

Case Study

Modifications in B-Lymphocyte Number and Phenotype in the Course of Pregnancy in a Woman with Persistent Polyclonal B-Cell Lymphocytosis: A Flow Cytometric Study

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Persistent polyclonal B-cell lymphocytosis (PPBL) is a rare clinical condition, characterized by a persistent, generally moderate lymphocytosis, generally due to stimulation of central memory B-lymphocytes, and by a moderate increase of polyclonal IgM. In some patients, slight or moderate splenomegaly is observed. A variable percentage of circulating, bone marrow and splenic lymphocytes display an abnormal nucleus (generally bilobated) or are binucleated. The clinical course is benign in most cases and transformation into splenic B-cell lymphoma occurs in few cases. In the current paper we report the first case of pregnancy in PPBL. Our patient became pregnant 18 months after diagnosis. In the course of pregnancy, a marked down-regulation of lymphocytosis (from $6 \times 10^9/L$ to $2.1 \times 10^9/L$) and a decrease in B-lymphocyte number was observed (from $3.6 \times 10^9/L$ to $1 \times 10^9/L$), mainly due to a marked reduction in the percentage and absolute number of central memory B-cells. Such modifications were similar to those described in normal pregnant women. One year after the delivery of a healthy female baby, the number of total lymphocytes and B-lymphocytes showed an inverse behavior, with a new expansion of central memory B-cells. Our case shows that a normal pregnancy can occur in patients with PPBL and that pregnancy can induce marked modifications in B-lymphocyte kinetics and phenotype. [*J Clin Exp Hematop* 55(1) : 77-82, 2015]

Keywords: B-lymphocytes, flow cytometry, persistent polyclonal B-cell lymphocytosis, pregnancy

INTRODUCTION

Persistent polyclonal B-cell lymphocytosis (PPBL), initially described by Gordon *et al.* in 1982,¹ is a rare and peculiar clinical condition, characterized by a persistent, generally moderate increase in circulating lymphocytes (i.e. $> 4 \times 10^9/L$) and moderate increase of polyclonal IgM. PPBL is more frequent in young and middle-aged women, often smokers, with a median age at presentation of about 40-50 years.²

A variable percentage of circulating lymphocytes are binucleated or show atypical nucleus (bilobate, bishaped).²⁻⁴ Immunophenotyping shows a clear increase of B-cells reacting with CD19, CD20, CD22, and expressing both κ and λ

immunoglobulin light chains, with a polyclonal behavior.^{2,3} In most cases, B-lymphocytes show the phenotype of central memory cells, being positive for CD27, IgM and IgD.^{3,5,6} However, Feugier *et al.*⁷ reported five cases negative for IgD, but always positive for IgM, thus showing that some variant cases can be observed. Karyotyping shows the presence of extra chromosome 3 long arm i(3q) in several patients.⁸ The *IgH* gene is not clonally rearranged, but virtually all cases show a *BCL2/IgH* rearrangement.⁹ However, the accumulation of B-lymphocytes in PPBL is probably not related to overexpression of the BCL2 protein.¹⁰

Bone marrow morphology, evaluated on trephine biopsy sections, shows a slight or moderate infiltration, with both interstitial and intravascular pattern.⁷ Infiltrating B-lymphocytes are positive for CD20 and BCL2, and show the same morphologic properties as circulating cells. Histologic sections obtained from splenectomy show infiltration of both white and red pulp, with enlargement of the marginal-zone of splenic follicles, and atypical lymphocytes in the splenic sinusoids.¹¹⁻¹³ Such morphologic findings could lead to a misdiagnosis of splenic B-cell lymphoma.⁷

Taken together, the above findings support the hypothesis that B-lymphocytes in PPBL derive from the marginal zone of

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the spleen, circulate in the peripheral blood and migrate to the bone marrow.

From a clinical point of view, PPBL is asymptomatic and diagnosis is casual in most cases. Some patients complain of weakness or fatigue. Splenomegaly seems to be present in about 10% of cases.² The clinical course is benign and only very few cases show transformation into a B-cell lymphoma. To the best of our knowledge, about 200 cases have so far been described and the most numerous case studies, carried out in 111 subjects with a median follow-up of 4.4 years, showed a benign course and transformation into malignant lymphoma in only 4 subjects.¹⁴ The absolute number of circulating lymphocytes remain stable over time and may show a decrease after splenectomy.¹²

In the present paper we report the first case of a successful pregnancy in a woman affected by PPBL. Interestingly, kinetics of circulating B-lymphocytes was characterized by down-regulation of absolute lymphocyte number and by modifications in B-lymphocyte immunophenotype. One year after delivery, an almost complete reversal of the latter findings could be observed.

