Unusual sequence of vdj rearrangement revealed by molecular analysis in a patient with indolent lymphoma

We report a unique case of indolent lymphoma with an unusual VDJ rearrangement. Polymerase chain reaction (PCR) analysis of bone marrow at the time of diagnosis was positive for both BCL-2/JH and CDRIII rearrangements. After treatment, the patient achieved complete remission (CR) with slow disappearance of both rearrangements (CDRIII and then BCL-2/JH). Subsequently, two new CDRIII rearrangements were detected in bone marrow, peripheral blood, and lymph node tissue. After this conversion, fluorescent activated cell sorting (FACS) analysis demonstrated monoclonal disease, suggesting that both CDRIII rearrangements originated from one cell. Histological evidence of a B-cell small lymphocytic lymphoma (B-SLL) infiltrate in the bone marrow became evident approximately 1 year after the two CDRIII rearrangements appeared. Direct sequencing revealed that one of the CDRIII sequences consisted of a VDVDJ rearrangement. This is the first report of such a rearrangement in a case of indolent lymphoma. This type of rearrangement has been described to result from a secondary VDJ recombination in childhood acute lymphoblastic leukemia (ALL) leading towards oligoclonality and poorer prognosis. Our observations suggest that such a finding in an indolent lymphoma patient may precede transformation into an aggressive disease. Early detection by PCR could have substantial impact on the prognosis of such patients.

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Introduction. B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL/SLL) and Follicular lymphoma (FL) both typically represent distinct entities with different morphology, immunophenotype, histology and genetics. However, FL occasionally, can show morphological features of both FL and monocytoid B-cell lymphoma (e.g. marginal-zone lymphoma) making the diagnosis challenging. [1,2] These lymphomas have been called composite lymphomas. [3]The molecular pathogenesis of B-CLL/SLL remains unknown. There is no typical chromosomal translocation associated with this pathological entity. High level of *BCL-2* expression that is consistently seen in B-CLL/SLL results from other mechanism (e.g. oncogene hypomethylation).[4] B-CLL/SLL is characterized by an accumulation of neoplastic B-cells positive for CD5, CD23, and CD19 and negative for surface CD22 and FMC7 with a low proliferative rate. Based on studies of mutational status B-CLL/SLL can be divided into cases with unmutated and mutated VH genes. Cells with mutated VH genes have gone through the germinal center (GC) and are connected with more favorable course of disease.[5-7] Chromosomal translocation t(14;18)(q32;q21) that occurs between the BCL-2 protooncogene and the JH immunoglobulin gene region is a hallmark of follicular lymphoma (FL) and can be detected in 85% to 90% of FL.[8] The lymphoma cells originate from germinal center that means they have already encountered with antigen. On the cell surface they express CD19, CD20, CD22, CD24, CD10, immunoglobulins and are CD5 negative. Clinically FL and B-CLL/SLL belong to low grade lymphomas with median survival approximately 10 years. Up to 70% of low grade lymphomas tend to convert into an aggressive lymphoma with a diffuse large cell architecture over time.[8,9] Detection of BCL-2/JH translocation and/or the CDRIII of immunoglobulin heavy chain gene rearrangement by PCR is commonly used for diagnostic purposes and can be monitored for minimal residual disease (MRD) evaluation during the posttreatment follow-up.

Methods. The material tested by PCR was represented by bone marrow samples (BM), peripheral blood samples (PB) and lymph node (LN) obtained from patient. The DNA was extracted using standard procedures and the usual precautions to avoid cross-contamination. The presence of the BCL-2/JH translocation in the major breakpoint region (MBR) of the BCL-2 gene was examined using a touchdown PCR (TD-PCR)[10] modified for our conditions. The presence of the clonal immunoglobulin heavy chain gene rearrangement (CDRIII, complementary determining region) was examined by polymerase chain reaction (PCR), as described previously.[11] Each reaction contained positive and negative control. Amplified products were visualized on 2% polyacrylamide or 3% Metaphor agarose (FMC Bioproducts, Rockland, ME, USA) gels stained with ethidium bromide. Results were confirmed by repeat PCR at least once. The sensitivity was routinely better then 10 positive cells in 10^5 normal cells as determined by comparative PCR and also real time PCR.[12] PCR products were extracted from 3% Metaphor agarose gel slices using the crush and soak technique[13] and purified with Microcon-100 (Millipore) purification columns, sequenced with BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and analyzed on ABI Prism 310 Genetic Analyzer (PE BioSystems).Sequences obtained from each sample were compared with germ line sequences in the EMBL/GenBank and current databases (V-BASE sequence directory; I.M. Tomlinson, MRC Center for Protein Engineering, Cambridge, UK) and the closest sequence was assigned.[14] Attribution of the D segments was based on the identification of at least 6 consecutive bases without mismatches. The nomenclature proposed by Corbett *et al*. was adopted.[15]

