Case Report

Composite Mantle Cell Lymphoma and Chronic Lymphocytic Leukemia/Small Cell Lymphoma with 17p Deletion: A Case Study

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Here we describe a case of a composite mantle cell lymphoma (MCL) and chronic lymphocytic leukemia/small cell lymphocytic lymphoma (CLL/SLL) with 17p deletion in the MCL. The patient presented with 3 years of progressive lymphocytosis. Cervical lymph node biopsy showed morphologic features of nodular MCL and internodular CLL/SLL, two populations of CD5+ cells by flow cytometry, a t(11;14) translocation with a deletion in chromosome 11 by FISH, and biclonal IGH gene rearrangement. In the peripheral blood, flow cytometry showed a single population of CD5+ cells; FISH showed a t(11;14) translocation. Peripheral blood IGH gene rearrangement confirmed a single B cell monoclonal population identical to one of the two lymph node clones. Peripheral blood karyotyping detected 17p deletion, attributed to the MCL, the sole B cell clonal population in the peripheral blood. The diagnosis was concurrent MCL and CLL/SLL in the lymph node with peripheral MCL harboring a 17p deletion.
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INTRODUCTION

Mantle cell lymphoma (MCL) constitutes approximately 3-10% of non-Hodgkin lymphomas. It occurs in middle aged to older patients with a median age of 60 years with a male preponderance. Morphologically, MCL shows a monotonous population of small often angulated B-cells with a vaguely nodular, diffuse, mantle zone or rarely follicular growth pattern. It is CD5 positive and CD23 negative by immunohistochemistry (IHC) or flow cytometry phenotyping. The genetic hallmark of this disease is the recurrent chromosomal abnormality t(11;14) (q13;q32), which brings the cyclin D1 (*CCND1*) gene under the influence of the enhancer of the immunoglobulin heavy chain (*IGH*) gene, leading to cyclin D1 overexpression.[1,2] This translocation is usually detected by fluorescence in situ hybridization (FISH), and almost invariably correlates with unequivocal expression of CyclinD1 upon immunohistochemical analysis (IHC).[2]

Chronic lymphocytic leukemia/small cell lymphoma is the most common adult leukemia in North America and Western Europe with an incidence of 2-6 cases per 100,000 persons per year. The mean age of diagnosis is 65 years. The nodal morphology in CLL/SLL demonstrate architectural effacement, often with a pseudofollicular pattern containing larger cells in a dark background of small cells, known as proliferation centers. The neoplastic cells are small B lymphocytes with clumped chromatin typically characterized by a CD5+/CD23+ immunophenotype.[1,3,4]

Composite MCL and CLL/SLL is an uncommon entity with only a handful of cases reported in the literature. A multi-center study of a large series of 11 cases using molecular techniques found that although these two entities share similar morphologic and immunophenotypic characteristics, they are clonally distinct at the molecular level. The occurrence of composite MCL and SLL/SLL implies it is necessary to rule out the other when a morphologic diagnosis of either MCL or CLL/SLL is made.[5]

Here we report a diagnostically difficult case of MCL with concurrent CLL/SLL. We also suggest an explanation for the occurrence of high single fusion count for the reciprocal t(11;14) in MCL. We also demonstrate the unrelated clonality of the two entities without the use of microdissection separation eliminating the potential of confounding contamination. We demonstrate how molecular analysis can be used to attribute prognostic cytogenetic aberrations to either of the two entities.

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CASE HISTORY

This was a 61-year-old male with progressive lymphocytosis who had been monitored for 3 years prior to the recent presentation. He presented with increasing lymphocytosis without B symptoms. He was a smoker with a history of 2-3 alcoholic drinks per night. Physical examination showed no palpable nodes.

Laboratory features showed a hemoglobin of 125 g/L, platelets 201 $x10^9$/L, white blood cells 138.1 $x10^9$/L with lymphocytes of 132.6 $x10^9$/L. Serum protein electrophoresis were negative for M-protein. The most recent CT scan revealed diffuse lymphadenopathy showing multiple sites of nodal enlargement and increased number of nodes, the largest were a right jugular node (1.8 x 1.6 cm) and a posterior cervical node (1.9 x 1.6 cm). There was moderate splenomegaly at 17.2 cm. A CT guided biopsy of iliac lymph nodes was suggestive of concurrent MCL and CLL/SLL. A left cervical lymph node excisional biopsy with lymphoma work up was performed.

