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PATHOLOGY ♦ LABORATORY MEDICINE ♦ LABORATORY MANAGEMENT

Large B-cell lymphoma with *IRF4* rearrangement of retroperitoneal lymph node in an elderly male with concomitant high-grade B-cell lymphoma without *IRF4r* masquerading as a gastric ulcer

CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from Henry Ford Health. If you would like to submit a case report, please send an email to the AMP at amp@amp.org. For more information about the AMP and all previously published case reports, visit www.amp.org.

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Large B-cell lymphoma with *IRF4* rearrangement (LBCL-*IRF4r*) was recognized as a provisional entity in the revised fourth edition of the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* in 2017.[1] This condition is also now acknowledged as a distinct entity in the International Consensus Classification. It predominantly occurs in children and young adults. However, *IRF4* rearrangements are also found in about two percent of adult-onset large B-cell lymphomas. The clinical, pathological, immunophenotypic, and cytogenetic characteristics of older patients with LBCL having concurrent *IRF4* rearrangement have not been well described.[2,3] Here we present a case of an elderly male with LBCL-*IRF4r* of retroperitoneal lymph node as well as a concomitant large B-cell lymphoma without *IRF4* rearrangement presenting as a gastric ulcer, and we discuss the molecular genetic alterations seen in this rare entity using next-generation sequencing. This case explores the complexity of molecular pathogenesis of this rare entity.

Case. A 74-year-old male with a history of smoking for more than 50 pack-years had a screening CT chest for lung cancer, which showed emphysema, right upper lobe nodules, and pleural effusion. Right thoracentesis yielded 1,250 mL bloody fluid, exudate, lymphocyte predominant, with negative cytology. Because of these findings, a right video-assisted thoracoscopic surgery, total decortication, and mediastinal lymph node biopsy and pleural biopsy were done, which showed focal lymphohistiocytic inflammation.

PET scan showed hypermetabolic adenopathy in bilateral neck with small and mildly enlarged lymph nodes, mediastinal lymphadenopathy, axillary lymphadenopathy, splenomegaly, and multiple pleural-based densities in lungs, extensive on the right side with hypermetabolism, likely relating to malignancy. Axillary lymph node biopsy showed reactive lymphoid hyperplasia, fatty replacement, and black-colored pigment-laden histiocytes and negative for histopathologic evidence of lymphoma or metastatic neoplasm. CT showed an increase in the size and number of retroperitoneal lymph nodes, as compared with a previous scan, and were suggestive of malignant neoplasm.

Needle biopsy of retroperitoneal lymph node showed high-grade B-cell lymphoma, morphologically consistent with diffuse large B-cell lymphoma (DLBCL), germinal center type (CD10+, BCL6+, and MUM1+), as shown in **Fig. 1A–E** (next page). There were frequent mitoses and apoptotic bodies. MIB1 proliferation index was 90 percent. FISH was positive for *IRF4* gene rearrangement (55 percent). When the *IRF4* gene is intact, the 5'*IRF4* (red) and 3'*IRF4* (green) are close or together, so merged signal (yellow) or green and red signals very close to each other are seen. In this sample, isolated red signals were seen, indicating that the 5'*IRF4* is remaining but 3'*IRF4* is missing, and rearrangement was present, as shown in **Fig. 1F**. FISH analysis was performed for *IGH* (14q32) but not for *IGK* or *IGL* (because they are not routinely performed in our lab, as they are less common compared with *IGH* rearrangement in B-cell lymphoma). An integrated diagnosis of large B-cell lymphoma with *IRF4* rearrangement was made. The tumor was negative for *MYC* and *BCL2* rearrangement.

