

Occurrence of Non-Hodgkin Lymphoma after the Diagnosis of Persistent Polyclonal B-cell Lymphocytosis

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Abstract

Persistent polyclonal B cell lymphocytosis (PPBL) is a rare indolent condition characterized by a polyclonal expansion of B cells associated with binucleated lymphocytes observable on blood smears. Though polyclonal, recurrent genetic abnormalities have been described in PPBL, supernumerary isochromosome 3q (+i(3)(q10)) being the most frequently described. We report here two clinical observations showing the occurrence of NHL after the diagnosis of PPBL. These observations lead us to recommend a closer follow-up of PPBL patients and raise the question of a relationship between PPBL and NHL.

Keywords: Persistent polyclonal B-cell lymphocytosis; Splenic marginal zone lymphoma; Diffuse large B-cell lymphoma

Introduction

Persistent polyclonal B cell lymphocytosis (PPBL) is a rare but rather indolent condition [1], affecting mainly middle-aged smoking women, and characterized by a polyclonal expansion of B cells associated with binucleated lymphocytes [2] and an increased polyclonal IgM serum level. Though polyclonal, recurrent genetic abnormalities have been described in PPBL, supernumerary isochromosome 3q (+i(3)(q10)) being the most frequently described [3]. We report the cases of two patients with PPBL, where B-NHL occurred after PPBL diagnosis, splenic zone marginal lymphoma (SMZL) and diffuse large B-cell 41 lymphoma (DLBCL), leading us to raise the question of a relationship between PPBL and B-42 NHL.

Material and Methods

Multiparameter flow cytometry (MFC)

Lymphocytes were isolated from blood samples by gradient density. For splenic samples, cell suspension was obtained after mechanical dissociation of tissue. Lymphocytes were immunophenotyped by using 4-color direct immunofluorescence on a BD FACS CANTO II (Becton Dickinson, Franklin Lake, NJ, USA).

PCR amplification, cloning, and sequencing analysis

DNA was extracted from samples using routine procedures. PCR analysis of B-cell clonality was performed following the recommendations of BIOMED 2 protocols [4].

Cytogenetics

Conventional cytogenetics and interphase Fluorescence In Situ Hybridization (FISH) were performed on peripheral blood and on spleen lysates as described elsewhere [5].

First case: Occurrence of pulmonary DLBCL 13 years after PPBL diagnosis

The first patient is a 42-year-old man, who smoked 30 cigarettes each day. The lymphocytosis ($8.5 \times 10^9/L$) was fortuitously discovered

on a routine blood count in 1988. A mild thrombopenia, constantly above $100 \times 10^9/L$ was iteratively reported on blood count since 1996, and binucleated lymphocytes were described on blood smears since 1997. The patient reported no clinical symptomatology. Clinical examination at diagnosis revealed a splenomegaly. There was no hepatomegaly or enlarged lymph nodes. On blood count, hemoglobin was 131 g/L, with $123 \times 10^9/L$ of platelets and $12 \times 10^9/L$ leucocytes (Polymorphonuclear neutrophils (PMN): $3.36 \times 10^9/L$, eosinophils: $0 \times 10^9/L$, basophils: $0 \times 10^9/L$, lymphocytes: $8.52 \times 10^9/L$, monocytes: $0.12 \times 10^9/L$). Examination of the blood smear 3 confirmed the presence of binucleated lymphocytes. MFC analysis of blood lymphoid cells confirmed the polyclonal B cell proliferation (Figure 1). An increased polyclonal IgM (17.3g/L) was observed. Cytogenetic analysis revealed a supernumerary isochromosome 3q (47,XY,+i(3)(q10)[1] / 47,XY,+18[1] / 48,XXY,+i(3)(q10),+8,-20[1] / 46,XY[47]).

