Case Study

Unusual extranodal composite CD5 negative mantle cell lymphoma and CD5 positive marginal zone lymphoma

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Abstract: Composite lymphomas composed of two non-Hodgkin small B cell lymphomas are infrequent and composite mantle cell lymphoma and marginal zone lymphoma is extremely rare with 3 cases reported to date. The case presented herein is the first report of extranodal composite CD5 negative mantle cell lymphoma and partially CD5 positive marginal zone lymphoma. The identification of these two histologically indistinct and immunophenotypically unconventional lymphoma components requires a comprehensive study including careful interpretation of immunohistochemical results and the fluorescence *in-situ* hybridization (FISH) analysis of t(11;14). Clinically, the diagnosis of this composite lymphoma is compatible with patient's aggressive disease course. Molecular studies for immunoglobulin gene rearrangement indicate that the mantle cell lymphoma and marginal zone lymphoma components are derived from different clones, supporting the notion that composite lymphomas of non-Hodgkin small B cell lymphomas are often biclonal.

Keywords: composite lymphoma, mantle cell lymphoma, marginal zone lymphoma, CD5 negative mantle cell lymphoma

Introduction

Composite lymphoma, a rare entity, is defined as two or more morphologically distinct types of lymphomas at the same anatomic site, occurring either synchronously or metachronously. The most common combinations that have been reported include two histologically distinct types of non-Hodgkin lymphoma, or the rare association of Hodgkin lymphoma with either chronic lymphocytic leukemia (CLL) or follicular lymphoma [1, 2]. The vast majority of the reported composite lymphomas showed a sequential occurrence of Hodgkin lymphoma or diffuse large B cell lymphoma following treatment of prior low-grade B cell lymphoma [3, 4]. The B cell immunoglobulin gene rearrangement studies of these previously reported composite lymphomas supported a clonal relationship among the components of the composite lymphomas, and provided molecular evidence of the B cell derivation of Reed-Sternberg cells in classical Hodgkin lymphomas [5].

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Composite lymphomas with two phenotypically different small B cell lymphoma components are infrequent, with only a small number of cases reported [6–11]. The molecular analyses in these reported cases revealed unrelated clonal rearrangements in composite lymphoma components, indicative of the biclonal origin of the composite small B cell lymphomas [6, 9].

Our study demonstrates an unusual extranodal composite CD5 negative mantle cell and partially CD5 positive marginal zone lymphoma, containing histologically indistinct but immunophenotypically distinct cell populations. The molecular analysis results suggest that the two components are biclonal, indicating a collision tumor with two unrelated B cell lymphoma clones, rather than divergent differentiation within a single neoplastic process.

Case Report

Clinical information

66-year-old woman presented with a mass lesion in the right upper eyelid, with CT scan of the head showing a lobular solid mass in the anterosuperior right orbit adjacent to superior rectus muscle. An excisional biopsy of the eyelid mass was performed, which was diagnosed as composite mantle cell and marginal zone lymphoma, based on the integrated analyses including morphology assessment, immunohistochemical work-up, molecular and cytogenetics studies. Further CT/PET scan showed multifocal pulmonary involvement without intrathoracic lymphadenopathy, extensive predominantly subcapsular lymphomatous encasement of the left kidney, and scattered retroperitoneal lymphadenopathy with the largest node measuring 2 cm. The needle core biopsy of the renal mass showed involvement by composite mantle cell and marginal zone lymphoma. The bone marrow staging biopsy revealed focal involvement by B cell lymphoma, comprising both components.

