cause of transient atypical lymphocytosis.<sup>39,40</sup> Clinical signs include a maculopapular rash with fever, adenopathy, hepatosplenomegaly, myocarditis or even death. Laboratory findings comprise abnormal liver or renal function, eosinophilia, monocytosis and high lactate dehydrogenase levels. The delay between the start on the

**Table 2. Causes of benign polyclonal non-neoplastic lymphocytosis.**

Infectious <sup>20</sup>	Epstein Barr virus <sup>24</sup>
Pertussis	Type VI herpes virus <sup>26</sup>
Rickettsia <sup>23</sup>	Varicella Zoster virus
Syphilis	Cytomegalovirus <sup>24,25</sup>
Brucellosis	Viral hepatitis
Shigella	Diffuse infiltrative lymphocytosis syndrome <sup>33,34,51,64</sup>
Tropical splenomegaly <sup>35,38</sup>	Acute infectious lymphocytosis <sup>29,30,65</sup>
Babesiosis <sup>27</sup>	Measles
Toxoplasmosis <sup>28</sup>	Mumps
Drugs <sup>20,39,40</sup>	Interleukine <sup>2</sup>
Allopurinol <sup>39</sup>	Isoniazid <sup>39</sup>
Atenolol	Mexiletine
Captopril	Mesalazine <sup>69</sup>
Carbamazepine <sup>39,66</sup>	Minocycline <sup>39,43</sup>
Catecholamines <sup>62</sup>	Norfloxacin <sup>70</sup>
Chlorpropamide	Para-amino salicylic acid <sup>20</sup>
Dapsone <sup>39</sup>	Phenobarbital <sup>39</sup>
Diltiazem	Phenytoin <sup>24,39</sup>
Enalapril <sup>39</sup>	Sulphasalazine <sup>39,71,73</sup>
Fenoprofen <sup>39</sup>	
Halothane <sup>67</sup>	
Insulin <sup>68</sup>	
Paraneoplastic	
Malignant thymoma <sup>44,46</sup>	
Nodular lymphocyte predominance Hodgkin disease <sup>47</sup>	
Genetic	
Autoimmune lymphoproliferative syndrome <sup>48,49</sup>	
Miscellaneous	
Hyposplenic states <sup>50-53</sup>	
Chronic natural killer lymphocytosis <sup>56,59</sup>	
Persistence binucleated polyclonal B lymphocytosis <sup>1,5,7,11</sup>	
Exercise-induced and stress <sup>60,62</sup>	
Post-perfusion syndrome <sup>64,55</sup>	



drug and the onset of the picture is about 3 weeks and there is usually an improvement after drug withdrawal. Some drugs have been involved, most typically being anticonvulsants as phenytoin or carbamazepine, some times with cross reaction. Other drugs that may cause lymphocytosis are detailed in Table 2. However, this issue is more complicated, as actual malignant lymphoproliferative diseases (Hodgkin and non-Hodgkin lymphoma) occur in patients receiving phenytoin.<sup>41,42</sup> There is little information about phenotyping in these hypersensitivity reactions, although MacNeil *et al.*<sup>43</sup> have found an expansion of CD4 DR<sup>+</sup> T-cells during treatment with mycyclyline.

*Malignant thymoma* is composed of neoplastic epithelial cells and small lymphocytes. Patients with thymoma rarely present peripheral T cell lymphocytosis.<sup>44-46</sup> The benign or malignant nature of these lymphocytes has been discussed. Medeiros *et al.*<sup>44</sup> and Smith *et al.*<sup>46</sup> have reported 3 cases that present a mixture of small mature CD4 and CD8 lymphocytes without clonal TCR rearrangement and an ALC ranging between 4.9 and 24×10<sup>9</sup>/L. Hypotheses include a spillover of tumor lymphocytes into the circulation and an immunoregulatory disorder because of a thymic hormone imbalance. However, Lishner *et al.*<sup>45</sup> found a patient with an increase in monoclonal T, CD4 negative, CD8 negative lymphocytes with clinical features of malignant lymphoma. An increase in polyclonal B atypical lymphocytes with a narrow thin cytoplasm and indented nuclei reaching an ALC of 7×10<sup>9</sup>/L was discovered in a patient with *nodular lymphocyte predominance Hodgkin's disease*.<sup>47</sup>