METHODS

IHC: The histopathological features were noted, and IHC was performed on formalin-fixed, paraffin-embedded (FFPE) material using the following commercial antibodies: CD20 (L26 clone), cyclin D1 (EP12 clone), CD23 (MHM6 clone), CD21 (1F8 clone) and Ki67 (MIB1) LEF1 (clone EPR2029Y) (Dako, Carpinteria, California), CD5 (4C7 clone) (Leica, Concord, Ontario), CD3 (2GV6 clone) (Ventana, Tucson, Arizona). These stains were performed using automated autostainer (Ventana).

Flow Cytometry: lymph node and peripheral blood (PB) were processed according to established protocol and data were collected on a Beckman Coulter flow cytometer (Beckman, Coulter, Miami, FL).

FISH: Two different FISH probes: Vysis LSI IGH/CCND1 XT and Vysis LSI IGH/CCND1 (Abbott Molecular, Illinois) were used. The CCND1 XT probe spans a 942kb genomic region on chromosome 11 compared with the 378 kb of CCND1 probe.

IGH Gene Rearrangement: Genomic DNA was extracted from lymph node, bone marrow and PB using Qiagen kit (Qiagen, Valencia, California). Polymerase chain reactions (PCR) were performed using utilizing primers from Invivo Scribe technologies (San Diego, California). The PCR products were examined using a high resolution fragment lengthy analyzer (ABI 3130 Genetic Analyzer, Applied Biosystems/Life Technologies, Carlsbad, California). DNA from normal polyclonal B-cells was used as control.

Cytogenetic Studies: trisomy 12, 11q, 13q and 17p deletion were performed on PB sample using FISH probes according to established protocol.

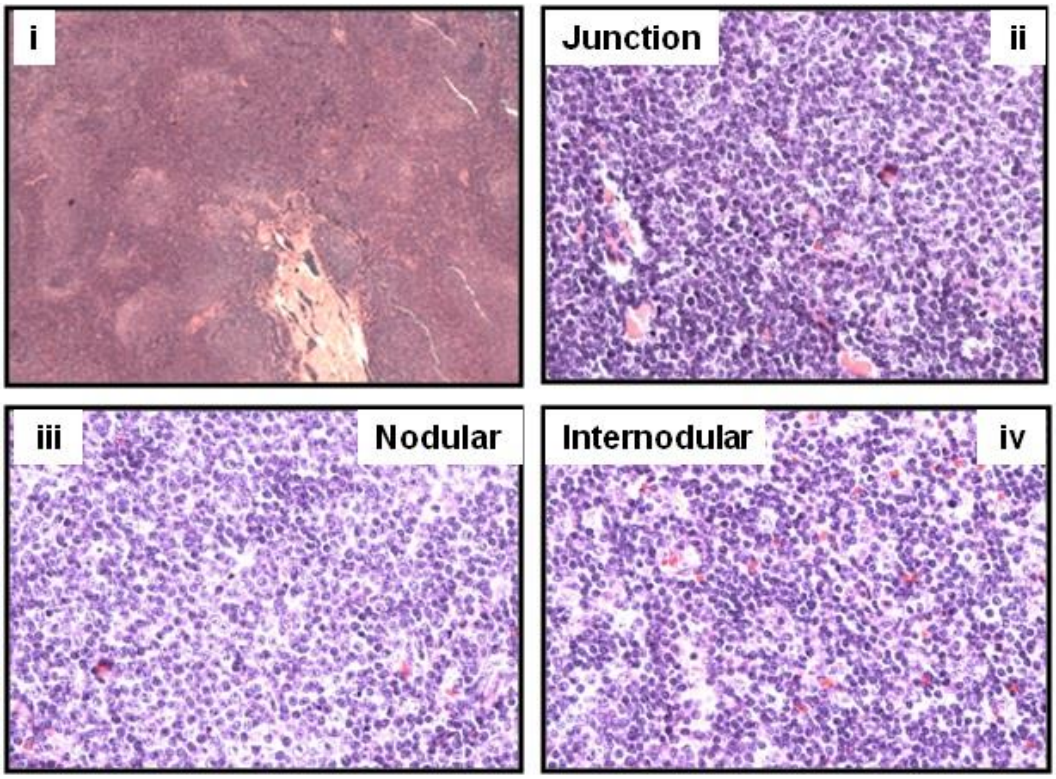

Figure 1. CERVICAL LYMPH NODE BIOPSY: (**i**) H & E showing effacement of nodal architecture with a vague nodular pattern. (**ii**) Junction: show presence of two different lymphoid population nodular areas and internodular areas. (**iii**) Nodular regions showing small monotonous lymphocytes with clumped chromatin. (**iv**)Internodular region showing densely packed small lymphocytes with irregular nuclei.