Histological and Immunohistochemical Findings

The eyelid mass biopsy shows diffuse proliferation of small atypical lymphoid cells displaying monotonous appearance, clumped nuclear chromatin, and moderate abundant clear cytoplasm admixed with scattered immunoblasts, histiocytes and clusters of plasma cells. There are scattered aggregates of small atypical lymphoid cells contain less prominent cytoplasm [Figure 1A- 1C]. Immunohistochemically, the atypical lymphoid cells are positive for CD20, PAX5 and BCL-2 [Figure 1D]; partial weakly positive for CD5 [Figure 1E, 1F]; and negative for CD10 and BCL6. Notably, the atypical B lymphoid cells show focal strong expression of Cyclin D1, and the Cyclin D1 positive cells are almost entirely negative for CD5 [Figure 1G]. The Cyclin D1 positive cells form scattered nodular aggregates, interwoven with Cyclin D1 negative B cell lymphoma, accounting for 25% of the entire B cell lymphoma. Further, the double immunostains using CD138 and Cyclin D1 antibodies confirm the absence of Cyclin D1 expression within plasma cells [Figure 1G]. Kappa and lambda immunostains reveal the kappa restriction in plasma cells [Figure 1H, 1I, 1J]. CD21 and CD23 stains demonstrate focal residual follicular dendritic cell meshworks [Figure 1K] and show absence of CD23 expression in the B-cells. Ki-67 stain shows an overall proliferation index of less than 10%.

Examination of the scanty left renal needle core biopsy specimen reveals diffuse proliferation of small lymphoid cells [Figure 2A]. Further morphological assessment of the lymphoid cells is nearly impossible, due to the needle core biopsy specimen sample processing related artifacts. Immunohistochemically, the lymphoid cells are mainly positive for B cell markers, CD20 and PAX5 [Figure 2B, 2C]. Cyclin D1 immunostain reveals scattered aggregates of positive B lymphoid cells, which account for 20% of all B lymphoid cells [Figure 2D]. Ki-67 stain shows a proliferation index of less than 10%.