By FISH analysis, 65 percent of cells showed two *IGH* probe signals and one *BCL2* probe signal, suggesting loss of *BCL2* (65 percent). Next-generation sequencing using a lymphoid neoplasm sequencing panel revealed tier 1/2 mutations in *MYD88* p.(Met232Thr) (AF=33.9 percent), *GNA13* p.(Trp110*) (AF=36 percent), and *MEF2B* p.(Tyr69His) (AF=35.3 percent). Variants of uncertain significance mutations were seen in *CARD11* p.(Lys215Gln) (AF=nine percent), *MYC* p.(His374Arg) (AF=34.7 percent), and *GNA13* p.(Asp155Gly) (AF=31.7 percent).

After two weeks, the patient underwent esophagogastroduodenoscopy for abdominal pain, which showed a nonbleeding ulcer. Biopsies of the gastric ulcer showed high-grade B-cell lymphoma, with features of DLBCL, germinal center phenotype, as shown in **Fig. 2A–E** (next page).The present tumor from the GI biopsy appeared to be immunophenotypically and genetically different from the previously diagnosed high-grade lymphoma diagnosed from the lymph node. Compared with the LBCL-*IRF4r* lymphoma, the gastric lymphoma did not express CD10 and MUM1. There were frequent mitoses and apoptotic bodies. The MIB1 proliferation index was greater than 90 percent. It was morphologically similar to the previously diagnosed lymphoma in the retroperitoneal lymph node.

FISH studies did not identify the previously detected *IRF4* gene rearrangement. It was negative for *MYC* gene rearrangement, but loss of *MYC* was present (60 percent). It was negative for *IGH*::*BCL2* gene rearrangement. It was positive for aneuploidy of *IGH* and *BCL2* (72.5 percent). It was positive for atypical *BCL6* gene rearrangements with loss of 5′*BCL6* (65 percent). The result was abnormal and indicated that about 65 percent of cells have atypical *BCL6* gene rearrangements; losses of chromosomes 6, 8, 14, and 18; as well as low-level gain of *IGH*. The *BCL6* result consisted of one or two copies of the 3′*BCL6* gene with loss of both 5′*BCL6* gene regions, possibly indicating involvement of one or both *BCL6* genes. These results suggested a hypodiploid cell population with unbalanced *BCL6* gene rearrangements. The patient underwent four cycles of R-CHOP treatment, and a subsequent PET scan revealed an overall improvement in the disease condition.

Discussion. Large B-cell lymphoma with *IRF4* rearrangement is a de novo mature B-cell lymphoma with follicular and/or diffuse growth pattern. It is defined by strong expression of *IRF4* (MUM1) usually due to an *IG*::*IRF4* translocation. These are rare tumors, constituting less than one percent of large B-cell lymphomas. It typically involves the cervical lymph nodes (Waldeyer's ring) and is less common in intestinal lymph nodes.[1,4] The immunophenotype of LBCL-*IRF4r* shows mature B cells that are positive for the protein encoded by the *IRF4* gene (MUM1), CD10, and BCL6. Gene expression profiling demonstrates a germinal center phenotype in most of the cases.[5]