The diagnosis of PPBL was retained. During follow-up, the patient did not describe any new symptom. Platelet count steadily decreased below $100 \times 10^9/L$ and the spleen enlarged to a maximal size of 22 cm and became slightly painful. IGHV gene analysis, immunophenotype and karyotype did not detect any clonal evolution in peripheral blood. A splenectomy was performed in December 2008. Histological features of spleen analysis revealed a nodular pattern of lymphoid cells infiltrating the marginal zone and an intrasinusoidal infiltration by lymphoid B-cells (Figure 2). However, immunophenotype, karyotypic analysis and IGHV gene rearrangement performed on the spleen failed to demonstrate the clonality of B-cells (Figures 1 and 2). After splenectomy, the blood count parameters came back within the range

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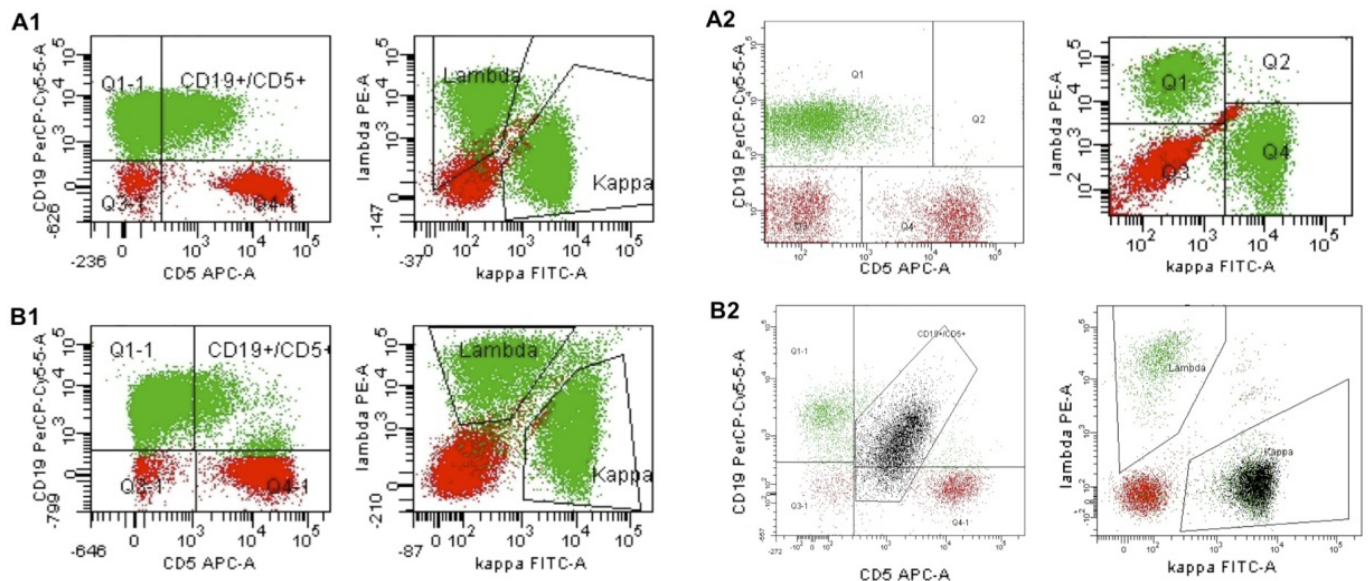


Figure 1: Flow cytometry graph of circulating lymphoid B-cells (CD45+ gating / CD19+ B4 cells in green / CD19+ CD5+ B-cells in black) by the time of diagnosis **A** for patient 1 (A1) and 2 (A2), and before splenectomy **B** for patient 1 (B1) and 2 (B2).