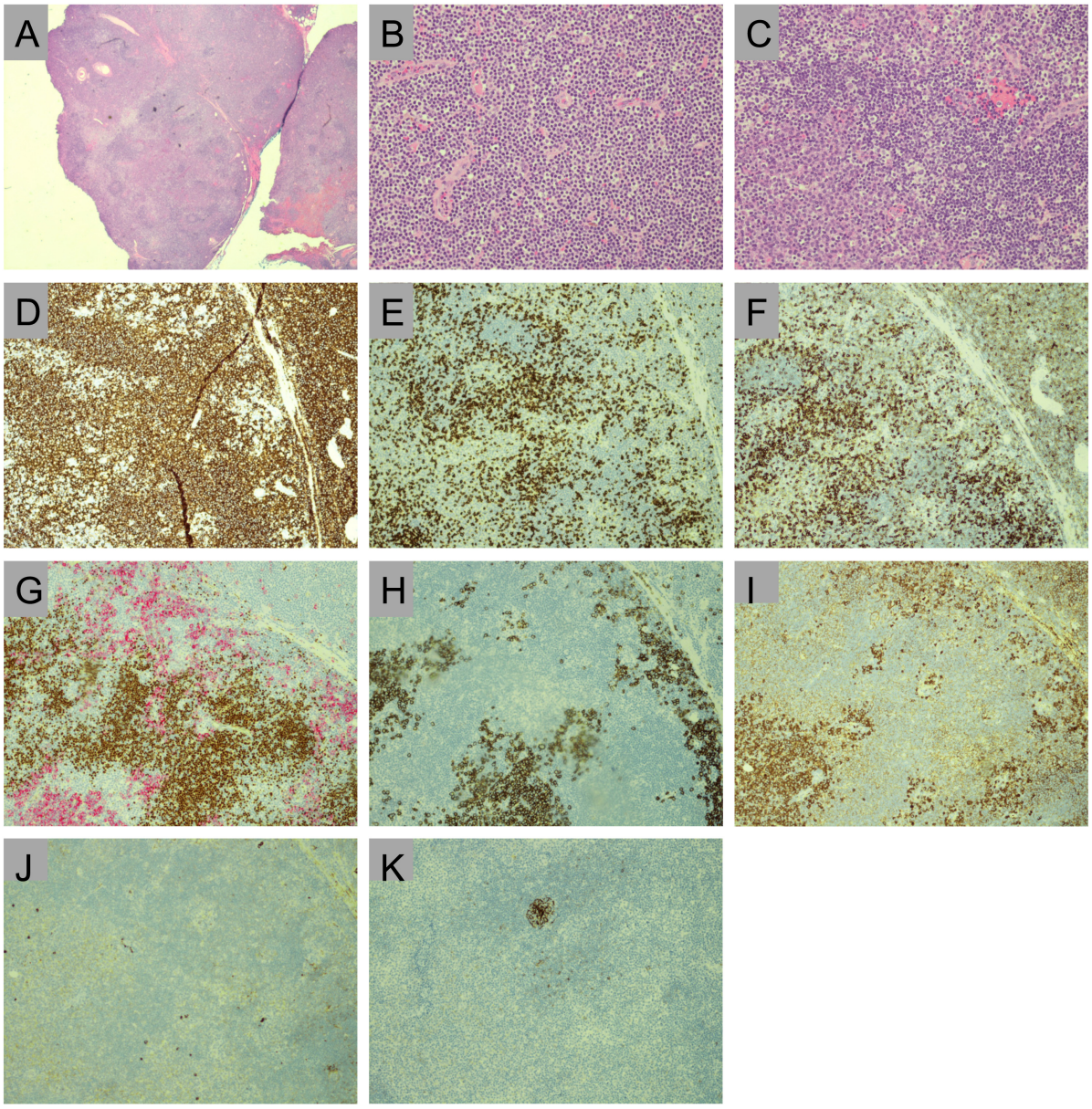

Figure 1: Histological and immunophenotypic findings in the excisional biopsy of the eyelid mass. A. H&E (low-power field, X20) shows diffuse proliferation of monotonous appearing small atypical lymphoid cells. B and C. H&E (high-power field, X200) show focal aggregates of plasma cells, surrounded by small atypical lymphoid cells. D. CD20 immunostain confirms the majority of the cells are B lymphoid cells. E and F. CD3 and CD5 immunostains show background scattered T lymphocytes. CD5 also stains weakly some B lymphoid cells. G: CD138 (membrane stain in red color) and Cyclin D1 (nuclear stain in brown color) double stain demonstrates that Cyclin D1 expression is not within plasma cells. The localization of Cyclin D1 positive cells is consistent with that of some B lymphoid cells. By comparison of CD5 and Cyclin D1 immunostain patterns, the Cyclin D1 positive cells are negative for CD5. H, I and J. CD138 immunostain highlights nodular aggregates of plasma cells, which are kappa-restricted by both kappa and lambda immunostains. K. CD23 immunostain shows residual follicular dendritic cell meshworks.

