IHC Antibody Test Selection Using a Panel Approach

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IHC Panels as an Aid in Diagnostic Decision Making

Diagnostic Use of Tumors Using Algorithms
- Utilizes a panel of antibodies intended to solve a diagnostic problem
- Many diagnostic algorithms exist
- The panel of antibodies selected should be based on the morphological appearance of the tissue
Diagnosis of Tumors using Algorithms

- A diagnostic algorithm is a method which utilizes a panel of antibodies intended to solve a diagnostic problem
- Many different diagnostic algorithms exist and are available in journals and textbooks
- A diagnostic algorithm is followed by a selective markers for tumor sub-classification
- The panel of antibodies selected should be based on the morphological appearance of the tissue and the patient's clinical history provided by the physician.

Why are panels important?

- Increase the number of diagnostic tools for the pathologists
- See a macroview of the disease state
- Faster turn around time
- No single antibody is 100% sensitive and specific
- More specific diagnosis leads to more specific treatment

CD Antibodies for Phenotyping
(Formalin-Fixed Paraffin-Embedded Tissues)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Neoplasm and/or Cell Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>Pan T-cell</td>
</tr>
<tr>
<td>CD5</td>
<td>T-cell and mantle cell</td>
</tr>
<tr>
<td>CD4</td>
<td>T-cell (helper/inducer)</td>
</tr>
<tr>
<td>CD8</td>
<td>Cell-mediated cytotoxicity</td>
</tr>
<tr>
<td>CD15</td>
<td>Hodgkin's (Reed Sternberg)</td>
</tr>
<tr>
<td>CD20</td>
<td>B-cell lymphoma</td>
</tr>
<tr>
<td>CD21</td>
<td>B-cell, follicular lymphoma</td>
</tr>
<tr>
<td>CD22</td>
<td>B-cell lymphoma</td>
</tr>
<tr>
<td>CD23</td>
<td>Small lymphocytic and follicular lymphoma</td>
</tr>
<tr>
<td>CD30</td>
<td>Anaplastic large cell and Hodgkin's</td>
</tr>
</tbody>
</table>
CD Antibodies for Phenotyping
(Formalin-Fixed Paraffin-Embedded Tissues)

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<thead>
<tr>
<th>Antibody</th>
<th>Neoplasm and/or Cell Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD43</td>
<td>Pan T-cell and low grade B-cell</td>
</tr>
<tr>
<td>CD45</td>
<td>Pan marker for lymphoma</td>
</tr>
<tr>
<td>CD45RB</td>
<td>Pan marker for lymphoma</td>
</tr>
<tr>
<td>CD45RO</td>
<td>Pan T-cell</td>
</tr>
<tr>
<td>CD68</td>
<td>Macrophage and histiocytic</td>
</tr>
<tr>
<td>CD74</td>
<td>B-cell lymphoma</td>
</tr>
<tr>
<td>CDw75</td>
<td>B-cell lymphoma</td>
</tr>
<tr>
<td>CD79a</td>
<td>Pan B-cell</td>
</tr>
</tbody>
</table>

CD Markers

- Most commonly used markers (CD = cluster designation)
  - **B-cell** - CD10, CD19, CD20, CD22, CD23, CD24, CD79b, CD160, PAX-5, kappa, lambda, CD200, cytoplasmic kappa, cytoplasmic lambda
  - **T-cell** - CD1, CD2, CD3, CD4, CD6, CD8, TCR a/β, TCR γ/δ, cytoplasmic CD3
  - **Myeloid/monocyte** - CD11b, CD13, CD14, CD15, CD33, CD64, CD117, myeloperoxidase
  - **Miscellaneous** - CD11c, CD16, CD25, CD30, CD34, CD38, CD41, CD42b, CD45, CD56, CD57, CD61, HLA-DR, glycoporphin, TdT, bcl-2

CD Markers

- CD1a, CD207: Langerhan cell histiocytosis cells
- CD2, CD3, CD4, CD5, CD7, CD8: T cells
- CD10: Early pre-B cells (immature B cells)
- CD11c, CD25, CD103, CD123: Hairy cell leukemia cells
- CD13, CD33, CD17: Myeloid cells
- CD14, CD64: Monocytic cells (positive in AML-M4 and AML-M5)
- CD15: Reed-Sternberg cells, neutrophils
- CD16, CD66: Natural killer cells
- CD19, CD20, CD21, CD22: B cells
- CD23 and CD5: Chronic lymphocytic leukemia/small lymphocytic lymphoma
- CD23 negative and CD5 positive: Mantle cell lymphoma cells
CD Markers