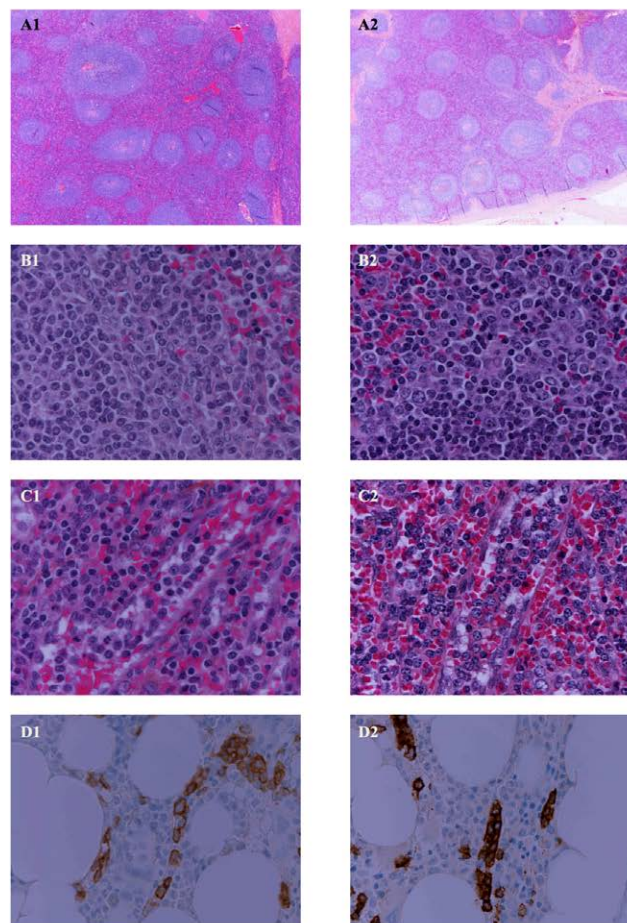


Figure 2: Histopathological examination of the spleen for patient 1 and 2. **A.** Nodular pattern of the lymphoid infiltrates with target-like extension of the marginal zone. HES staining, 2x magnification. **B.** Polymorphous cytology of the marginal zone with association of small irregular cells and scattered larger cells. HES staining, 40x magnification. **C.** Splenic intrasinusoidal infiltration of lymphoid cells. HES staining, 40x magnification. **D.** Bone marrow trephine biopsy. Medullar intrasinusoidal infiltration by CD20+ lymphoid cells.

of normality and binucleated lymphocytes disappeared from blood smear. This patient developed 2 years after splenectomy (13 years after PPBL diagnosis) a pulmonary diffuse large B cell lymphoma (DLBCL). Neither karyotypic nor FISH analysis of pulmonary DLBCL did reveal isochromosome 3q(45-47, XY, add(5)(q35),+7,del(8)(q13q22),del(10)(p11),t(14;18)(q32;q22),add(16)(q24) [cp 9]). No clonal relationship between PPBL and DLBCL could be demonstrated. The patient died of infection.