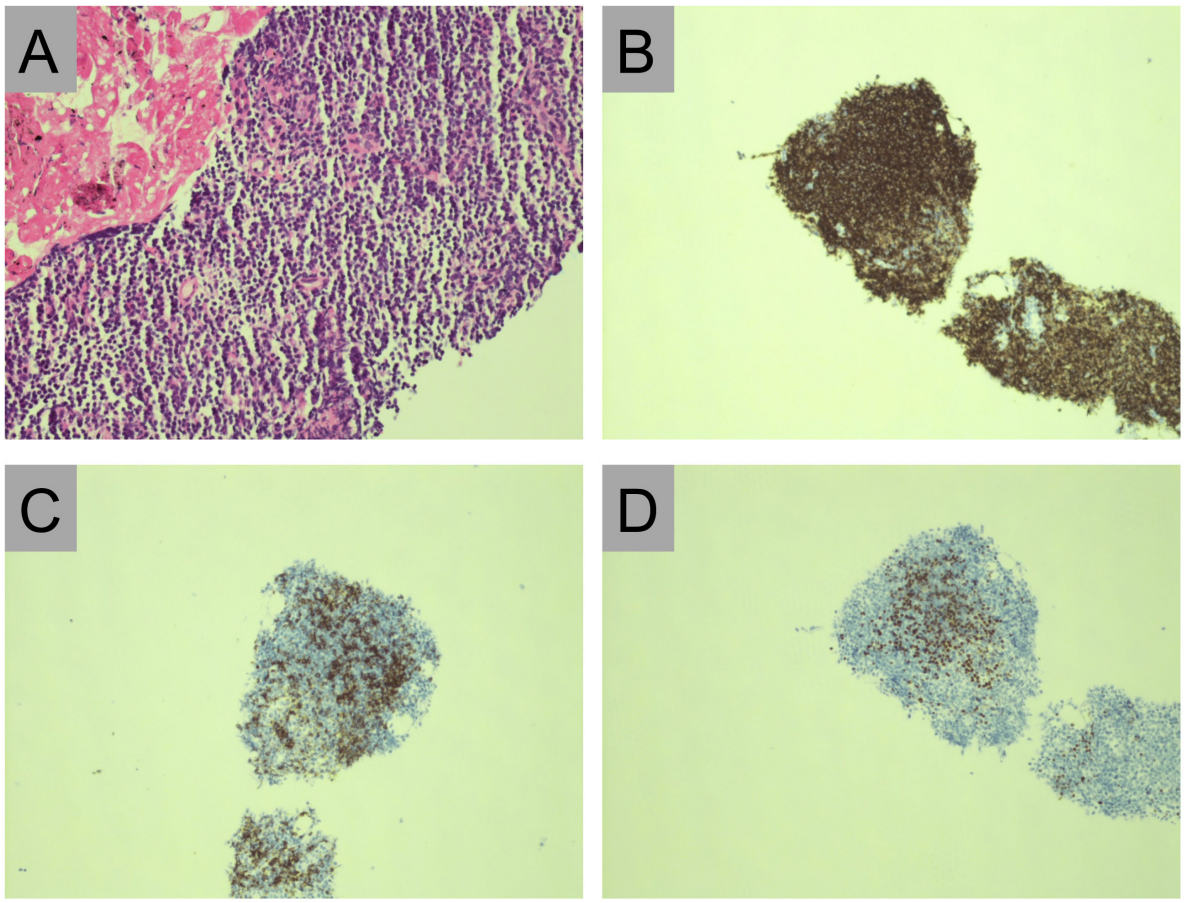

Figure 2: Histological and immunophenotypic findings in the needle core biopsy of renal mass. A. The needle core specimen contains scanty tissue. H and E (high-power field, X200) shows diffuse proliferation of small lymphoid cells. B. CD20 immunostain shows the predominance of B lymphoid cells. C. CD3 immunostain shows the admixed T lymphocytes. D. Cyclin D1 immunostain shows approximately 20% positive B lymphoid cells, consistent with mantle cell lymphoma component.

Lastly, the staging bone marrow biopsy demonstrates mildly hypercellular marrow with two small interstitial nodular lymphoid aggregates, which are predominantly composed of small lymphoid cells [Figure 3A]. Immunohistochemical work-up confirms the aggregates are mostly composed of B lymphoid cells [Figure 3B], which are partially positive for CD5 and negative for Cyclin D1 (rare cells with weak positivity). The corresponding flow cytometric analysis demonstrates the presence of kappa restricted monotypic B cell population with partial weak CD5 expression consistent with B cell lymphomatous involvement, which accounts for 5% of the total marrow cellularity.

Molecular and Cytogenetics Findings

B cell immunoglobulin heavy chain gene rearrangement PCR analysis performed on the eyelid mass specimen shows clonal amplification, confirming the presence of B cell lymphoma. Three of the five reactions targeting immunoglobulin heavy chain gene and the two reactions for the light chain genes demonstrate two to three clonal peaks, most com-

