IHC Classification of undifferentiated tumors – the primary panel

Mogens Vyberg
Professor of Clinical Pathology
Director of NordiQC
Aalborg University Hospital, Aalborg, Denmark
Tumours of unknown origin: Histology

Brain tumour - biopsy
Tumours of unknown origin: Immunohistochemistry

Pan-CK

S-100

CD45

VIM
UPT: A tumour appearing in metastatic setting without a histologically proven primary tumour.

UPT pose an increasing challenge for the pathologist - due to the progress in surgical and oncological treatment possibilities.
New, relatively specific antibodies give the pathologist more and better diagnostic tools.

But the diagnostic work also become more complex in terms of planning, optimization of protocols, interpretation of reaction patterns and error trapping.
- **IHC classification of the Unknown Primary Tumour**

10 - 15% of cancers remained UPTs

+ ??% uncertain if primary or metastatic

  - liver, lung, bone, lymph nodes, brain, peritoneum . . .

‘Undifferentiated’ neoplasms (5-10%)

  - carcinomas, sarcomas, melanomas, germ cell tumours
  
    - malignant lymphomas

- **Adenocarcinomas (80-90%)**

  - lung, breast, prostate, colorectum, ovary, pancreas ... 

- **Squamous cell carcinomas (5-10%)**

  - lung, esophagus, uterine cervix ...
Differences in prognosis

Differences in treatment regimes
- malignant lymphomas
- carcinomas (breast, prostate, ovary . . .)
- sarcomas (GIST, synovial sarcoma . . .)
- germ cell tumours

Pathology tests cost effective

Pathology tests save patient discomfort

The patient’s ‘right to know’

The risk of hereditary cancer
IHC classification of the Unknown Primary Tumour

- Most likely diagnoses
- Relevant differential diagnoses

Optimal selection of antibodies for a diagnostic algorithm
- Primary and secondary antibody panels
- Turn-around-time
- Laboratory expenses
- IHC classification of the Unknown Primary Tumour

Pathologist
- knowledge, acceptance, skill

Tumour material
- diagnostic markers

Antibodies available
- applic. in diagnostic algorithms

Methods
- protocol:
  - sensitivity, specificity, reliability
- interpretation:
  - cut-off level for positivity
  - clinical relevance

GIST: S-100B Protease
GIST: S-100B MWO
IHC classification of the Unknown Primary Tumour

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- protocol:
  sensitivity, specificity, reliability
- interpretation:
  cut-off level for positivity
  clinical relevance
An immunohistochemical vade mecum

**

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South Manchester

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version date October 2012
Planning diagnostic immunohistochemistry

Histopathology

Small to medium-sized blast cells with scanty cytoplasm. Nucleoli are inconspicuous.

- Bone marrow: the blasts are relatively uniform with round/oval indented, sometimes convoluted, nuclei. Nucleoli are variable but usually inconspicuous. Mitotic figures are less common than in T-ALL>

- Lymph nodes in B-LBL, there is usually diffuse involvement but sometimes paracortical infiltration. Cytology as for the bone marrow. Mitoses usually frequent. There may focally be "starry sky" pattern.

Immunohistochemistry 80%-90% of cases show an immature B cell immunophenotype

| CD10 | most cases, except for t(4;11) (q21;q23) ALL which is usually negative |
| CD13 | may be positive |
| CD19↑ | almost always |
| CD20 | variable |
| CD22 | variable |
| CD24 | most cases, except for t(4;11) (q21,q23) ALL which is frequently negative |
| CD33 | may be positive |
| CD45 | variable |
| CD79a | almost always |
| HLA-DR | + |
| Surface Ig | rarely positive |
| Surface Ig | rarely positive |
CD45

top
Also called leukocyte common antigen (LCA).
An essential regulator of T and B cell antigen χ
The target of immunosuppressive antibody treat
Major component of glycolyx

