**CASE REPORT**

# Classical and molecular methods in differentiation of mantle cell lymphoma and small lymphocytic lymphoma in composite lymphoma: a case report

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## Abstract

Composite lymphoma (CL) is a very rare phenomenon. To date, only few cases of composite mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL) have been reported by Sun et al. (Cytometry B Clin Cytom 2018;94(1):148-50), Hoeller et al. (Hum Pathol 2013;44(1):110-21), and Papathomas et al. (Hum Pathol 2012;43(4):467-80). In the majority of CL cases, the clonality relationship was not examined or could not be proven. So far, there is no optimal treatment strategy for CL. However, it has been a commonly accepted rule that the more aggressive component should be treated first. Thus, it is of vital importance to precisely distinguish the components of CL and to determine the response of a particular component to treatment. This may be done by monitoring the molecular minimal residual disease (mMRD) as reported by Pott et al. (Blood 2010;115(16):3215-23) and Pott (Semin Hematol 2011;48(3):172-84). This report presents the possibility of determining clonality of each CL component by using flow cytometry in parallel with specific molecular markers. Molecular analysis was performed by quantitative real-time PCR (qPCR) using two markers: *IGH* rearrangement as reported by van Dongen et al. (Leukemia 2003;17(12):2257-317) and *SOX11* expression by Hamborg et al. (Eur J Haematol 2012;89(5):385-94) and Szostakowska et al. (Med Oncol 2018;35(4):49). The *SOX11* expression allowed us to distinguish MCL and SLL subclones in a 60-year-old male with multifocal MCL/SLL as this marker is not detected in SLL as reported by Swerdlow et al. (2001) and Mozos et al. (Haematologica 2009;94(11):1555-62). To our best knowledge, this is the first example of the use of mMRD to distinguish CL components.

**Keywords** Composite lymphoma · Mantle cell lymphoma · Small lymphocytic lymphoma · Flow cytometry · Minimal residual disease · Quantitative real-time PCR

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## Introduction

Composite lymphoma (CL) is defined as the coexistence of two different types of lymphoma in the same organ or tissue site [1]. It can be composed of either non-Hodgkin lymphoma (NHL) (B cell or T cell) or Hodgkin lymphoma. Here, we describe a case of composite mantle cell lymphoma (MCL) and small lymphocytic lymphoma (SLL).

MCL is a rare (5–8% of all NHL) and incurable B-NHL characterized by a multiple relapsing and aggressive course in most cases. The hallmark of the disease is a translocation t(11;14)(q13;q32) resulting in overexpression of cyclin D1 [2]. In rare cyclin D1 negative cases, the presence of SOX11 may allow the correct final diagnosis [3]. The MCL immunophenotype is common to the B lymphocytes: CD19+/CD20+/CD22+/cyclin D1+/CD200− with coexpression of

CD5+/CD43+ (characteristic for the T cell linage) and sIgM+/sIgD± with λ > k monoclonal restriction. Malignant cells are usually negative for CD23, CD11c, CD10, and BCL6 [2] although there are some CD5−, CD10+, and CD23+ cases (e.g., splenic type). The disease usually presents as a generalized lymphadenopathy, with common bone marrow (BM), peripheral blood (PB), and gastrointestinal tract involvement. There is no defined therapeutic standard; however, the goal of the first line therapy is to achieve complete remission [4], most preferable at a molecular level assessed by qPCR analysis [5–8].

CLL and SLL are the most common types of adult leukemia/lymphoma which account for approximately 30% of all NHL [9]. According to the 2016 World Health Organization (WHO) lymphoma classification, although CLL and SLL are the same disease, the term SLL is used for cases with a circulating SLL cell count $<5 \times 10^9$/L and documented nodal, splenic, or other extramedullary involvement. Lymph nodes are infiltrated by small CD19+/CD20+$^{dim}$/CD22−/CD23+$^{dim}$/cyclin D1−/CD200+ with coexpression of CD5+/CD43+ and sIgM+/sIgD± B lymphocytes [10–12]. SLL/CLL has a highly variable clinical course: from aggressive forms (with a complex karyotype and TP53 mutations/deletions) which should be treated at diagnosis to indolent forms that do not require treatment for many years and require only a watch-and-wait approach [13].