RESULTS

Histomorphology and IHC Studies

1. Lymph node: the lymph node morphology demonstrates architectural effacement by small atypical lymphoid cells in a nodular pattern. The nodules are composed of relatively monotonous small to intermediate sized lymphocytes with clumped chromatin. Rare larger cells are seen. The interfollicular regions are composed of densely packed small lymphoid cells with irregular nuclear outlines and clumped chromatin (**Figure 1 i-iv**). No proliferation centers are noted. IHC showed the nodular population to be CD20 +/ CD5 dim/cyclin D1 positive, whereas the internodular cells are CD20 dim/CD5/cyclin D1 negative (**Figure 2 i-iv**). The follicular dendritic network is highlighted by CD21 and CD23; however CD23 expression was seen in a minority of B-cells. LEF-1 stained mainly in internodular areas (**Figure 2 vi**). Ki-67 proliferative index stained 15% of cells with no specific zonal pattern.

2. Bone marrow: Bone marrow biopsy shows several peri- and intertrabecular dense nodules of small lymphocytes which are CD5 and CD20 positive with cyclin D1 expression in a subset of cells and patchy LEF-1 staining (**Figure 3 i-vii**).

3. PB: Examination of the PB smear confirms marked lymphocytosis consisting predominantly of small to medium-sized cells with mature clumped chromatin. Rare large cells with slightly more open chromatin are seen. Occasional smudge cells are also present.

