Immunoglobulin Variable Heavy Chain Somatic Hypermutation Testing in a Patient with Small Lymphocytic Lymphoma and Multiple Myeloma

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CASE DESCRIPTION

The patient is a 54-year-old man who was initially diagnosed with small lymphocytic lymphoma (SLL) 4 years earlier, presenting now with an increased sense of fatigue. At diagnosis, his symptoms were limited to self-noted inguinal lymphadenopathy and back pain. Complete blood counts were within normal range. Computed tomography (CT) scans of the abdomen and pelvis revealed widespread lymphadenopathy involving the mesenteric, retroperitoneal and inguinal lymph node (LN) groups. Subsequent inguinal LN biopsy showed histopathology consistent with SLL/chronic lymphocytic leukemia (CLL). This diagnosis was based on the morphologic features of the clonal appearing cells and the expression of CD5, CD23, and lambda immunoglobulin light-chain (LC) restriction on the CD20-positive lymphocytes. No cytogenetic abnormalities were noted by fluorescence in situ hybridization testing. CT scans of the chest also showed mediastinal, paraesophageal, and axillary lymphadenopathy.

During the current evaluation, CT scans of the chest and abdomen were performed and revealed widespread lymphadenopathy involving multiple LN groups above and below the diaphragm and massive confluent mesenteric and retroperitoneal adenopathy with longest dimensions measuring 19 cm and 22 cm, respectively. Positron emission tomography (PET)/CT scan images also showed markedly increased fluorodeoxyglucose avidity in all enlarged LNs with highest uptake noted in the retroperitoneal group. A core needle biopsy was obtained from the most fluorodeoxyglucose-avid retroperitoneal node. Histopathologic features were consistent with SLL/CLL with no evidence of large cell transformation (Fig. 1).

Serum chemistries revealed markedly elevated total serum protein levels and normal albumin levels, which prompted additional workup including serum protein electrophoresis to assess for the presence of paraproteinemia. A large 4.8 g/dL M-spike was found and typed as IgG-kappa by immunofixation. As the patient's SLL cells were lambda LC restricted, these findings were therefore

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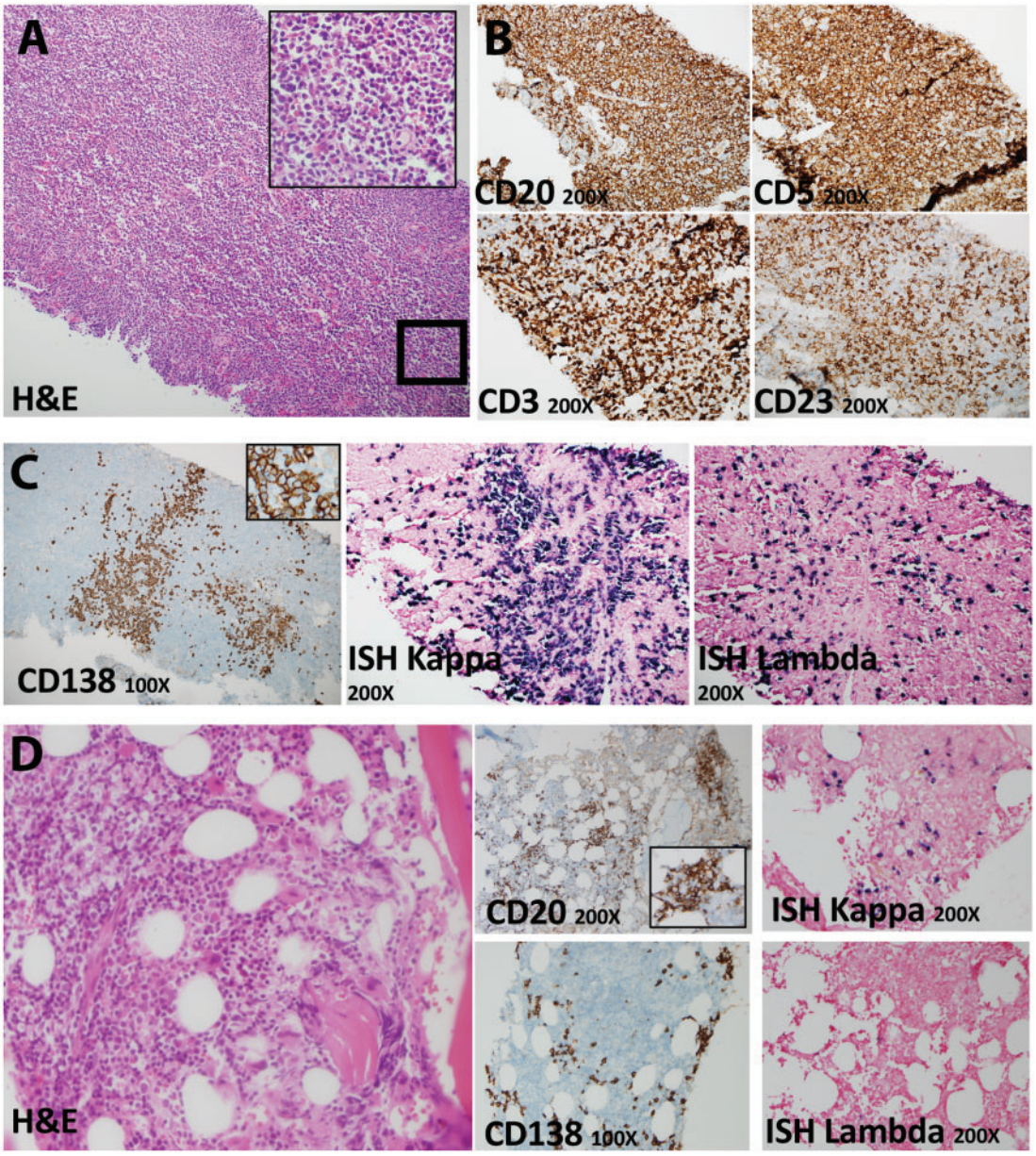