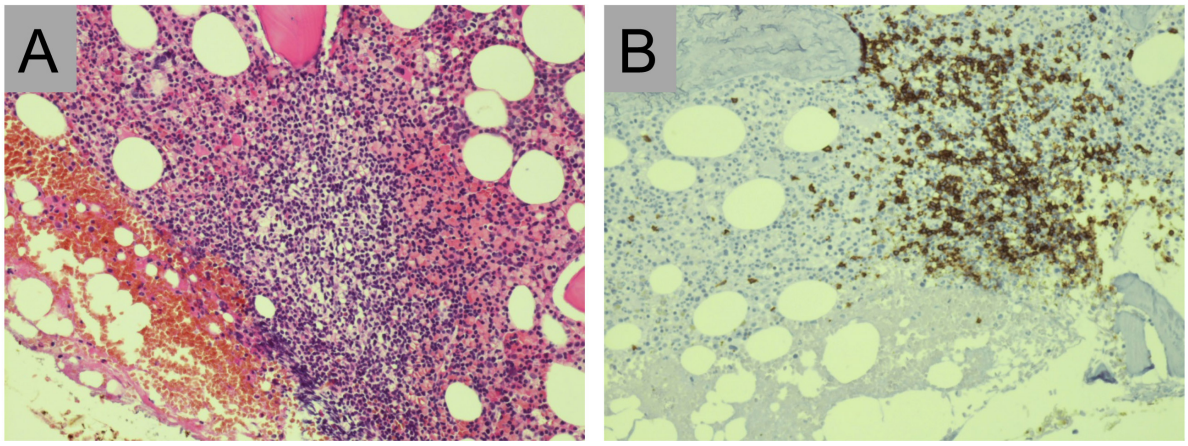

Figure 3: Histological and immunophenotypic findings in the staging bone marrow biopsy. A. H and E (high-power field, X200) shows an interstitial nodular lymphoid aggregate, which is predominantly composed of small lymphoid cells. B. CD20 immunostain shows the lymphoid cells are predominantly B lymphoid cells. The corresponding flow cytometric analysis of the bone marrow aspirate confirms the kappa restriction of the B lymphoid cells. The PCR analysis for t(11;14)(q13;q32) is positive, indicating the presence of mantle cell lymphoma component.

patible with biclonal origin of the two lymphoma components [Figure 4A and 4B]. In addition, PCR analysis for t(11;14)(q13;q32) demonstrates positive BCL1/JH t(11;14)(q13;q32) gene rearrangement in both eyelid mass and staging bone marrow specimens.

Further FISH analysis performed on the eyelid mass specimen was reported as 17% cells demonstrating typical abnormal signal patten of IgH and *CCND1* gene rearrangement, which indicates the presence of t(11;14) translocation, associated with mantle cell lymphoma, in a small population of lymphoma cells.

Discussion

Composite lymphomas comprising two histologically indistinguishable but immunophenotypically different small B cell lymphomas are very rare. The reported composite small B cell lymphomas are commonly composed of follicular lymphoma and another type of B cell lymphomas - often chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and occasionally mantle cell lymphoma. Recently, a study containing eleven composite mantle cell lymphoma and CLL/SLL cases was reported [10]. Among these cases, ten cases show nodal composite lymphomas, and the mantle cell lymphoma components are all conventionally CD5 positive. To date, only three cases of composite mantle cell lymphoma and marginal zone lymphoma were reported [7, 8, 11], with two cases composed of nodal marginal zone lymphoma and mantle cell lymphoma and one case of composite splenic marginal zone lymphoma and mantle cell lymphoma. In contrast, our case demonstrates an extranodal composite mantle cell and marginal zone lymphoma, with two lymphoma components intermingled with each other. Imaging study revealed that this composite lymphoma involves eyelid soft tissue, lung and kidney - the sites predisposed to acquisition of acquired lymphoid neoplasms upon appropriate antigen stimulation.

There are several diagnostic challenges in the composite lymphoma case described here. First, morphologically the diffuse proliferation of monotonous small atypical B lymphoid cells, in conjunction with the scattered nodular aggregates of plasma cells, is

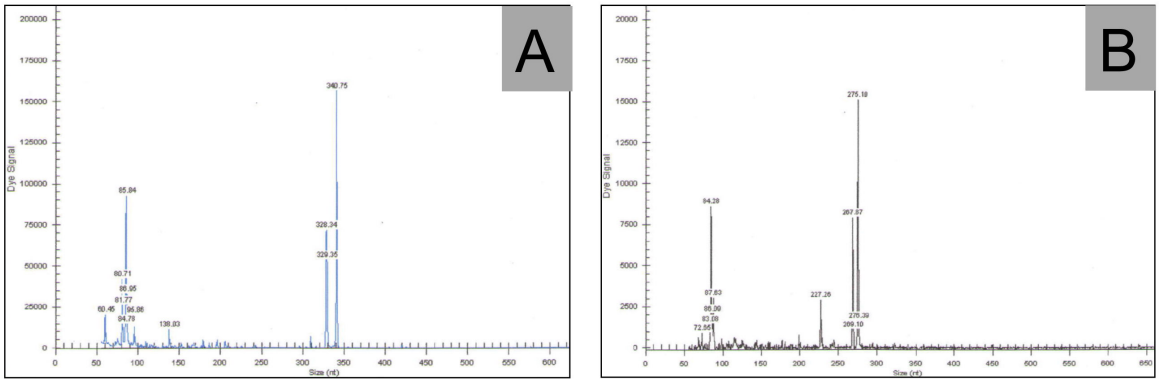

Figure 4: Molecular analysis results. Representative results for two pairs of primers targeting immunoglobulin heavy chain gene are shown. Both demonstrate at least two clonal peaks.