Case history I. A 58-year-old man was diagnosed with a stage IV nonhodgkin lymphoma which was described as foliculla (FL) type in 1993. The PCR revealed positivity for BCL-2/JH translocation in BM. The BM was also positive for immunoglobulin heavy chain gene rearrangement (CDRIII). The patient received 6 cycles of ProMACE-MOPP and then 4 cycles of ProMACE-CYTABOM (Prednisone, Doxorubicin, Cyclophosphamide, Etoposide, ara-C, Bleomycine, Vincristine, Methotrexate, Leucovorin) and reached complete remission. He remained PCR positive for BCL-2/JH and CDRI-II rearrangements. Then first CDRIII disappeared followed by BCL-2/JH clearance. This was due to more sensitive PCR technique (see sample from 1995 in table 1). The next PCR test (see sample from 1996 in table 1) revealed surprising result as the patient converted from positivity for BCL-2/JH and CDRIII into BCL-2/JH negativity and CDRIII double positivity in BM. These findings were consistent also in subsequent samples and other tissues (PB and LN). The two distinct CDRIII rearrangements were confirmed by direct sequencing. Later on also the histology and the FACS analysis was consistent with BM infiltration with a B-CLL/SLL infiltrate. Slow progression in BM occurred in XI/1999, approximately 3 1/2 years after the molecular conversion. Then the patient presented with generalized lymphadenopathy within the matter of weeks. Upon histological evaluation a large cell infiltrate in BM sugested a

Table 1: Summary of the clinical laboratory and molecular biology results during the course of disease. [PCR- polymerase chain reaction, FACS- fluorescein activated cell sorting, histol- histology, BCL2- translocation BCL-2/JH, CDRIII-complementarity determining region III, CDRIIIx- two CDRIII's detected, pos- positive, neg- negative, transf-transformation.]

	PCR	PCR	PCR	PCR	FACS	histol
date	LN	BM	PB	PBPC	BM	BM
2/9/1994		BCL2+,CDRIII-				
3/4/1994			MBR+,CDRIII-			
4/25/1994		MBR+,CDRIII-				
4/28/1994			MBR+,CDRIII-			
6/27/1994			MBR+,CDRIII-			
6/27/1994				MBR+,CDRIII-		
6/28/1994				MBR+,CDRIII-		
6/29/1994				MBR+,CDRIII-		
6/30/1994				MBR+,CDRIII-		
7/1/1994				MBR+,CDRIII-		
2/1/1995		MBR+,CDRIII-			neg	neg
2/28/1996		MBR+,CDRII-b			neg	neg
5/6/1997		MBR+,CDRII-b			pos	pos
2/18/1998		MBR+,CDRII-b			pos	pos
6/11/1998		MBR+,CDRII-b			pos	neg
6/11/1998			MBR+,CDRII-b			
10/1/1998		MBR+,CDRII-b			pos	pos
8/31/2000		MBR+,CDRII-b			pos	pos
8/31/2000			CDRIII-b1		pos	pos
1/22/2001			CDRIII-b1		pos	pos
2/9/2001			CDRIII-b1		pos	pos
2/19/2001	CDRIII-b1				pos	histol
3/2/2001			CDRIII-b1		pos	pos
4/24/2001		CDRIII-b1			pos	pos
8/3/2001			CDRIII-b1		pos	pos

Table 2A: Sequence of short CDRIII rearrangement obtained from PCR positive samples. FR3A (blue)- framework region 3, D (green)- diversity segment, JH (blue)- joining gene, N nucleotides (black)- sequence between rearranged VH, D and JH genes; primer sequences underlined.

5'- AC-ACG-GCV-STG-TAT-TAC-TGTGCG-AGA-GAG-G-
FR3A primer — 3' end of VH gene

-AT-ATT-GTA-CTA-ATG-GTG-TAT-GCC-ATC-CGC TGG-GGC-
D 2-8 segment — (N- nucleotides)

-TAC-TAC-GGT-ATG-GAC-GTC-TGG-GGC-CAA-GGG-ACC-ACG-
JH6b sequence

-GTC-ACC-GTC-TCC-TCA-G- 3'
JH primer

Table 2B: Sequence of long CDRIII rearrangement obtained from PCR positive samples. FR3A (blue)- framework region 3, D (green)- diversity segment, JH (blue)- joining gene, VH (red)- variable gene, N nucleotides (black)- sequence between rearranged VH, D and JH genes; primer sequences underlined.