CASE REPORT

A 33-year-old woman was sent by her general practitioner in September 2011 because of leucocytosis discovered casually about six months before. Her clinical history was silent, but smoking of about 1 package of cigarettes per day was referred. At presentation, the following blood count parameters were registered: Hb 12.6 g/dL; WBC $12 \times 10^9/L$; peripheral mononuclear cells $5.5 \times 10^9/L$; lymphocytes $6 \times 10^9/L$; monocytes $0.4 \times 10^9/L$; eosinophils $0.1 \times 10^9/L$; platelets $168 \times 10^9/L$. At physical examination, a slight splenomegaly was found. This finding was confirmed by abdominal

ultrasound and computed tomography, which showed a longitudinal diameter of 14 cm. Blood films were observed under a light microscope and several atypical lymphocytes were found, generally characterized by atypical nucleus (binucleated, bilobate) (Fig. 1). Blood chemistries showed a mild increase of serum IgM concentrations (600 mg/dL; normal value 40-230). IgM were found to be polyclonal at serum immunofixation. Viral and autoimmune diseases were excluded and there was no evidence for neoplastic diseases.

Flow cytometric immunophenotyping was carried out both on peripheral blood and on bone marrow samples using a FacsCanto II cytometer (Becton Dickinson) equipped with three lasers (405, 488 and 633 nm). Samples were stained with fluorochrome-conjugated monoclonal antibodies (MoAbs) specific for the following antigens: CD3, CD4, CD8, CD5, CD16-56, CD19, CD20, CD22, CD23, CD27, CD79b (purchased from Becton Dickinson), rabbit F(ab')₂ polyclonal antibodies directed to δ and μ immunoglobulin heavy chains and κ and λ immunoglobulin light chains (purchased from Dako). A multiparametric method with either seven or eight fluorescences was used for each tube, associating MoAbs conjugated with FITC, PE, PerCP-Cy5.5, PE-Cy.7, APC, APC-Cy.7, Horizon V-450 and Horizon V-500. Lymphocytes were gated using CD45 expression and right angle scatter, and 100,000 events/tube were acquired.^{15,16}

Immunophenotyping of peripheral blood showed a high percentage of B-lymphocytes (60%; absolute number: $3.6 \times 10^9/L$), positive for CD19, CD20, CD22, CD79b, and with polyclonal expression of surface immunoglobulin κ and λ light chains (ratio: 1.2). Most of B-lymphocytes (72%; absolute number: $2.59 \times 10^9/L$) showed the immunophenotype of central memory cells (CD27⁺, IgD⁺, IgM⁺), while a lower percentage of naïve B-lymphocytes (27%; absolute number: $0.97 \times 10^9/L$) was found (Fig. 2A-2C). Very low percentages

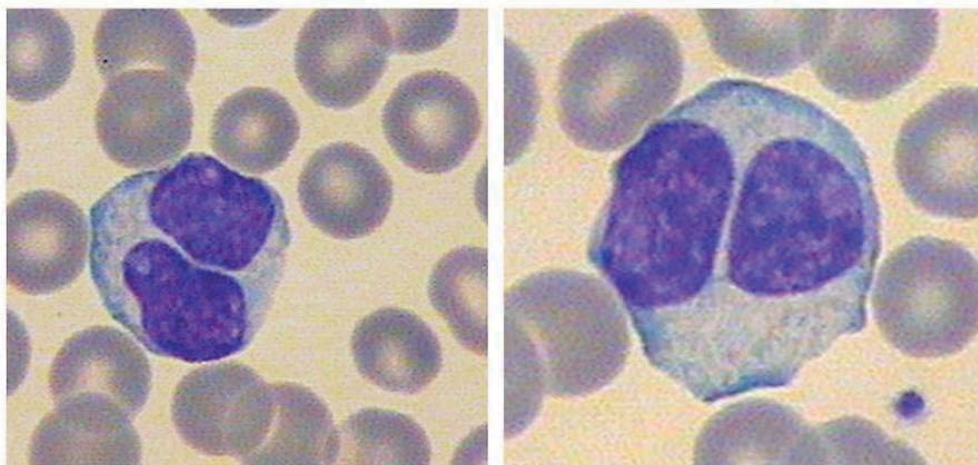


Fig. 1. Circulating atypical lymphocytes. *Left:* binucleated lymphocyte. *Right:* bilobated lymphocyte. May-Grünwald-Giemsa, magnification $\times 1,000$.