Relapse due to undetermined minimal residual disease (MRD) is the main cause of death in lymphoma patients; therefore, monitoring of MRD is crucial in the process of treatment. Recent studies have shown that consistent monitoring of MRD can improve treatment decisions [6]. Moreover, in patients who are in clinical remission, MRD status estimated in PB was prognostic for their overall survival (OS) [14]. Therefore, consistent monitoring of MRD could allow improvements to current treatments and predict clinical relapse, progressive-free survival (PFS), and OS. In this clinical case, we aimed to use mMRD assessment as an indication for deciding on treatment specific for relapsing subclone of the CL.

## Clinical history

Here, we report a case of a 60-year-old male with multifocal MCL/SLL composite lymphoma who presented with unspecified pain in the lower part of the abdomen, periodic diarrheas and rectal hemorrhages, night sweats, and weight loss over a period of 6 months. The patient was also diagnosed with diabetes type 2, persistent atrial fibrillation, and arterial hypertension. He had no other medical history. In the local hospital, he was diagnosed for colon polyposis or inflammatory bowel disease. The colonoscopy revealed multifocal flat polyps. The histopathology examination indicated the possibility of MCL presented as lymphomatoid polyposis. The patient was transferred to the Department of Lymphoid Malignancies at the Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland. Physical examination revealed numerous enlarged superficial lymph nodes (armpits, groins, neck, supraclavicular, and submandibular). He had no palpable organomegaly. The flow cytometry (FCM), classical cytogenetics (CC), and fluorescence in situ hybridization (FISH) examinations of fine needle aspiration biopsy (FNAB) of an enlarged 3-cm axillary lymph node (LN) and BM/PB revealed MCL as well as SLL. To confirm the diagnosis, a right neck lymphadenectomy was performed. We received 4 swollen and enlarged LNs, each of them approximately 2 cm long.

The diagnosis results are shown in Table 1 and Figs. 1 and 2. Histopathology and immunohistochemistry of this CL case are shown in Fig. 1. Cervical LN biopsy showed a coexistence of SLL and MCL. Both clones expressed CD20, and the SLL component was CD23+, cyclin D1, and SOX11 negative, while the MCL component was CD23−, cyclin D1, and SOX11 positive. The Ki-67 index was about 5% for SLL and about 40% for MCL. Detailed results are summarized in Table 1.

Figure 2 shows flow cytometric immunophenotypic analysis of cells obtained by FNAB from an enlarged lymph node. Analysis of the expression of selected antigens shows the coexistence of two separate neoplastic clones originating from B cells. Cells belonging to both subpopulations, marked with the R1 gate in the scattergram A, are not different from each other in terms of size (forward scatter, FSC) and granulation (side scatter, SSC).

The phenotype of the MCL subpopulation demonstrates expression of surface antigens most commonly encountered in routine FCM diagnostics as previously described [2]. Considering differences in its median fluorescence intensity (MFI) levels, this phenotype may be characterized by having the following: high CD20 expression of bright type, reduced CD19 expression in comparison to normal B cells, medium CD5/CD45 coexpression (weaker than that of T cells), CD23−, high CD79b expression, low CD25 coexpression, CD200−, medium-high CD38 expression, low IgD expression, medium-high IgM expression, and CD71 expression in nearly 50% of cells.

Unlike in MCL, the phenotype of the coexisting SLL subpopulation is different from that in the typical form of this disease. The coexisting SLL phenotype has bright, medium CD20 expression (in the classic forms, CD20 expression is weak and dim), low peak IgD expression, and lacks IgM expression (in typical SLL, the accumulation of both chains is most commonly of low intensity). The absence of CD25 coexpression which is relatively uncommon in SLL is also observed. Moreover, there is no CD38 expression, characteristic of the nonmutated SLL form associated with a less favorable prognosis. Furthermore, CD200+, CD5+, CD23+, CD19+, CD79b±, and CD71+ occur in a low percentage of cells. This immunophenotype clearly indicated the diagnosis of SLL as previously described [11].