5'- AC-ACG-GCV-STG-TAT-TAC-TGT-GCG-AGA-GAT-
FR3A primer — 3' end of VH gene

-TGG-GAT-TAC-TAT-GAT-AGT-AGT-GGT-TAT-TTA-CCG-GGC-GAG -
(N- nucleotides) — D 3-22 segment

-AGT-CCA-TCA-AAA-ACT-CAT-GCC-TGG-GAG-CCT-CCC-ACC-ACA-GCC-

-CTC-CCT-GCG-GGG-GAC-CGC-TGC-ATG-CCG-TGT-TAG-GAT-TTT-GAT-
Stop codon

-CGA-GGA-CAC-GGC-GCC-ATG-GGT-ATG-GTG-GCT-
VH 4-4 gene (variable)

-TCG-TTG-AAC-GAG-GGT-GGG-ACC-TTG-GGT-
(N- nucleotides) — D 2-21 segment

-TGA-CTA-CTG-GGC-CAG-GGA-ACC-CTG-GTC-ACC-GTC-TCC-TCA-G- 3'
JH4b sequence — JH primer

disease transformation. The patient obtained 1 cycle of salvage chemotherapy (ESAP), but further progressed. The treatment was then changed to fludarabine and cyclophosphamide together with rituximab (anti-CD20). The disease remained resistant to therapy and the patient died due to disease progression.

Results and discussion. This is a first report of patient with indolent lymphoma bearing a secondary VDJ rearrangement. This report discusses also a rare case of indolent lymphoma with both alleles for IgH rearranged in the malignant clone. Sequencing revealed two distinct CDRIII rearrangements (117 bp and 252 bp, see table 2) in BM, PB as well as LN. The result of each PCR can be evaluated as a duplex PCR. Using software analysis we observed equal ratio between fluorescent intensities of short and long CDRIII from different samples. This result was another proof of monoclonality. We wanted to sequence the BCL2-JH translocation and CDRIII rearrangement from the diagnosis samples. However, we were not able to successfully amplify any of those archival samples due to lack of DNA. That the two CDRIII rearrangements originated from single malignant clone was confirmed by FACS analysis. The immunophenotype of tumor cells was characterized by monoclonal surface expression of CD 19, CD 5, CD 23, CD 20, CD 11c, and immunoglobulin light chain kappa, FMC 7 and small % CD 79b. The histology resembled B-CLL/SLL at that time so one explanation for presence of two CDRIII rearrangements in the patient's BM could be that those result from lack of allelic exclusion. Rassenti *et al.* presented that up to 5% of CLL cases lack allelic exclusion. [16] Aberrant expression of CD79b was previously suspected to lead to lack allelic exclusion, but no difference in CD79b expression was observed between CLL samples that expressed more than one IgH allele and those with normal IgH allelic exclusion. [17] We compared both CDRIII sequences with the corresponding germline sequences using the GenBank/EMBL database computer-based search. The shorter CDRIII resembled a usual V(♀) - D2-8 - JH6b gene rearrangement and was within reading frame and productive (see table 2A). The longer CDRIII consisted of V(♀) - D3-22 - VH 4-4 - D2-21 - JH4b segments and we found a stop codon TAG (unproductive rearrangement; see table 2B). The comparison of CDRIII sequences with the germ line sequences did not suggest mutation. However mutational status of VH genes was not evaluated in detail. Without the initial BCL-2/JH and CDRIII sequences from the diagnosis it is rather difficult or speculative to explain whether this molecular conversion resulted from a clonal evolution or if our patient had a composite lymphoma (the originally dominant FL clone was eradicated and the minor clone of B-SLL expanded). However, the presented data still suggest a presence of secondary VDVDJ recombination that is unusual for indolent lymphoma. The V(D)J recombinase possesses latent transponase activity. Errors of V(D)J recombination may generate chromosomal translocations consistent with those present in lymphomas and leukemias. Lymphomas bearing t(14;18) have been classified by phenotype and genotype as malignant disorders originating from germinal center (GC) B cell. The GC is a site of affinity maturation where B cells undergo V(D)J hypermutation, Ig class switch and may also support secondary V(D)J recombination. The BCL-2/JH translocation is typical for tumor cells of GC B origin and remains stable even after the transformation occurs.[18] In our patient this marker disappeared as the patient reached remission. The CDRIII is often used as molecular marker in B cell lymphoproliferative disorders. Our patient was originally positive for one CDRIII, then was shortly CDRIII negative. Later on we detected two CDRIII rearrangements. This was followed by the disease progression and transformation. It is known that in 30-50% of cases of childhood B precursor acute lymphoblastic leukemia the CDRIII may change along the course of the disease leading to oligoclonality. This is believed to result from a secondary V gene to DJ rearrangement.[19] There is also some evidence about receptor revision through secondary VH gene rearrangements in normal human B-lymphocytes.[20] We are not

aware of data about such phenomena in indolent lymphomas or leukemias. It is believed that secondary rearrangements are driven by somatic hypermutation, which takes place in GC. It is difficult to explain the VDVDJ rearrangement in presented case since we did not observe any mutations within both CDRIII sequences. Our observation suggests that occurrence of a secondary rearrangement is possible also in indolent lymphomas. Since it may be followed by transformation into aggressive disease, detection of such CDRIII sequence should be considered seriously and the patient managed carefully before a full-blown clinical relapse/progression of aggressive disease develops.

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