Fig. 1. **(A) Hematoxylin and eosin stain of retroperitoneal LN core needle biopsy section showing diffuse clonal appearing small-sized lymphocytes. Square is magnified region with increased plasma cells. (B) Immunophenotyping of LN lymphocytes. Note positivity for CD5 and CD23 in CD20-positive regions. (C) Areas of increased CD138-positive plasma cells in the LN tissue and corresponding regions of increased Kappa LC detected by in situ hybridization. (D) Hematoxylin and eosin stain of bone marrow core biopsy section showing infiltrates small-sized lymphocytes. Immunostains of bone marrow sections showing aggregates of CD20-positive lymphocytes and increased CD138-positive plasma cells. Note Kappa LC restriction of the increased bone marrow plasma cells.**

suggestive of the presence of a separate clonal process, likely a plasma cell dyscrasia given the high concentration of the paraproteinemia. A bone marrow aspirate and biopsy were then obtained, which revealed about 15% involvement by clonal kappa LC-restricted CD138-positive plasma cells (Fig. 1) and nearly 30% involvement by nonparatrabecular lymphoid aggregates. Flow cytometric analysis was consistent with lambda LC-restricted SLL phenotype and additionally demonstrated high expression of the adverse prognostic markers CD38 and ZAP70 (70% and 64%, respectively). Importantly, there was no radiologic or clinical evidence of lytic bone lesions or end-organ damage. These findings were therefore consistent with a smoldering multiple myeloma (MM) diagnosis and also suggest the co-occurrence of 2 distinct malignant clones.

Analysis of immunoglobulin variable heavy chain (*IGHV*) somatic hypermutation in the bone marrow by next-generation sequencing detected 2 different clonal *IGHV* rearrangements based on their relative abundance with 1 unmutated and 1 mutated (64.62% and 4.79% of total merged reads, respectively) (Fig. 2). However, at that point it was not possible to definitively attribute either clonal *IGHV* rearrangements to the SLL/CLL or myeloma processes. In an attempt to delineate this finding, similar analysis was performed on genomic DNA extracted from the biopsied retroperitoneal LN. The unmutated *IGHV* clonal rearrangement, which had been found in higher abundance in the bone marrow, was the sole rearrangement detected above the threshold for positivity, hence indicating that this clone is representative of CLL cells (Fig. 2). It is important to note that the second clone identified in the bone marrow was also found in the LN, but at an abundance level less than the validated reportable cutoff. These findings reflect the different abundance of CLL and myeloma plasma cells in LN and bone marrow. Intriguingly, increased plasma cells were also noted in LN biopsy with predominant kappa LC restriction as highlighted by messenger RNA in situ hybridization. Taken together, the concurrent *IGHV* hypermutation analysis of the bone marrow and LNs tissues enabled the identification of the molecular *IGH* clones of SLL and myeloma disease in our patient.