Second case: occurrence of SMZL 6 years after PPBL diagnosis

The second patient was a 45-year-old woman, who smoked 20 cigarettes each day. The lymphocytosis ($6.12 \times 10^9/L$) was fortuitously discovered on a routine blood count in 2004. The patient reported a mild chronic asthenia, without any other symptom. Clinical examination at diagnosis revealed a splenomegaly. There was no hepatomegaly or enlarged lymph nodes. On blood count, hemoglobin was 139 g/L, with $143 \times 10^9/L$ of platelets and $8.4 \times 10^9/L$ leucocytes (PMN: $2.44 \times 10^9/L$, eosinophils: $0.08 \times 10^9/L$, basophils: $0 \times 10^9/L$, lymphocytes: $6.12 \times 10^9/L$, monocytes: $0.76 \times 10^9/L$). Examination of the blood smear revealed binucleated lymphoid cells, accounting for 1% of all lymphocytes. MFC analysis of blood lymphoid cells revealed 75% of B lymphoid cells, CD19+, CD22+, FMC7+, CD79b+, CD5-, CD10-, without clonal restriction or abnormal phenotype (Figure 1). An increased polyclonal IgM (5.94 g/L) was observed. Cytogenetic analysis, revealed a supernumerary isochromosome 3q, associated with genetic instability: 46,XX,der(6)t(6;?)(q27;?),del(6)(q15q26)[06] / 46,XX,t(1;14)(p12;q32)[02] / 47,XX,+i(3)(q10)[02] / 46,XX [20]. After one year of PPBL diagnosis, a clonal rearrangement of IGHV gene was detected in peripheral blood, without karyotypic changes. Nevertheless, spleen size, lymphocytosis and clinical symptomatology remained stable. Two years after, a monoclonal IgM was detected (monoclonal peak: 7 g/L). In February 2010, though lymphocytosis decreased to normal range ($2.24 \times 10^9/L$) and spleen size did not increase, a clonal B-cell population was detected in blood by MFC (Figure 1). B-cells accounted for 60% of whole blood lymphocytes, and 17% of these cells were CD19 low, CD20/CD22/CD79b+, CD5+, CD10-, CD23/CD43/FMC7/CD38+ with a κ isotypic restriction. Karyotypic analysis revealed a clone carrying del(6)(q15q26) and involving MYB gene locus: 47,XX,+X,del(6)(q15q26)[4] / 46,XX,del(6)(q15q26),der(6)t(6;6)(q21;q23)[cp6]/46,XX,t(1;14)(p12;q32)[2] / 49,XX,+X,del(2)(p11),+i(3)(q10),+8,t(1;14)(p12;q32)[1] / 46,XX[03]. Isochromosome 3q was detected in 4% of cells by interphase FISH, but was not detected in cells with del(6). These biologic findings suggested the uprising of a clonal B lymphoid malignancy associated with PPBL. A splenectomy was performed in November 2010. Histologic analysis of the spleen revealed a nodular pattern of lymphoid cells infiltrating the marginal zone and an intrasinusoidal infiltration of lymphoid B-cells. This aspect was compatible with SMZL (Figure 2), with κ restriction and a phenotype comparable to the circulating abnormal B-cell population, confirmed by MFC (Figure 1). Karyotypic analysis revealed the same clone as previously described in blood sample, i.e. carrying del(6)(q15q26), but no isochromosome 3q (46,XX,del(6)(q15q26)[2] / 47,XX,+X,del(6)(q15q26)[3] / 48,idem,+8[4] / 46,XX,del(1)(p12),der(14)t(1;14)(p31;q32)[2] / 46,XX[4]). FISH analysis confirmed the loss of MYB gene locus (6q23) in 77% of spleen cells but did not reveal isochromosome 3q. Thirty months after splenectomy and 9 years after PPBL diagnosis, lymphocytosis decreased at $3.7 \times 10^9/L$. However, by MFC, polyclonal B-cell population, accounting for 40% of all lymphocytes, is still detectable in blood but remains stable. Some binucleated lymphocytes are still observed on the blood smear.

Discussion

Both reports illustrate the necessity of a close follow-up of PPBL

patients and remind that B-cell malignancies emergence in PPBL history is not uncommon [6]. These observations lead us to recommend a careful follow-up of patients with PPBL. To date, the proof for a clonal evolution from PPBL has never been established. Nevertheless, some features could suggest a link between PPBL and SMZL. Both entities share same histologic patterns: 1/expansion of the white pulp of the spleen, with an enlargement of the marginal zone area of the follicles, and an intrasinusoidal infiltration of the red pulp [7,8] and 2/ intrasinusoidal infiltration by polyclonal B-cells in bone marrow [7,9]. Phenotypical characterization by MFC is also similar between the two entities, with the expression of surface IgM, CD19, CD20 and CD79b, but lacking the expression of CD5, CD10, CD23, CD43 and CD103 (WHO classification, 2008). It has been hypothesized that PPBL and marginal zone lymphocytes originate from memory B-cells. B-cells in PPBL share a rearranged IGHV gene and a common phenotype with normal B-cells of the marginal zone compartment [10]. The discovery of a specific immunophenotypic pattern, close to the memory B-cells has recently been completed and distinguished from the other B lymphoid malignancies [11]. The lack of specific molecular characterization of these entities makes difficult to establish a link between PPBL and SMZL. In conclusion, despite common features between PPBL and SMZL, still no strong argument has demonstrated a clonal evolution of PPBL toward SMZL.

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