**Table 1** Histopathology/immunohistochemistry and flow cytometry immunophenotyping/karyotype/FISH of composite lymphoma

Immunohistochemistry / FCM (LN):

| | Histopathological/immunohistochemical, LN | | | FCM, LN | |
|---|---|---|---|---|---|
| | Cytomorphology, growth pattern | SOX11 | Cyc D1 | Ki-67 [%] | neo. [%] |
| SLL | Pseudofollicular pattern | – | – | ~5 | 45 |
| MCL | Classical, nodular | + | + | ~40 | 47 |

FCM markers (LN):

| | 19 | 20 | 22 | 23 | 5 | 43 | 200 | 79β | 62L | 25 | 11c | 38 | 10 | 52 | 71 [%] | κ | λ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SLL | +↓ | +↓ | +↓ | + | + | +↓ | +↓ | +↓ | +↓ | +↓ | – | ± | – | – | 40 | ± | – |
| MCL | +↓ | +↑ | +↑ | – | + | +↓ | +↑ | +↑ | +↑ | +↑ | ± | – | – | +↑ | 60 | + | – |

| | HLADR | BCL2 | FMC7 | IgD | IgM | IgG |
|---|---|---|---|---|---|---|
| SLL | + | +↑ | ± | + | – | – |
| MCL | +↓ | +↓ | + | + | + | – |

FCM, PB, BM:

| | PB [%] | BM [%] |
|---|---|---|
| SLL | +1.7 | +9 |
| MCL | +4.2 | +1.2 |

Classical cytogenetics — Karyotype:

| | Karyotype |
|---|---|
| SLL | 46,XY,del(11)(q21)[15]/46,XY[2] BM |
| MCL | 44,XY,add(1)(p36),t(11;14)(q13;q32),-13,-18[4] LN |

FISH, LN:

| | 13q14 del | TP53 del | Trisomy 12 | ATM del | CCND1/IGH |
|---|---|---|---|---|---|
| SLL | + | – | + | + | – |
| MCL | + | – | – | – | + |

SLL small lymphocytic lymphoma, MCL mantle cell lymphoma, Cyc D1 cyclin D1, FCM flow cytometry; study material: LN lymph node, PB peripheral blood, BM bone marrow. Antigens (45/19/20/22/23/5/43/200/79β/62L/25/11c/38/10/52/71) identified by the cluster of differentiation system; +, 100% expression; –, no expression (<20% neoplastic cells); ±, 20–99% of cells; +↑, positive expression but higher than in SLL cells or MCL cells or normal lymphocytes; +↓, positive expression but weaker than in SLL or MCL cells or normal lymphocytes; neo [%], percentage of neoplastic cells in fine needle aspiration biopsy: 13q14deletion, TP53 deletion, trisomy 12, ATM deletion, and CCND1-IGH fusion, respectively

The karyotype demonstrated the presence of two different clones: one with 11q deletion in BM and the second with a t(11;14)(q13;q32) in LN (Table 1). However, FISH performed on LN confirmed both above aberrations in neoplastic cells: ATM (11q22.3) deletion and CCND1-IGH, observed in 15% and 23% of cells, respectively. Moreover, FISH revealed 13q14 deletion in 83% of cells. CC and FISH results confirmed occurrence of two subpopulations: SLL with ATM deletion and MCL with CCND1-IGH, both carrying 13q14 deletion. Standard PB examinations revealed grade 2 [according to the Common Terminology Criteria for Adverse Events (CTCAE)] anemia and a mild hyperleukocytosis with an elevated lymphocyte count. The lactate dehydrogenase (LDH) was elevated (276 IU/L, upper normal range 227 IU/L). The baseline PB examination revealed 6.73 G/L WBC with a pathological MCL population at a level of about 280 cells in 1 mm$^3$ of PB (4.1% of PB nucleated elements and 0.28 G/L) and SLL subclone at a level of about 115 cells in 1 mm$^3$ of PB (1.7% of nucleated blood cells and 0.11 G/L).

The patient was in good general condition [grade 1 according to Eastern Cooperative Oncology Group (ECOG) performance status scale]. Whole body computed tomography (CT) revealed numerous enlarged lymph nodes in the cervix, the armpit, and near the stomach as well as enlarged mesenteric, external, and inguinal lymph nodes. Massive infiltration of the stomach wall, duodenum, jejunum, ileum, caecum, and rectum as well as an enlarged spleen was also visible in CT. MCL was diagnosed as stage IVB according to Ann Arbor classification with a high Mantle Cell International Prognostic Index (MIPI) score predicting a shorter and more aggressive disease course. SLL was classified as stage IVB. Due to bulky disease, the patient started treatment with fractionated doses of RCHOP (rituximab, cyclophosphamide, adriamycin, vincristine, and prednisone). The patient received 6 courses of alternating RCHOP and RIVAC (rituximab, ifosfamide, etoposide, and cytarabine) immunochemotherapy. After the fourth course of induction (second RIVAC), two successful aphaereses were performed. The midterm evaluation revealed partial remission according to the Cheson response criteria. The patient then became eligible for autologous hematopoietic cell transplantation (auto-HCT). Unexpectedly, just before the auto-HCT, the disease progression of SLL was detected. The colonoscopy revealed massive infiltration of the large intestine by SLL. The FCM of PB/BM confirmed SLL progression without features of Richter syndrome transformation [11] and MCL partial remission. The patient died due to SLL rapid progression.

