Pangenomic study in patients with persistent polyclonal binucleated B-cell lymphocytosis (PPBL)

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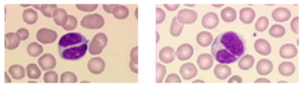
Background

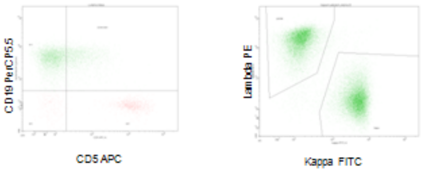
Initially described in 1982 (1), Persistent Polyclonal B-cell Lymphocytosis (PPBL) is a chronic, stable, persistent and polyclonal B-cell lymphocytosis observed more often in women (82% of patients), with a median age of 40 years and smokers in 98% of cases (2). Physical examination is usually within normal limits, except for a mild splenomegaly in 10% of cases. A polyclonal increase in serum immunoglobulin M (IgM) is associated.

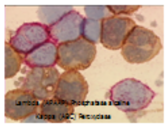
PPBL is diagnosed when blood smear analysis revealed the presence of binucleated lymphocytes, whatever the lymphocyte count. At time of diagnosis, 83% of patients present with mild lymphocytosis with a lymphocyte count over 4x10⁹/L (median at 5.45x10⁹/L, range 2.45-20.52x10⁹/L).

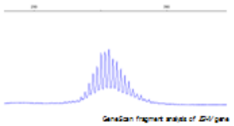
Blood smear analysis revealed the presence of binucleated lymphocytes in the peripheral blood (median=4%, range 1-40%)



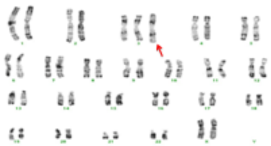
Multiparameter flow cytometry showed an expansion of the CD19⁺ CD5⁻ B lymphocytes pool. Both kappa and lambda light chain are expressed, confirming the B-cell polyclonal expansion. Polyclonality has been confirmed by immunocytochemistry and by molecular clonality analysis (analysis of *IGHV* gene rearrangements by PCR).

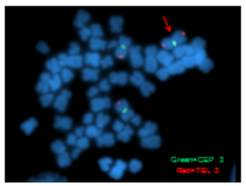






We identified a cytogenetic profile by conventional cytogenetic analysis (CCA) and FISH (3). Supernumerary isochromosome 3q, +i(3)(q10), is observed in 71% of PPBL cases. Moreover, cytogenetic instability is often observed (4).





Objective

Our purpose is to study cytogenetic abnormalities in PPBL with a high resolutive pangenomic method based on the last DNA chips technology.

Materials & Methods

Peripheral blood from 7 patients with typical PPBL was analyzed.

After separation of blood leukocytes by Ficoll gradient, we performed immunomagnetic sorting (Miltenyi™ AutoMACS Pro Separator®) of CD19⁺ B-cells versus CD19⁻ non B-cells.

DNA extraction was proceeded on the two sorted fraction and hybridization on Affymetrix™ Cytogenetics Whole Genome 2.7M Arrays® was performed according the manufacturer's recommendations.

Data analysis was then performed with Chromosome Analysis Suite® (ChAS®) software using the following settings. Copy number aberrations (gains and losses) and mosaicism were defined when a minimum of 50 copy number (CN) markers corresponding in a size of more than 25 kilobases (kb) were detected. Gene copy number (GCN) aberrations were compared with the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation/) to study only non variant GCN aberrations.

Patient	Age	Sex	Karyotype	FISH	Analyzed fraction(s)
1 BRA	59	F	46-47,XX,+i(3)(q10)[3]/ 46,XX,del(2)(q22),-17,+mar [1]/ 45,X,-X [1] / 46XX [40]	+3q(6%) / +3(6%)	CD19⁺
2 RAB	58	M	47,XY,+i(3)(q10)[08] / 48,XY,+i(3)(q10),+12 [01] / 46,XY,t(14;18)(q32;q22)[01] / 47,XY,t(11;14)(q13;q32),+mar [01] / 46,XY,add(3)(p26)[1] / 47,XY,+22[01] / 49,XY,+i(3)(q10),+5,+mar[01] / 46,XY [39]	+3q(7%)	CD19⁺ - CD19⁻
3 GAN	44	F	46,XX [45]	+3q(4%)	CD19⁺ - CD19⁻
4 PIT	51	F	47,XX,+X,del(6)(q15q26)[01] / 46,XX,del(6)(q15q26),der(6)t(6;6)(q21;q23)[08] / 46,XX,del(1)(q12),der(14)t(1;14)(p22;q32)[02] / 46,XX[39]	+3q(2%)	CD19⁺
5 BER	40	M	47,XY,+i(3)(q10) [3] / 46,X,der(Y)t(Y;7)(q12;?) [3] / 46,XY [12]	+3q(12%) / +3(21%)	CD19⁺
6 KRA	45	F	46,XX,del(6)(q21q24) [6] / 46,XX,der(5)t(3;5)(q11;q11),der(17)t(17;?)(p11;?) [2] / 46,XX,del(17)(p11) [2] / 46,XX,t(1;6)(q24;q21) [1] / 46,XX,der(14)t(14;?)(p25;?) [1] / 46,XX,dup(3)(p13p26) [1] / 46,XX,der(4)t(4;?)(p16;?) [1] / 46,XX [28]	+3q(11%)	CD19⁺ - CD19⁻
7 SPA	47	F	47,XX,+18 [2] / 47,XX,+3 [1] / 46,XX [37]	+3q(4%) / +3(14%)	CD19⁺ - CD19⁻

Results

Recurrent aberrations

The most recurrent GCN gains were detected on the long arm of chromosome 3 (3q), only in CD19⁺ B cells (5/6 patients). We identified GCN aberrations for all the genes on 3q or non consecutive genes. We also observed mosaicism phenomenon on 3q (gain) for 2 patients.

Other recurrent GCN aberrations were observed, 5 on Xp (3 patients) and 16 on 6q (2 patients).

Non recurrent aberrations

Non recurrent GCN aberrations involved the whole genome, except chromosomes 5 and 22.

One gene were frequently amplified on 3q, only in CD19⁺ B-cells

MDS1 and *EVI1* complex locus (*MECOM*) was amplified in 5/6 patients, with a common minimal amplified sequence (28 kb, 85 CN markers) of *MECOM* concerning exon 2 of *MDS1*.

Discussion

Our aim was to study genetic abnormalities observed in PPBL. Since +i(3)(q10) has been described as the cytogenetic profile of PPBL, we focused our study on aberrations detected on 3q.

+i(3)(q10) identified by CCA is not a true complete duplication of 3q. The presence of recurrent GCN amplifications is restricted to CD19⁺ B-cells. Most recurrent GCN aberrations concerned the long arm of chromosome 3 and a few aberrations chromosomes 6q and Xp.

Interestingly, *MECOM* gene, implicated in myeloid malignancies, was totally or partially amplified, with a common minimal amplified sequence of 28 kb implicating exon 2 of *MDS1*. We are getting *MDS1* quantitative PCR to confirm our observations.

Genetic instability in PPBL is confirmed by pangenomic study, particularly the occurence of non recurrent GCN aberrations involving the whole genome, requiring a long term follow-up of PPBL patients.

The impact of *MECOM* abnormalities (overexpression, rearrangement) is clearly identified in myeloid malignancies but remains to be clarified in lymphoid malignancies.

References

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