reminiscent of marginal zone lymphoma with plasmacytic differentiation. However, the focal weak CD5 expression by B lymphoid cells raises the possibility of atypical CLL/SLL. Given the lack of CD23 expression by the B lymphoid cells, the presence of monocytoid B-cells with background monoclonal plasma cells and extensive extranodal involvement, a definitive diagnosis of marginal zone lymphoma component with plasmacytic differentiation is indicated. Secondly, the focal strong Cyclin D1 expression within the B lymphoid cells indicates the presence of immunophenotypically distinct second B cell lymphoma population. The Cyclin D1 positive B lymphoid cells are entirely CD5 negative. Thus, based on the immunohistochemical profile, the differential diagnoses include CD5 negative mantle cell lymphoma (so called "marginal zone variant" of mantle cell lymphoma) versus marginal zone lymphoma with focal Cyclin D1 expression. Cyclin D1, as an important component of cell cycle and potential oncogenic protein, is expressed in a number of B cell lymphomas. Real-time quantitative polymerase chain reaction (RT-PCR) of *CCND1* (the gene encoding Cyclin D1) RNA expression demonstrates that mantle cell lymphoma has the highest level of *CCND1* expression while the other B cell lymphomas have the moderate *CCND1* expression [12]. The translocation abnormality t(11;14)(q13;q32), which involves the *CCND1* breakpoint and leads to overexpression of Cyclin D1, is detected with great incidence (>95%) in mantle cell lymphoma by fluorescence in situ hybridization (FISH) [13]. With the exclusion of plasma cell neoplasms that sometimes harbor t(11;14)(q13;q32), t(11;14) appears to be very specific for differentiating mantle cell lymphoma from other mature B cell neoplasms, based on the observations that some B cell lymphomas such as hairy cell leukemia show elevated levels of *CCND1* mRNA and protein but with the absence of gene rearrangements involving *CCND1* locus [14]. As illustrated in our case, the positive t(11;14) FISH analysis signals detected in 17% cells eventually enables the second component of this composite lymphoma to be distinguished from the marginal zone lymphoma.

Literature review shows two case reports of CD5 negative mantle cell lymphoma resembling extranodal marginal zone lymphoma of mucosa-associated lymphoid tissues, which pose diagnostic dilemmas [15, 16]. In both cases, the patients were initially diagnosed with CD5 negative marginal zone lymphomas, which appeared to be incompatible with the subsequent clinical courses, leading to the repeated tissue examinations that reveled CD5 negative mantle cell lymphomas. The accurate diagnosis

of mantle cell lymphoma component in marginal zone lymphoma has prognostic and therapeutic implications, particularly when considering the therapeutic targets. In our composite lymphoma case, the patient presents large renal mass, multiple lung lesions and positive staging bone marrow, indicating an aggressive clinical course that necessitates an appropriate clinical management.

The immunoglobulin gene rearrangement analysis results demonstrate presence of two to three clonal peaks in multiple reactions, which is highly suggestive of presence of two clonal processes. Theoretically, mantle cell lymphoma cells are derived from mature B cells with unmutated variable-region genes that are not related to geminal centers or post-germinal centers; while the putative cell origins of marginal zone lymphoma are memory B cells of post germinal centers. In fact, all B-cell non-Hodgkin lymphomas, with the exception of majority of the mantle cell lymphoma and some CLL/SLLs, carry hyper mutated IgV genes [17]. The difference in cell origin may explain that all three reported cases of composite marginal zone lymphoma and mantle cell lymphoma demonstrated two independent tumor clones.

In summary, we report a unique case of composite extranodal marginal zone lymphoma and mantle cell lymphoma with aberrant expression of CD5 in the marginal zone lymphoma while loss of CD5 positivity in the mantle cell lymphoma component, highlighting the importance of a systemic approach in evaluating lymphomas.

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