DISCUSSION

The coincidence of SLL/CLL and MM is relatively rare. In 1 study, only 28 patients concurrently had SLL/CLL and MM among 10 725 MM and 7700 CLL patients (1). Importantly, there were no differences in overall survival or causes of death in MM patients with or without SLL/CLL. However, the same study estimated that prevalence of CLL is higher in MM patients (0.26%) compared to the general population (0.05%). This raises the intriguing questions of whether both tumors could arise from the same progenitor cells or represent independent B-cell clones and if they could be driven by shared or distinct genetic alterations and/or antigenic exposures. In 1 study relying on *IGHV* mutation status, single-nucleotide polymorphism arrays to detect chromosomal copy number changes and fluorescence in situ hybridization studies, biclonal origin for CLL and MM was established in 4 out 5 patients (2). Interestingly, MM patients treated until negative for minimal residual disease were still identified to have somatic mutation in CD34-positive lymphopoietic progenitors, implying that such mutations may predispose to B-cell and plasma cell oligoclonality susceptible to subsequent secondary oncogenic genetic events (3).

The early discovery that somatic hypermutation of *IGHV*, defined as ≥2% difference from the closest germline reference, is robustly associated with better outcomes and durable responses to chemoimmunotherapy than unmutated *IGHV* in CLL patients, and the adoption of *IGHV* sequencing methods as a prognostic marker for CLL led to

