

Immunohistochemistry

Then and Now

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This special issue is devoted to practical diagnostic immunohistochemistry (IHC) of different organ systems and infections. It is not meant to be a comprehensive review but a handy, practical, desktop reference tool with its many illustrations and tables. The articles in this issue also highlight newer markers that are now maturing for use in routine practice.

I had the good fortune to witness all the changes that occurred in IHC during the last 3 decades, which enables me to appreciate the monumental effort and progress that we have made in IHC today. I briefly review the major milestones that led to the current role of IHC in surgical pathology.

Coons and colleagues¹ did not know how widespread the use of IHC would be in the next half of the century when they introduced immunofluorescence for the detection of antigens in frozen tissue. However, the science of antigen detection via visibly tagged antibodies did not catch on until the advent of readily available monoclonal antibodies in the early 1980s. This is when I took charge of the IHC and flow cytometry laboratory at the Bronx VA Medical Center in New York. At first the staining was weak and the signal-noise ratio was high. The horseradish peroxidase detection system was soon replaced by the more sensitive avidin-biotin system. The chromogen, diaminobenzidine, remained the same and continues to be useful today with further refinement using tyramide amplification. Tyramide is a highly sensitive polymer-based labeling system. Another big hurdle that we faced in those days was that many antibodies worked only on frozen tissue sections with inferior morphology. Another giant step was taken in 1991 when Shi et al² discovered heat-induced epitope retrieval, which involved use of a microwave/pressure cooker in conjunction with a metal salt-buffered solution. This revolutionized immunohistochemical staining because many more antigens could be detected on routine formalin-fixed archival tissue. The incubation time with the primary antibody was also shortened to less than 1 hour for most antigens as opposed to overnight. Frozen sections were no longer necessary for

workup of lymphomas. The markers for lymphocytes, T or B, were now stainable on formalin-fixed tissue as opposed to 5 to 10 years before. The morphology is so much better as a result of this. In parallel, automation of IHC made large-volume work feasible, although old-fashioned manual staining is still preferred in some commercial laboratories. Some anatomic pathology information systems are now looking to link automated IHC systems to track down the status of the staining. Because of automation, for the first time it is possible to standardize staining for quantitation, although this continues to be a problem and needs more work. In an effort to standardize staining, effect of fixation time on preservation of tissue antigen is recorded routinely in our laboratory when immunohistochemistry matters such as the case with HER-2 IHC. In my opinion, another giant step was taken when the ImmunoQuery (now STATdxPathIQ) became available. It is a valuable resource for looking up immunoprofiles of tumors online. This has been a free resource until recently. In the future, we will have evidence-based meta-analysis available in conjunction with tissue microarrays, which will facilitate rapid evaluation of antibody profiles on thousands of cases. The “art” of immunohistochemistry is rapidly developing into a “science.”

Specific examples of the impact of IHC in different settings follow.

In the last 5 years, the availability of immunostains for myoepithelium has helped delete breast diagnoses such as “highly suspicious for microinvasion” from our lexicon to the more definitive “microinvasion identified/not identified.” In addition, the use of immunohistochemical markers in breast cancer has transformed pathology from simple diagnoses to predictive and prognostic necessities. Immunohistochemistry has assisted in guiding adjuvant therapy decisions and sentinel node staging; subtyping a carcinoma as ductal or lobular, basal (cytokeratin [CK] 5/6) or luminal; distinguishing invasive carcinoma from mimics; and establishing that a metastatic carcinoma of unknown primary site has originated in the breast or elsewhere. In the thyroid, CK19 has been considered a useful ancillary to diagnosing papillary carcinoma of thyroid especially in cytology specimens with a high sensitivity and specificity with the caveat that proper sample and controls need to be used when applying IHC to cytology. Another marker that is useful, although not sensitive and not entirely specific, in detection of thyroid malignancies, is HBME-1. HBME-1 detects an unknown antigen on microvilli of mesothelioma cells and elsewhere.