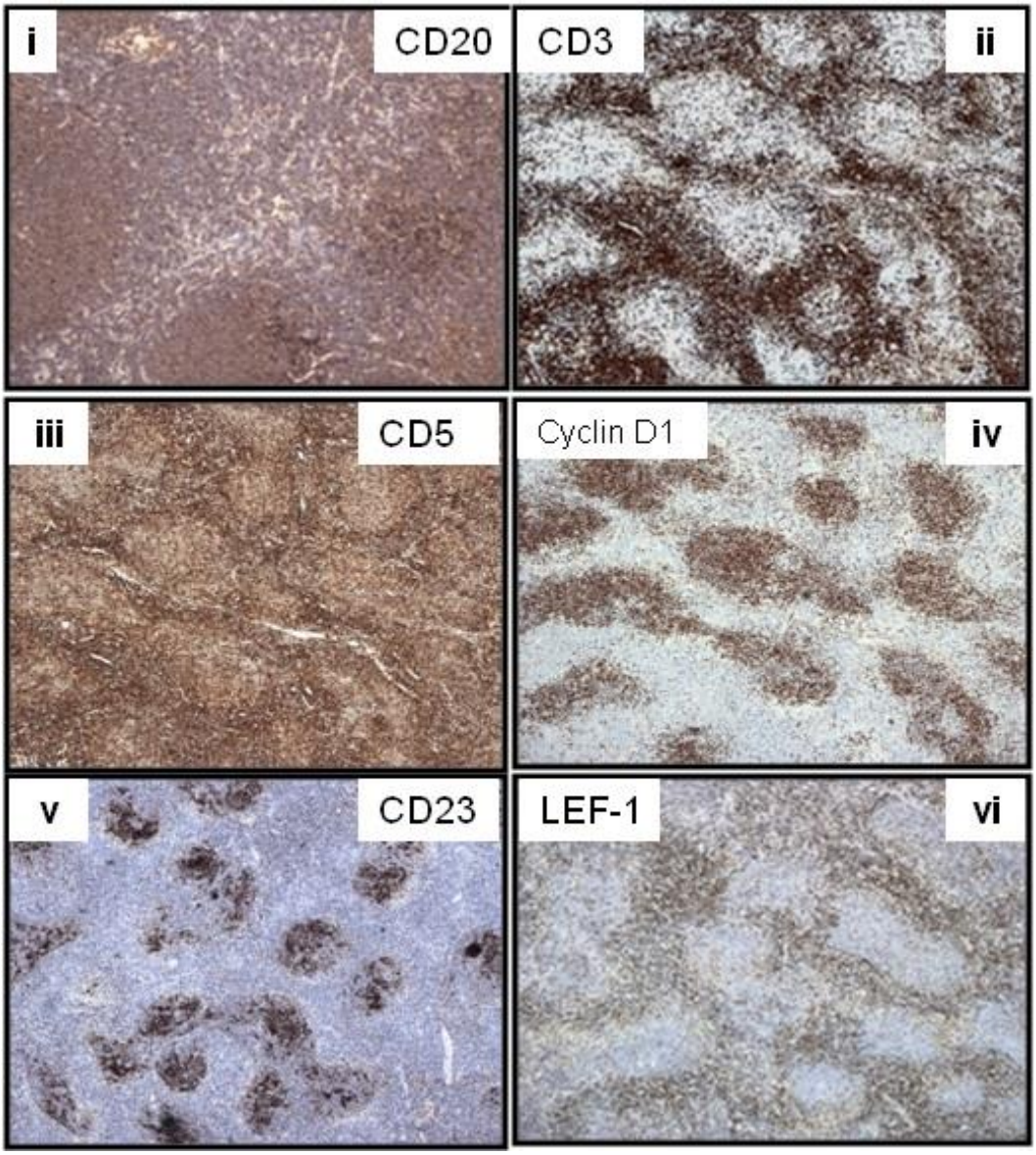

Figure 2. IMMUNOHISTOCHEMISTRY OF CERVICAL LYMPH NODE (**i-vi**): Nodular regions show strong positivity for CD20 and cyclin D1 with dim CD5, while internodular areas show dim CD20, scattered CD3 positive cells, LEF-1 positivity and cyclin D1 negativity. CD23 highlight the follicular dendritic cells.

