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Clinical course and features of persistent polyclonal B-cell lymphocytosis with BCL-6 amplification during pregnancy

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Abstract. – BACKGROUND: Persistent polyclonal B-cell lymphocytosis is a rare nonmalignant disorder characterized by mild persistent lymphocyte proliferation with possible evolution to aggressive lymphoma. Its biology is not well known, but it is characterized by a specific immunophenotype with rearrangement of the *BCL-2*/IGH gene, whereas amplification of the *BCL-6* gene has rarely been reported. Given the paucity of reports, it has been hypothesized that this disorder is associated with poor pregnancy outcomes.

CASE REPORT: To our knowledge, only two successful pregnancies have been described in women with this condition. We report the third successful pregnancy in a patient with PPBL and the first with amplification of the *BCL-6* gene.

CONCLUSIONS: PPBL is still a poorly understood clinical condition with insufficient data to demonstrate an adverse effect on pregnancy. The role of *BCL-6* dysregulation in the pathogenesis of PPBL and its prognostic significance are still unknown. Evolution into aggressive clonal lymphoproliferative disorders is possible and prolonged hematologic follow-up is warranted in patients with this rare clinical disorder.

Key Words:
B lymphocytes, Clonal evolution, Flow cytometry, Gene expression, PPBL, Pregnancy, *BCL-2*, *BCL-6*.

Introduction

Persistent polyclonal B-cell lymphocytosis (PPBL) is a rare, nonmalignant lymphoproliferative disorder, characterized by persistent mild lymphocytosis and a moderate increase in polyclonal IgM. Possible evolution to aggressive B-cell lymphoma is reported in up to 10% of cases[1,2].

This disorder, more commonly diagnosed in young or middle-aged smoking females, usually shows an indolent course, but careful long-term follow-up is strongly recommended[2-4].

Polyclonal lymphocytosis is the main feature of this hematologic disorder, hypothetically due to stimulation of central memory B lymphocytes[5].

The distinct atypical morphological appearance of lymphocytes is characterized by a large and slightly basophilic cytoplasm with round or irregular nuclei and a variable percentage of binucleate lymphocytes that are typical enough to be distinctive. The chromatin is dense, and nucleoli are often evident[2].

Despite a polyclonal rise in IgM is often observed, a monoclonal component is not detected in most cases[2].

The immunophenotype, mandatory for diagnosis, demonstrates a population of polyclonal B lymphocytes (CD19+, CD20+, CD22+) expressing both kappa and lambda immunoglobulin light chains. In addition, the B lymphocytes co-express IgM, IgD, and CD27, suggesting a possible origin from memory-unswitched B lymphocytes of the marginal zone[6].

An additional long arm of chromosome 3 as isochromosome i(3)(q10) is often found[7].

A valid scientific hypothesis identifies PPBL as a stepwise process that probably begins with the appearance of *BCL-2/IGH* gene rearrangements[8,9], while tetrasomy of the *BCL-6* gene locus is rarely reported[10]. These aberrations could cause genetic instability which would also lead to the emergence of different clones of B cells exhibiting the translocation t(14;18)[8,11]. HLA typing disclosed the presence of at least one DR7 antigen in 70% of patients[2,12]; moreover, PPBL has a familial clustering, suggesting an underlying genetic disorder[11].

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PPBL is generally asymptomatic or mildly symptomatic[2]. A recent analysis[12] based on the French Registry for PPBL showed that approximately 60% of PPBL patients may present with a wide range of clinical symptoms, such as fatigue with severe impairment, post-exercise malaise, cognitive dysfunction, and orthostatic intolerance, meeting the criteria for the diagnosis of the recently defined "systemic exertion intolerance disease".

Sometimes there is splenomegaly, which assumes considerable dimensions and often mimics splenic lymphoma, and this condition may need to be treated. There have been reports of the use of monoclonal antibodies (e.g., rituximab) or, in some cases, splenectomy[13,14].

To date, less than 200 cases of PPBL have been described worldwide, mostly in case reports or collective series[12,15,16]; but, to our knowledge, only two cases of pregnancy in women with PPBL have been reported[5,17]. In this paper we describe a case of PPBL diagnosed during pregnancy in a young woman whose diagnosis was confirmed after 2 years of follow-up.

