

AMP case report: Role of lymphoma sequencing panel in diagnosis of pediatric-type follicular lymphoma

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November 2020—Pediatric-type follicular lymphoma (PTFL) is a rare form of lymphoma that was recognized as a new diagnostic entity in the revised 2016 *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. The classic features of PTFL include male predominance, localized stage I lymphadenopathy, blastoid morphology, high proliferation index, and exceedingly good response rate to local excision.¹ Because PTFL patients have an excellent prognosis, accurate and reliable identification of PTFL is important to minimize the prospect of unnecessary treatment.

Here we report an uncommon PTFL case that was diagnosed initially as “follicular lymphoma, WHO grade 1–2 of 3, with follicular growth pattern.” Using a massively parallel (next-generation) lymphoma sequencing panel, we were able to identify two missense variants in *MAP2K1* and *TNFRSF14*. Although these variants are not unique to PTFL and have been reported in rare cases of other B-cell lymphomas, they provide an objective parameter to support the final diagnosis of PTFL.²

Case presentation. A 22-year-old male with no significant past medical history presented with a two-month history of painless swelling of the left neck. Physical examination showed a localized, rubbery, and non-tender left cervical nodule. CT imaging studies demonstrated a 1.8 × 1.6 × 2.4 cm solid mass in the upper left neck superficial to the sternocleidomastoid muscle. A PET/CT detected two hypermetabolic left cervical lymph nodes. An ultrasound-guided fine-needle aspiration biopsy of the mass showed an increased proportion of medium-sized to large lymphocytes in a background of small lymphocytes, and flow cytometry demonstrated a CD10+ CD5– mature B cell neoplasm. The patient subsequently underwent an excisional left cervical lymph node biopsy. Gross examination revealed a 3.5 × 1.7 × 0.6 cm tan-white soft tissue fragment, which was consistent with a lymph node with tan-pink cut surfaces.

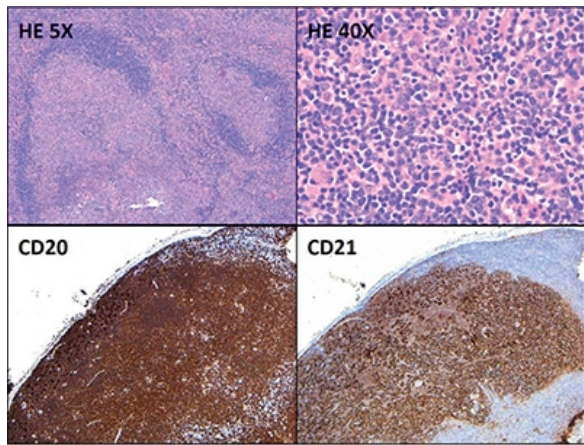


Fig. 1. HE 5x: The lymph node architecture is subtotally effaced by large irregular and expansile follicles (5x objective, x50 total magnification). HE 40x: The germinal centers are composed predominantly of small to medium-sized centrocytes and few larger cells morphologically compatible with centroblasts (40x objective, x400 total magnification). The majority of the cells in the follicles are positive for CD20 (5x objective, x50 total magnification). CD21 highlights expanded and disrupted follicular dendritic cell meshworks associated with the follicles (5x objective, x50 total magnification).

Hematoxylin-and-eosin-stained sections showed profiles of a lymph node with subtotal effacement of the architecture by a nodular infiltrate composed predominantly of small to medium-sized lymphoid cells with ovoid to irregular nuclei, clumped chromatin, inconspicuous nucleoli, scant cytoplasm, and an overall centrocytic morphology. Larger lymphoid cells morphologically compatible with centroblasts accounted for <15/high-power field within the nodules. No diffuse areas, sheets of large cells, or necrosis were seen. Immunohistochemistry showed that the majority of the cells within the nodules were positive for CD20, PAX5, CD10, BCL6 (partial), and BCL2 (partial, weak) (**Figs. 1 and 2**). CD21 and CD23 highlighted expanded and disrupted follicular dendritic cell meshworks. The proliferative index (Ki-67) was 20 to 30 percent within the nodules and 10 to 20 percent in the internodular areas. CD3 and CD5 highlighted an abundant number of small background T cells. Flow cytometry performed on a portion of the lymph node identified a surface lambda-restricted CD19+, CD20+, CD10+, and CD5-mature B cell population (11 percent of total events) (**Fig. 3**).

Fluorescence in situ hybridization was negative for *BCL2* and *BCL6* rearrangements and 1p36 deletion. A lymphoma sequencing panel (Illumina TruSight Sequencing Panel), designed and validated at the Center for Personalized Diagnostics at the University of Pennsylvania, was used for sequence analysis of 40 genes commonly altered in lymphomas (*ATM*, *B2M*, *BIRC3*, *BRAF*, *BTB*, *CARD11*, *CD79A*, *CD79B*, *CIITA*, *CREBBP*, *CXCR4*, *EGR2*, *EZH2*, *GNA13*, *ID3*, *IDH2*, *JAK3*, *KLF2*, *MAP2K1*, *MYD88*, *NFKBIE*, *NOTCH1*, *NOTCH2*, *PLCG1*, *PLCG2*, *POT1*, *RHOA*, *RPS15*, *RRAGC*, *SF3B1*, *SOC3*, *STAT3*, *STAT5B*, *TCF3*, *TET2*, *TNFAIP3*, *TNFRSF14*, *TP53*, *TRAF3*, *XPO1*). Two missense variants were identified: *MAP2K1* (NM_002755.3) c.308T>A, p.I103N (16 percent variant allele frequency) and *TNFRSF14* (NM_003820.3) c.362G>A, p.C121Y (14 percent variant allele frequency) (**Fig. 4**). No variants were detected in any of the other 38 genes included in this panel.