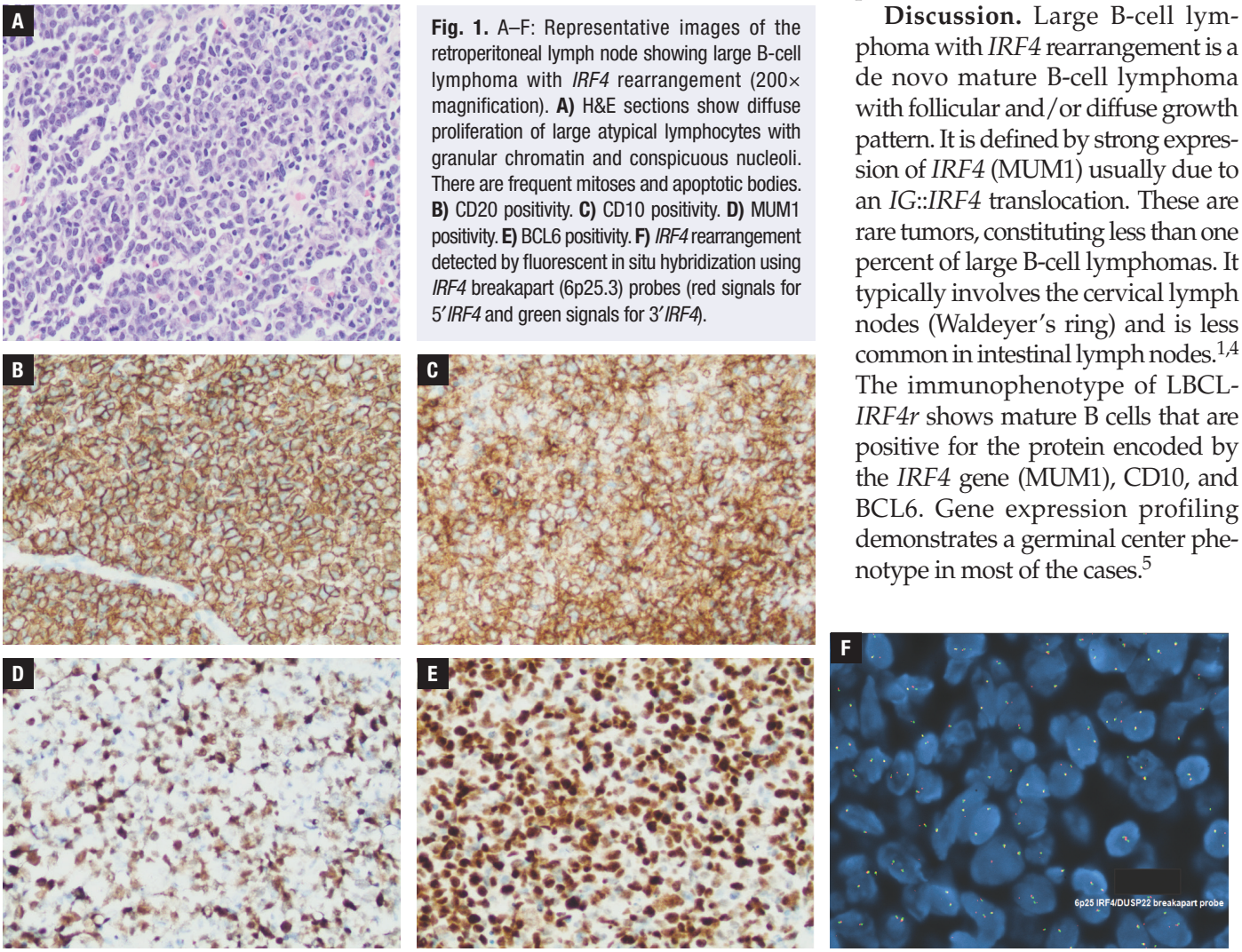

Fig. 1. A–F: Representative images of the retroperitoneal lymph node showing large B-cell lymphoma with *IRF4* rearrangement (200× magnification). **A)** H&E sections show diffuse proliferation of large atypical lymphocytes with granular chromatin and conspicuous nucleoli. There are frequent mitoses and apoptotic bodies. **B)** CD20 positivity. **C)** CD10 positivity. **D)** MUM1 positivity. **E)** BCL6 positivity. **F)** *IRF4* rearrangement detected by fluorescent in situ hybridization using *IRF4* breakapart (6p25.3) probes (red signals for 5′*IRF4* and green signals for 3′*IRF4*).

They are more common in children and young adults and are very rare in the older population. Unlike in younger individuals, where it commonly appears in the Waldeyer's ring and clinical stages I/II, in adults it tends to present more frequently as nodal disease and in advanced clinical stages. Despite these differences, the prognosis is typically favorable. Our patient also experienced an improvement in nodal disease following chemotherapy.[6,7] They constitute six to 20 percent of pediatric mature B-cell lymphomas with a morphology of DLBCL or follicular large cell lymphoma.[1]