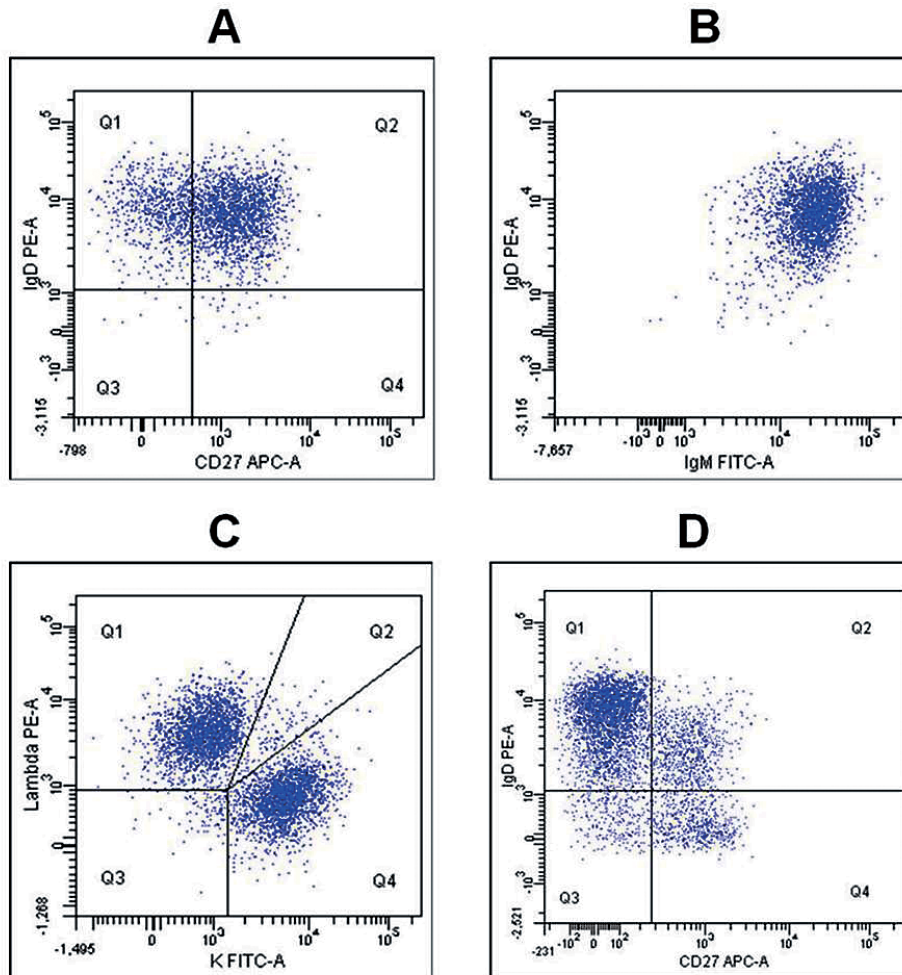


Fig. 2. Immunophenotyping of circulating B-lymphocytes. (2A-2C) Evaluation at diagnosis. (2A) CD27/IgD staining, showing a prevalent population of central memory B-cells (Q2). Q1: naïve B-cells. (2B) Positivity for both IgD and IgM. (2C) Polyclonal expression of surface κ and λ immunoglobulin light chains. (2D) Immunophenotyping during pregnancy, showing a prevalent population of naïve B-lymphocytes (Q1). Q4: effector memory B-cells. Q3: double-negative memory B-cells.

of effector memory and double-negative memory B-lymphocytes (i.e. CD27⁻, IgD⁻) were found (0.7 and 0.3%, respectively). The reference values of our laboratory were: 64 ± 7 naïve B-lymphocytes; 12 ± 7 memory B-cells; 13 ± 4 effector memory B-cells; 10 ± 3 double-negative B-lymphocytes).