Negative regulator of IgE class switch recombination (J Biol Chem 2002;277:28836)
Mutations with loss of CD45 cause severe combined immunodeficiency - autosomal recessive, T cell negative, B cell positive, NK cell positive (OMIM 608971) patients have a defect in function or B and T cell development, lymphopenia, and deficiency in humoral and cell-mediated immunity.
77C to G mutation may increase intensity of T cell receptor signaling (J Immunol 2006;176:3931), and cause some cases of systemic sclerosis (Genes Immun 2003;4:168), multiple sclerosis (controversial), Nat Genet 2000;25:495 and autoimmune hepatitis (Genes Immun 2003;4:79).
Loss of CD45 activity in lymphocytes of elderly may cause T cell dysfunction in elderly (Mech Ageing Dev 2003;124:191)
Necrotic lymphomas are still CD45+, but necrotic carcinomas may also be CD45+ (AJCP 1998;110:521).
Different subsets of hematopoietic cells express different CD45 isoforms due to variable exon splicing, which can change in response to cytokines:
CD45RA - naive/resting T cells, medullary thymocytes
CD45RO - memory/activated T cells, cortical thymocytes
Uses: confirm presence of inflammatory cells, including intestinal intraepithelial lymphocytes (Archives 2002;126:897); confirm hematopoietic nature of tumors; classify lymphomas and leukemias (AJCP 1998;110:797).

Micro images: normal - liver with CD45+ Kupffer cells and lymphocytes; small intestine with CD45+ intraepithelial lymphocytes; splenic lymphocytes; thymus; tonsil
lymphoma - B cell lymphoma-unusual CD45 negative case (figure 3A); T cell: #1 - urine cytology; Hodgkin's-Reed-Sternberg cells are CD45 neg (figure 3B); intravascular (figure 1); primary bone lymphoma (figure 1B).
other - lymphoepithelioma-like carcinoma #1 of stomach (CD45+ lymphocytes); #2 of es.
Flow cytometry images: transient myeloproliferative disorder with erythroid differentiation.
Virtual slides: diffuse large B cell lymphoma
Positive staining (normal): hematopoietic cells (including monocytes, macrophages / histiocytes, platelets and megakaryocytes, dendritic cells, fibrocytes (J Immunol 1998;160:419), thymus (me

References: OMIM 151460

NordiQC
Enter a search phrase to select a Diagnosis Group (and repeat for a 2 or 3 Dx Group search), set Sensitivity and Minimum Refs, then click Build Panel button.

- **Adenocarcinoma CK07 positive CK20 Negative**
  - Mesothelioma, NOS
- **Mesothelioma, All**
  - Mesothelioma, Biphasic; Proliferation, Mesothelial, NOS;
  - Mesothelioma, Sarcomatoid; Mesothelioma, NOS;
  - Mesothelioma, Epithelioid
- **Mesothelioma, benign proliferations**
  - Proliferation, Mesothelial, NOS
- **Mesothelioma, lymphohistiocytoid**

**Selected Dxs:** none selected

- **Set Sensitivity:** 1 2 3
- **Set Minimum Refs:** All > 1 > 5

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**Open Cases**

- Start date
- Case Description
- View Panel
- Analyze Results
- Delete

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**Diagnosis Group and Antibody Education**

Enter a Diagnosis Group or Antibody search phrase and select the desired item.

**Learn About a Diagnosis Group:**

**Learn About an Antibody:**
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<th>Author(s)</th>
<th>Article</th>
<th>Publication</th>
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<td>2008</td>
<td>Lyons-Boudreaux V, Mody DR, Zhai J, Coffey D</td>
<td>Cytologic malignancy versus benignancy: how useful are the &quot;newer&quot; markers in body fluid cytology?</td>
<td>ARCH PATHOL LAB MED. 132:23-28</td>
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Open Cases

Start date                                      Case Description

View Panel   Analyze Results   Delete

Diagnosis Group and Antibody Education

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Learn About a Diagnosis Group:

Learn About an Antibody:
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<td>MOC-31</td>
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<td>BER-EP4</td>
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<td>97% 99</td>
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<td>S-100 (cytoplasmic/nuclear)</td>
<td>5% 208</td>
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<td>TAG-72</td>
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<td>RCC</td>
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Enter a search phrase to select an Antibody (and repeat for a 2 or 3 Antibody search), then click Build Panel button.