A recent study shows that although it is rare, LBCL-*IRF4r* should be considered in older patients and at locations other than the head and neck.[5] Our patient was a 74-year-old male, with retroperitoneal lymph node showing LBCL-*IRF4r*. A gastric ulcer discovered two weeks later showed high-grade B-cell lymphoma, with features of DLBCL but without *IRF4* gene rearrangement. It is uncertain whether these were two different tumors or whether there was gastrointestinal involvement by the previously diagnosed lymphoma that had undergone clonal evolution, while losing its *IRF4* rearrangement, or if these could have been two concurrent lymphomas. The result of "B-cell gene rearrangement" for clonality testing was indeterminate in both tumors, as neither a monoclonal nor a polyclonal population of B lymphocytes was identified. Hence the clonal relationship of the two tumors remained inconclusive.

Intriguingly, LBCL-*IRF4r* had pathogenic or likely pathogenic mutations in *MYD88*, *MEF2B*, and *GNA13* genes. *MYD88* is a NF-κB-pathway-related gene, and mutations of NF-κB-pathway-related genes (*CARD11*, *CD79B*, *MYD88*) are observed in about 35 percent of cases of LBCL-*IRF4r*, as seen in this patient.[1] However, *MYD88* mutations are also more frequent in activated B-cell like (ABC) subtype of DLBCL.[8] *MEF2B* mutation was also present in LBCL-*IRF4r*. *MEF2B* encodes a transcriptional activator and is mutated in approximately 11 percent of DLBCLs. Mutations in *MEF2B* led to dysregulated expression of *BCL6*, thereby contributing to lymphomagenesis in DLBCL.[9] Interestingly, whole exome sequencing has identified recurrent copy number gains in *MEF2B* proto-oncogene in high-grade B-cell lymphoma with *MYC* and *BCL2* with or without *BCL6* rearrangements.[8] *GNA13* mutation, another genetic alteration seen in germinal-center–derived DLBCL,[10] was also present in LBCL-*IRF4r*.

Aside from the possibility of clonal evolution involving the entire tumor, it is possible that this may have reflected intratumoral genetic heterogeneity (55 percent *IRF4* rearrangement), with the *IRF4*-rearrangement-negative subset having undergone clonal evolution seen in the lymphoma involving the gastric ulcer. It could also represent spatial or temporal tumoral heterogeneity, with the *IRF4* rearrangement-positive portion predominating in the node and the *IRF4* rearrangement-negative portion predominating in the stomach. This is an important consideration because it indicates that an *IRF4*-rearranged lymphoma at one site may have a synchronous or subsequent DLBCL at a different site. While the consideration of clonal and subclonal tumor evolution emphasized the established notion of intratumoral genetic heterogeneity, which can occur stochastically,[11] the new gastric tumor offered a rare