A myeloaspirate showed a mild infiltration (25%) by polyclonal B-lymphocytes, with the same immunophenotypic features as the circulating counterpart. The patient underwent a bone marrow trephine biopsy. The histologic sections were subjected to H&E staining and immunohistochemistry, and a moderate infiltration by CD20⁺ lymphocytes was observed, with a clear intravascular localization. Such lymphocytes often showed binuclearity or bilobate nucleus, and positivity for the BCL2 protein (Fig. 3). Bone marrow karyotyping

(carried out by Dr. Maria Immacolata Ferreri, Division of Medical Genetics, AOUP, Pisa) yielded normal metaphases. PCR assays, carried out as described elsewhere,^{17,18} showed a polyclonal *IgH* gene pattern and the presence of the *BCL2/IgH* translocation.

Diagnosis of PPBL was established and the patient underwent periodical (about every six months) controls of blood count, blood chemistries, immunophenotyping, abdomen ultrasound. All findings remained stable for about 18 months.

In April 2013 the patient became pregnant and during her pregnancy periodical evaluations of blood count were available. In addition, two lymphocyte immunophenotype analyses were performed. In the course of pregnancy, a significant decrease of lymphocyte absolute number was registered ($2.1 \times 10^9/L$) mainly due to a drastic reduction of B-

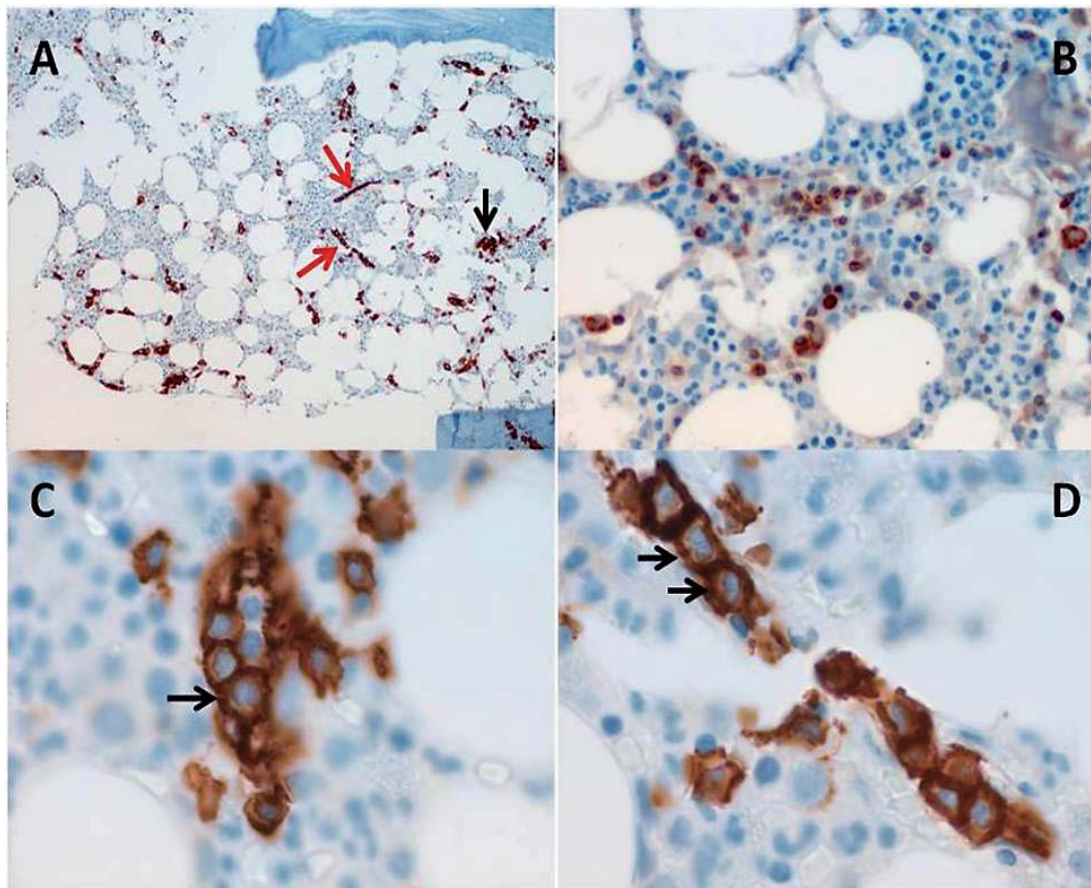


Fig. 3. Bone marrow evaluation by trephine biopsy. (3A) CD20 immunostaining. Mild infiltration by positive B-lymphocytes with both interstitial (*black arrow*) and intrasinusoidal pattern (*red arrows*). $\times 40$. (3B) positivity for BCL2 protein. $\times 100$. (3C & 3D) CD20-positive binuclear (3C, *arrow*) and bilobated (3D, *arrows*) lymphocytes. $\times 1,000$.