Case Report

In February 2019 a 30-year-old nonsmoking woman was referred to our hematology department because of lymphocytosis that had occurred during the first trimester of pregnancy. The patient reported a history of atopic dermatitis that had been treated with continuous low-dose steroid and cyclosporine six years earlier.

During this period mild lymphocytosis (5.2 x 10^9/l) was detected, with a subsequent increase after discontinuation of cyclosporine, reaching 8.0 x 10^9/l lymphocytes.

A progressive decrease to 6.4 x 10^9/l lymphocytes was observed during the following year. Subsequently, neither blood count checks nor lymphocyte typing were performed.

At presentation, the patient was six weeks pregnant, in good clinical condition, with no reactivation of dermatitis and no constitutional symptoms.

The blood count showed hemoglobin Hb 12.4 g/dL, white blood cells WBC 15.6 x 10^9/l, neutrophil count 4.7 x 10^9/l, lymphocytes 9.8 x 10^9/l, monocytes 0.7 x 10^9/l, eosinophils 0.1 x 10^9/l, platelets 114 x 10^9/l.

Clinical examination revealed small, subcentimeter-laterocervical lymph nodes, and abdominal organomegaly was not detected.

Blood chemistry showed a polyclonal increase in IgM without organ damage or monoclonal gammopathy. Screening for autoimmune and viral diseases was negative.

In particular, a considerable number of large lymphocytes with atypical features was seen in the peripheral blood smear. The lymphocytes had a rounded and sometimes irregular nucleus with a large basophilic cytoplasm devoid of granules and, as a very important feature, binucleated cells were observed, accounting for approximately 30% of the lymphocyte count (Figure 1).

Immunophenotyping (peripheral blood) highlighted an expansion of B lymphocytes CD45+, CD5+, CD10- with a polyclonal expression of surface light chains kappa and lambda (ratio 1.8), which accounted for about 80% of the total lymphocytes.

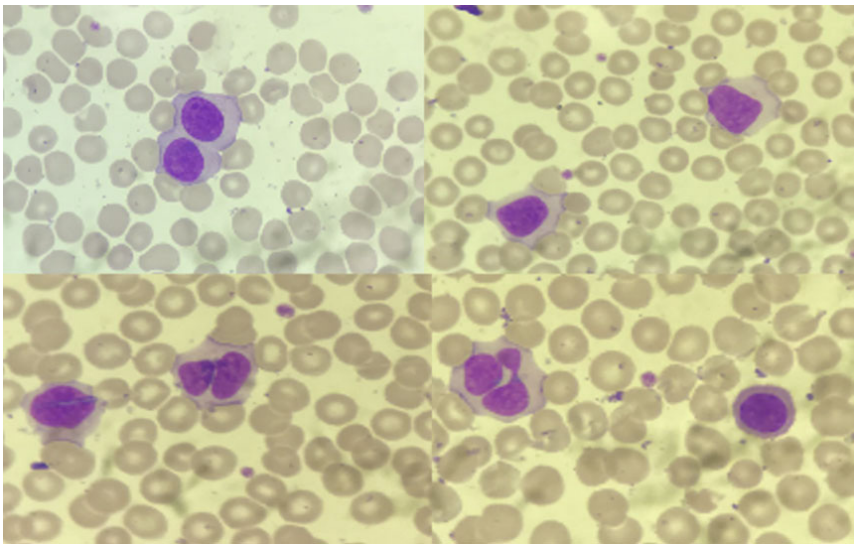

Figure 1. PPBL peripheral smear (magnification 100x). Several lymphocytes with atypical features can be observed, some binucleate with round or irregular nuclei and large, weakly basophilic cytoplasm without granules.