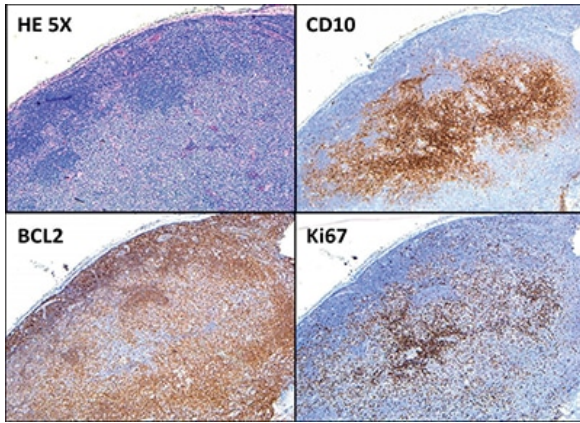


Fig. 2. The B cells within the follicles are positive for CD10 and BCL2 (partial, weak). The proliferative index (Ki-67) is 20 to 30 percent within the nodules and 10 to 20 percent in the internodular areas (all 5× objective, ×50 total magnification).

Discussion. Recognized as a distinct diagnostic entity in the revised 2016 WHO classification, PTFL is a localized germinal-center-derived B cell neoplasm and has clinical, histopathologic, and genetic features distinct from typical follicular lymphoma. PTFL mainly affects young males and its prognosis is excellent. Studies have shown that local excision of PTFL diagnosed in children may be sufficient treatment, with no evidence of subsequent progression or relapse.¹ Therefore, it is important to distinguish PTFL from typical follicular lymphoma to avoid unnecessary treatment.

Morphologically, the architecture of the involved lymph nodes is totally or subtotally effaced by large expansile follicles, often with a serpiginous growth pattern. The neoplastic follicles usually show a starry-sky pattern and thin or absent mantle zones.³ On high-power magnification, PTFL follicles consist of a monotonous population of intermediate-sized blastoid cells with frequent mitotic figures and inconspicuous nucleoli. Neoplastic cells in PTFL are usually positive for germinal center markers BCL6 and CD10, and negative or weakly positive for BCL2. The proliferative index (Ki-67) is usually high (greater than 30 percent). There is significant histologic and immunophenotypic overlap between PTFL and typical follicular lymphoma, sometimes making it difficult to differentiate between these two entities. In such situations, genetic studies may be helpful.

At the genetic level, in contrast to typical follicular lymphoma, PTFL lacks rearrangements of *BCL2*, *BCL6*, *IRF4*, or *IGH*, or amplification of *BCL2*, and has a near absence of mutations in epigenetic modifiers. The two most frequently mutated genes in PTFL are *MAP2K1* and *TNFRSF14* (observed in 43 percent and 29 percent of PTFL, respectively), whereas variants in epigenetic modifiers commonly found in follicular lymphoma (e.g. *KMT2D*, *CREBBP*, *EZH2*) are rare.^{1,4} In our case, no variants were identified in epigenetic modifiers such as *EZH2* and *CREBBP*, which are commonly mutated in follicular lymphoma.¹

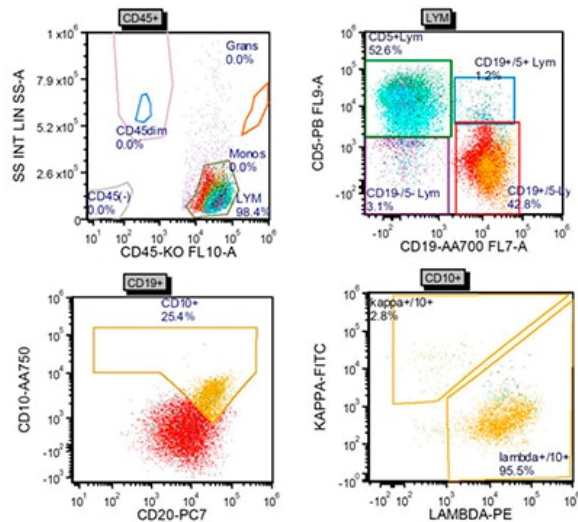


Fig. 3. Flow cytometry performed on a portion of the lymph node identified a surface lambda-restricted CD19+, CD20+, CD10+, and CD5-mature B cell population.

