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CD5+ follicular lymphoma rapidly transformed to high-grade B-cell lymphoma with double-hit: from *BCL2* to *MYC* disruption

TO THE EDITOR: CD5+ follicular lymphoma (CD5+FL) is an infrequent disease that has been associated with poor prognosis and a rapid transformation to diffuse large B-cell lymphoma (DLBCL) [1, 2]. We herein describe a patient diagnosed with this entity that evolved to a high-grade B-cell lymphoma with double hit (DH-HGBCL) and appealing molecular abnormalities five months after diagnosis.

A 42-year-old male was admitted because of malaise. Blood test revealed hemoglobin 144 g/L, lactate dehydrogenase (LDH) 445 U/L (normal range, 208–378) and beta-2 microglobulin 3.90 mg/L (normal range, 1–2.40). A CT scan showed generalized lymph node enlargement. A bone marrow (BM) aspirate and biopsy showed an interstitial infiltrate of atypical lymphocytes (Fig. 1A). A lymph node biopsy showed an infiltrate of centrocytes with a follicular pattern (Fig. 1B). Immunophenotype of lymph node disaggregate was CD19+, CD10+(weak), CD20+, CD38+(MFI: 187), CD25+, FMC7+, CD5+, Bcl2+, CD45+low, CD23 negative and lambda clonality (Fig. 2). Immunohistochemistry confirmed the expression of CD5; MUM1 was negative. A normal karyotype was obtained, but FISH revealed 94% nuclei with Bcl-2 rearrangement (MetaSystems Translocation/DF Probe, Medford, MA, USA) (Fig. 1C). Bcl-6 and c-Myc were negative. FR1 and FR2 clonality was noted by using IdentiClone IGH Gene Clonality Assay (Invivoscribe Technologies, San Diego, CA, USA) (Fig. 1D). PET-CT showed increased glycidic metabolism of supra and infradiaphragmatic lymph nodes (SUVmax 7.9), and diffuse increased metabolism of several bone structures (SUV 4.3). A diagnosis of CD5+FL, histology grade 2, Ann Arbor stage IV-B and either IPI 2 and FLIPI-2 3 was established. The patient was treated with O-CHOP [Obinutuzumab 1,000 mg IV infusion, administered on Day 1, 8 and 15 during Cycle 1, and on Day 1 of subsequent cycles, for 6–8 cycles; Cyclophosphamide 750 mg/m² IV, Doxorubicin 50 mg/m² IV, Vincristine 1.4 mg/m² (maximum 2 mg) IV on Day 1 of each 21-day cycle, and Prednisone 100 mg administered orally on Days 1–5 of each 21-day cycle for six cycles].

After 6 cycles, he was readmitted because of shortness of breath. LDH was 17,000 U/L (normal range, 208–378). Reevaluation PET-CT showed partial metabolic response in lymph nodes but pleural progressive disease and bilateral pleural effusion (SUVmax 4.6).

A new BM aspirate showed an infiltrate (15%) of great sized lymphocytes with high N/C ratio, irregularly shaped nuclei with immature chromatin and conspicuous nucleoli. Cytoplasm was hyperbasophilic and contained a number of unstained vacuolae (Fig. 1E). A thoracentesis showed