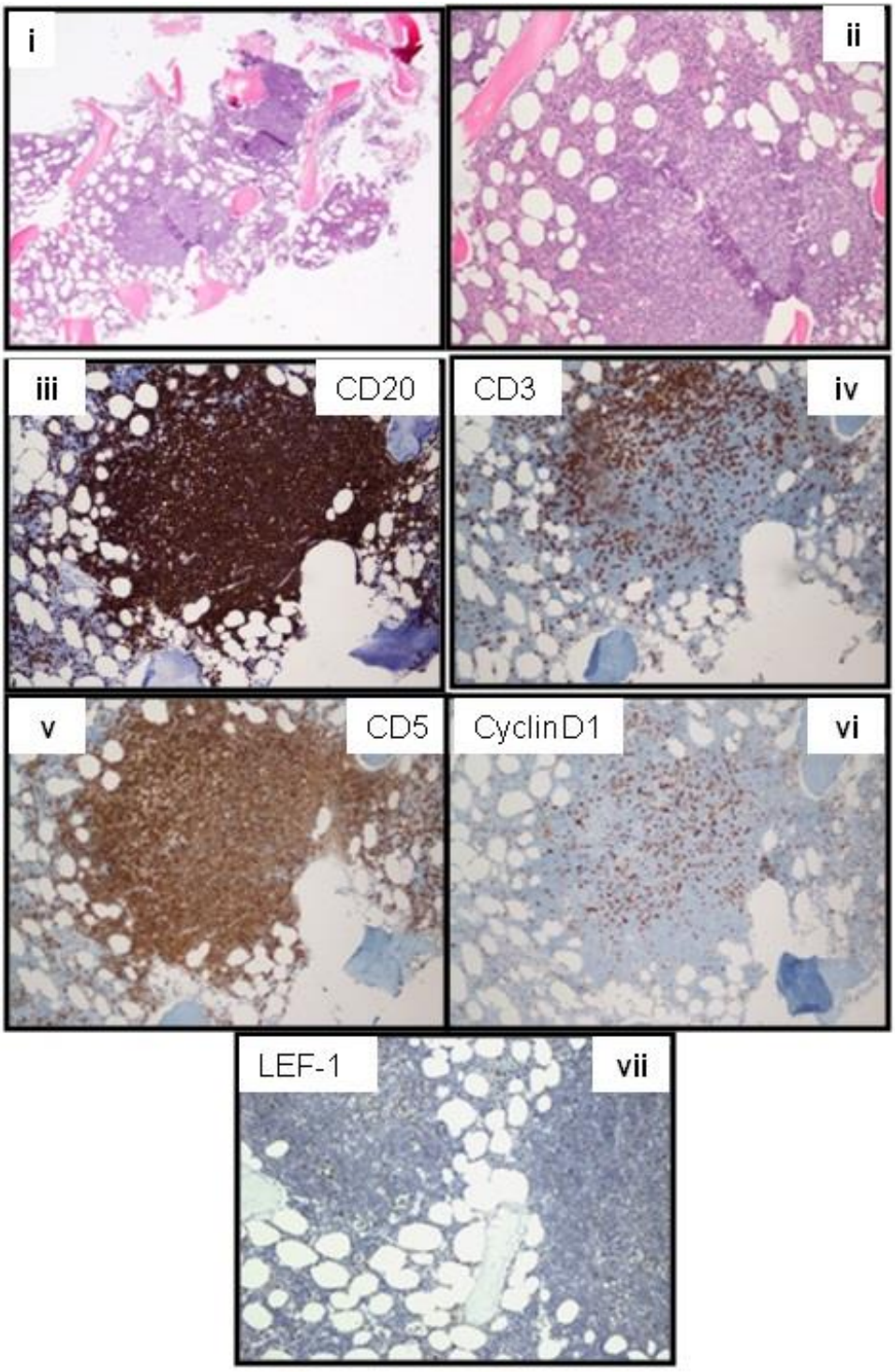

Figure 3. BONE MARROW BIOPSY (**i, ii**): H &E showing nodules of small densely packed lymphocytes, (**iii-v**): Nodules show CD20, CD5, CD3 (note that areas stained by CD3 & CD5 do not coincide, confirming CD5 co-expression in B cells). Cyclin D1 and LEF-1 show patchy positivity in different areas.

Flow Cytometry

1. Lymph node: Flow cytometric analysis identified a monoclonal kappa- restricted CD19-positive B cell population accounting for 73% of the overall gated lymphoid cells. There is uniform coexpression of CD5, CD20, CD22, CD 79b, and CD43 with equivocal expression of FMC7, and no expression of CD10. The B cells comprise of two populations; the first one, accounting for 44% of B lymphocytes, shows brighter kappa expression and negative CD23 expression, the second one, accounting for 56% of B lymphocytes shows dim kappa expression and is positive for

CD23 (**Figure 4, i**).

2. PB: Flow cytometric analysis identified monoclonal (bright kappa-restricted) CD19 positive B cells accounting for 94% of the gated events. There is coexpression of CD19, CD20, CD5 and CD79b and absent CD10, CD23, CD11c, CD 103, CD 25, surface IgM and surface IgG (**Figure 4, ii**).

