A Practical Approach to the Diagnosis of Common Hematopoietic and Solid Tumors of Childhood

USCAP 2007
Short Course #63

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Course Objectives
• To provide participants with basic concepts regarding the morphology, immunophenotype, molecular genetics, clinical features and biologic behavior of tumors encountered commonly in pediatric patients.
• To provide participants with a practical approach to the differential diagnosis of pediatric solid and hematopoietic neoplasms.

Hematopoietic (CD45-Positive) Tumors of Childhood

Lymphoid
• Lymphoblastic lymphoma/leukemia
• Other Non-Hodgkin lymphomas
• Hodgkin lymphoma

Myeloid
• Acute myeloid leukemia/Myeloid sarcoma
• Chronic myeloproliferative disorders
• Myeloproliferative/Myelodysplastic syndromes

Histiocytic
• Monocyte/macrophage tumors
• Acute monoblastic leukemia/Monoblastic sarcoma
• Histiocytic sarcoma
• Langerhans cell tumors (benign/malignant)
• Langerhans cell
• Dendritic reticulum cell

Hematopoietic Malignancies

Children
• MDS & MPD
• CML
• Hodgkin
• ALL
• NHL (Burkitt, A.L.L.)

Adults
• MDS & MPD
• AML
• CML
• CLL
• Hodgkin
• Multiple Myeloma
• NHL (Follicular and Diffuse Large Cell)
Non-Hodgkin Lymphomas Most Common in Children

- Lymphoblastic lymphoma/acute lymphoblastic leukemia
- Burkitt lymphoma
- Anaplastic large cell lymphoma (ALK-positive)
- Diffuse large B-cell lymphomas
- Primary mediastinal large B-cell lymphoma

Tissue Triage for Lymphoma Work-up

**Fresh tissue:**
- Flow cytometry
- Conventional cytogenetics
- Metaphase FISH
- Molecular analysis (fresh or snap frozen)
  - DNA-based (PCR, Southern blotting)
  - RNA-based (RT-PCR)

**Formalin-fixed tissue:**
- Morphologic examination
- Immunohistochemistry
- In situ hybridization (Interphase FISH)
- Molecular testing
  - DNA-based (PCR, Southern blotting)
  - RNA-based (RT-PCR)

Practical approach:
- If enough tissue available:
  - One portion in formalin
  - One portion of fresh tissue to flow cytometry and/or cytogenetics
  - One portion frozen for potential molecular analysis
  - +/- One portion (sterile) for Microbiology
  - Fix all in formalin

Handling of the sample:
- Drop in fixative as soon as obtained (do not stretch on tissue pads) – communicate with surgeon/radiologist
- Lift the tissue, do not pinch

Tissue processing:
- Cut multiple unstained levels upfront and stain every 5th level
- Many unstained sections available without facing the block again
- Pick specific levels for special stains (where cells of interest are present)
Case 5
Clinical History:
- 15-year-old boy
- Fever for three weeks, not responding to antibiotics, and isolated left inguinal lymphadenopathy.
- Excisional lymph node biopsy
  - The lymph node measured 2.5 cm in greatest dimension.
- Staging work-up: no additional disease

Case 5
Microscopic Findings:

Low magnification:
- Complete obliteration of the interfollicular (paracortical) area with preserved follicles
- Diffuse growth pattern
- “Starry-sky” appearance

High magnification:
- Large monotonous lymphoma cells with immunoblastic morphology
- Tingible-body macrophages
1. Burkitt lymphoma
2. Diffuse large B-cell lymphoma
3. Peripheral T-cell lymphoma, unspecified
4. Anaplastic large cell lymphoma, monomorphic variant.

Differential Diagnosis

Case 5 Immunohistochemical Stains

- CD20
- CD3
- CD30
- ALK

Case 5 Cytogenetics/Molecular Analysis

- The t(2;5)(p23;q35) translocation present in several metaphases.
- RT-PCR positive for the NPM-ALK fusion transcript

Diagnosis:

- Anaplastic large cell lymphoma of T/null cell lineage, ALK-positive (systemic type) - Monomorphic variant

Clinical History

- 10-year-old boy
- Left cervical lymphadenopathy.
- Excisional lymph node biopsy was performed.
  - The lymph node measured 2.3 cm in greatest dimension.
- Staging work-up: additional lymphadenopathy in the submandibular, paratracheal, axillary and inguinal areas.
Case 6
Microscopic Findings:

Low magnification:
- Complete effacement of normal architecture

Growth patterns:
- Diffuse
- Nodular
- Fibrosis, vascular proliferation

High magnification
- Polymorphous inflammatory cell population (histiocytes, plasma cells, small lymphocytes)
- Fibrosis and vascular proliferation
- Scattered areas with large immunoblastic cells
- Rare “hallmark cells”
**Case 6**

**Differential diagnosis**

1. Peripheral T-cell lymphoma, unspecified
2. Anaplastic large cell lymphoma, lymphohistiocytic variant
3. Angioimmunoblastic T-cell lymphoma (T-cell lymphoma, AIL-like)
4. Hodgkin lymphoma, classical type
5. Reactive lymphadenopathy (infectious process).

**Case 7**

**Immunohistochemical Stains**

- CD45
- CD3
- CD43
- CD45RO
- CD30
- ALK

**Case 6**

**Diagnosis**

- Anaplastic large cell lymphoma of T/null cell lineage, ALK-positive (systemic type) - Lymphohistiocytic variant

**Anaplastic Large Cell Lymphoma (ALCL)**

- **Definition:**
  - T-cell lineage (immunophenotype/genotype)
  - Large anaplastic (‘hallmark’) lymphoma cells
  - CD30 expression
Anaplastic Large Cell Lymphoma (ALCL)

- Categories:
  - Systemic
    - ALK-positive
    - ALK-negative
  - Primary cutaneous (ALK-negative)

- Demographics:
  - ALCL: 20-30% of childhood NHL
  - ALK+ tumors >90% of ALCL in children
  - Male : Female 6.5 : 1.

- Clinical Features:
  - Sites of involvement:
    - Nodal
    - Extranodal:
      - Skin, bone, soft tissue, lung (discrete lesions or diffuse), liver, rarely paranasal sinuses or brain
    - Systemic symptoms: fever, malaise, respiratory distress.

- Histologic variants -ALK positive:
  - Common (classic)
  - Small cell
  - Leukemic presentation (peripheral blood involvement)
  - Lymphohistiocytic
  - Monomorphic
  - Giant-cell rich
  - Sarcomatoid

- ALK-negative: Neutrophil/eosinophil-rich, Signet-ring cell variant.