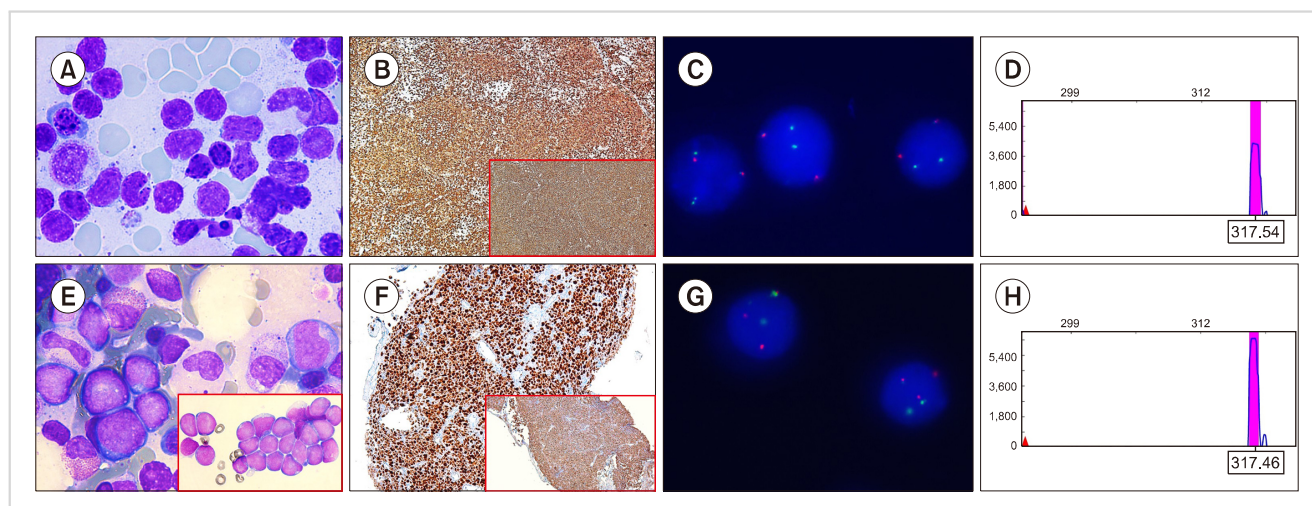


Fig. 1. At diagnosis: **(A)** Bone marrow aspiration, May Grunwald-Giemsa (MGG) staining, $\times 100$. **(B)** Lymph node biopsy, CD5 (main) and Bcl-2 (small panel) detection by immunohistochemistry staining, $\times 10$. **(C)** Bcl-2 detection by in situ hybridization (DC/DF Metasystems). **(D)** FR1 clonality by Immunoglobulin heavy chain gene clonality assay (IdentiClone). At high-grade B-cell lymphoma phase: **(E)** Bone marrow aspiration (main) and pleural fluid (small panel), MGG, $\times 100$. **(F)** Retroperitoneal mass biopsy, Myc (main) and Bcl-2 (small panel) detection by immunohistochemistry staining, $\times 10$. **(G)** c-Myc detection by in situ hybridization (DC/DF TC Metasystems). **(H)** FR1 clonality by Immunoglobulin heavy chain gene clonality assay (IdentiClone).

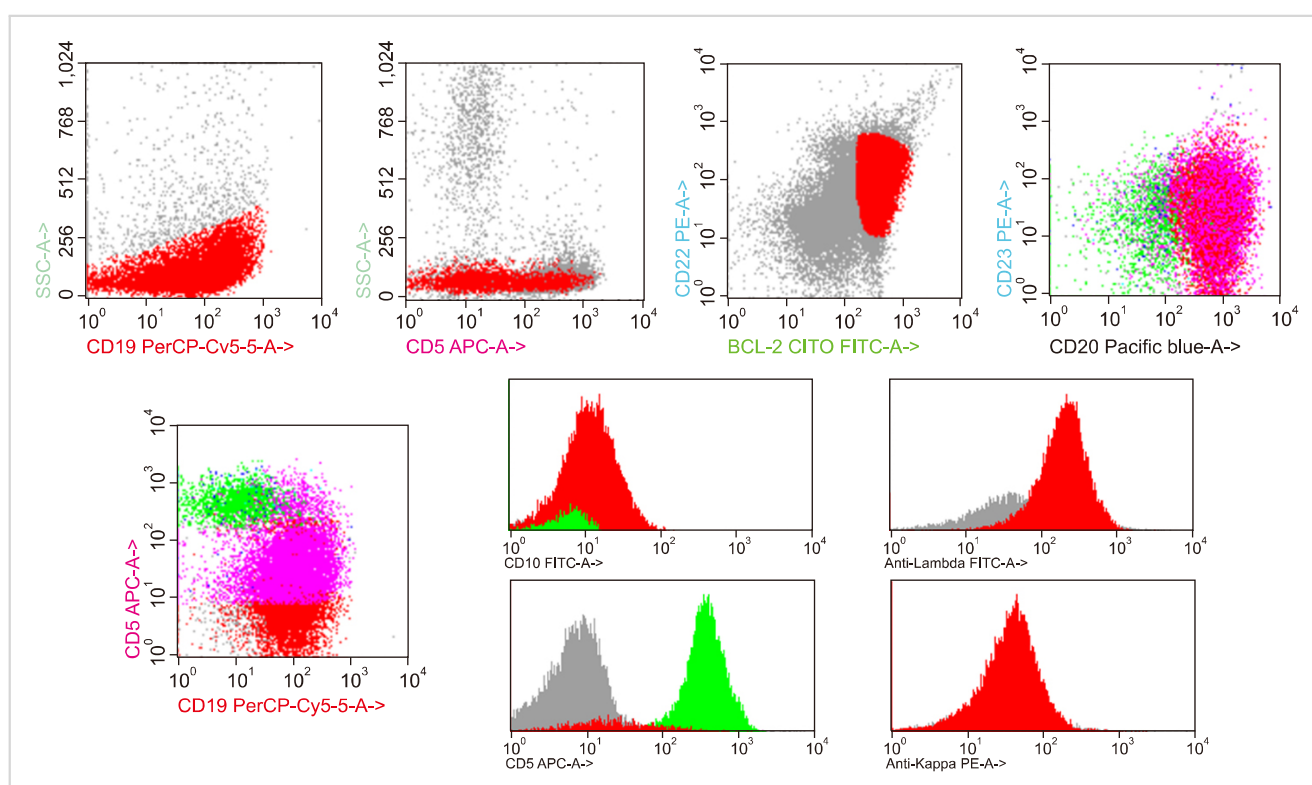


Fig. 2. Immunophenotype of lymph node at diagnosis.

a massive infiltrate (Fig. 1E), and a core needle biopsy of the retroperitoneal conglomerate revealed a diffuse infiltration by those cells. Immunophenotype was CD19+, CD10+, CD20-, CD38++(MFI: 2,895), FMC7-, CD25-, Bcl-2+, TdT- and PAX5+ (Fig. 1F). Karyotype from the pleural effusion sample showed the following formula: 47,Y,t(X;9)(q28;q22),

add(1)(p36),der(6)t(1;6)(q23;q21), t(8;22)(q24;q11), t(14;18)(q32;q21),+21 [13]/46,XY [7]. FISH revealed 82% nuclei positive for Bcl-2 and 52% for c-Myc (Fig. 1G). FR1 and FR2 amplicons of the same size as at diagnosis were obtained (Fig. 1H). A diagnosis of DH-HGBCL, Ann Arbor stage IV was achieved.