## Molecular analysis

Gene Scan molecular analysis of IGH amplified sequences was performed on PB and BM samples at the time of diagnosis. This examination demonstrated two peaks: 341 bp and

**Fig. 1** Histopathology and immunohistochemistry of composite lymphoma. **a** Cervical lymph node biopsy showing the coexistence of small lymphocytic lymphoma (SLL) (red stamp in the lower right corner) and mantle cell lymphoma (MCL) (green stamp in the upper left corner). Diffuse growth of SLL small lymphocytes and a slightly larger monotonous population of medium-sized centrocytic cells with scant cytoplasm. Scattered "pink" macrophages in MCL component (green arrows in the upper left corner (hematoxylin and eosin)). **b–d** SLL (on the right) and MCL (on the left): CD20 expression stronger in MCL component than in SLL; CD23 positive in SLL but negative in MCL and cyclin D1 negative in SLL comparing to positive in MCL. Original magnification ×100
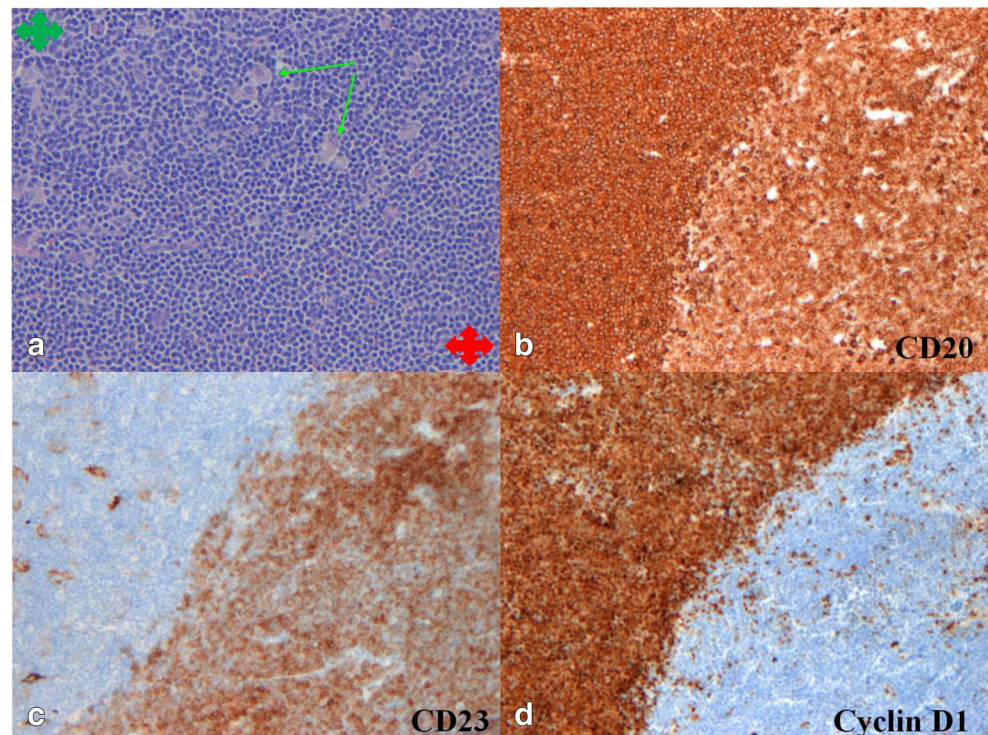


349 bp, confirming the presence of two different clonal rearrangements: VH3, JH4 and VH4, JH6 which indicated lack of a clonality relationship.