Located on chromosome 1p36.32, *TNFRSF14* encodes a member of the TNF (tumor necrosis factor) receptor superfamily. The encoded protein functions in signal transduction pathways that activate inflammatory and inhibitory T cell immune response. As one of the most commonly mutated genes in PTFL, *TNFRSF14* variants are also frequently seen in typical follicular lymphoma (www.genecards.org).⁵ The observed *TNFRSF14* variant (c.362G>A, p.C121Y) in our case has been described previously in other typical follicular lymphoma patients.⁶

MAP2K1 is located on chromosome 15q22.31 and its encoded protein is a member of the dual specificity protein kinase family, which acts as a mitogen-activated protein (MAP) kinase. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), act as an integration point for multiple biochemical signals. As an essential component of MAP kinase signal transduction pathway, this kinase is involved in many cellular processes, such as proliferation, differentiation, and transcription regulation and development (www.genecards.org).⁵ *MAP2K1* variants have been described as driver mutations in hairy cell leukemia variant, conventional hairy cell leukemia, Langerhans cell histiocytosis, and less frequently chronic lymphocytic leukemia, splenic marginal zone lymphoma, and splenic diffuse red pulp lymphoma.⁷ However, *MAP2K1* alterations, to the best of our knowledge, have not been evaluated previously in typical follicular lymphoma cases.

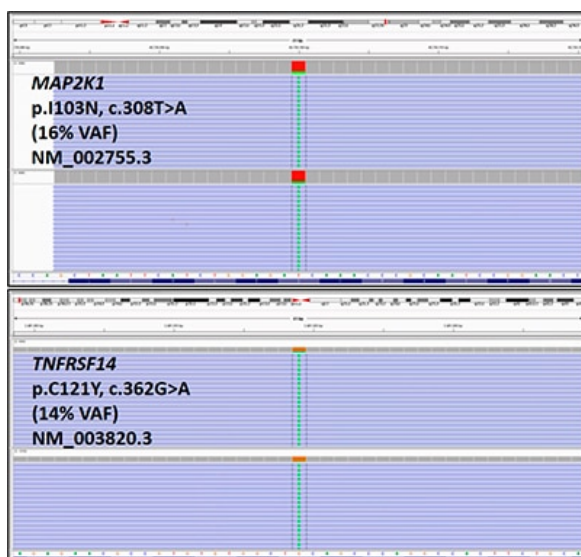


Fig. 4. *MAP2K1* and *TNFRSF14* variants were identified using a lymphoma sequencing panel (see text for additional assay details). The variants are displayed using the Integrative Genomics Viewer.¹¹ Reference DNA and protein sequences are displayed on the bottom of each figure. Sequencing reads are displayed as individual purple bars, with the altered base pairs displayed as green “A”s in the center of the reads. The lymphoma sequencing panel used is a dual pool assay, and both pools displayed each variant. (VAF: variant allele frequency)

The *MAP2K1* variant identified in the index case (p.I103N, c.308T>A) lies within the protein kinase domain of the *MAP2K1* protein and is predicted to confer a gain of function as demonstrated by increased *MAP2K1* auto-phosphorylation in cell culture.⁸ This *MAP2K1* variant has been reported previously in classical hairy cell leukemia.^{9,10} In addition, a *MAP2K1* variant on the same codon (103) has been detected in one other case of PTFL.⁷ Based on these lines of evidence, this variant is likely disease-associated in the setting of this patient’s PTFL.

Conclusion. The absence of *BCL2* and *BCL6* rearrangements and variants in epigenetic modifiers, the identification of *MAP2K1* and *TNFRSF14* mutations, and the clinical presentation (young male with cervical lymphadenopathy) all contributed in this case to the refinement of the initial diagnosis of typical follicular lymphoma to PTFL. This report emphasizes the importance of gene sequencing studies in the diagnostic workup of clinically challenging lymphoma cases.

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Dr. Yang was a hematopathology fellow (at the time the case report was written), Dr. Caponetti is assistant professor of clinical pathology and laboratory medicine, Dr. Roth is assistant professor of clinical pathology and laboratory medicine, Dr. Elenitoba-Johnson is the Peter C. Nowell, MD, professor and director of the Center for Personalized Diagnostics and of the Division of Precision and Computational Diagnostics, and Dr. Lim is professor of pathology and laboratory medicine and director of hematopathology—all at the University of Pennsylvania Perelman School of Medicine and the Hospital of the University of Pennsylvania. Dr. Yang is now an instructor, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School.

Test yourself

- Here are three questions taken from the case report.
- Answers

1. Which statement is true about pediatric-type follicular lymphoma?

- a. Its treatment usually involves only local excision.
- b. Its prognosis is usually poor.
- c. It usually harbors *BCL2* and *BCL6* rearrangements.
- d. It mainly affects females.

2. Which of the following hematopoietic neoplasms commonly have *MAP2K1* variants as driver mutations?

- a. Hairy cell leukemia variant.
- b. Langerhans cell histiocytosis.
- c. Hairy cell leukemia.
- d. All of the above.

3. Which statement is false about *TNFRSF14*?

- a. It is one of the most common variants observed in PTFL.
- b. Its encoded protein is a member of the TNF (tumor necrosis factor) receptor superfamily.

- c. Its variants are specific for PTFL.
- d. Its encoded protein activates the inflammatory and inhibitory T cell immune response.

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