Table 1. Characteristics of somatic mutations from whole exome sequencing at high-grade B-cell lymphoma phase.

Gene ID	Chr	POS	REF	ALT	VAF (%)	Nucleotide change	Aminoacid change	Chromosome band	ACMG classification
<i>BCL2</i>	18	60985750	GG	AA	6 ^{a)}	c.149_150delinsTT	p.Ser50Phe	18q21.33	VUS
<i>KSR1</i>	17	25910028	A	C	10.5	c.466A>C	p.Ser156Arg	17q11.2	VUS
<i>GRHPR</i>	9	37424928	G	A	12.1	c.170G>A	p.Cys57Tyr	9p13.2	VUS
<i>KRT2</i>	12	53045600	A	T	32.4	c.327T>A	p.Ser109Arg	12q13.13	VUS
<i>BMP7</i>	20	55841130	G	C	10.8	c.49C>G	p.Leu17Val	20q13.31	VUS

^{a)} At diagnosis: 30%.

Abbreviations: ACMG, American College of Medical Genetic and Genomic; ALT, alternative nucleotide; Chr, chromosome; POS, chromosome position; REF, reference nucleotide; VAF, variant allele frequency; VUS, variant of unknown significance.

Whole exome sequencing (WES) was performed on bone marrow samples at diagnosis of CD5+FL and DH-HGBCL by using Novaseq 6000 (Illumina, San Diego, CA, USA). Initial prioritization of both somatic variants was performed using a proprietary pipeline, eliminating intronic, synonymous and those with a minor allelic variant greater than or equal to 1%. The somatic variants were defined as recurrently mutated variants in lymphoproliferative disorders that had been reported by more than one author in the main databases (COSMIC, IARC TP53, CLINVAR). Finally, we filtered for genes known to be involved in pathogenesis of lymphoma, including follicular lymphoma and HGBCL.

Samples from either the CD5+FL and the DH-HGBCL phase of the disease revealed a *BCL2* mutation (p.Ser50Phe) with a variant allele frequency (VAF) of 30% and 6%, respectively. *KSR1* (p.Ser156Arg), *GRHPR* (p.Cys57Tyr), *KRT2* (p.Ser109Arg) and *BMP7* (p.Leu17Val) mutations were only detected in the DH-HGBCL phase (Table 1). To ensure whether HGBCL came from the original B-cell of follicular lymphoma, we compared DNA sequence of several B-receptors genes of the both samples and their allele frequency to reach the conclusion about the transformation of the original tumor disease.

DISCUSSION

CD5+FL has been associated with leukemic phase, expression of CD25 and MUM1, a lesser frequency of t(14;18) (q32;q21) a worse overall survival [1], a higher IPI, a higher rate of transformation into DLBCL and a shorter disease-free survival [2]. The pathogenesis of CD5+FL is unclear. CD5 induces an anergic state in lymphocytes and contributes to B-cell survival and interleukin-10 production. Mayson *et al.* [3] discussed that CD5 positivity was a marker of activation and differentiation with inappropriate response to cytokine networks.

Our patient showed initially extranodal involvement. CD5 was detected by both FC and immunochemistry. CD25, rare in FL, was detected [4]. MUM1 was negative. IGH/BCL2 translocation was detected only by FISH analysis. Both the MBR and MCR rearrangements were negative, though WES showed *BCL2* mutation.

Transformation was more rapidly than that reported for

CD5+FL transformation into DLBCL [2]. CD5 and CD25 were lost and a marked overexpression of CD38 was noted, the latter being recently reported in HGBCL [5].

WES showed an acquired *BCL2* variant both at baseline and transformation. However, at diagnosis it was the prevailing gene aberration whilst a decrease VAF was noted when the two-hit clone Bcl-2/c-myc was predominant. Additional mutations in either *GRHPR*, *BMP7* and *KSR1*, unusual in a likely setting [6-8], were noted in the transformation. *GRHPR* has been described as a partner of *BCL6* in the transformation from FL, and has been related with refractoriness in DLBCL [9]. *BMP7* has been described as a driver gene in Burkitt lymphoma [10]. *KSR1* deletion attenuates the Ras/Mapk pathway leading to Myc underexpression [11]. Our finding of a *KSR1* missense variant in the transformation rise the possibility for a c-Myc double disruption.

To the best of our knowledge this is the first report of the transformation of the very rare CD5+FL into DH-HGBCL. To be highlighted: the transition from a *BCL2* mutated clone to a multiple chromosomal and single nucleotide mutated variants.

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