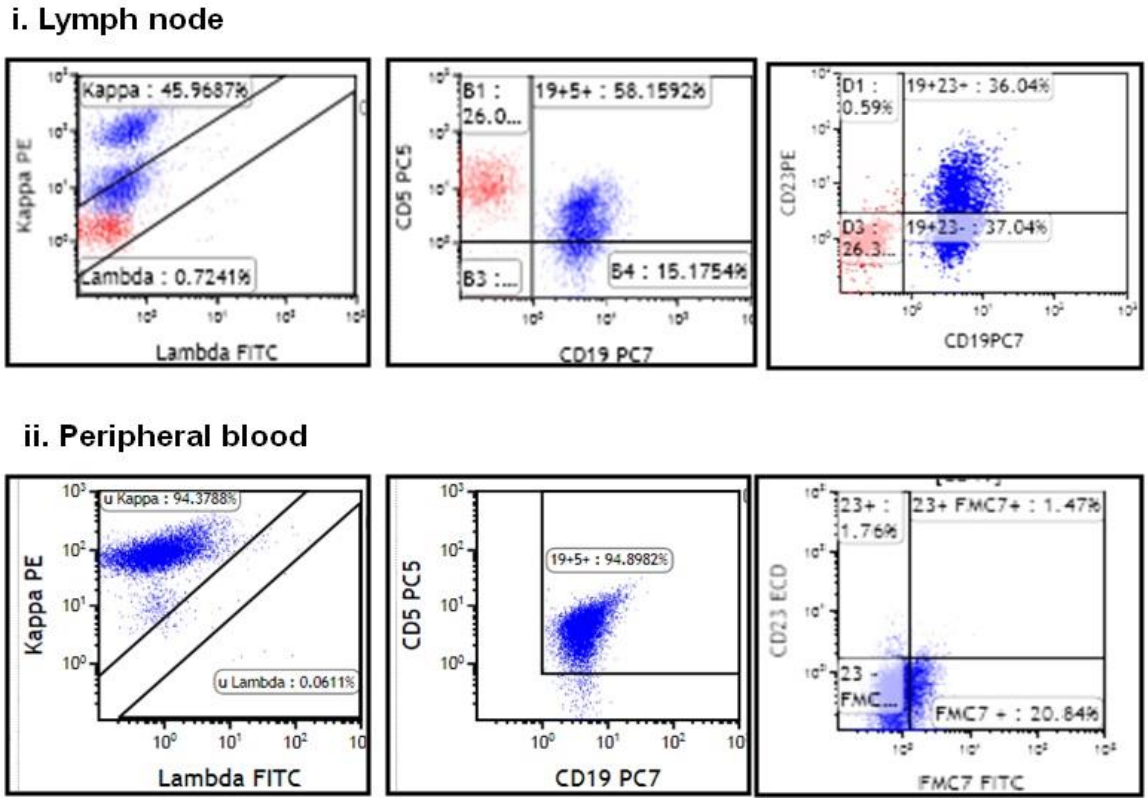

Figure 4. FLOW CYTOMETRY (**i**) lymph node: show 2 populations of B-cells (**ii**) peripheral blood: show 1 population of B-cells.

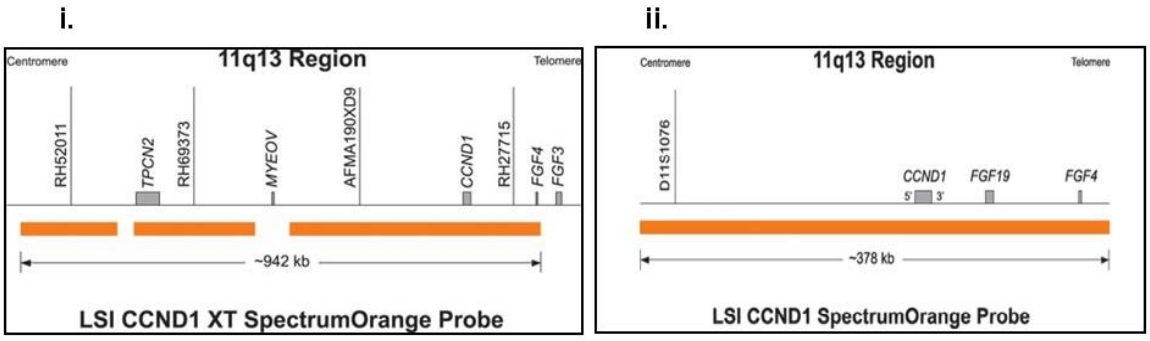

Figure 5. FISH PROBE MAPS (**i**) LSI CCND1 XT probe (**ii**) LSI CCND1. The LSI CCND1 XT probe spans a larger genomic region.

FISH

1. *Lymph node:* While both probes identify a t(11;14) translocation, the LSI IGH/CCND1 probe shows a high single fusion count (40%) with low double fusion counts (6%). The LSI IGH/CCND1 XT shows high double fusion count (36%) with low single fusion counts (2%). The signal count disparity between the two probes suggests a deletion in the region of the *CCND1* gene on one of the chromosome 11 pair, probe maps are shown (**Figure 5**).

2. PB smear: Similar to the lymph node, the PB was investigated by two FISH probes Vysis LSI IGH/CCND1 and Vysis LSI IGH/CCND1 XT. Findings are similar to those in the lymph node. Both probes demonstrate the t(11;14) translocation in the PB. Whereas the LSI IGH/CCND1 probe shows high single fusion and low double fusion counts (67% versus 7%), the LSI IGH/CCND1 XT shows a high double fusion count and low single fusion counts (90% versus 10%). Comparison of the two probes suggests a deletion in the

region of the *CCND1* gene on one of the chromosome 11 pair.
3. Bone marrow: FISH probe Vysis LSI IGH/CCND1 XT showed the t(11;14) translocation in 15% of cells.

***IGH* Gene Rearrangement**
1. Lymph node: Two prominent peaks of distinct sizes were identified and assessed to be biclonal (**Figure 6 i**).

2. Bone marrow: Two prominent peaks of distinct sizes were identified and assessed to be biclonal (**Figure 6 ii**).

3. PB smear: PB *IGH* gene rearrangement demonstrated a single monoclonal peak of identical size to one of two peaks identified in the lymph node (**Figure 6 iii**). The assay analytic sensitivity of 5% suggested significant blood involvement by only one of the two disease entities.

Cytogenetics
FISH on PB for genetic prognostic markers confirmed 17p deletion present in 93% of cells examined.