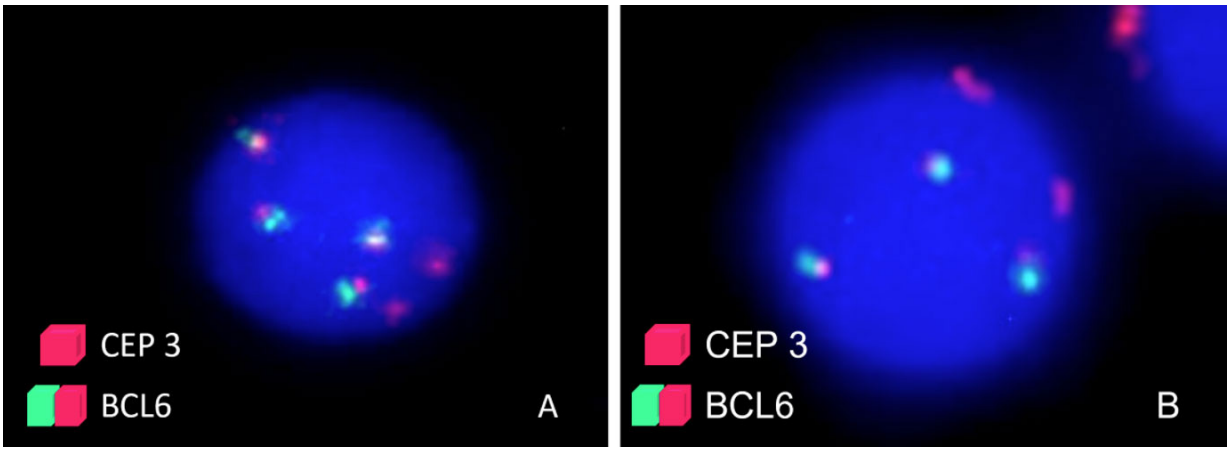

Figure 2. PPBL FISH (magnification 100x) Image (**A**) shows *BCL6* tetrasomy (4 red/green and 2 red centromere signals), image (**B**) shows *BCL6* trisomy (3 red/green and 2 red centromere signals).

Almost all CD19+ B lymphocytes (98%) exhibited a memory-unswitched phenotype CD27+/IgD+/IgM+. Karyotyping of peripheral blood revealed normal metaphases while Fluorescent in situ hybridization (FISH) in peripheral blood revealed a hybridization pattern consistent with *BCL6* gene amplification (Figure 2).

Blood count and lymphocyte subpopulations were closely monitored during pregnancy, and a constant decline in lymphocytosis was noted pecking at the time of delivery in the 39th week (Figure 3).

It is of particular importance to point out that the gradual decrease in lymphocyte count affected mainly the B-cell compartment, with the unswitched memory lymphocytes showing a proportionally greater decrease, from 99% of the total B-cell percentage at diagnosis to 87% at the time of delivery. On the other hand, there was a slight expansion in the T-cell compartment, especially the CD4+ T-cell compartment. Any noteworthy complication was observed during pregnancy and at the time of delivery. A healthy male of 2,900 g was delivered in September 2019, his blood count showed Hb 18.7 g/dL, WBC 7.76 x 10^9/l, neutrophil count 3.56 x 10^9/l, lymphocytes 3.18 x 10^9/l, platelets 161 x 10^9/l. After pregnancy a significant increase in lymphocyte count was observed in the patient, reaching values similar to those at the time of diagnosis.

More than 12 months after delivery blood values were: Hb 12.7 g/dL, WBC 14.6 x 10^9/l, neutrophils 4.4 x 10^9/l, lymphocytes 9.8 x 10^9/l, monocytes 0 x 10^9/l, eosinophils 0.3 x 10^9/l, basophils 0.1 x 10^9/l, platelets 123 x 10^9/l.

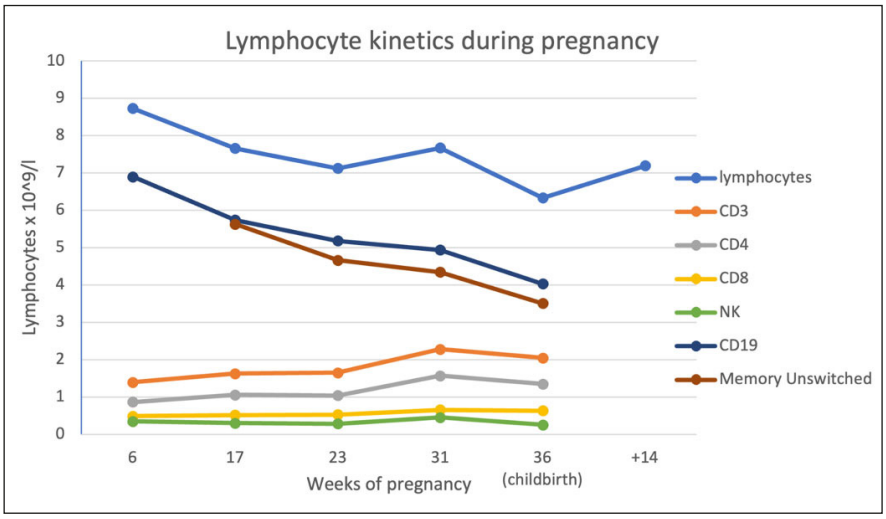

Figure 3. PPBL: lymphocyte kinetics during pregnancy. NK: Natural Killer cells.