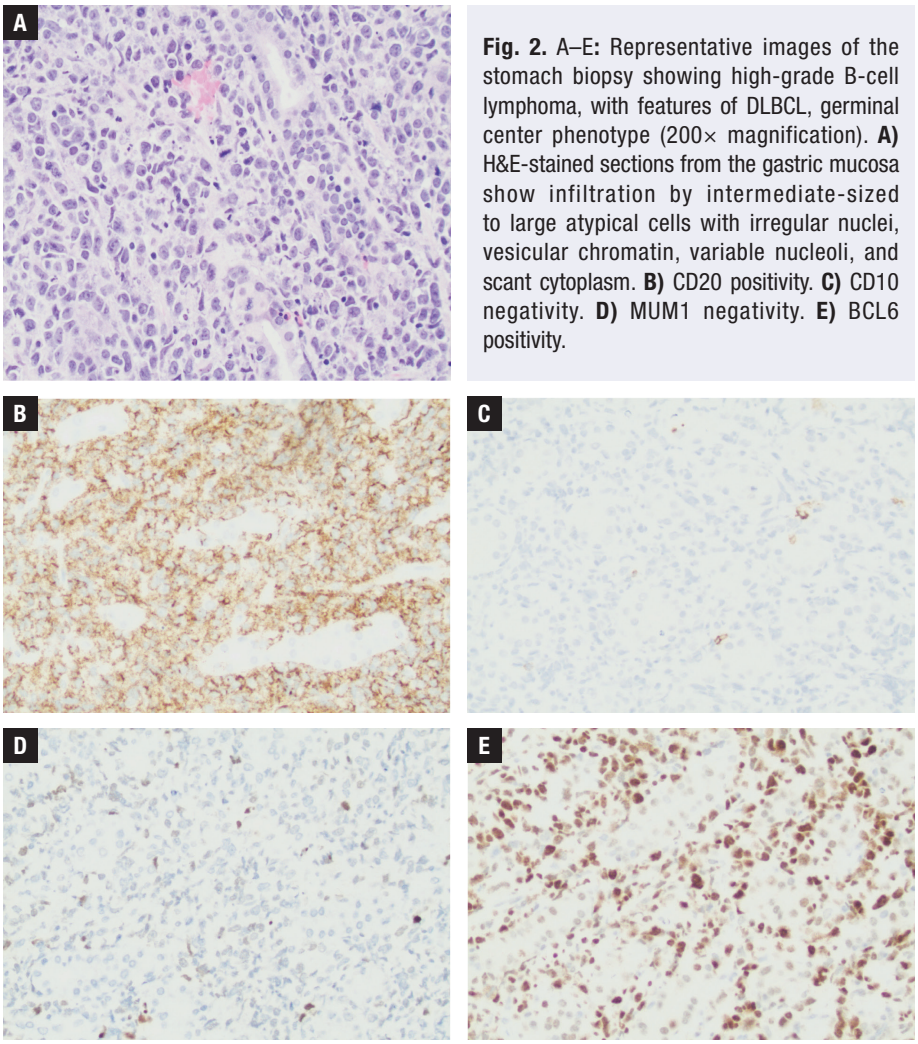

Fig. 2. A–E: Representative images of the stomach biopsy showing high-grade B-cell lymphoma, with features of DLBCL, germinal center phenotype (200× magnification). **A)** H&E-stained sections from the gastric mucosa show infiltration by intermediate-sized to large atypical cells with irregular nuclei, vesicular chromatin, variable nucleoli, and scant cytoplasm. **B)** CD20 positivity. **C)** CD10 negativity. **D)** MUM1 negativity. **E)** BCL6 positivity.

chance to gain further insights into tumor evolution.[12]

In summary, LBCL-*IRF4r* is a recently recognized entity commonly seen in young age and associated with a favorable prognosis, with *IRF4r* having an independent beneficial prognostic influence.[6] This case report provides a rare opportunity to gain insights into the molecular genetic alterations in LBCL-*IRF4r* in the elderly population. It also alerts the physician to the possibility of a lymphoma masquerading as a gastric ulcer. It helps in the understanding of the pathogenesis of evolution of lymphomas and opens avenues for therapeutic targeting. □

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Test yourself

Here are three questions taken from the case report. Answers are online now at www.amp.org/casereports *and will be published next month in* CAP TODAY.

1. Which of the following about large B-cell lymphoma with *IRF4* rearrangement is false?

a. It predominantly occurs in children and young adults.
b. It commonly appears in the Waldeyer's ring.
c. The prognosis is poor in adults.
d. Gene expression profiling demonstrates a germinal center phenotype in most of the cases.

2. Large B-cell lymphoma with *IRF4* rearrangement typically involves which group of lymph nodes?

a. Inguinal
b. Axillary
c. Cervical
d. Retroperitoneal

3. Which of the following statements about large B-cell lymphoma with *IRF4* rearrangement is false?

a. Mutations of NF-κB-pathway-related genes are observed.
b. When the *IRF4* gene is rearranged, the 5′*IRF4* (red) and 3′*IRF4* (green) are separated on FISH.
c. These are rare tumors, constituting less than one percent of large B-cell lymphomas.
d. LBCL-*IRF4r* should be considered in older patients, especially in the head and neck location.