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Very few markers are specific to the lung and lung tumors. The best marker that we have now is thyroid transcription factor 1 (TTF-1), which is widely used in differentiating a lung primary from other neoplasms, including mesothelioma. However, it is not without its shortcomings and is close to desired specificity but lacks sensitivity, being absent or sparsely positive in poorly differentiated lung cancers and primary squamous cell carcinomas of the lung. Another promising marker on the horizon that appears to complement TTF-1 in detecting a lung primary is napsin A. In our study of more than 1000 carcinomas of diverse origin, we found that napsin A was complementary to TTF-1 in the detection of lung primary and more sensitive than TTF-1 (I. Sainz, P. T. Cagle, MD, J. Jagirdar, MD, unpublished data, September 2007). Napsin A is a functional aspartic proteinase that is expressed in the normal lung parenchyma in type II pneumocytes and in the proximal and convoluted tubules of the kidney. It is weakly expressed in pancreatic tumors and is well expressed in some renal tumors and thyroid carcinomas. Until now there were no good positive mesothelial markers, and a diagnosis of mesothelioma was one of exclusion and depended on having several negative epithelial markers. Now we have calretinin, which is the single best sensitive marker for mesothelial differentiation. The source of calretinin is important, with Zymed antibodies (Zymed Laboratories, South San Francisco, Calif) outperforming the others. Cytokeratin 5/6 is another positive marker for mesothelioma. The issue of separating benign mesothelial proliferation from mesothelioma still relies on morphology. However, it is aided by desmin, which is most frequently positive in benign mesothelial proliferations, whereas epithelial membrane antigen and p53 are positive in malignant ones.

Immunostaining using a panel of markers in the differential diagnosis of trophoblastic lesions is another recent major contribution in gynecologic pathology. The algorithm begins with immunostaining with CK18, p16, and HLA-G antibodies. If CK18 and HLA-G are both diffusely positive and p16 is negative, the lesion is trophoblastic. If p63 is selectively stained in mononucleate trophoblast corresponding to cytotrophoblast and human chorionic gonadotropin is selectively stained in syncytiotrophoblast, the lesion is choriocarcinoma. If p63 is negative and human placental lactogen is diffusely positive, the lesion is either an exaggerated placental site or a placental site trophoblastic tumor. These lesions can be distinguished based on the Ki-67 labeling index. If p63 is diffusely positive and only focally positive for human placental lactogen, the lesion is either a placental site nodule or an epithelioid trophoblastic tumor. These lesions can be distinguished based on the Ki-67 labeling index. The uterine stromal tumors are positive for CD10, and serous carcinomas are positive for p53.

Immunohistochemistry has proven to be a useful tool in the diagnosis of infectious diseases in tissue samples. It is especially useful in the identification of microorganisms that are present in low numbers, stain poorly, are fastidious to grow, are noncultivable, or exhibit an atypical morphology. A caveat to remember when staining pathogens is that there may be widespread occurrence of common antigens among bacteria and pathogenic fungi and both monoclonal and polyclonal antibodies must be tested for possible cross-reactivity with other organisms. Examples of some newer pathogens that can be immunostained

now are Hantavirus, parvovirus B19, Rocky Mountain spotted fever, *Candida*, *Aspergillus*, and mycobacteria.

By using IHC in selected cases, the rate of false-negative and false-positive diagnoses can be reduced in the genitourinary tract, with some patients getting more specific or effective therapy. Prostate-specific antigen remains one of the best organ-specific markers with sensitivity available for the prostate. Uroplakin III is specific for terminally differentiated urothelial cells and is present in 60% of bladder cancers. α -Methylacyl CoA racemase (clone P504S) was identified using expression profiling and was found preferentially in prostatic carcinoma as compared with normal tissue. It is also positive in prostatic intraepithelial neoplasia and less commonly in nodular hyperplasia, atrophic glands, and nephrogenic adenoma. Cocktails of high-molecular-weight keratin, keratin 903, p63 (a basal cell marker), and α -methylacyl CoA racemase is used in confirming a morphologic diagnosis of minimal prostatic carcinoma. In testicular tumors, hematopoietic marker CD30 is used to distinguish seminoma (except spermatocytic seminoma and intratubular germ cell tumor) and embryonal carcinoma from other germ cell tumors. In seminomas, the staining is focal. CD117, a transmembrane tyrosine kinase receptor protein, is also strongly positive in seminoma as opposed to other nonseminomatous germ cell tumors. Conversely, CAM 5.2 is positive in nonseminomatous germ cell tumors. Eighty-five percent of clear cell renal cell carcinomas are positive for CD10, another hematopoietic marker. Inhibin is a highly specific marker for ovarian and testicular sex cord tumors and a sensitive marker for adrenal cortical neoplasms.