After 3 years of follow-up, the patient was in good clinical condition, hematologic parameters were stable compared with before, and immunoglobulin titration and serum electrophoresis confirmed polyclonality. Immunophenotyping of peripheral blood showed the same features as at the time of diagnosis. The child was in good health and his growth was normal; no significant changes were noted in the blood count.

Discussion

To our knowledge, we report the first case of safe pregnancy in a patient with PPBL and *BCL-6* amplification, while only two other cases of pregnancy in PPBL have been reported. Our observations confirm the previous findings of Carulli et al[5] regarding the kinetics of lymphocyte subsets during pregnancy in a case of PPBL with *BCL-2* amplification.

The clinical significance of these findings is not entirely clear, but the variation in lymphocyte count is probably related to hormonal changes during pregnancy, which may determine morphologic and functional changes in lymphocyte subsets. Preclinical data deriving from pregnancy models in mice confirmed that, at an early stage of B lymphopoiesis, the formation of B-cell precursors in the hematopoietic niche is selectively suppressed, which is controlled by estrogens produced during pregnancy[18].

To date, no data have been reported in the literature to indicate that PPBL may adversely affect pregnancy in terms of predisposing to an increased risk of complications, for both the mother and the unborn child. However, sporadic observations of multiple cases in some families strongly suggest that pregnant women with a history of PPBL, should be monitored in order to make an early diagnosis of PPBL and prevent the occurrence of clonal lymphoproliferative disorders.

PPBL has been associated with a group of symptoms that may be due to systemic dysregulation of the immune system[12]. In this case, it can be rightly assumed that the condition of polyclonal lymphocytosis was already present at the time of diagnosis of atopic dermatitis which could represent an epiphenomenon of PPBL itself. We can also hypothesize that PPBL may be the expression of immunologic dysregulation that is not always transient. This hypothesis is further sustained by the fact that immunosuppressive therapy (i.e., steroids, calcineurin inhibitors) leads to depletion of lymphocytes without achieving complete remission.

Hyperplasia of the marginal zone compartment, resembling splenic MZL, is found in both bone marrow and spleen. However, despite the presence of *BCL2/IGH* rearrangements no evidence of clonality has been found[19]. The *BCL6* gene has long been known to be an important oncogene in B-cell lymphomas and encodes a transcription factor critical for the development of B-cells in the normal germinal center[20]. The role of *BCL6* dysregulation in the pathogenesis of PPBL and its prognostic significance are still unknown.

Limitations

The rarity of this condition means that there is a lack of data in the literature. Information comes mainly from case reports and data collection from small cohorts. There is a need for studies with a higher level of evidence, such as observational studies.

Conclusions

In summary, PPBL remains a poorly understood clinical condition. Clinical presentation is variable, ranging from asymptomatic disease to conditions requiring therapy[13,14]. This feature confirms that this disorder represents an exceptional model for the study of B-cell homeostasis.

Evolution to aggressive clonal lymphoproliferative disorders is possible and a prolonged hematologic follow-up is indicated in patients with this rare clinical disorder[2-4].

Although the effects of PPBL on pregnancy remain unclear, it is possible that women with PPBL can safely carry a pregnancy to term, and there is no evidence of adverse pregnancy effects.

Ethics Approval

Ethical approval was not required, as the patient in this case report was not involved in any study.

Informed Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying data and images.

Data Availability

All data generated or analyzed are included in this case report. Further inquiries can be directed to the corresponding author.

Conflict of Interest
All the authors declare no conflicts of interest.

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Authors' Contributions
A.G. wrote the manuscript. G.C., R.M. and L.B. collected the data and revised the manuscript. M.G. performed immunophenotype analysis. A.A and V.M.L performed FISH. All authors read and approved the final manuscript.

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