- Antigens commonly expressed:
  - ALK, CD30, CD45, CD43, EMA
  - CD2, CD4
  - Cytotoxic antigens: TIA-1, perforin, granzyme B

- Antigens expressed in <50% of cases:
  - CD3, CD5, CD7, CD8, CD45RO

- Antigens expressed in 40-50% of cases:
  - Myeloid antigens: CD11b, CD13, CD15, CD33
Common Translocations Involving the ALK Gene in ALCL

<table>
<thead>
<tr>
<th>Chromosomal translocation</th>
<th>Fusion product</th>
<th>Fusion partner</th>
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</thead>
<tbody>
<tr>
<td>t(2;5)(p23;q35)</td>
<td>NPM-ALK</td>
<td>Nucleophosmin</td>
</tr>
<tr>
<td>t(1;2)(q21;p23)</td>
<td>TPM3-ALK</td>
<td>Tropomyosin 3</td>
</tr>
<tr>
<td>t(2;3)(p23;q21)</td>
<td>TFG-ALK</td>
<td>TFG (Tropomyosin receptor kinase-fused gene)</td>
</tr>
<tr>
<td>t(2;22)(p23;q11)</td>
<td>CLTCL-ALK</td>
<td>Clathrin heavy chain</td>
</tr>
<tr>
<td>inv(2)(p23;q35)</td>
<td>ATIC-ALK</td>
<td>ATIC (pur H gene)</td>
</tr>
</tbody>
</table>

Anaplastic Large Cell Lymphoma
The NPM-ALK Transcript

ALK (Anaplastic Lymphoma Kinase - receptor tyrosine kinase) - ALK gene on 2p23:

- Ribosomes
- Extracellular domain
- Transmembrane domain
- Tyrosine kinase domain
- Cytoplasm

Nucleophosmin – NPM gene on 5q35:

- Nucleolus
- Ribosomes

Anaplastic Large Cell Lymphoma
The NPM-ALK Transcript

Dimerization domain

Cytoplasmic kinase domain

Antibodies Specific for ALK
Available for Immunohistochemical Staining

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Type</th>
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<tr>
<td>p80</td>
<td>NPM-ALK (kinase domain)</td>
<td>polyclonal</td>
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<tr>
<td>ALK-11</td>
<td>ALK, cytoplasmic portion</td>
<td>polyclonal</td>
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<tr>
<td>ALK-1</td>
<td>ALK, cytoplasmic portion</td>
<td>monoclonal</td>
</tr>
<tr>
<td>ALK-1c</td>
<td>ALK, cytoplasmic portion</td>
<td>monoclonal</td>
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Differential Diagnosis of ALK+ ALCL

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<th>CD30</th>
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<th>CD7</th>
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<td>+/−</td>
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<td>+</td>
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<td>+/−</td>
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<td>+</td>
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<tr>
<td>DLBCL</td>
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<td>+/−</td>
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<td>+/−</td>
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<td>−</td>
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<tr>
<td>ALK+ DLBCL</td>
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<td>−</td>
<td>−</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
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<td>+</td>
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<td>+/−</td>
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<td>−</td>
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<td>−</td>
<td>−/−</td>
<td>+</td>
<td>+/−</td>
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<td>−</td>
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<td>+/−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Case 7
Clinical History

- 18-year-old boy
- Blunt trauma to the abdomen with persistent pain; Clinical diagnosis – Appendicitis; Operative procedure: Appendectomy.
- Intra-operative findings: purulent ascites fluid; massively enlarged appendix (18 cm in length, ~4 cm in diameter); extensive small bowel involvement by tumor.
- Staging work-up: extensive bone marrow involvement (ALL, L3; many blasts present in the peritoneal fluid).
Case 7
Gross Findings (Appendix):

Low magnification:
- Extensive infiltration of the appendiceal wall; mucosal ulceration/necrosis
- Diffuse growth pattern
- “Starry-sky” appearance

Microscopic Findings (Appendix):

High magnification:
- Intermediate size ("small non-cleaved") cells
- Tingible body macrophages
- Scattered large cells with cleaved nuclei
- High mitotic rate
**Case 7**

**Microscopic Findings:**

[Images of bone marrow and peritoneal fluid]

**Differential Diagnosis**

1. Burkitt lymphoma, atypical variant
2. Diffuse large B-cell lymphoma
3. Precursor B-cell/T-cell lymphoblastic lymphoma/leukemia

**Immunohistochemical Stains**

- CD20
- Ki-67
- CD3
- CD10

**Immunohistochemical Stains/ In situ hybridization**

- Ig kappa
- Tdt
- Ig lambda

**Cytogenetics/Molecular Analysis**

- Conventional cytogenetics: t(8;14)(q24;q32) present in several metaphases
- Fluorescence in situ hybridization (FISH): c-myc translocated to 14 q32 (confirming the involvement of this gene in the translocation).
**Case 7 - Conventional Cytogenetics**

-46,XX,del(1)(p36.3),add(10)(p11.21)

**Case 7**

**Diagnosis:**

- Burkitt lymphoma
  - Atypical (Burkitt-like) variant

**Burkitt Lymphoma**

- **Definition**
  - Mature B-cell phenotype (CD20+, Tdt-)
  - Medium-sized, monomorphic lymphoma cells
  - MYC translocation present leading to a high proliferation rate (Ki-67+ in nearly 100% of the cells)

**Burkitt Lymphoma Epidemiology**

<table>
<thead>
<tr>
<th>Sites of involvement</th>
<th>Endemic Burkitt Lymphoma</th>
<th>Sporadic Burkitt Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph nodes</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Ovary</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peak age (years)</td>
<td>4-7</td>
<td>4-7</td>
</tr>
<tr>
<td>Male : Female ratio</td>
<td>2:1</td>
<td>2:3:1</td>
</tr>
<tr>
<td>EBV (%)</td>
<td>96-98</td>
<td>58-60</td>
</tr>
</tbody>
</table>

**Burkitt Lymphoma Molecular Pathology**

- **MYC gene overexpressed as a result of one of the following translocations:**
  - t(8;14)(q24;q32) – MYC/IgH
  - t(2;8)(q11;q24) – MYC/Ig kappa
  - t(2;22)(q11;q11) – MYC/Ig lambda

**Burkitt Lymphoma – c-myc/IgH breakpoints**

- c-myc / IgH breakpoints
**Burkitt Lymphoma**

**Histologic Features**

- **Variants:**
  - Classical Burkitt lymphoma
  - Atypical Burkitt/Burkitt-like lymphoma
  - Burkitt lymphoma with plasmacytoid differentiation

**Immunophenotype**

- Mature B-cell phenotype, post-germinal center
- Positive for:
  - CD20 (strong), CD19, CD22, CD24, CD79a, surface Ig with light chain restriction
  - CD10, BCL-6
  - Ki-67 in >95% of the lymphoma cells (proliferation rate)
- Negative for:
  - CD5, CD23, BCL-2, TdT
- Sparse mature T-lymphocytes infiltrating the tumor (CD3+, CD5+, BCL-2++).