The recently described *autoimmune lymphoproliferative syndrome*<sup>48,49</sup> affects children who present moderate to massive splenomegaly and lymphadenopathy, hypergammaglobulinemia, and autoimmunity as hemolytic anemia, idiopathic thrombocytopenic purpura, positive ANA or Guillain-Barré syndrome. There is a B cell lymphocytosis and an expansion of CD4<sup>+</sup> CD8<sup>-</sup> T cells with the α/β TCR. This picture, easily confused with malignant lymphoma, seems to be due to abnormal lymphocyte apoptosis, which may be related to mutations of the Fas gene.<sup>48</sup>

Leukocytosis is a well recognized sequel of splenectomy. Immediately following a splenectomy, leukocytosis results mainly from neutrophilia, but lymphocytosis and monocytosis develop within weeks. The causes of the *hyposplenic state* may be various: surgery, atrophy or infiltrative disease.<sup>50-53</sup> It is a benign condition with an ALC between 4 and 5×10<sup>9</sup>/L, indistinguishable from normal lymphocytes. It appears after a median of 6 years and tends to remain indefinitely. Increments in either CD8 T cells<sup>50,51</sup> or NK cells<sup>50,53</sup> have been reported.

*Post-perfusion syndrome* was referred to as the appearance of atypical lymphocytes, fever and splenomegaly within approximately 30 days after receiving large volumes of blood during cardiopulmonary bypass. Most cases are due to CMV infections,<sup>54</sup> although occasion-

al cases have been linked to EBV.<sup>55</sup>

*Chronic large granular lymphocytes* can be phenotypically divided into CD3<sup>+</sup> (T cell) and CD3<sup>-</sup> (NK cell).<sup>56</sup> Most patients with CD3<sup>+</sup> LGL have a clonal disorder proved by the presence of TCR rearrangements. LGL proliferations of NK cells that present clonal cytogenetic abnormalities and/or an aggressive lymphoproliferative disorder with multi-organ involvement and short survival times, are referred as *NK-LGL leukemia/lymphoma*. By contrast, most patients with increased numbers of CD3<sup>-</sup> LGL have a chronic course with associated diseases such as pure red cell blood aplasia, recurrent neutropenia, vasculitis syndromes and humoral abnormalities, such as the presence of auto-antibodies including rheumatoid factor, hypergammaglobulinemia and monoclonal paraprotein.<sup>57,58</sup> The ALC in this chronic CD3<sup>-</sup> LGL is about 5×10<sup>9</sup>/L with an absolute leukocyte count that is below the upper normal value and it may be associated with bone marrow granulomas.<sup>59</sup>

A quick transient atypical mild lymphocytosis may be observed in patients with *emergency medical conditions* such as cardiac arrest, seizure disorders, anaphylactic reactions status asthmaticus, hypertensive crisis, acute pancreatitis or non surgical trauma.<sup>20,60,61</sup> This increase in lymphocyte count may be due to an increase in plasma catecholamine levels in response to stress.<sup>62</sup>

In conclusion, we presented the case of a young woman with persistent lymphocytosis, who displayed multiple bcl-2/IgH rearrangements. Although most of similar cases have had a benign course, the over-expression of bcl-2 advise to keep these patients under observation and to follow a *watch and wait* policy. Special efforts must be made in order not to confuse this disorder with a malignant lymphoproliferative neoplastic disease and prescribe chemotherapy treatment. Even so, care must be taken not to overlook these rare cases that could be more frequent than expected so far.

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PLL made the molecular analysis by means of PCR. PF and FO worked in the cytology laboratory and they were the first who realized that this was an unusual entity. RA is the responsible of project FIS no. 95/0203, which has funded the primers for the molecular studies. RC and JFT work in clinical management of this case. IP looked for some of the references named in the bibliography. EG wrote the case report and the review of the different entities and JMFR co-ordinate all the works.

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### Disclosures

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