Based on the collated data, the diagnosis was composite MCL and CLL/SLL in the lymph node and bone marrow and MCL harboring a 17p deletion in the peripheral blood. A summary of the different studies comparing lymph node, bone marrow and PB are shown in **Table 1**.

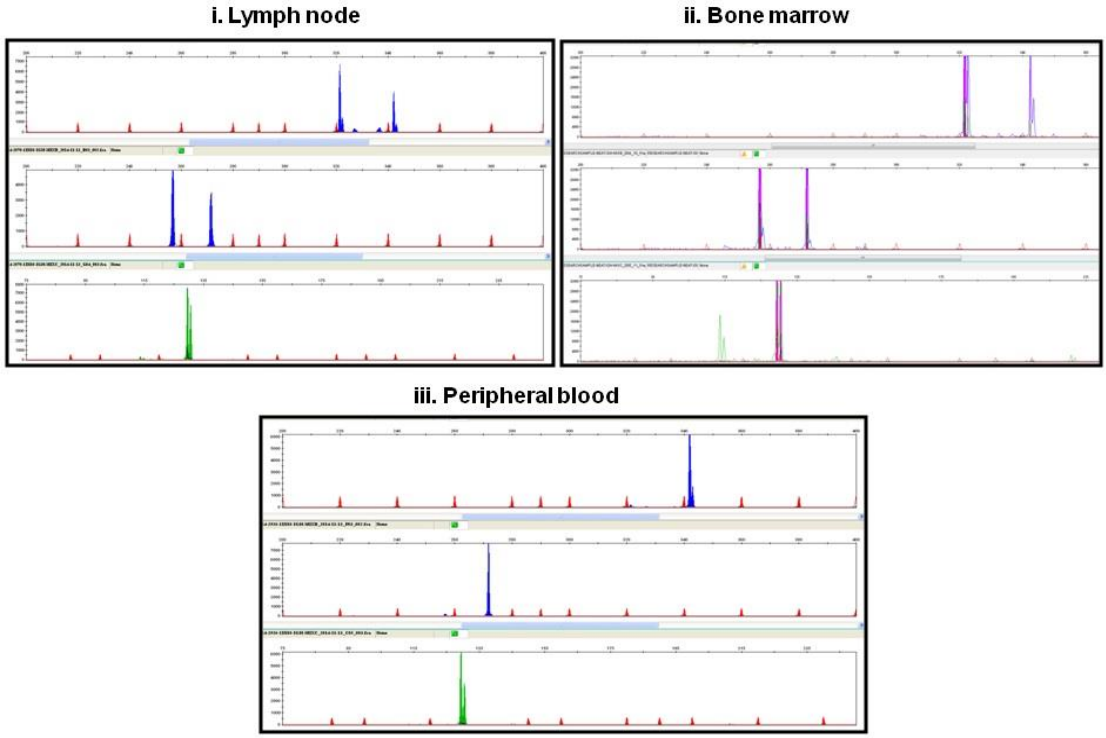

Figure 6. IGH CLONALITY ELECTROPHEROGRAM (**i**) lymph node: showing biclonality, (**ii**) Bone marrow: showing biclonality, (**iii**) Peripheral blood showing monoclonality with a single peak of identical size to one of the peaks in the lymph node and bone marrow.

Table 1. Morphologic, immunophenotypic, cytogenetic and molecular characterization of lymph node, peripheral blood and bone marrow biopsy.

	Lymph Node	**Peripheral Blood**	**Bone Marrow**
H&E	Nodules of MCL Internodular CLL/SLL	MCL lymphocytosis (small to medium sized cells with mature clumped chromatin)	Small lymphocytes in nodular pattern (peritrabecular and inter -trabecular)
IHC	MCL (CD20+/CD5 dim/cyclinD1+) CLL/SLL (CD20 dim/CD5dim/cyclin D1-)	N/A	CD20+/CD5+ B-cell with multifocal cyclin D1 expression
Flow	Two distinct cell populations of CD5+ cells	Single cell population of CD5+ cells	Not done
FISH	t(11;14) translocation in 36% of cells	t(11;14) translocation in 90% of cells	t(11;14) translocation in 15% of cells
IGH	Biclonal	Monoclonal	Biclonal