**Differential Diagnosis**

- Diffuse large B-cell lymphoma
  - Centroblastic - for the atypical variant
  - Immunoblastic - for the plasmacytoid variant
- Precursor B-cell lymphoblastic lymphoma
- ALCL, monomorphic variant

**Clinical History**

- 12-year-old boy
- Shortness of breath; cervical, axillary and inguinal lymphadenopathy. Imaging studies: Large anterior mediastinal mass.
- Operative procedure: Percutaneous needle-biopsy of a left cervical lymph node.
- Further staging work-up: Bone marrow negative for lymphoma; CBC/Diff normal.

**Microscopic Findings:**

**Case 8**

- Low magnification:
  - Complete effacement of normal architecture
  - Diffuse growth pattern
Case 8

Microscopic Findings:

- High magnification:
- Monotonous small to intermediate blast cells
- Finely dispersed nuclear chromatin
- Inconspicuous or absent nucleoli

Differential Diagnosis

1. Lymphoblastic lymphoma (precursor B-cell or precursor T-cell)
2. Other blastic neoplasms (myeloid sarcoma)
3. Blastic variant of mantle cell lymphoma (in adults only)
4. Burkitt lymphoma

Immunohistochemical Stains

- CD45
- CD3
- CD79a
- Tdt

Diagnosis:

- Precursor T-cell lymphoblastic lymphoma/leukemia
Acute Lymphoblastic Leukemia & Lymphoblastic Lymphoma

**Definitions**
- B-cell or T-cell lineage
- Blastic morphology
- Immature (precursor) phenotype (Tdt+, CD34+, HLA-DR+)

- Lymphoma vs. Leukemia (arbitrary clinical cut-off):
  - Lymphoma: no bone marrow involvement or <25% bone marrow involvement
  - Leukemia: >25% bone marrow involvement

**Demographics**
- Most common childhood malignancy
- >75% in children <6 years of age
- Male:Female 1.3:1
- ALL: 80-85% B-lineage
- LBL: 90% T-cell lineage

**Clinical Presentation**
- **B-cell ALL:**
  - Cytopenias, marked leukocytosis
  - Fever, fatigue, bone/joint pain, abdominal pain
  - Hepatosplenomegaly, lymphadenopathy

- **B-cell LBL:**
  - Solitary or multiple lesions
  - Scalp, lymph nodes, soft tissue, bones of the head/neck

- **T-cell ALL/LBL:**
  - Frequent anterior mediastinal mass with signs of mediastinal compression (shortness of breath, SVC syndrome)
  - Signs of systemic involvement similar to B-cell ALL