lymphocytes, which were 1 and $1.1 \times 10^9/L$ in two different instances (Fig. 4A). In addition, the phenotypic features of B-lymphocytes changed, since a reduction of percentage of central memory cells was found; in turn, the percentage of naïve B-lymphocytes increased and was similar to that of normal controls. In fact, immunophenotyping showed: 70% naïve B-lymphocytes (absolute number: $0.7 \times 10^9/L$); 20% central memory B-lymphocytes (absolute number: $0.2 \times 10^9/L$); 4% effector memory B-lymphocytes; 6% double-negative memory B-lymphocytes (Fig. 2D). Therefore, while the absolute number of naïve B-lymphocytes remained substantially unchanged, the absolute number of central memory B-lymphocytes showed a very marked decrease.

Pregnancy was normal and healthy female baby was delivered in January 2014. A new complete evaluation (blood count, blood chemistries, abdomen ultrasound, immunophenotyping) was carried out one year after delivery. At this time (39 months after diagnosis) the number of total lymphocytes and B-lymphocytes were found to be more similar to those registered in the period before pregnancy (Fig.

4A). Main blood count parameters were: Hb 12.5 g/dL; WBC $8.13 \times 10^9/L$; PLT $176 \times 10^9/L$; neutrophils $3.3 \times 10^9/L$; lymphocytes $4.4 \times 10^9/L$; monocytes $0.3 \times 10^9/L$; eosinophils $0.1 \times 10^9/L$; basophils $0.03 \times 10^9/L$. The spleen did not show any modification in terms of both dimensions and ultrasound features. Immunophenotyping showed: 51% naïve B-lymphocytes (absolute number: $2.24 \times 10^9/L$), 36% memory B-lymphocytes (absolute number: $1.58 \times 10^9/L$), 6% effector memory B-lymphocytes, and 7% double-negative memory B-lymphocytes. Kinetics of B-lymphocyte sub-populations is shown in Fig. 4B.

DISCUSSION

The present paper deals with the first case of pregnancy reported in a patient with PPBL. The first interesting point is that a normal, successful pregnancy occurred, with the delivery of a healthy baby. The second point is represented by the peculiar behavior of peripheral lymphocytes. In fact, the absolute number of total lymphocytes showed a marked de-

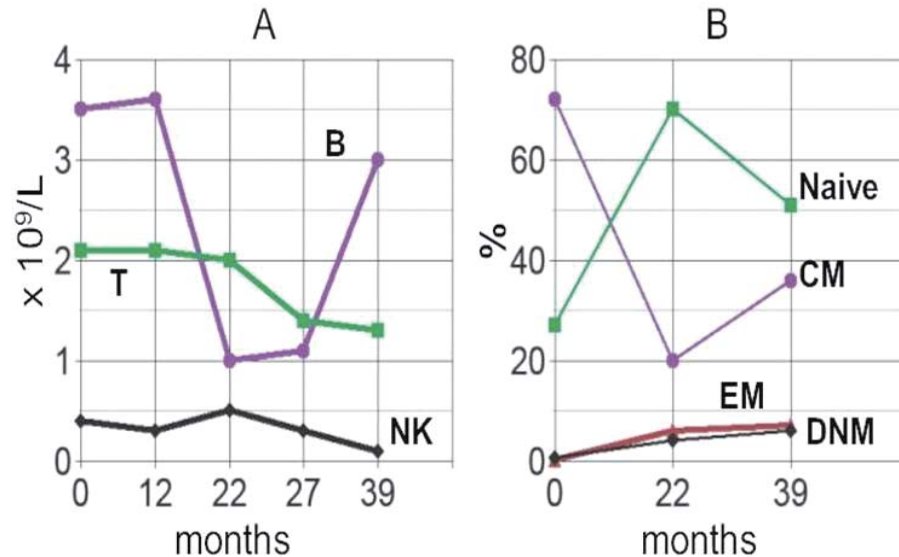


Fig. 4. Kinetics of circulating leukocytes during the clinical observation, from diagnosis (month 0) to the last observation (month 39). (**4A**) Kinetics of T-, B- and natural killer (NK) lymphocytes, evaluated by flow cytometry. (**4B**) B-lymphocytes subset evaluated in three different instances. CM, central memory B-cells; EM, effector memory B-cells; DNM, double-negative memory B-cells. Month 22 and 27 represent flow cytometric analyses in the course of pregnancy.

