

AP116 Evidence-Based Immunohistochemistry: Technical Validity and Diagnostic Relevance

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Immunostaining for Cytokeratins 7 and 20: Are We Wasting Resources? A Critical View of Application in Immunohistochemistry with Consideration of Their Utility in Carcinoma of Unknown Primary Evaluation

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- I. Course Objectives
 - A. To review the current practice relating to the utilization of CK7 and CK20
 - B. To critically evaluate the evidence basis for this practice
 - C. To postulate any changes based on any changes in philosophy resulting from evaluating the evidence base
 - D. To consider the utility of these two markers in the context of evaluation of carcinoma of unknown primary
 - E. To potentially establish new practice habits based on a better understanding of the evidence basis for the utilization of CK7 and CK20

- II. Basis for Good IHC Practice in an Evidence Based Context
 - A. Suitable and appropriate utilization of positive and negative controls in all cases
 - B. Reliance on a panel of antibodies to apply to a histomorphologic differential diagnosis
 1. Should be exceptionally rare circumstance when only one antibody should be utilized
 2. Never order a test that you would have trouble explaining away an unexpected/aberrant result (Don't ask for the data point if you don't want to deal with it)
 - C. IHC is an ancillary test and only an ancillary test
 1. It is NOT a substitute for poor histopathologic diagnostic acumen
 2. Ancillary tests occasionally have unexpected or aberrant results (think 1 in 20 CP tests)...expect the unexpected
 - D. Two Cardinal Rules of IHC
 1. A fool with a tool is still a fool
 2. If the immuno does not fit you must ignore it

- III. Basis for Utilization of CK7/CK20 in Practice

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- A. Existence of 30 subtypes of intermediate filament/cytoskeletal protein cytokeratin, 20 of these are the so-called “soft” variety
 - B. Distinction based on Moll number related to molecular weight and electrophoretic differences
 - C. Many investigations point toward differential expression of various cytokeratins in various specific types of epithelium
 - D. Cytokeratin 7
 - 1. Low/Intermediate molecular weight cytokeratin found on chromosome 12
 - 2. Expressed rather ubiquitously in “non-pavement” type epithelium
 - E. Cytokeratin 20
 - 1. Low molecular weight cytokeratin found on chromosome 17
 - 2. Expression pattern relatively restricted to gastrointestinal type epithelium and Merkel cells in skin
 - F. Based on differential expression the theory is that one can differentiate different expression profiles to help determine differentiation of neoplasm
 - G. Cannot definitively determine “site of origin” as IHC only determines differentiation toward a particular tissue type and not where a neoplastic cell originates from
- IV. Evidence Basis for Utilizing CK7 and CK20
- A. Recent PubMed search shows over 200 manuscripts in literature utilize these two antibodies as a means to differentiate between different types of neoplasms
 - B. Best known of these include:
 - 1. Wang, et al. Coordinate Expression of Cytokeratins 7 and 20 Defines Unique Subsets of Carcinomas (*Appl Immuno* 1995;3(2):99-107)
 - 2. Chu, et al. Cytokeratin 7 and Cytokeratin 20 Expression in Epithelial Neoplasms: A survey of 435 cases (*Mod Pathol* 2000;13(9):962-972)
 - 3. Chu, et al. Keratin expression in human tissues and neoplasms (*Histopathol* 2002;40:403-439)
 - C. First two have similar findings; latter is a review article
- V. Summary of Literature Findings
- A. Mirroring expression in histologically normal tissues, Cytokeratin 7 routinely expressed in many neoplasms
 - 1. Long list of adenocarcinomas arising from a variety of sites
 - 2. Greater than 50% of the following types:
 - a. Salivary gland; lung; esophagus; gastric; breast; pancreatico-biliary; non-seminomatous testicular; ovarian epithelial; endometrial; cervical
 - 3. Less than 50% of the following types:
 - a. ENT SCCa; Lung SCCa; Colo-rectal;urothelium

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- B. Cytokeratin 20 with a much more restricted expression profile
 - 1. Colo-rectal; Urothelium; Merkel Cell Carcinoma

VI. Examples

- A. Case #1 - Patient is a 53 year old female with a painful pre-auricular swelling.
- B. Case #2 - Patient is a 62 year old male with a 1.5 cm firm supraclavicular lymph node
- C. Case #3 - Patient is a 74 year old woman with ascites and an omental mass
- D. Case #4 - Patient is a 53 year old woman with an axillary mass
- E. Case #5 - Patient is a 47 year old male with an incidental 3.0cm renal mass
- F. Case #6 - 67 year old male with mediastinal lymphadenopathy
- G. Case #7 - A 58 year old male with excruciating penile pain, an elevated PSA, and hematuria
- H. Case #8 - A 53 year old male with firm 1.0 cm subcutaneous nodule on his left cheek

VII. The Utilization of CK7/CK20 in Work-Up of Metastatic Carcinoma of Unknown Primary

- A. Increasing demand to evaluate small tissue specimens from patients with no known primary lesion containing a poorly differentiated malignant neoplasm
- B. IHC is a reasonably effective tool and cost efficient tool in this context
- C. IHC can only point toward differentiation and not origin although the two are commonly linked
- D. For IHC to be an effective tool in this regard, pathologist must utilize a panel of antibodies and analyze the results in an algorithmic manner based on probabilities and positive predictive values
- E. There are many examples of such algorithms proposed by many authors
- F. Some of the algorithms utilize CK 7 and CK 20 while others only employ CK20
- G. Rationale for only utilizing CK20 is that any discriminatory value that the two antibodies hold is based only in CK20 and that CK7 is superfluous given its ubiquitous expression pattern and adds no further information

VIII. Some Questions and Perhaps Some Answers

- A. Based on the evidence in the literature, do we gain anything from using CK7, especially in a routine manner?
 - 1. *Despite what seems to be support in the literature, critical review and examination would suggest that we do not need the data point*
- B. Which of the two antibodies provides discriminatory power, especially when considering the work up of metastatic carcinoma of unknown primary?
 - 1. *CK20 with its selective expression provides more discriminatory power*

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2. *Can, for all practical purposes, utilize a negative CK20 as a presumptive positive CK7*
- C. Can CK7 be utilized as a “screening”cytokeratin?
 1. *Yes, that could be possible but only as a component of a “cocktail” with other cytokeratin subtypes since it is ubiquitously but not universally expressed*
- D. Does CK7 have any utility?
 1. *There are **rare** occasions where the data point does have utility*
 - a. *Co-expression of CK7/20 in a differential of urothelial versus poorly differentiated colo-rectal*
 - b. *Expression of CK7 versus CK5/6 in metastatic versus primary skin adnexal*
 - c. *Expression of CK7 in non-clear cell variants of renal carcinoma*

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