**Morphology/Cytology**
- Morphologic classification (FAB):
  - ALL, L1
  - ALL, L2
  - ALL, L3 (Burkitt)

- Cytoplasmic vacuoles

- ```image```
Acute Lymphoblastic Leukemia Immunophenotype

- Precursor B-cell ALL/LBL:
  - CD45 – weakly positive or negative
  - B-lineage antigens: CD19, CD20 (dim+/-), CD22, CD79a;
  - Immunoglobulin: IgMu only (cytoplasm and/or surface; no light chain restriction)
  - Other: CD10, CD34, HLA-DR, TdT
  - Aberrant expression of myeloid antigens (CD13, CD33)

- Precursor T-cell ALL/LBL
  - CD45 – weaker than normal lymphocytes
  - T-lineage: CD3 (cytoplasmic +/- surface), CD2, CD4, CD5, CD7, CD8, CD1a (CD4+/CD8+ CD2+);
  - Other: CD10, CD34, HLA-DR, TdT (10-20% negative for CD34/TdT/HLA-DR)
  - Rarely myeloid antigens: CD13, CD33, CD117

Cytogenetic and Molecular Groups Important in Risk Stratification in Precursor B-cell Acute Lymphoblastic Leukemia (60-70% of cases)

<table>
<thead>
<tr>
<th>Cytogenetic Abnormality</th>
<th>Molecular Lesion</th>
<th>Prognostic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>BCR-ABL (‘ALL-type’)</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>t(12;21)(p13;q22)</td>
<td>TEL-AML1</td>
<td>Favorable</td>
</tr>
<tr>
<td>t(1;19)(q23;p13.3)</td>
<td>PBX-E2A</td>
<td>Standard risk</td>
</tr>
<tr>
<td>t(4;11)(q21;q23)</td>
<td>AF4-MLL</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>Hyperdiploid (&gt;50 chromosomes)</td>
<td>Not known</td>
<td>Favorable</td>
</tr>
<tr>
<td>Hypodiploid (&lt;46 chromosomes)</td>
<td>Not known</td>
<td>Unfavorable</td>
</tr>
</tbody>
</table>

Acute Lymphoblastic Leukemia

Differential Diagnosis

- Benign B-cell and T-cell precursors:
  - B-cell: Hematogones (bone marrow, lymph nodes, tonsil)
  - T-cell: Normal thymocytes (mediastinal location only).

- Other blastic hematopoietic neoplasms

Benign B-cell Precursors (Hematogones)

Normal Thymus

- Benign B-cell and T-cell precursors:
  - B-cell: Hematogones (bone marrow, lymph nodes, tonsil)
  - T-cell: Normal thymocytes (mediastinal location only).

- Other blastic hematopoietic neoplasms

- Acute Lymphoblastic Leukemia

- Cytogenetic and Molecular Groups Important in Risk Stratification in Precursor B-cell Acute Lymphoblastic Leukemia (60-70% of cases)

- Acute Lymphoblastic Leukemia Immunophenotype

- Benign B-cell Precursors (Hematogones)
Differential Diagnosis of Hematopoietic Neoplasms with Blastic Morphology

<table>
<thead>
<tr>
<th></th>
<th>CD45</th>
<th>Tdt</th>
<th>CD79a</th>
<th>CD3</th>
<th>MPO</th>
<th>Lys</th>
<th>CD43</th>
<th>CD20</th>
<th>Cyclin D1</th>
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<tbody>
<tr>
<td>B-LBL.</td>
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<td>+</td>
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<tr>
<td>T-LBL.</td>
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<td>Myeloid sarcoma</td>
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<tr>
<td>Monoblastic sarcoma</td>
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<tr>
<td>Mantle cell lymphoma</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>−+/−</td>
<td>−+</td>
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</tr>
</tbody>
</table>

Case 9
Clinical History:
- 17-year-old previously healthy boy
- Small bowel obstruction. Abdominal CT: terminal ileal mass.
- Operative procedure: ileal-cecal resection.
  - The resected mass: 8 cm in greatest dimension; situated 13-14 cm proximal to the ileal-cecal valve.
- No additional sites of disease were found at staging work-up.

Case 9
Microscopic Findings:
Low magnification
- Extensive involvement of the ileal wall by lymphoma; mucosal ulceration
- Growth patterns:
  - Diffuse
  - Nodular (focal, <10% of the tumor)

Case 9
Microscopic Findings:
High magnification:
- Relatively monotonous large cells
  - Large or cleaved nuclei
  - Coarsely clumped and margined nuclear chromatin
  - Small nucleoli
1. Diffuse large B-cell lymphoma
2. Burkitt lymphoma, atypical variant

**Case 9**

**Immunohistochemical Stains**

- CD3
- Ki-67
- CD20
- BCL-2
- CD10
- BCL-6

Positive for: CD10, CD19, CD20, CD23, and surface immunoglobulin (lambda light chain restricted).

Negative for: CD34 and Tdt.

**Flow Cytometry:**

- Positive for: CD10, CD19, CD20, CD23, and surface immunoglobulin (lambda light chain restricted).
- Negative for: CD34 and Tdt.

**Cytogenetic Analysis**

45,X,-Y, del(6)(q14),del(17)(p13)[13]/
57, idem x2,
+1,+3,+5,+9,+10,+15,+16,+20,+20,+22[1]/
46,XY[6].
Case 9
Diagnosis:
- Diffuse large B-cell lymphoma
  - Centroblastic variant

Case 10
Clinical History:
- 14-year-old girl
- Large anterior mediastinal mass and cervical lymphadenopathy.
- Operative procedure: incisional biopsy of the cervical lymph node.
- Additional staging work-up: no other disease sites identified.

Case 10
Microscopic Findings:
Low magnification:
- Total effacement of the normal architecture
- Diffuse growth pattern
- Areas of interstitial fibrosis

High magnification:
- Monotonous large cells
  - Abundant pale/clear cytoplasm
  - Cleaved nuclei (some “flower-like”)
  - Rare Reed-Sternberg-like cells
1. Diffuse large B-cell lymphoma
2. Anaplastic large cell lymphoma (common or monomorphic variant).
3. Hodgkin disease, nodular sclerosis, syncitial
4. Metastatic non-hematopoietic tumor (e.g. germ cell tumor).

**Case 10**
**Differential Diagnosis**
1. Diffuse large B-cell lymphoma
2. Anaplastic large cell lymphoma (common or monomorphic variant).
3. Hodgkin disease, nodular sclerosis, syncitial
4. Metastatic non-hematopoietic tumor (e.g. germ cell tumor).

**Case 10**
**Diagnosis:**
- Diffuse large B-cell lymphoma
  - Features suggestive of the mediastinal subtype

**Diffuse Large B-cell Lymphoma**
- **Definitions**
  - Mature B-cell neoplasm (CD20+)
  - Large lymphoma cells (larger than normal macrophage nuclei)
  - Diffuse growth pattern

- Mediastinal (thymic) large B-cell lymphoma:
  - DLBCL with primary anterior mediastinal location and distinctive clinicopathologic features

**Immunohistochemical Stains**
- CD45
- CD30
- CD20
- CD15
Diffuse Large B-cell Lymphoma

Demographics
- ~10% of pediatric NHL
- Male:Female 1.2:1
- Mediastinal lymphoma: marked female predominance

Clinical Features
- Lymphadenopathy
- Extranodal (40%): -
  - GI tract (gastric or ileocecal), +/- Helicobacter pylori
  - +/- MALT lymphoma
- Mediastinal lymphoma
  - Bulky anterior mediastinal mass with associated symptoms
  - Dissemination predominantly to extranodal/extramedullary sites (kidney, skin, lungs)

Biological Insights:
- De novo or as progression of a low-grade lymphoma (follicular center cell)
- Complex chromosomal abnormalities
- Two prognostic groups:
  - Follicular center cell differentiation (CD10+, BCL-6+)
  - Activated B-cell differentiation (CD10-, BCL-6-, MUM1+)
- Mediastinal lymphoma:
  - Alterations on chromosomes 9p (REL gene), 6p (HLA class I genes)
  - Closer to Hodgkin lymphoma than to DLBCL by gene expression profiling.