Sequence analysis using an IMGT/V-QUEST tool (www.imgt.org) of junctional regions [13] showed that one of the clones had developed by recombination of IGHVH3-21*01F, D3-9*01F, and JH4*02F regions while the second one by recombination of IGHVH4-59*01F, D3-22*01F, and JH6*02F.

| 341 bp peak | | | | |
|---|---|---|---|---|
| tgtgcgag | gaggcc | attacgatattttgactggttattat | gaa | actactttgactactgg |
| 3′ VH3 | N1 | D3 region | N2 | 5′ JH4 region |
| 349 bp peak | | | | |
| tgtgcgagag | ctcgtgcgg | attactatgatagtagtggttat | ctcc | actactacacggtatggacgtctgg |
| 3′ VH4 | N1 | D3 region | N2 | 5′ JH6 region |

## Monitoring of mMRD during follow-up

Apart from midterm clinical evaluation, an analysis of the level of mMRD was performed in order to determine the effectiveness of chemotherapy. Molecular markers have greater sensitivity than the FCM [6]; therefore, in this study, they were chosen as the standard method for MRD monitoring. For this purpose, in follow-up samples, an *IGH* rearrangement with 2 different starter sets was analyzed by qPCR [7] and *SOX11* expression was estimated. The SOX11 marker is not expressed in SLL [16]; therefore, it is detectable only in MCL. The regression analysis for the V3J4 and V4J6 rearrangements with *SOX11* expression showed a relationship between V4J6 and SOX11 ($R^2 = 0.9966$) indicating that this *IGH* rearrangement is a characteristic of MCL (Fig. 3). Based on this analysis, we considered the V4J6 rearrangement specific for MCL.

The MRD was also set by standard FCM, using the starting FCM immunophenotypic features of both CL components, at three time points: at the time of diagnosis, during treatment, and at the end of the treatment. The MRD assessment during the patient's treatment showed decreasing levels of MCL and SLL. In one PB sample taken in the middle of the treatment, the qPCR analysis showed an increased level of SLL (Fig. 4, top, left). This unexpected finding is difficult to explain when looking at the other results from that timepoint which

**Fig. 2** Fine needle aspiration biopsy/flow cytometry analysis of composite lymphoma. Coexistence of small lymphocytic lymphoma (SLL) cells (red), mantle cell lymphoma (MCL) cells (green), normal B cells (black dots in boxes), and T lymphocytes. **a** Forward scatter (FSC)/side scatter (SSC) scattergram showing only one cluster of cells in terms of size in R1 region. **b** and **d–l** Scattergrams showing the different patterns of expression of analyzed markers in SLL and MCL. **c** Showing the same CD5/CD19 expression on SLL and MCL cells
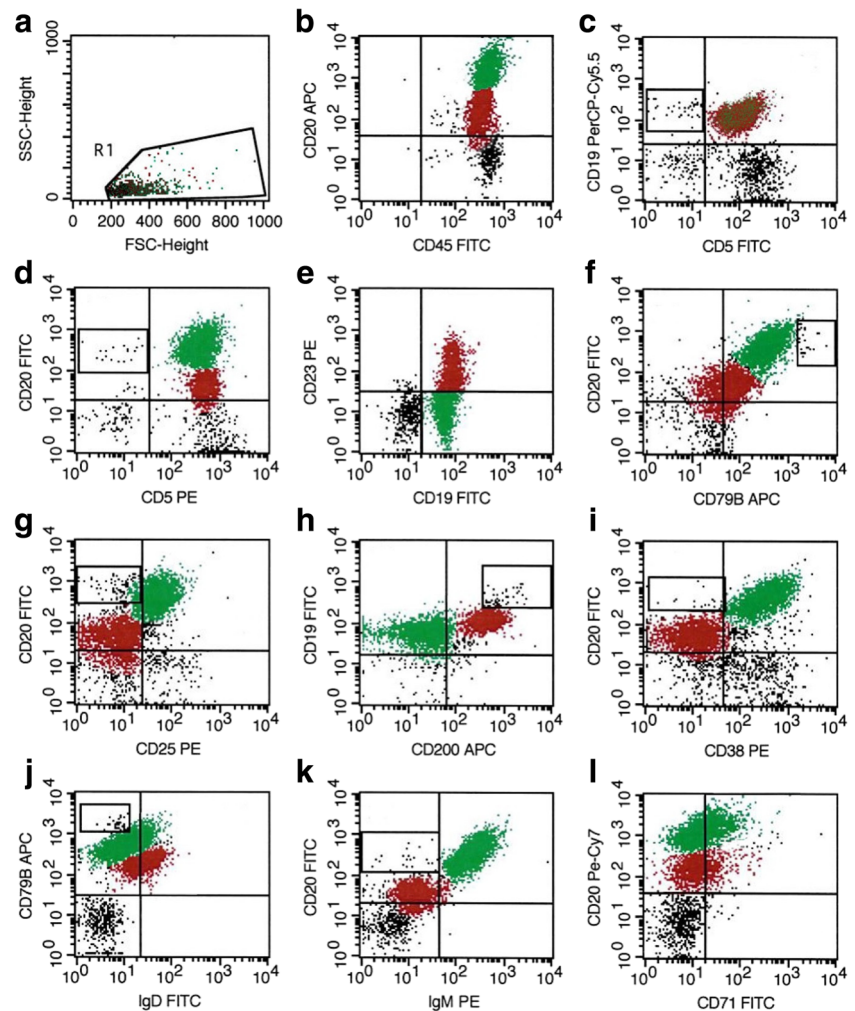