- KERATIN-HMW
- KERATIN-LMW
- KERATIN-PAN

Selected Abs:
- VIMENTIN
- KERATIN-PAN

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<td>0%</td>
<td>1</td>
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<td>&quot;Real&quot;</td>
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<tr>
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<td>+/(-)</td>
<td>-(+)</td>
<td>-(+)</td>
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<tr>
<td>Epithelial neoplasms</td>
<td>-</td>
<td>+(−)</td>
<td>−/+</td>
</tr>
<tr>
<td>Mesothelial neoplasms</td>
<td>-</td>
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<td>−</td>
</tr>
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<td>-/(+)</td>
<td>-/+</td>
</tr>
<tr>
<td>Non-neuronal neuroepithelial neoplasms</td>
<td>−</td>
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<td>+</td>
</tr>
<tr>
<td>Germ cell neoplasms</td>
<td>-</td>
<td>−/+</td>
<td>−/+</td>
</tr>
</tbody>
</table>
CD45 - Leucocyte common antigen (LCA)

- Transmembrane protein tyrosin phosphatase essential for haematopoietic signal transduction and cell activation
- Membrane associated component: 5 isotypes
- Intracellular component: one common type
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- Transmembrane protein tyrosin phospatase essential for haematopoietic signal transduction and cell activation
- Membrane associated component: 5 isotypes
- Intracellular component: one common type

- Large majority of haematolymphoid cells
- Lost in maturing erythocytes, megakaryocytes and plasmacells
- "Never" found in non-haematolymphoid cells
CD45 - Leucocyte common antigen (LCA)

Normal lymph node

Malignant lymphoma
CD45 - Leucocyte common antigen (LCA)

Liver

Brain

Critical assay performance control
Which is best?
CD45 – NordiQC run 37 2013

Optimal

Insufficient
CD45 - Leucocyte common antigen (LCA)

- CD45 RO ~ T-cells
- CD45 RA ~ B-cells

Lymph node/Tonsil
Cytokeratin-Positive, CD45-Negative Primary Centroblastic Lymphoma of the Adrenal Gland

A Potential for a Diagnostic Pitfall

Ludvik R. Donner, MD; PhD; Frank E. Mott, MD; Isaac Tafur, MD

- We report a case of cytokeratin-positive, CD45-negative primary polymorphic centroblastic lymphoma of the adrenal gland. Additional immunostaining, which demonstrated positivity for CD20 and κ light chain, as well as detection of the monoclonal rearrangement of the immunoglobulin heavy chain gene, helped to establish the diagnosis of lymphoma and to rule out an initially favored diagnosis of poorly differentiated carcinoma.

(Arch Pathol Lab Med. 2001;125:1104–1106)
CD45 - Leucocyte common antigen (LCA)

Molecular Biologic Findings
Monoclonal rearrangement of the immunoglobulin heavy chain gene was identified by polymerase chain reaction (data not shown).

Figure 2. Light microscopic appearance of the tumor (Giemsa stain, original magnification ×100, inset ×250).

