Q&A column, 2/15

Editor: Frederick L. Kiechle, MD, PhD

Submit your pathology-related question for reply by appropriate medical consultants. CAP TODAY will make every effort to answer all relevant questions. However, those questions that are not of general interest may not receive a reply. For your question to be considered, you must include your name and address; this information will be omitted if your question is published in CAP TODAY.

Submit a Question

Q. Can our laboratory use ALK immunohistochemistry in lung adenocarcinoma to select patients for targeted therapy?

A. ALK gene rearrangements (the most common of which results in expression of the EML4-ALK fusion protein) are found in approximately five percent of lung adenocarcinomas, and these ALK-rearranged tumors show marked clinical response to the tyrosine kinase inhibitor crizotinib.[1] At this time in the United States, crizotinib treatment is available only for patients who test positive for the ALK gene rearrangement with the Food and Drug Administration-approved companion fluorescence in situ hybridization diagnostic test, a commercially available FISH break-apart probe kit.[2] Notwithstanding this current requirement, immunohistochemical staining for the ALK protein as a screening tool to select cases warranting confirmatory testing by FISH is an increasingly accepted practice that offers pathology laboratories benefits in cost-effectiveness, use of technical time and resources, and turnaround time, without sacrificing sensitivity or specificity.[3]

Should your laboratory wish to pursue ALK IHC screening of lung cancers, antibody clone selection, test validation, and stain interpretation are critically important. The ALK protein is not expressed at sufficient levels in most lung adenocarcinomas with ALK rearrangements to be detected by conventional IHC methods using the antibody clone ALK1 (mouse monoclonal), widely (and effectively) used in the diagnosis of anaplastic large cell lymphoma. Newer commercially available clones 5A4 (mouse monoclonal), D5F3 (rabbit monoclonal), and anti-ALK (rabbit monoclonal) have shown high sensitivity and specificity in lung cancer in large studies of 100 or more tumors.4 While methods and automated platforms have varied across several studies, many studies emphasize enhanced signal amplification as a technique to improve sensitivity. Validation of these highly sensitive antibodies should follow standard IHC validation theory and methods. Most studies of ALK IHC testing report strong and diffuse cytoplasmic staining in 10 percent or more tumor cells of ALK-rearranged tumors with the cautionary note that faint cytoplasmic stippling may be seen in alveolar macrophages, and further warning that high background staining may be occasionally seen, especially in mucinous/signet ring and possibly neuroendocrine tumors.[4]

Standalone ALK IHC testing is now commonplace in European and Asian countries, with at least one commercially available companion diagnostic kit. Given the high sensitivity and specificity of the ALK antibodies described here and the decreased cost and turnaround time of ALK IHC, it is likely that ALK IHC companion diagnostic testing will become more widespread in the near future. In anticipation of increased ALK IHC testing in lung cancer, the CAP has already incorporated ALK IHC into its Immunohistochemistry Survey series (PM5), with mailings to be shipped in April 2015.

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Kristin Jensen, MD
Assistant Professor of Pathology
Assistant Service Chief, Anatomic Pathology
Stanford University Hospital
and Palo Alto Veterans
Affairs Health Care System
Palo Alto, Calif.
Member, CAP Immunohistochemistry Committee

Q. Why does cryoprecipitated antihemophilic factor from blood groups A and B have higher levels of factor VIII than cryoprecipitated AHF from blood group O?

A. Cryoprecipitated antihemophilic factor (AHF) is the cold-insoluble precipitate produced upon thawing a unit of frozen plasma at 1°–6° C. The proteins most susceptible to cold-induced precipitation are those of large molecular weights, including von Willebrand factor. The size of vWF molecules can vary, but large and ultra-large multimers can reach in excess of > 10 × 106 Da. VWF plays many roles in hemostasis, one of which is as a carrier protein for factor VIII. Plasma levels of factor VIII are stabilized by its noncovalent interaction with vWF, and factor VIII is rapidly degraded in the absence of vWF.1

The ABO blood group antigens are not only expressed on red blood cells but also found on other tissues (such as platelets and endothelial cells) and plasma proteins, including vWF. A well-recognized association between an individual's ABO blood group and both vWF and factor VIII levels has been noted.1 VWF has N-linked glycans to which ABH determinants are added.2 How the ABO blood group influences the vWF and factor VIII levels is not well understood; however, susceptibility of vWF to cleavage by ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 repeats-13) may be an important mechanism.3,4 Protection of vWF degradation by the H carbohydrate moiety is less effective than AB moieties;5 blood group O individuals are consistently found to have lower vWF and factor VIII levels than non-group O individuals. Similarly, fresh frozen plasma collected from group O individuals is observed to have lower factor VIII levels than that of non-group O individuals.6 It stands to reason that if the starting vWF levels are higher in non-group O donor plasma, the cryoprecipitate collected from that plasma would similarly show increased concentrations of vWF–factor VIII complex accordingly.

It is worth noting that while the original intent for cryoprecipitated AHF was as a source of factor VIII for patients with hemophilia A, virally inactivated and recombinant factor VIII concentrates are considerably safer for these patients. Therefore, cryoprecipitate is a second-line therapy for hemophilia A and should be used only when pathogen-reduced products are not available.7

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Jeffrey Lawrence Winters, MD
Professor of Laboratory Medicine and Pathology
Mayo Clinic College of Medicine
Rochester, Minn.
Member, CAP Transfusion Medicine
Resource Committee

Evelyn Lockhart, MD
Associate Medical Director
University of New Mexico Transfusion Service
Associate Professor, Department of Pathology
University of New Mexico, Albuquerque
Member, CAP Transfusion Medicine
Resource Committee

Nicole Dodge Zantek, MD, PhD
Medical Director, Special Coagulation Laboratory Division of Transfusion Medicine, Department of Laboratory Medicine and Pathology
University of Minnesota, Minneapolis
Liaison, CAP Transfusion Medicine
Resource Committee