indicated a decreasing level of MRD. At the end of the treatment, both qPCR and FCM showed the lowest levels of MRD indicating a good response to the treatment. Unfortunately, before the next planned MRD assessment, the patient was diagnosed with the progression of SLL. The SLL progression was confirmed by qPCR. No samples were taken for FCM at that moment. Due to the very rapid and unexpected progression of the less aggressive SLL subclone (Fig. 4, bottom, left), the previously planned early detection of the molecular progression as well as the prompt start of SLL treatment could not be initiated.

## Materials and methods

### Histology and immunochemistry

After fixation in neutral buffered formalin, the lymph node was embedded in paraffin and sectioned following routine methods. The sections were stained with hematoxylin and eosin (HE). Immunohistochemical staining with anti-CD20,

CD23, cyclin D1, SOX11, and Ki-67 antibodies was performed according to a standardized automated protocol.

### Flow cytometry

Immunophenotype was also determined by FCM of cellular suspension obtained by FNAB, PB, and BM. All samples were prepared by the standard technique and were stained for 4–5 color immunophenotyping with a broad panel of monoclonal antibodies conjugated with one of the following fluorochromes: FITC/PE/RPE/PerCP/PerCP-Cy-5.5/PE-Cy7/APC (Becton Dickinson, BD) as previously described [11]. Data was gathered from the FACSCanto II flow cytometer and processed using the BD CellQuest Pro software (BD).

### Classical cytogenetics and FISH

LN cells obtained by FNAB and BM cells were fixed directly and cultured for 24 h without mitogen or for 72 h with DSP-30 (2 μM; TIB MolBiol, Berlin, Germany) together with IL-2 (200 U/ml; R&D Systems, Minneapolis, MN, USA). These
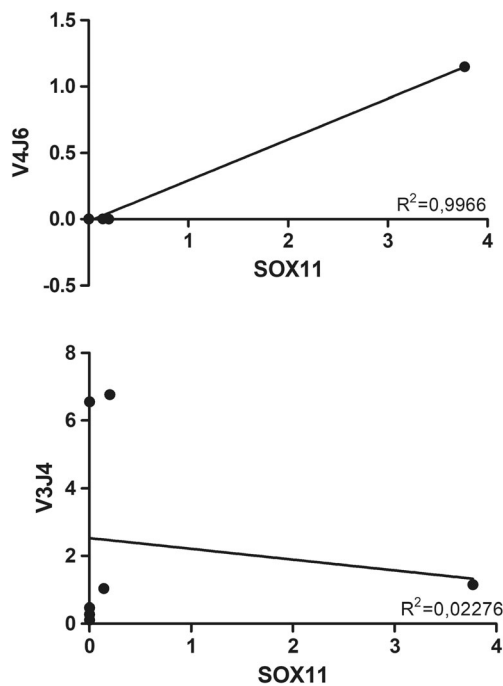
**Fig. 3** The regression analysis graphs for V3J4 and V4J6 rearrangement with *SOX11* expression. For V4J6 and SOX11 ($R^2 = \sim 1$; $p < 0, 05$), changes in *SOX11* expression explain changes in V4J6. For V3J4 ($R^2 < 0, 05$; $p > 0, 05$)—changes in *SOX11* expression are not associated with changes in V3J4 rearrangement

cultured cells were used for CC and FISH. The G-banding was performed by standard methods. FISH was performed with the use of probes: D13S319, TP53, ATM, CEP12, and CCND1/IGH (Vysis Abbott Molecular, Downers, Grove, IL, USA).