Morphologic Features
- Variants:
  - Centroblastic (<90% immunoblasts)
  - Immunoblastic (<90% immunoblasts)
  - Anaplastic (anaplastic cells, centroblasts, immunoblasts)
- Mediastinal lymphoma
  - Any of the above
  - Clear-cell morphology

Immunophenotype
- Mature B-cell tumors:
  - CD45 (strong)
  - B-lineage: CD19, CD20 (strong), CD22, CD79a, Immunoglobulin (light chain restricted) (some lymphomas lack Ig expression, but have rearranged Ig genes)
  - Subset: CD10+, BCL-6+, BCL-2+ ("follicular")
  - Subset: CD5+

Mediastinal lymphoma:
- CD45+, CD20+, CD30+
- CD5-, CD10-, BCL-2-, BCL-6-, Ig-, HLA class I-.
Differential Diagnosis of B-lineage Lymphomas Common in Children

<table>
<thead>
<tr>
<th></th>
<th>eCD10</th>
<th>eCD4</th>
<th>eCD8</th>
<th>ly/Scy/s</th>
<th>Mu chain</th>
<th>Ki-67</th>
<th>MEL T-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkitt</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>g+</td>
<td>+</td>
<td>(100%) Rare</td>
</tr>
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<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>DLBCL</td>
<td>++</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/+</td>
<td>+</td>
<td>(70-90%) Freq.</td>
</tr>
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</tr>
<tr>
<td>B-LBL</td>
<td>+/-</td>
<td>++</td>
<td>+/-</td>
<td>+</td>
<td>cy/s Mu chain</td>
<td>+</td>
<td>(60-70%) Rare</td>
</tr>
</tbody>
</table>

Case 1

Case history

- 5 year old girl presented to her pediatrician with hematuria
- Physical examination showed a left sided abdominal mass
- Radiographic studies showed a large left renal mass
- A left radical nephrectomy was performed
Case 1
Gross examination

- 341 gm, 8.9 x 7.0 x 6.0 cm kidney
- No capsular defects
- No adrenal gland attached to the specimen
- Irregular tumor thrombus protruding from renal vein was noted
- External surface inked
- Ureteral and vascular margins sectioned

Identify margins (vessels)
Ink

Weigh Measure Inspect +/- Photograph external surface

Case 1 - Gross examination

- 7.2 x 6.4 x 5.9 cm mass
- Well circumscribed and encapsulated
- Hemorrhage and necrosis
- Apparent gross extension through renal capsule with peritumoral hemorrhage
- Margins grossly uninvolved
- Uninvolved kidney has poorly demarcated tan discoloration
- Para-aortic lymph nodes submitted separately were grossly normal

Bisect
Measure mass
Photograph
Take Samples for biologic studies
Fix overnight

Tumor protruded through renal capsule
Case 1
Diagnosis

- Wilms tumor with anaplasia
- Metastatic tumor involving one lymph node in periaortic region
- Stage III
- Margins free of tumor
- Intralobar nephrogenic rest
**Wilms tumor**

- **Gross Pathology**
  - Well demarcated
  - Non infiltrative growth
  - Pseudocapsule
  - Soft, mucoid, gray, yellow, tan
  - Hemorrhage and necrosis

---

**Pediatric Renal Tumor**

**Gross Examination**

"Make your life easier"

- Don’t let the surgeon cut it open
- Don’t forget to identify and inspect vessels and potential sites of capsular rupture before inking
- Don’t forget to ink before bisecting
- Absolutely don’t shave margins or strip capsule
- Don’t forget to wash blade between cuts and keep bench clean
- Use a photograph or diagram to indicate where your sections came from

---

**Pediatric Renal Tumors**

**Key elements for staging**

- Take most sections from the periphery of the tumor
  - Show relationships between tumor and normal kidney, hilum and margins
  - Submit hilar fat to assess for vascular invasion

- Look for lymph nodes in hilum
  - If none found, submit all fatty hilar tissue
- Submit sections of uninvolved kidney and nephrogenic rests

---

**Location of sections to best demonstrate capsular penetration**
Wilms tumor

- **Histology**
- Primitive cells recapitulating developing fetal kidney
- Variety of patterns: blastemal, stromal, epithelial
- Heterologous elements can be seen

Nodule of blastema with background stroma

3 patterns of blastemal growth
- Serpentine
- Nodular
- Diffuse
  - Aggressive growth
  - But highly chemosensitive

Diffuse blastemal Wilms
Note lack of capsule

Skeletal muscle differentiation in stroma is relatively common in Wilms tumor
Wilms tumor
Differential diagnosis

- Nephrogenic rests
- Mesoblastic nephroma
- Clear cell sarcoma
- Rhabdoid tumor
- Metanephric adenoma
- Papillary renal cell carcinoma
- Lymphoma
- PNET
- Neuroblastoma
- Desmoplastic small round cell tumor
- Hepatoblastoma
- Rare miscellaneous tumors

Clear cell sarcoma

1-6 year-old patients
Peculiar predilection for bone metastases
Grossly well circumscribed
Variety of patterns
Cells with bland nuclei, dispersed chromatin
Cellular fibrous bands containing tubules
Cytokeratin negative
Vimentin positive
CD10 positive
Lymph nodes often positive
Metanephric adenoma
- Pure epithelial tumor
- Wide age range
- Circumscribed but not encapsulated
- Bland nuclei, absent nucleoli, no mitoses
- IHC:
  - CK7+ (patchy)
  - WT1+
  - EMA negative

Wilms tumor
Differential diagnosis
- Neuroblastoma
  - Negative for epithelial markers
  - More likely to have calcification on x-ray
- Desmoplastic small round cell tumor
  - Negative for the N-terminal antibody to WT1

Wilms tumor
Histologic classification
- Favorable histology
  Usual Wilms tumor (90% of cases)
- Unfavorable histology
  Anaplastic Wilms tumor (10% of cases)

Definition of anaplasia
- Increase in nuclear diameter at least 3x that of an adjacent nucleus of the same cell type
- Nuclear hyperchromatism
- Enlarged, abnormal, usually multipolar mitoses
  - Each component of the mitotic figure is as large or larger than a normal metaphase
  - Indicative of abnormal DNA content

** All three must be present
Anaplastic Wilms tumor post chemotherapy

Wilms tumor
Histologic classification
- Anaplasia can be focal or diffuse
  - Most are diffuse
- Criteria for focal anaplasia
  - Area of anaplasia has to be circumscribed
  - Surrounded on all sides by non-anaplastic tumor or normal kidney
  - Cannot extend to the edge of a slide unless you have an adjacent section on tumor map
  - Anaplasia cannot be located outside kidney or in a vessel
  - No moderate or severe nuclear unrest in the remainder of the tumor
  - Cannot be present in a pre-operative needle biopsy specimen

Wilms tumor
Histologic classification
- Severe "nuclear unrest"
  - Background nuclear enlargement and cytologic atypia and histologic disarray similar to that seen in anaplastic tumors
  - NO enlarged, multipolar mitotic figures
- Poor interobserver variability has led to some controversy over whether or not this is a valid category
  - Milder forms of unrest can be seen in most Wilms tumors
- Currently grouped with other favorable histology tumors

Wilms tumor
Histologic classification
- Anaplastic WT are thought to arise from accumulation of genetic damage in favorable histology tumors
- Anaplastic WT are characterized by p53 dysfunction and resistance to chemotherapy