DISCUSSION
MCL is a non Hodgkin lymphoproliferative neoplasm characterized by the t(11;14) translocation that involves the juxtaposition of the CCND1 gene to the IGH promoter resulting in the overexpression of cyclin D1 protein.[2] The detection of this translocation for clinical diagnosis is usually done by the use of dual color dual fusion FISH in PB specimen or by cyclin D1 IHC in FFPE samples. FISH diagnosis requires the detection of a double fusion signal using a dual color dual fusion probe that targets the genomic regions for the IGH gene promoter and CCND1 gene. Dual

fusion signal detection confers higher specificity for MCL than the detection of a single fusion alone. Cases of MCL with high single fusion signals and absent double fusion signal have been reported.[6,7] Such findings may result from deletions in the targeted regions of chromosome 11 or 14 to which the probe binds. High single fusion counts have also been reported in a number of other low grade lymphoproliferative disorders, including chronic lymphocytic leukemia, follicular lymphoma etc. making the use of single fusions a less specific criteria for MCL diagnosis. However, very high single fusion counts are more commonly associated with MCL. These likely occur as a result of atypical breakpoints with a deletion in the probe binding region.[7]

In the current case, FISH for t(11;14) with the LSI IGH/CCND1 on the PB and lymph node sample showed a high single fusion but low double fusion signals. These types of abnormalities may be indicative of MCL but do not rule out other more indolent low grade lymphoma like CLL/SLL, marginal zone lymphoma and follicular lymphoma. Confirmatory FISH for t(11;14) using the LSI IGH/CCND1 XT demonstrated diagnostic high double fusion counts confirming the presence of the translocation. Mapping of the different genomic regions targeted by the two probes resolved the discrepancy in double fusion signals between the two probes. Both probes possess genomic regions of overlap, while the CCND1 XT probe has a unique targeted region. Correlation of the genomic probe maps and the single-double signal discrepancies is consistent with the presence of a small deletion in one of the two chromosomes 11 of the tumor genome that is targeted by the XT probe but not the LSI IGH/CCND1 probe. This finding could also explain the reported cases of high single fusion FISH results for t(11;14) in some cases of MCL. In our case, the FISH assay showed similar results in both blood and the lymph node samples indicating blood involvement by the MCL. The FISH results were consistent with the flow cytometry finding of a MCL phenotype found both in the lymph node sample and the blood. However, IGH gene rearrangement studies of the lymph node identified two dominant peaks indicative of biclonality corresponding to two phenotypically distinct populations, while in the blood only a single dominant peak (with a size similar to one of the peaks that was detected in the lymph node) and a corresponding phenotypic profile (highlighting only one of the lymph node subsets) were identified. We concluded that only one lymphoma type was present in the blood, and that this PB component is best characterized as MCL.

In addition to the diagnostic translocation t(11;14) in MCL, other additional cytogenetic abnormalities, including numeric and recurrent structural abnormalities have been reported to occur in this disease entity. Examples include trisomies of chromosome 3,7,9,10,12,13,17,21, monosomies of chromosome 3,7,8,9,10,12,13,15,17,21, specific additions, specific deletions, and translocations.[8] In MCL 17p deletions do not have a significant prognostic impact and do not alter disease management.[9]

In CLL/SLL, cytogenetic features are important for prognosis and therapeutic decision making. Chromosomal aberrations occur in chromosomes 6, 11, 12, 13, and 17. In contrast to MCL, aberrations in chromosome 17p occurring in CLL/SLL are suggestive of p53 mutation and are associated with a poorer prognosis demanding a more aggressive treatment.[10] Whereas 17p deletion can occur in both CLL/SLL and MCL, its prognostic significance differs based on the disease context in which it occurs. It is therefore important for clinical purposes to distinguish which lymphoma entity harbors the deletion when diagnostic phenotypic features suggest CLL/SLL and/or MCL. In the current case, 17p deletion was detected in the lymphoma population in blood which was characterized as MCL. This information is important for prognostic and therapeutic purposes as a deletion in CLL may require bone marrow transplantation while if present in MCL, chemotherapy remains the recommended treatment protocol.[10]

In summary, this case report demonstrates a diagnostic dilemma posed by concurrent lymphoma types and its resolution using a combination of flow cytometry and molecular studies including gene rearrangement and FISH studies to determine the lymphoma cell of occurrence. We also highlight the opportunity of using alternate FISH probes for the diagnosis of MCL, especially in cases with high single fusion counts with the routine probe. Also, the critical difference in the significance of the 17p deletion in MCL versus CLL/SLL was discussed as it applies to this case.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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