## Molecular studies

*IGH* rearrangement and *SOX11* expression analysis were performed for PB and BM samples. White blood cells were isolated using a standard gradient separation protocol. DNA and RNA were isolated according to the manufacturer's protocols using the Sherlock AX isolation kit (A&A Biotechnology) and the GeneMATRIX Universal Purification Kit (EURx), respectively. For the DNA studies, a standard qPCR reaction was performed according to the BIOMED-2 Concerted Action BMH4-CT98-3936 protocol [7]. The expression studies were performed in a two-step RT-qPCR reaction according to a previously published protocol [16, 17]. Sensitivity of molecular markers was defined by the lowest possible dilution detected by qPCR. The quantitative measurement range was set up as the lowest detected dilution which had a Ct lower than that of the negative controls background by three cycles. MRD level was calculated to allow comparisons between molecular markers to be made. Results from diagnostic samples were set to a 1 (100%) MRD level, while MRD level in follow-up samples was calculated as derived proportions.

## Discussion

The correct diagnosis of composite MCL and SLL is very important and challenging. Distinction between the components of CL is crucial, since MCL, which is the more aggressive disease, should be treated first. This type of CL is very rare, which only few cases having been reported [1, 15, 18]. Some authors suggest that biopsies containing CLL-type cells but lacking proliferation centres and with non-enlarged or only slightly enlarged lymph nodes on imaging represent a very indolent disease that may be considered a tissue equivalent of monoclonal B cell lymphocytosis rather than overt SLL [12]. However, in our CL case, we received 4 enlarged lymph nodes, each of them approximately 2 cm long. In addition, FNAB/FCM was performed from an enlarged 3-cm axillary lymph node. This type of CL may be underdiagnosed as the two neoplasms under consideration have many common features. Both malignancies are CD20/CD19/CD5 positive small B cell neoplasms, and the correct distinction between MCL and SLL by FCM is possible using a broad panel of antibodies as previously described [1, 2, 11]. Additionally, during the treatment, some immunophenotypical markers (e.g., CD20) might be missing on tumor cells making correct detection of the residual disease by FCM difficult. Recently published work recommends the use of two standard panels for FCM and IHC to distinguish MCL from SLL in CL [1]. As we have shown here and previously [2, 11], FNAB is a highly efficient method of obtaining tumor cells for FCM, CC, FISH, and molecular studies in MCL and SLL.

The importance of MRD assessment in therapy decision has been increasing over the last years [6]. Molecular PCR-based MRD estimation presents higher sensitivity ($10^{-5}$) comparing to standard FCM ($10^{-3}$) and is highly standardized [6].