- Anaplastic WT also may be inherently more aggressive
  - Comparing Stage I tumors without adjuvant therapy, anaplastic tumors do worse than favorable histology tumors (preliminary data)
Wilms tumor
Histologic classification

Does nuclear unrest predict an adverse outcome compared to favorable histology?

- Tumors with nuclear unrest have a 5-year event free survival that appears in between that of favorable histology and anaplasia (p=0.10)
  - Favorable histology: 87.1%
  - Nuclear unrest: 74.1%
  - Anaplasia: 59.1%

- Tumors with nuclear unrest have an overall survival comparable to favorable histology (p=0.0347)
  - Favorable histology: 91.1%
  - Nuclear unrest: 88.9%
  - Anaplasia: 63.6%

Tumors with nuclear unrest while more likely to relapse retain chemosensitivity and overall survival comparable to favorable histology.

Key points: 1) Tumors with nuclear unrest are categorized as favorable histology 2) Nuclear unrest in a tumor can be used to get from focal anaplasia to diffuse anaplasia.

Wilms Tumor
Staging

- **Stage I** Confined to kidney and completely resected
  - Tumor cannot penetrate renal capsule
  - Tumor not seen in vessels of the renal sinus
  - No biopsy before nephrectomy
  - No tumor at margins
  - No lymph node metastases

Stage I Wilms Tumor

- **Stage II** – Tumor extends beyond kidney but completely resected
  - Tumor extends through renal capsule
  - Invasion of renal sinus vessels or renal vein
  - Specimen margins uninvolved
  - Tumors that extend into adjacent organs as long as completely excised en bloc can still be regarded as Stage II

Stages I and II favorable histology Wilms tumors are treated with the same chemotherapy regimen (except for tiny tumors).
Stage II Wilms Tumor

Wilms Tumor Staging

Stage III Gross residual tumor
- Involved surgical margin
- Transected tumor in renal vein or tumor thrombus firmly attached to or invading vein wall at margin where vein was severed
- Tumor removed in more than one piece
- Local infiltration into vital structures not resectable
- Tumor implants on peritoneal surface or invasion through peritoneal surface
- Tumor in regional lymph nodes
- Tumor spill before or during surgery of any degree
- Prior core or open biopsy

Stage III favorable histology WT and all tumors with anaplasia receive radiation therapy in addition to a more aggressive chemotherapy regimen

Stage III Wilms Tumor

Wilms Tumor Staging

Stage IV
- Hematogenous metastasis or nodal metastases outside the abdomen

Stage V
- Bilateral tumors
- Whenever possible, the substage of each tumor should be determined with the final designation indicating the highest substage lesion (Stage V, substage II)

Wilms tumor

Summary of New staging updates
- Stage I tiny tumors arm
  - lymph nodes must be sampled and be negative
- Prior core or open biopsy or any spillage before or during surgery now = Stage III
- Bilateral WT can be treated with chemotherapy without biopsy if radiographic evidence is classic
- Post treatment response will be used to guide further therapy

Wilms tumor

Nephrogenic rests

- What is a nephrogenic rest?
  - Region of persistent embryonal tissue in renal parenchyma
  - Found in 30-44% of kidneys removed for Wilms tumor
  - Prevalence is directly proportional to the care with which renal parenchyma is sampled

- Nephroblastosomatosis = multiple or diffusely distributed nephrogenic rests
Wilms tumor
Nephrogenic rests

- Two fundamental categories of nephrogenic rests based on topography
  - Intralobar - represents earlier developmental abnormality
  - Perilobar - represents a later developmental abnormality

<table>
<thead>
<tr>
<th>Feature</th>
<th>Perilobar rests</th>
<th>Intralobar rests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site in lobe</td>
<td>Periphery, subcapsular</td>
<td>Parenchymal</td>
</tr>
<tr>
<td>Usually numerous, can be diffuse</td>
<td>Usually sparse</td>
<td></td>
</tr>
<tr>
<td>Margins</td>
<td>Circumscribed</td>
<td>Indistinct, interdigitating</td>
</tr>
<tr>
<td>Relation to renal parenchyma</td>
<td>Demarcated, no nephrons within rest</td>
<td>Dispersed between nephrons</td>
</tr>
<tr>
<td>Composition</td>
<td>Blastema or tubules</td>
<td>Blastema, tubules</td>
</tr>
<tr>
<td>Scant or sclerotic stroma</td>
<td>stroma usually predominates</td>
<td></td>
</tr>
<tr>
<td>Associations</td>
<td>BWS, Hemihypertrophy, Perlman Trisomy 18, 13</td>
<td>WAGR, Denys-Drash</td>
</tr>
<tr>
<td>GU anomalies</td>
<td>Contralateral WT</td>
<td>Contralateral WT</td>
</tr>
</tbody>
</table>

Diffuse perilobar nephroblastomatosis

Diffuse perilobar nephroblastomatosis
Intralobar nephrogenic rest

Parenchymal
Usually sparse

Indistinct, interdigitating margins
Dispersed between nephrons
Stroma usually predominates

Wilms tumor
Nephrogenic rests

Primary diagnostic considerations

- Hyperplastic nephrogenic rests can be a problem diagnostically

Both hyperplastic rests and WT
  - Can produce large masses
  - Can demonstrate rapid proliferation and growth
  - Can be multifocal
  - Are composed of primitive nephrogenic elements
  - Can be better distinguished by serial radiographic studies than by needle biopsy

Favor Wilms tumor
  - Spherical growth
  - Conforms to shape of kidney (perilobar)
  - Has a fibrous pseudocapsule
  - Associated with normal renal structures
  - No normal renal elements within lesion

Favor Nephrogenic rest
  - Multifocal
  - Usually sparse
  - PRAME positive
  - Associated with primitive tubules
  - No pseudocapsule between embryonal tissue and normal kidney elements

Wilms tumor
Nephrogenic rests

Primary diagnostic considerations

- Type of rest has implications for risk of developing Wilms tumor in contralateral kidney
  - Risk for developing Wilms tumor in opposite kidney applies to patients < 12 months of age at diagnosis of first WT
    - If perilobar rests are present, risk = 10%
    - If intralobar rests are present, risk = 5%
    - If both perilobar and intralobar rests are present, risk = 15%
    - Most second WT occur within 3 years (range 0-13 years)


Fates of nephrogenic rests

- Adenomatous rest
- Hyperplastic rest
- Nephrogenic rest
- Regressing rest
- Obsolescent rest

Neoplastic rest

Chemotherapy

Modified from Beckwith 1990


Case 2
Case history
- 14-month-old girl who presented with a large abdominal mass
- Radiographic studies showed a left adrenal mass with focal calcification
- Bone marrow biopsies were normal
- A left adrenalectomy was performed

Case 1
Gross examination
- 35 gm, 3.0 x 3.0 x 2.5 cm mass
- Adrenal gland at one aspect
- External surface inked
- Sections show nodular, relatively homogeneous grey tissue with focal necrosis and calcification

Take Samples for biologic studies

Weigh → Measure → Inspect → Ink → Section → Photograph

Photograph → Take Samples for biologic studies
Case 2
Diagnosis

Neuroblastoma, stroma poor, differentiating (Favorable histology)

**Neuroblastoma**

- Tumor composed of neuroblasts