- CD30 and CD15: Reed-Sternberg cells
- CD30 positive and CD15 negative: Anaplastic large cell lymphoma
- CD31: Endothelial cells (positive in angiosarcoma)
- CD33: Myeloid cells and precursors
- CD34: Stem cells (also positive in angiosarcoma)
- CD41, CD61: Megakaryocytes and platelets (positive in AML-M7)
- CD45: All leukocytes (except Reed-Sternberg cells)
- CD45 RO: Memory T cells
- CD45 RA: Naive T cells
- CD68: Histocytes (positive in malignant fibrous histiocytosis)
- CD99: Ewing's sarcoma cells
- CD117: Gastrointestinal stromal tumor (GIST) cells, mast cells (positive in mastocytosis), myeloid cells

CD Markers

- CD71: All proliferating cells; erythroid precursors through reticulocytes, capillary endothelium in brain
- CD123: Acute Myeloid Leukemia
- CD138: B cell precursors, plasma cells, stratified squamous epithelium

B-cell lymphoproliferative disorders

Probesite if immunoglobulin light chain restriction is demonstrated by surface typing of kappa or lambda

B-cell CLL or mantle cell lymphomas (MCL) are suspected if CD5 is positive and CD10 is negative

Circulating MCL can be mistaken morphologically for B-cell CLL or B-cell prolymphocytic leukemia (B-PLL)

MCL is the following:
- CD20, CD19 (strong intensity)
- Surface immunoglobulin (strongly expressed)
- CD23 (absent)

Diagnosis: Molecular and FISH testing

Reactive (11;14) translocation demonstration

CLL is more likely when:
- CD20 - weak intensity
- Surface immunoglobulin - weakly expressed
- CD23 - present
- CD5 - present
B-cell lymphoproliferative disorders

Circulating germinal center cell-derived lymphoma is probable if CD10 is positive and CD5 is negative

Germinal center lymphomas – follicular, Burkitt lymphoma, diffuse large B-cell lymphoma (DLBCL)

Marginal zone lymphoma should be considered if both CD5 and CD10 are negative

Hairy cell leukemia (HCL) has a characteristic phenotype that is CD5+, CD10-, CD11c+, CD22+, CD25+, and CD103+

CD103 antigen (also known as B-lyt) is present in virtually all cases

CD11c and CD25 are less specific but present in almost all cases of hairy cell leukemia

HCL variant can be considered in otherwise typical cases of HCL when CD25-

Examples of common panels of Antibodies Used

Generic T-cell Vs B-Cell: CD3, CD20, CD45

Follicular Lymphoma Vs Hyperplasia: CD10, CD21, CD3, CD10, CD20

Low Grade B Lymphoma: CD3, CD20, CD21, CD23, CD45, Bcl2, Bcl6

MALT Lymphoma: CD3, CD8, CD20, Bcl2, IGH Kappa and Lambda

Hodgkin's Lymphoma: CD3, CD15, CD20, CD30, CD45

Myeloma: CD138, IGH Kappa and Lambda

Carcinoma Vs Lymphoma: CD4, CD20, CD45, PanCK

Metastatic Carcinoma: CD4, CD20, TTF-1

GIIB: CD117, CD34, S100, Desmin, SMA

Mesothelioma: PanCK, CD56, Calret, TTF-1, CEA, CD45

If metastatic, add TCA, IHC and 16q12

Sometimes the staining pattern of a single stain could be different in different diagnostic contexts – CD3 (T-cell marker)

Cytoplasmic positivity – in precursor T cell neoplasms

Membranous positivity – in peripheral T cell neoplasms
Immunophenotypic Findings in Classical Hodgkin’s Lymphoma
- positive for CD15 and CD30 and
- negative for LCA (CD45) and EMA.
- B-cell antigens—such as CD20, CD79A, PAX-5/BSAP, and MUM1/IRF4—are expressed in a subset of cases.
- CD20 expression is often weak.
- T-cell antigens are usually not expressed by the neoplastic cells.
- BCL-2 is positive in up to half the cases and has been correlated with poorer prognosis.
- EBV is common in the Reed-Sternberg and Hodgkin cells of classic HL

Mantle Cell Lymphoma
- Mantle Cell Lymphoma has a generally poor prognosis
- Median survival is 3 to 4 years
- MCL is not curable by conventional chemotherapy and most patients succumb to organ dysfunction due to tumor infiltration