The methods of distinguishing both malignancies are normalized for diagnostic purposes. However, MRD monitoring of MCL and SLL separately during treatment is still not commonly used. MRD monitoring enables to detect molecular relapse and improve treatment decision [19], and PCR-based methods are " the gold standard" for measuring MRD. The most widely used molecular marker for analyzing MRD in mature B cell lymphomas is *IGH* rearrangement [7]. This method is highly specific for the identification of a certain fraction of mature B cells (including B-NHLs). However, it is impossible to distinguish which *IGH* rearrangements are specific to SLL and MCL in CL without a separate molecular analysis of each of the CL components, which is not possible in PB. Our analysis of *IGH* rearrangement revealed two clones of malignant mature B cells: with V4J6 rearrangement and with V3J4 rearrangement. The V4 recombinants are found in 29% of MCL and 10% of SLL, while V3 recombinants are found in 46% of MCL and 30% of SLL [7]. As both rearrangements are found in MCL and SLL, for proper distinction of these clones, we used an additional molecular
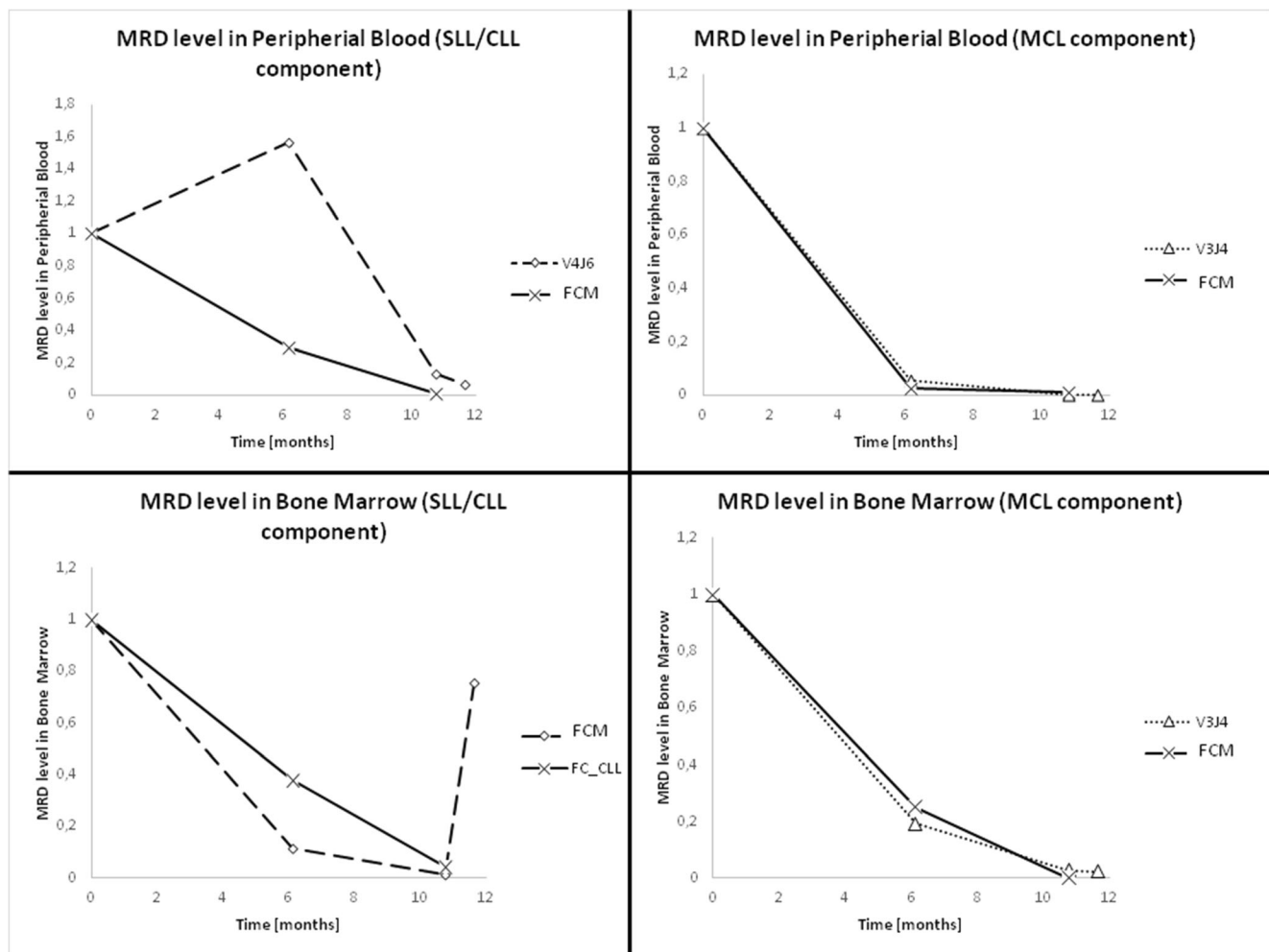
**Fig. 4** Minimal residual disease level SLL and MCL components estimated by different molecular markers in peripheral blood and bone marrow. As minimal residual disease (MRD) estimated by molecular markers and flow cytometry had different range of values, the resulting MRD levels were averaged: at the time of diagnosis, the MRD level was set as 1, and the follow-up values were calculated as a percentage of diagnostic sample

marker highly specific for MCL. *SOX11* expression is found in over 90% of MCL cases [3, 20] and can be used as an MRD monitoring marker, as we previously reported [16].

In this report, we have shown that qPCR analysis may help to differentiate the CL components, especially when supported by *SOX11* gene expression analysis. Applying the SOX11 marker allowed us to distinguish the clones and further monitor the disease by standard molecular markers. To our best knowledge, this is the first example of the use of mMRD estimation for the differentiation of the CL components. However, it should be noted that challenges may also occur using molecular methods. In some cases, the proper distinction between clones by *IGH* rearrangements is impossible due to the polyclonal background, close locations of loci, or inability to detect the t(11;14)(q13;q32) translocation specific for MCL using PCR, as it was in this case. While this translocation is detectable in 80 to 90% of MCL by FISH, PCR is able to detect it in only 25 to 40% [7]. Therefore, it seems

crucial to support the classical diagnostic methods by applying genetic and molecular methods in the process of diagnosis and monitoring. As we have shown, applying additional molecular marker SOX11 may allow proper differentiation of the clones in CL.

## Compliance with ethical standards

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