Figure 3. Note immunoreactivity of the lymphoma cells for cytokeratin (A) and CD20 (C) but not CD45 (B) (original magnification ×100, inset ×250).
MATERIALS AND METHODS

We performed immunohistochemical stains for cytokeratin (AE1/AE3, Cell Marque, Austin, Tex; CAM5.2, Becton Dickinson, San Jose, Calif; cytokeratins 5/6, Zymed, San Francisco, Calif; cytokeratin 7, Dako Corporation, Carpinteria, Calif; cytokeratin 20, Dako; 34Beta12, Enzo, New York, NY), CD3, CD20, CD30, CD45RO, CD68, κ light chain, λ light chain, myeloperoxidase, epithelial membrane antigen, neuron-specific enolase, synaptophysin, S100 protein, HMB-45 (Dako), and chromogranin A (Cell Marque) on a TechMate 500 with a ChemMate Secondary Detection Kit–Peroxidase/DAB (Ventana Medical Systems, Tucson, Ariz). The histologic sections were pretreated by steaming in citrate buffer solution (Target Retrieval Solution, Dako) for 30 minutes at 99°C.

The monoclonal antibodies AE1/AE3 (working concentration, 0.4 μg of protein/mL) were applied for 25 minutes at room temperature. The immunostaining was repeated twice, each time with identical results.
### Primary panel for the unknown primary tumour

<table>
<thead>
<tr>
<th></th>
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<th>CK</th>
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</tr>
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<tbody>
<tr>
<td><strong>Haematolymphoid neoplasms</strong></td>
<td>+/(−)</td>
<td>−/(+)</td>
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<td>+/(−)</td>
</tr>
<tr>
<td><strong>Epithelial neoplasms</strong></td>
<td>−</td>
<td>+/(−)</td>
<td>−/+</td>
<td>−/+</td>
</tr>
<tr>
<td><strong>Mesothelial neoplasms</strong></td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><strong>Mesenchymal and neuronal neoplasms</strong></td>
<td>−</td>
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<td>+</td>
</tr>
<tr>
<td><strong>Non-neuronal neuroepithelial neoplasms</strong></td>
<td>−</td>
<td>−/(+)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Germ cell neoplasms</strong></td>
<td>−</td>
<td>−/+</td>
<td>−/+</td>
<td>+</td>
</tr>
</tbody>
</table>
Cellular filaments

# Microfilaments: (6 nm)

# Intermediate filaments (7-11 nm)

# Microtubuli (23 nm)
Intermediate filaments

- Group of mainly cytoplasmic filaments 7 – 11 nm in diameter
- Part of the cytoskeleton in virtually all cells, creating as meshwork and connecting nuclear membrane with cell membrane
- Often associated with microfilaments (6 nm) and microtubules (23 nm)
- Important for mechanical strength and cellular functions
Intermediate filaments – tetrameric units

Central core formed by eight tetramers

Nagle, AJSP 1988, 12:4
Intermediate filaments - 5 classes

- I acidic cytokeratins
- II basic-neutral cytokeratins
- III vimentin, desmin, glial fibrillary acidic protein, peripherin
- IV neurofilament protein, α-internexin, nestin
- V lamins
Cytokeratins as tonofilaments

Cytokeratin intermediate filaments attached to desmosomes

Drochmans et al.
Cytokeratins (CKs) belong to the most fundamental markers of epithelial differentiation

CKs comprise a large family of subtypes. Different cell types express different patterns of CK subtypes.

Cancers generally express CK patterns that at least in part represent the pattern of the putative cell of origin.

Metastases express CK patterns fairly concordant with those of the primary tumours.
Micrometastases identified by cytokeratin
Carcinoma in frozen section identified by cytokeratin
Low molecular weight cytokeratins in carcinomas

- Carcinomas “always” LMW-CK-positive, except some cases of
  - Renal cell carcinoma
  - Adrenal cortical carcinoma
  - Small cell carcinoma

CK8: Adrenal cortical carcinoma
CK8: Renal cell carcinoma
### Primary panel for the unknown primary tumour

<table>
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<th>Neoplasm Type</th>
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<td>Germ cell neoplasms</td>
<td>-</td>
<td>-/+</td>
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</table>
Cytokeratins in non-epithelial tumours