Hairy Cell Leukemia
- CD20+
- CD5- 
- CD10- 
- CD23- 
- CD11c++
- CD25+ 
- CD103+ 
Chronic Lymphocytic Leukemia

- CD20+ (dim)
- CD5+
- CD23+
- Cyclin D1-
- CD3-

Chronic Lymphocytic Leukemia

- The most common leukemia of adults in the Western world.
- Tumor cells resemble a small subset of circulating B-cells that express CD5.
- Most patients are males over 50 years of age
- Patients with CLL are often asymptomatic
- When symptoms are noticed, they are often non-specific (fatigue, weight loss)

T-cell lymphoproliferative disorders

Most show abnormalities of pan T-cell antigens CD2, 3, 5, or 7

T-cell disorders

- Proliferating lymphocytes are usually positive for CD3
- Most common form is large granular lymphocytosis

Large granular lymphocytosis is suspected if percentage of CD16+, CD56+, or CD57+ T cells is >50% or if absolute count of these cells >2,000/μL

Angioimmunoblastic lymphoma has characteristic CD10+ and CD4+, and CD52-, CD56-, and CD16-

Anaplastic large cell lymphoma - CD30+ and ALK+ (+)
- Some pan T-cell antigens are frequently deleted
Immunohistochemistry
Cytokeratins (CKs)
- 20 sub-types of CK with different molecular weight and different expressions are seen in various cancers and cell types.
- Monoclonal antibodies to specific CK sub-types are used classify tumors according to site of origin.
- CK7/CK20 are most useful and hence, most commonly used.
- CK7 is seen in lung, breast, ovary and endometrium
- CK20 is seen in lower GI tract
- CK20 is seen in GI epithelium, urothelium and Merkel’s cells.
- A pattern of CK20/CK7 strongly suggests GI neoplasm
- A pattern of CK20/CK7 strongly suggests ovarian cyst

Cytokeratin phenotype
- CK phenotype
- CK7 (-) / CK 20 (+)
- CK7 (+) / CK 20 (-)
- CK7 (-) / CK 20 (+)
- CK7 (+) / CK 20 (+)

Differential Diagnosis
<table>
<thead>
<tr>
<th>Bladder vs Prostate ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin 7</td>
</tr>
<tr>
<td>Cytokeratin 20</td>
</tr>
<tr>
<td>CEA</td>
</tr>
<tr>
<td>PAP</td>
</tr>
<tr>
<td>PSA</td>
</tr>
</tbody>
</table>
Infectious Organisms

- H. Pylori
- CMV
- HSV
- EBER

Squamous and Basal Cell Carcinoma

- Most common cancer in the US
- 1% of all cancer deaths
- Excellent prognosis if early
- Fatal if neglected
**Lung Adenocarcinoma vs. Squamous Cell Carcinoma**

<table>
<thead>
<tr>
<th>Napsin A</th>
<th>TTF-1</th>
<th>CK 5/14</th>
<th>Sox-2</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Well Differentiated Lung Adenocarcinoma</td>
</tr>
<tr>
<td>Napsin A</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Poorly Differentiated Lung Adenocarcinoma</td>
</tr>
<tr>
<td>Napsin A</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Neuroendocrine Tumor (verify w/NE marker)</td>
</tr>
<tr>
<td>Napsin A</td>
<td>+</td>
<td>+</td>
<td>Sox-2</td>
<td>Squamous Cell Carcinoma</td>
</tr>
</tbody>
</table>

**Napsin A**

- Clone: Polyclonal
- Visualization: Cytoplasmic
- Lung adenocarcinoma
- Multiple panel applications
- Higher sensitivity and specificity compared to TTF-1
- USCAP 2010, IAP 2010

**SOX-2**

- Clone: SP76
- Visualization: Nuclear
- Rabbit Monoclonal
- Differentiates lung squamous cell carcinoma from lung adenocarcinoma
- Distinguishes embryonal carcinoma from other germ cell tumors
- Useful in the identification of astrocytomas
- Important for general pathologists and GU pathologists
Master List for Immunohistochemistry