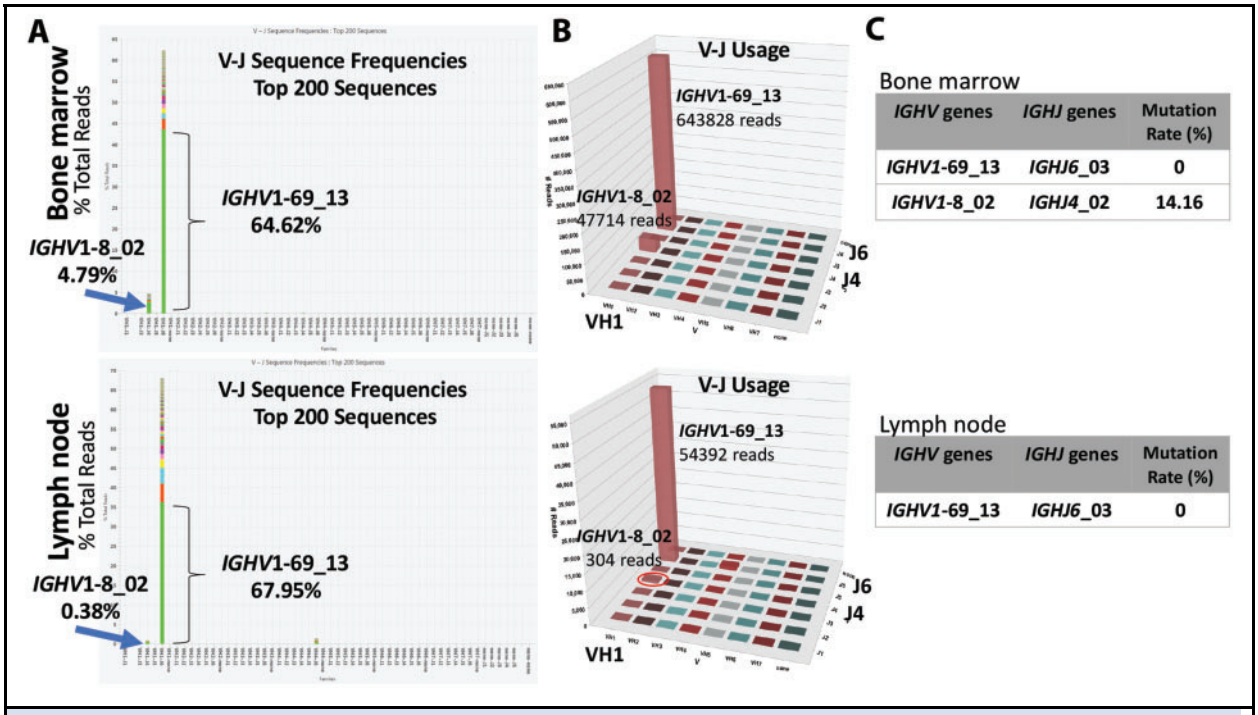

Fig. 2. (A) Charts generated by the LymphoTrack® (Invivoscribe, Inc.) clonality analysis software representative of the top 200 identified V-J sequence frequencies based on percentage total reads. Top row is bone marrow, and bottom is LN. (B) Bar charts of the number of reads for sequenced V-J sequences, generated by the LymphoTrack® Software (bone marrow top; LN bottom). (C) Table of reported (>2.5% of total reads) clonal V-J rearrangements in bone marrow and LN. The *IGHV1-69_13* and *IGHV1-8_02* represent SLL and MM clones, respectively.

the generation of large amounts of immunoglobulin sequencing data from CLL patients (4). Later on, it was realized that the usage of *IGHV* genes is biased in about one third of unrelated CLL patients, further enabling the grouping of these patients into major distinct subsets based on the relatedness of their heavy chain rearrangements (5). Interestingly, at least four of these SLL/CLL subsets were shown to be of different clinical and biological disease features. Adding to that, B-cell receptor of mutated and unmutated CLL cells have different binding affinities to autoantigens and microbial antigens (6). In our patient, the combined *IGHV1-69* and *IGHJ6* rearrangement has been reported to be highly prevalent in unmutated CLL and with no identified similar sequence counterparts in normal B-cell repertoires, further supporting its CLL origin (7). These findings argue that common antigenic pressure may shape and promote tumor development in a subset of patients. Nevertheless, autonomous B-cell receptor signaling also plays a role in CLL pathogenesis, which is the basis for some novel therapies such as Bruton's tyrosine kinase and phosphatidylinositol 3-kinase inhibitors. An example from a recent study that identified a point mutation (R110) in the *IGLV3-21* gene characteristic of the B-cell receptor subset 2 patients leading to autonomous activation and was found to be associated with poor prognosis independent of *IGHV* mutational status (8). On the myeloma side, LC variable gene sequencing of flow cytometry sorted

single-cell myeloma and normal B cells showed that the clonal myeloma gene rearrangements extend to a small population (about 1.2%) of normal phenotype B-cells, in agreement with previous reports (9). Whether these cells represent a reservoir of cancer stem cells remains unclear. Interestingly, a recent study also demonstrated biased *IGHV* gene usage in myeloma plasma cells and significantly prolonged progression-free survival associated with certain *IGHV* rearrangements and higher somatic hypermutation (10).

The current International Workshop on CLL guidelines only recommend assaying the somatic hypermutation status of *IGHV* for prognostication purposes. However, this test also provides clinicians with a highly sensitive tool to track clonal disease progression, response to therapy, and detection of minimal residual disease. In our CLL/MM patient, *IGHV* somatic hypermutation analysis identified the presence of the 2 distinct clones, and the concurrent bone marrow and LN analyses enabled deliniation of MM and CLL clones, thus providing the treating clinican with highly sensitive tool to track both disease clones.

TAKEAWAYS

- The co-occurrence of SLL/CLL and MM is uncommon.
- *IGHV* hypermutation remains a robust prognostic indicator in SLL/CLL.
- A recent study suggests that hypermutation may be a favorable prognosticator in subgroups of MM patients.
- The correlation of histopathologic features with the abundance and clonotype of identified V-J rearrangements may suggest which clonal *IGH* rearrangements represent concurrent CLL and MM processes.

In summary, *IGHV* hypermutation testing is an established prognostic marker in SLL/CLL and can also be a useful to tool to identify and monitor B-cell clones through disease course.

Nonstandard Abbreviations: SLL, small lymphocytic lymphoma; CT, Computed tomography; LN lymph node; CLL, chronic lymphocytic leukemia; LC, light-chain; PET, Positron Emission Tomography; MM, multiple myeloma.

Human Genes: immunoglobulin heavy chain variable region, IGHV; Immunoglobulin heavy chain region IGH

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