Traditionally, the practice of hematopathology has relied heavily on immunophenotyping. The diagnosis and classification of tumors of the lymphoid system is complex and based on multiple parameters including clinical, morphologic, immunophenotypic, and molecular features. During the last 2 decades, advances in gene cloning and genome-wide molecular analysis have greatly increased the number of antibodies available for diagnosis and for predicting prognosis. In particular, antibodies against genes that are normally quiescent or produced at low levels but are activated by translocation partners (eg, t(14;18), *IgH/BCL-2*) or fusion genes (eg, t(2;5), *NPM/ALK*) are used in routine practice. In the last 5 to 10 years, antibodies against transcription factors, important in cell differentiation and lineage determination, have become useful in differentiating Hodgkin lymphoma and non-Hodgkin lymphoma and in predicting prognosis, particularly in diffuse large B-cell lymphoma. The diagnosis and classification of leukemic disorders is becoming increasingly complex. Modern approaches now incorporate morphologic, immunohistochemical, laboratory, molecular, and clinical data to specifically categorize each unique entity as in lymphomas. Although sophisticated methodologies are frequently used to detect characteristic features conferring diagnostic, prognostic, or therapeutic information, a thorough microscopic examination remains essential to the pathologic evaluation. Detailed blast immunophenotyping can be performed with lineage- and maturation-specific markers. Although no one epitope is pathognomonic for one malignancy, a well-chosen panel of antibodies can efficiently aid the diagnosis and classification of leukemia.

In soft tissue tumors, the characteristic translocation in Ewing sarcoma/primitive neuroectodermal tumor, t(11;

22)(q24;q12) involving Ewing sarcoma (*EWS*) gene on chromosome 22 and the *FLI-1* gene on chromosome 11 results in overexpression of FLI-1 protein, which can be detected immunohistochemically in nuclei of ~70% of Ewing sarcoma/primitive neuroectodermal tumors. Another new abnormally expressed protein product of chromosomal aberration 2p23, ALK1(p80), is present in approximately 50% of pediatric inflammatory myofibroblastic tumors and in some other malignant soft tissue tumors. The staining is usually cytoplasmic in inflammatory myofibroblastic tumor as opposed to nuclear and cytoplasmic in anaplastic large cell lymphoma. Alveolar soft part sarcomas are now theorized to be related to muscle tumors because of their desmin positivity. However, staining with newer muscle markers such as MyoD1 and myogenin are negative. Recently, a characteristic X:17 translocation has been identified in alveolar soft part sarcomas resulting in an *ASPL-TFE3* fusion gene. Antibody TFE3 detects the presence of overexpressed gene product in alveolar soft part sarcomas. The same protein is also overexpressed in certain pediatric renal cell carcinomas.

Some liver lesions can be problematic. Differentiation between hepatic adenoma and focal nodular hyperplasia can be problematic and can now be aided by β -catenin,

which is found in hepatic adenomas. Hepatocellular carcinomas can be distinguished from cirrhotic nodules and benign liver tumors with the aid of a new marker glypican-3. Hepar-1 is a liver-specific antigen that can be used in the context of hepatocellular carcinoma look-alikes such as renal cell carcinoma and adrenal carcinoma. However, it is not specific for the liver and must be used with a panel of markers. Distinguishing an appendiceal adenocarcinoma from a colonic carcinoma is now possible via MUC5AC, which is positive in appendiceal carcinoma and not in colonic carcinoma, whereas β -catenin is negative in appendiceal lesions and almost always positive in colonic cancers. β -Catenin and villin are both highly unusual in ovarian mucinous carcinomas as compared with colonic mucinous carcinomas.

In summary, we will continue to make significant progress as more organ-specific markers emerge as a result of proteomics and genomics in conjunction with microarrays.

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Jaishree Jagirdar, MD, earned her medical degree from Calcutta Medical College, India, right before moving to New York and joining an anatomic pathology/clinical pathology residency at Mount Sinai Medical Center. After completion of her residency, she was appointed as a staff member in an affiliate institution, Bronx VA Medical Center, New York, in 1981. Soon after she joined the staff she was instrumental in establishing the immunohistochemistry and flow cytometry laboratory there. She obtained certification the first time the board examination for immunohistochemistry was offered. She then moved to Bellevue Hospital/ NYU Medical Center in New York as head of surgical pathology where she remained on the staff as tenured associate professor for a period of 10 years before moving to the University of Texas Health Science Center at San Antonio, as director of anatomic pathology and professor of pathology. She has held a lifelong interest in pulmonary pathology and immunopathology that is supported by her ~95 publications and many book chapters on these subjects. Dr Jagirdar also serves as a section editor for the *Archives of Pathology & Laboratory Medicine*.