crease (of about 55%), and this reduction was due to the marked decrease (about 70%) of B-lymphocytes. Moreover, a peculiar modification of B-cell immunophenotype was found, since a significant down-regulation of central memory B-lymphocytes, which were responsible for the persistent lymphocytosis before pregnancy, occurred. Interestingly, one year after delivery lymphocytosis showed relapse, but naïve B-lymphocytes were found to be higher than central memory B-lymphocytes, in terms of both percentage and absolute number.

Our findings are consistent with a possible interference of pregnancy with B-lymphocyte kinetics in PPBL. Studies published in the past have shown that the count of total lymphocytes decreases gradually during pregnancy, with nadir values at about 25-28 weeks of gestation.¹⁹ Moreover, the overall B cell compartment and its functions are suppressed partially during normal human pregnancy, and loss of responsiveness of B cells to mitogens and infectious can be observed.²⁰ Taken together, the above observations are consistent with a significant interference of normal pregnancy with B-lymphocyte number, kinetics and functions.²¹

It has been reported that, during murine pregnancy, the formation of B cell precursors is suppressed selectively in the bone marrow. This suppression occurs at the early stage of B lymphopoiesis and is driven by the pregnancy hormone oestrogens.²² However, oestrogens also have a positive effect on the survival of mature murine B cells,²³ suggesting a compensatory effect of oestrogens at different stages of devel-

opment to maintain a balance within the B cell compartment.

Our findings show that such particular behavior of the immune system during pregnancy may occur also in the peculiar B-lymphocyte activation such as that observed in PPBL, leading to a clear reduction in the absolute number of B-cells and to a reversal of central memory B-cell expansion that generally characterizes PPBL.

In conclusion, our observation shows that: normal pregnancy is possible in patient affected by PPBL; in the course of pregnancy, the expansion of B-lymphocytes may show a significant down-regulation, with a particular down-regulation of central memory B-lymphocytes; these phenomena are transient. Therefore, a direct interference of the hormonal modifications that occur during the gestation period can be hypothesized.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Gordon DS, Jones BM, Browing SW, Spira TJ, Lawrence DN:

- Persistent polyclonal lymphocytosis of B-lymphocytes. *N Engl J Med* 307:232-236, 1982
- 2 Troussard X, Cornet E, Lesesve JF, Kourel C, Mossafa H: Polyclonal B-cell lymphocytosis with binucleated lymphocytes (PPBL). *Onco Targets Ther* 1:59-66, 2008
 - 3 Schmidt-Hieber M, Burmeister T, Weimann A, Nagorsen D, Hofmann WK, *et al.*: Combined automated cell and flow cytometric analysis enables recognition of persistent polyclonal B-cell lymphocytosis (PPBL), a study of 25 patients. *Ann Hematol* 87: 829-836, 2008
 - 4 Lesesve JF, Gressot AL, Troussard X, Mossafa H, Cornet E: Morphologic features of binucleated lymphocytes to assess the diagnosis of persistent B-cell polyclonal lymphocytosis or other mature B-cell neoplasms. *Leuk Lymphoma* 55:1551-1556, 2014
 - 5 Himmelmann A, Gautschi O, Nawrath M, Bolliger U, Fehr J, *et al.*: Persistent polyclonal B-cell lymphocytosis is an expansion of functional IgD⁺CD27⁺ memory B cells. *Br J Haematol* 114:400-405, 2001
 - 6 Berkowska MA, Grosserichter-Wagener C, Adriaansen HJ, de Ridder D, Mirani-Oostdijk KP, *et al.*: Persistent polyclonal B-cell lymphocytosis: extensively proliferated CD27⁺IgM⁺IgD⁺ memory B cells with a distinctive immunophenotype. *Leukemia* 28: 1560-1564, 2014
 - 7 Feugier P, De March AK, Lesesve JF, Monhoven N, Dorvaux V, *et al.*: Intravascular bone marrow accumulation in persistent polyclonal lymphocytosis: a misleading feature for B-cell neoplasm. *Mod Pathol* 17:1087-1096, 2004
 - 8 Mossafa H, Malaure H, Maynadie M, Valensi F, Schillinger F, *et al.*: Persistent polyclonal B lymphocytosis with binucleated lymphocytes: a study of 25 cases. *Groupe Français d'Hématologie Cellulaire. Br J Haematol* 104:486-493, 1999
 - 9 Delage, R, Roy, J, Jacques, L, Darveau, A: All patients with persistent polyclonal B cell lymphocytosis present Bcl-2/Ig gene rearrangements. *Leuk Lymphoma* 31:567-574, 1998
 - 10 Lancry L, Roulland S, Roué G, Mossafa H, Salaün V, *et al.*: No BCL-2 protein over expression but BCL-2/IgH rearrangements in B cells of patients with persistent polyclonal B-cell lymphocytosis. *Hematol J* 2:228-233, 2001
 - 11 Sun P, Juskevicius R: Histological and immunohistochemical features of the spleen in persistent polyclonal B-cell lymphocytosis closely mimic splenic B-cell lymphoma. *Diagn Pathol* 7:107, 2012
 - 12 Del Giudice I, Pileri SA, Rossi M, Sabattini E, Campidelli C, *et al.*: Histopathological and molecular features of persistent polyclonal B-cell lymphocytosis (PPBL) with progressive splenomegaly. *Br J Haematol* 144:726-731, 2009
 - 13 Martinez-Lopez A, Montes-Moreno S, Mazorra F, Miranda-Vallina C, Ulibarrena C, *et al.*: Persistent polyclonal B-cell lymphocytosis with splenomegaly: histologic description of 2 cases. *Am J Surg Pathol* 37:1085-1090, 2013
 - 14 Cornet E, Lesesve JF, Mossafa H, Sébahoun G, Levy V, *et al.*: Long-term follow-up of 111 patients with persistent polyclonal B-cell lymphocytosis with binucleated lymphocytes. *Leukemia* 23: 419-422, 2009
 - 15 Carulli G, Ciancia EM, Azzarà A, Ottaviano V, Grassi S, *et al.*: Simultaneous presentation of Waldenström macroglobulinemia and multiple myeloma: multidisciplinary diagnosis, treatment and 30-month follow-up. *J Clin Exp Hematop* 53:29-36, 2013
 - 16 Carulli G, Baratè C, Marini A, Ottaviano V, Cervetti G, *et al.*: Reduced circulating B-lymphocytes and altered B-cell compartments in patients suffering from chronic myeloid leukaemia undergoing therapy with Imatinib. *Hematol Oncol* [Published online: September 25, 2014. DOI: 10.1002/hon.2156]
 - 17 Galimberti S, Brizzi F, Mameli M, Petrini M: An advantageous method to evaluate IgH rearrangement and its role in minimal residual disease detection. *Leuk Res* 23:921-929, 1999
 - 18 Galimberti S, Luminari S, Ciabatti E, Grassi S, Guerrini F, *et al.*: Minimal residual disease after conventional treatment significantly impacts on progression-free survival of patients with follicular lymphoma: the FIL FOLL05 trial. *Clin Cancer Res* 20:6398-6405, 2014
 - 19 Valdimarsson H, Mulholland C, Fridriksdottir V, Coleman DV: A longitudinal study of leucocyte blood counts and lymphocyte responses in pregnancy: a marked early increase of monocyte-lymphocyte ratio. *Clin Exp Immunol* 53:437-443, 1983
 - 20 Birkeland SA, Kristoffersen K: Lymphocyte transformation with mitogens and antigens during normal human pregnancy: a longitudinal study. *Scand J Immunol* 11:321-325, 1980
 - 21 Nguyen TG, Ward CM, Morris JM: To B or not to B cells-mediate a healthy start to life. *Clin Exp Immunol* 171:124-134, 2013
 - 22 Medina KL, Kincade PW: Pregnancy-related steroids are potential negative regulators of B lymphopoiesis. *Proc Natl Acad Sci U S A* 91:5382-5386, 1994
 - 23 Grimaldi CM, Cleary J, Dagtas AS, Moussai D, Diamond B: Estrogen alters threshold for B-cell apoptosis and activation. *J Clin Invest* 109:1625-1633, 2002