  - primitive cells that populate the sympathetic nervous system
- Heterogeneous tumors
  - spontaneously regress
  - spontaneously or treatment induced maturation
  - aggressively grow and metastasize
Neuroblastoma

• Histologic features are useful in predicting outcome

• International Neuroblastoma Pathology Classification (INPC) (Cancer 1999;86:364)
  – Updated Shimada system
  – Histopathologic features
  – Age of the patient

• Important for stratifying patients into risk categories

INPC System

• Four factors to consider in histologic classification
  – Stroma
  – Degree of differentiation
  – Measurement of proliferation and apoptosis
  – Age of the patient

Neuroblastoma

• Stroma contains spindled nuclei
• Neuropil expands space between nuclei and is actually abundant cytoplasmic processes projecting off the neuroblasts
Neuroblastoma
INPC System - Definitions

• Differentiation assessment
  – based on cytologic differentiation of neuroblasts toward ganglion cells
  – Undifferentiated neuroblasts are unrecognizable as neuroblasts on H&E
    • Need ancillary studies
  – A differentiating neuroblast has twice the cytoplasmic to nuclear volume

Neuroblastoma
INPC System - Differentiation

• Undifferentiated NB
  – Malignant small cell tumor
  – No neuropil or rosettes visible on routine stains
  – Requires ancillary studies
    • Immunohistochemistry
    • Electron microscopy
  – Urinary catecholamine levels usually helpful
  – Differential diagnosis
    • ES/PNET, ARMS, hematolymphoid tumors
Undifferentiated NB Immunohistochemistry

- Undifferentiated neuroblastomas will still show markers of neuroblasts
  - PGP9.5
  - NSE (this one is not specific and can be seen in hematolymphoid tumors)
  - Synaptophysin
  - Chromogranin
    - The latter two markers will be more variable and focal than PGP9.5
    - Can be positive for vimentin (poorly diff NB are typically negative)
    - Can be positive for CD99 (be careful here)

Neuroblastoma INPC System - Differentiation

Poorly differentiated NB
- Uniform round cells with speckled chromatin
- Abundant cytoplasmic processes (neuropil)
- Presence of Homer Wright pseudorosettes aid diagnosis

Differentiating NB
- 5% of neuroblasts show ganglion cell differentiation
- A differentiating neuroblast has twice the cytoplasmic to nuclear volume

Neuroblastoma INPC System - MKI

- Mitotic karyorrhectic index is the next assessment
  - Number of cells in mitosis
  - Number of cells in the process of karyorrhexis
    - Condensed and fragmented nuclear material
    - Dense eosinophilic cytoplasm
  - Denominator 5000 cells
  - Count should reflect an average from an assessment of several regions of the tumor

- Low MKI = < 2% (< 100/5000)
- Intermediate MKI = 2-4% (100-200/5000)
- High MKI = > 4% (> 200/5000)
- High correlation between high MKI and MYCN amplification
Poorly differentiated NB with high MKI

Arrows highlight some of the many karyorrhectic cells in the field

Cells with condensed but not fragmented nuclei do not count toward the MK index – don’t count these

Figure 2 from Shimada et al. International neuroblastoma pathology classification for prognostic evaluation of patients with peripheral neuroblastic tumors. Cancer 2001;92:2451-61 with permission John Wiley & Sons.

Neuroblastoma INPC System - Prognosis

Prognostic classification
For Stroma poor tumors

Differentiation
MKI
Age of patient
< 1.5 years
1.5 to 5 years
> 5 years

Stratified into favorable and unfavorable groups

Figure 2 from Shimada et al. International neuroblastoma pathology classification for prognostic evaluation of patients with peripheral neuroblastic tumors. Cancer 2001;92:2451-61 with permission John Wiley & Sons.

Neuroblastoma INPC System - Outcome

Favorable Histology (58% of cases)
EFS 90.4%
OS 97.8%

Unfavorable Histology (42% of cases)
EFS 26.9%
OS 35.6%
**Neuroblastoma**

INPC System - Stroma

- Amount of Stroma
  - < 50%
    - Neuroblastoma
      - Stroma poor
  - Essentially all
    - Ganglioneuroma
      - Stroma dominant
  - Ganglioneuroblastoma

**Ganglioneuroblastoma**

- Ganglioneuroblastoma
  - Nodular
    - Stroma poor
    - or
    - or
    - Stroma rich
  - Ganglioneuroblastoma
    - Intermixed
      - Stroma-rich

---

**Ganglioneuroblastoma, nodular**

- Formerly called composite ganglioneuroblastoma
- **Classic type**
  - Macroscopically visible, circumscribed neuroblastic nodule surrounded by stroma
- **Variant types**
  - Multinodular
  - Large nodular
  - No nodule

---

**Ganglioneuroblastoma, nodular, classic type**

- Neuroblastic nodule

---

**Ganglioneuroblastoma, nodular, classic type**

- Macrophotograph of ganglioneuroblastoma, nodular. Cancer 2003;98:2274-81. This material is used by permission of John Wiley & Sons, Inc.
Ganglioneuroblastoma, nodular

- Prognostic classification dependent on grading of neuroblastic component using the INPC scheme for stroma-poor tumors

- GNB with favorable nodules
  - OS 90.5%
  - EFS 86.1%

- GNB with unfavorable nodules
  - OS 33.2%
  - EFS 32.2%

Peuchmaur M, d’Amore ES, Joshi VV et al. Revision of the International Neuroblastoma Pathology Classification: confirmation of favorable and unfavorable prognostic subsets in ganglioneuroblastoma, nodular. Cancer 2003;98:2274-81. This material is used by permission of John Wiley & Sons, Inc.

Ganglioneuroblastoma, nodular

- Variant types:
  - Multinodular
    - Two or more macroscopically visible neuroblastic clonal nodules in a ganglioneuromatous background

Peuchmaur M, d’Amore ES, Joshi VV et al. Revision of the International Neuroblastoma Pathology Classification: confirmation of favorable and unfavorable prognostic subsets in ganglioneuroblastoma, nodular. Cancer 2003;98:2274-81. This material is used by permission of John Wiley & Sons, Inc.

Ganglioneuroblastoma, nodular

- Variant types:
  - Large nodular
    - Neuroblastic nodule has overgrown stromal component
    - A thin rim of stroma is seen at the periphery of the nodule or as the trabecular portion between neuroblastomatous nodules

Peuchmaur M, d’Amore ES, Joshi VV et al. Revision of the International Neuroblastoma Pathology Classification: confirmation of favorable and unfavorable prognostic subsets in ganglioneuroblastoma, nodular. Cancer 2003;98:2274-81. This material is used by permission of John Wiley & Sons, Inc.

Ganglioneuroblastoma, nodular

- Variant types:
  - No nodule
    - Primary tumor has features of GNB intermixed or GN but a metastatic site shows neuroblastoma

Peuchmaur M, d’Amore ES, Joshi VV et al. Revision of the International Neuroblastoma Pathology Classification: confirmation of favorable and unfavorable prognostic subsets in ganglioneuroblastoma, nodular. Cancer 2003;98:2274-81. This material is used by permission of John Wiley & Sons, Inc.
**Neuroblastoma**

**INPC System**

- **Ganglioneuroblastoma, intermixed**
  - Stromal rich, greater than 50% of tumor comprised of stroma
  - Intermixed GNB have a 93.2% event free survival and 100% overall survival