♀ 42 y, tumour infiltrating retroperitoneum

Malignant lymphoma!
### Primary panel for the unknown primary tumour

<table>
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Cytokeratins in malignant mesothelioma

CK8

CK5
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Cytokeratins in sarcomas

Synovial sarcoma

angiosarcoma
Cytokeratins in non-epithelial tumours

Leiomyosarcoma
### Primary panel for the unknown primary tumour

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Cytokeratins in malignant melanoma
## Primary panel for the unknown primary tumour

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<td>−/(+)</td>
<td>−/(+)</td>
<td>+/(−)</td>
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<tr>
<td>Epithelial neoplasms</td>
<td>−</td>
<td>+/(−)</td>
<td>−/+</td>
<td>−/+</td>
</tr>
<tr>
<td>Mesothelial neoplasms</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Mesenchymal and neuronal neoplasms</td>
<td>−</td>
<td>−/(+)</td>
<td>−/+</td>
<td>+</td>
</tr>
<tr>
<td>Non-neuronal neuroepithelial neoplasms</td>
<td>−</td>
<td>−/(+)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Germ cell neoplasms</td>
<td>−</td>
<td>−/+</td>
<td>−/+</td>
<td>+</td>
</tr>
</tbody>
</table>
Cytokeratins in germ cell tumours

CK8: Seminoma

CK8: Embr. carcinoma
Cytokeratins: retrieval causing false negativity

- AE1 detects CK8 after HIER only
- AE1 does not detect CK18
- AE3 does not detect CK8/CK18

HIER

Proteolysis

SCLC

Proteolysis
Cytokeratins: retrieval causing false negativity

HIER

Proteolysis

RCC

FN

TP
## Primary panel for the unknown primary tumour

<table>
<thead>
<tr>
<th></th>
<th>CD45</th>
<th>CK</th>
<th>S-100</th>
<th>VIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematolymphoid neoplasms</td>
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<td>-/(+)</td>
<td>+/-(-)</td>
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<tr>
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<td>+/(-)</td>
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<td>+</td>
</tr>
<tr>
<td>Germ cell neoplasms</td>
<td>-</td>
<td>-/+</td>
<td>-/+</td>
<td>+</td>
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</tbody>
</table>

Legend: 
- positive
+ positive
- negative
- negative
+ positive
S-100 protein

- Family of acid calcium binding proteins 9/13 kDa
- Located in nuclei, cytoplasm and cell membranes
- at least 10 $\alpha$-chains and one $\beta$-chain creating homo- and heterodimers

- S-100 $\beta$-chain mainly found in
  - Melanocytes
  - Glial cells
  - Langerhans’ cells / interdigitating reticulum cells
  - Fat cells
  - Myoepithelial cells
- Polyclonal antibodies primarily detects the $\beta$-chain
S-100 protein

brain

chondrocytes
S-100 protein

Tonsil
S-100 protein – pancreas
S-100 in malignant tumours

>90%

+++
S-100 protein

To HIER or not..
## Primary panel for the unknown primary tumour

<table>
<thead>
<tr>
<th>&quot;Real&quot;</th>
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<th>S-100</th>
<th>VIM</th>
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*Note: The table shows the expression patterns of various antibodies for different types of neoplasms.*
Vimentin

- Cytoplasmic intermediate filament, 57 kDa
- Present in all mesenchymal cells
- Present in early stages of all cells, replaced by other intermediate filaments in most non-mesenchymal cells
- Coexpressed with cytokeratin in some epithelia
  - Endometrium, renal tubules, thyroid gland …
- Coexpressed with cytokeratin in some non-epithelial cells
  - Mesothelium
Vimentin in normal tissue

Normal brain

HE

VIM
Vimentin in carcinomas

- renal cell carcinoma
- endometrioid carcinoma
Vimentin in non-epithelial tumours

mal. melanoma

mal. mesothelioma
IHC Classification of undifferentiated tumors –
the primary panel

Thank you for your attention

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