<table>
<thead>
<tr>
<th>Macrophage</th>
<th>CD68, CD163, MAC387, D11, LN5, HAM56, AAT, AACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>S100, HMB45, Melan-A (HMB45), Microphthalmia, PTF, Tyrosinase, NDHCB</td>
</tr>
<tr>
<td>Derry</td>
<td>CA120, PLAAP, CORT, Pan Melanoma Cocktail, MAGE-1, Survivin</td>
</tr>
<tr>
<td>Pancreas</td>
<td>NE, Chromogranin, Insulin, Sphatophysin, PDX-1, Muacin</td>
</tr>
<tr>
<td>Prostate</td>
<td>ACTH, FSH, TSH, LH, Prostate, Human Growth Hormone</td>
</tr>
<tr>
<td>Prostate</td>
<td>PSA, PSAAP, P53, PIN-1, PIN-4, Androgen Receptor, HBB-C1, CG67-1, TURF, C7, Vascular Endothelial Growth Factor (VEGF)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Pan Actin, Vimentin, Smooth Muscle Actin, Desmin, Myogenin, CD34, CD38, FH-1</td>
</tr>
<tr>
<td>Skin</td>
<td>Pan CK, HMW CK, Neurofilament, Factor XIIIa, S100, HMW 45, Cytokeratin</td>
</tr>
<tr>
<td>Thyroid</td>
<td>Thyroglobulin, TTF-1, Calcitonin, HMW CK, CK19</td>
</tr>
<tr>
<td>Vascular</td>
<td>Factor VIII, CD34 (Qbend10), CD31, VEGF, Ulex, CD105</td>
</tr>
</tbody>
</table>

Distribution of Cytokeratin

Cytokeratins are also expressed in pairs comprising of type I (9-20) and type II (1-8), and acidic and basic comprising of 1-6 (pH 7.3 to 7.8) and 7-20 (pH 4.9 to 6.0).

<table>
<thead>
<tr>
<th>Number</th>
<th>MW (kD)</th>
<th>Distribution in Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 &amp; 10</td>
<td>68, 65.5, 65.5</td>
<td>Cornifying stratified; skin, endocervix</td>
</tr>
<tr>
<td>3 &amp; 12</td>
<td>63, 55</td>
<td>Human cornea</td>
</tr>
<tr>
<td>4 &amp; 13</td>
<td>59, 58</td>
<td>Non-cornifying stratified squamous epithelia; tongue, esophagus</td>
</tr>
<tr>
<td>5 &amp; 14, 15</td>
<td>58, 50, 50</td>
<td>Simple stratified epithelium; epidermis, squamous cell carcinoma</td>
</tr>
<tr>
<td>6 &amp; 16</td>
<td>56, 48</td>
<td>Fast turnover cells; hair follicles, suprabasal cells, activated keratocytes</td>
</tr>
<tr>
<td>7 &amp; 19</td>
<td>54, 40</td>
<td>Simple and glandular epithelium; breast, lung, bladder</td>
</tr>
</tbody>
</table>

(Continued)
### Classification of Cytokeratin Antibodies

<table>
<thead>
<tr>
<th>Type</th>
<th>Clone</th>
<th>Moll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type: Pan Cytokeratin</td>
<td>AE1/AE3</td>
<td>1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 15, 16, 17, 19</td>
</tr>
<tr>
<td>Type: Pan Cytokeratin</td>
<td>AE1</td>
<td>10, 14/16, 16/17, 19</td>
</tr>
<tr>
<td>Type: Pan Cytokeratin</td>
<td>AE3</td>
<td>1, 2, 3, 4, 5, 6, 7, 8</td>
</tr>
<tr>
<td>Type: Pan Cytokeratin</td>
<td>Lu-5</td>
<td>1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 15, 16, 17, 18, 19</td>
</tr>
<tr>
<td>Type: Pan Cytokeratin</td>
<td>MAK-6</td>
<td>8, 14, 15, 18, 19</td>
</tr>
<tr>
<td>Type: LMW Cytokeratin</td>
<td>5D3</td>
<td>8/18</td>
</tr>
<tr>
<td>Type: HMW Cytokeratin</td>
<td>34ßE12 (903)</td>
<td>1, 2, 5, 10, 14/15</td>
</tr>
<tr>
<td>Type: HME Cytokeratin</td>
<td>DE-SQ</td>
<td>13, 14, 15, 16</td>
</tr>
</tbody>
</table>

### Living up to Life

Duchenne Muscular Dystrophy

In the early stages, Duchenne and Becker Muscular Dystrophy affect the pectoral muscles, the trunk, and the upper and lower legs. These weaknesses lead to difficulty in rising, climbing stairs and maintaining balance.

### DMD and BMD

In the early stages, Duchenne and Becker Muscular Dystrophy affect the pectoral muscles, the trunk, and the upper and lower legs. These weaknesses lead to difficulty in rising, climbing stairs and maintaining balance.
Summary

- Consider "multi-purpose" antibodies
- Avoid employing antibodies if not ordered greater than three times per week
- Avoid employing novel antibodies until specificity is sufficiently established