**Ganglioneuroma**

- **Ganglioneuroma, mature and maturing**
  - Stroma-dominant, nearly all stroma
  - Uniformly benign but may cause mass effect

**INPC Classification - Revised**

**Neuroblastoma**

Caveats to histologic typing

- Prognostic classification can be problematic when dealing with:
  - Metastatic sites
    - Evaluation of tumor from metastatic sites can yield equivalent information provided the sample is large enough
  - Small specimens (needle cores)
    - Tumors may be heterogeneous (Ganglioneuroblastoma, nodular)
  - Prior treatment
Post treatment Neuroblastoma

- Histologic description can be provided but prognostic classification does not apply
  - Histologic features may change with therapy
  - High risk biologic features (MYCN amplification) persist
- Treatment associated changes include calcification, necrosis and differentiation
- Dx: Post-treatment NB (see comment) – comment should include differentiation, mitotic activity, margin evaluation and lymph node evaluation

Neuroblastoma Clinical staging

<table>
<thead>
<tr>
<th>Stage</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Localized tumor with complete gross excision. Ipsilateral, nonadherent lymph nodes negative for tumor (lymph nodes adherent to the tumor and removed intact with the primary tumor may be positive)</td>
</tr>
<tr>
<td>2A</td>
<td>Localized tumor with incomplete gross excision. Ipsilateral, nonadherent lymph nodes are negative for tumor (lymph nodes adherent to the tumor and removed intact with the primary tumor may be positive)</td>
</tr>
<tr>
<td>2B</td>
<td>Localized tumor with or without complete gross excision. Ipsilateral, nonadherent lymph nodes are positive for tumor. Contralateral, nonadherent lymph nodes are negative for tumor.</td>
</tr>
<tr>
<td>3</td>
<td>Unresectable tumor infiltrating across the midline (tumor originating on one side infiltrating across the midline beyond the opposite border of vertebral column or midline tumor extending bilaterally beyond both borders of vertebral column or contralateral lymph node involvement rendering tumor unresectable)</td>
</tr>
<tr>
<td>4</td>
<td>Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin and/or other organs except as defined in Stage 4S</td>
</tr>
<tr>
<td>4S</td>
<td>Localized primary tumor (Stage 1, 2A or 2B) in an infant &lt; 1 year of age with dissemination limited to skin, liver and/or limited involvement of bone marrow (&lt;10% of total nucleated cells are neuroblasts)</td>
</tr>
</tbody>
</table>

Neuroblastoma Prognostic Factors

**Good**
- age < 1 year
- Stage 1, 2, or 4S (special)
- Extra adrenal location
- low serum ferritin,NSE, LDH
- high urine VMA/HVA ratio
- CD44 expression
- High TRKA expression

**Bad**
- age >1 year
- Stage 3 or 4
- adrenal primary
- MYCN amplification
- loss of 1p36, gain 17q
- BCL2 expression
- High TRKB expression

**MYCN**
- High level amplification (>10 copies per nucleus)
  - 22% of primary neuroblastomas
  - 40% of advanced stage neuroblastoma
- Amplification in localized tumors is not automatically predictive of poor outcome
- MYCN amplified tumors show high MKI and little differentiation
- Beware of calling high MKI in differentiating tumors

FISH for MYCN on formalin-fixed paraffin embedded tissue showing numerous red signals in neuroblast nuclei consistent with high MYCN amplification

Courtesy of Dr. Arie Perry
Neuroblastoma
Prognostic Factors

- DNA index
  - Tumors with a diploid DNA content (DNA index = 1) have a worse prognosis
  - Tumors with hyperdiploid DNA content fare better

- MYCN status and DNA index are used in conjunction with age, stage and INPC classification in risk stratifying patients for therapy

Neuroblastoma
Key points

- Know the difference between stroma and neuropil
- Know that “differentiation” used in classification is based on cytologic features of neuroblasts→ganglion cells
- Know how to do an MKI assessment
- Know that ganglioneuroblastoma, nodular now has a good prognosis (favorable histology) category
- Know where you keep your INPC cheat sheet

Case 3
Case history

- A 1 year-old male presented to his pediatrician with an enlarging buttock mass
- Physical examination showed a firm mass in the gluteal region
- A pelvic CT scan showed a 10.0 x 8.0 x 7.0 cm mass involving the gluteus muscle with extension into the pelvis
- An incisional biopsy was performed
- Several fragments of tan-white, fleshy firm tissue were received
- One of the fragments of tissue was snap frozen in liquid nitrogen and stored at -80°C C for biological studies.
Myogenin

Case 3
Diagnosis
Alveolar rhabdomyosarcoma

Rhabdomyosarcoma
- Most common soft tissue sarcoma in children
- Accounts for 15% of solid tumors treated at pediatric hospitals
- Typically sporadic
  - Some may be inherited as part of the Li Fraumeni syndrome (inherited p53 mutations)
  - Also an association with NF1
- Cooperative groups formed the Intergroup Rhabdomyosarcoma Study (IRS) in 1972

Rhabdomyosarcoma
Epidemiology
Two incidence peaks for RMS
1st decade
- Embryonal RMS

Adolescence
- Alveolar RMS

Median age 5 years; range 0-20
72% less than 10 years old
5% less than 1 year old
Rhabdomyosarcoma

Sites include:

- Head and neck 41%
- Genitourinary tract 31%
  - Bladder, prostate
  - Uterus, vagina
- Extremity 13%
- Retroperitoneum 7%
  - Including biliary tract/pancreas
- Trunk 5%
- Other 3%

Rhabdomyosarcoma

Gross examination

Rhabdomyosarcoma

Classification Schemes

- Horn and Enterline (1958)
  - Embryonal
  - Botryoid
  - Alveolar
  - Pleomorphic
- Palmer (1983)
- SIOP (1989)
- NCI (1992)
- WHO (1995)
- ICR (1994)

International Classification (ICR)

- Superior prognosis
  - Botryoid
  - Spindle cell
- Intermediate prognosis
  - Embryonal, NOS
- Poor prognosis
  - Alveolar rhabdomyosarcoma
  - Undifferentiated sarcoma
- Subtypes whose prognosis is not presently evaluable
  - Rhabdomyosarcoma with rhabdoid features

Superior prognosis category

Botryoid RMS
Variant of embryonal RMS
6% of all RMS
“Botryoid” refers to the gross appearance resembling grapes
This pattern seen in organs with hollow structures
Histologic diagnosis requires a distinct cambium layer

5-year survival of 95%
**Rhabdomyosarcoma Classification Schemes**

**Superior prognosis category**
- Spindle cell RMS
- Variant of embryonal RMS
- 3% of all RMS
- Commonly present in paratesticular site
- Low cellularity tumors consisting almost exclusively of spindle cells
- Collagen-poor and collagen-rich variants
- Spindle cells can be seen in association with other RMS subtypes

5-year survival 88%
- Improved survival seen exclusively in paratesticular and orbital examples
Spindle cell RMS
Differential diagnosis

- Embryonal RMS, NOS
- Leiomyosarcoma
- Fibrosarcoma
- Malignant fibrous histiocytoma (MFH)
- Rhabdomyoma
- Leiomyoma
- Nodular fasciitis

Rhabdomyosarcoma
Classification Schemes

Intermediate prognosis category

- Embryonal, NOS
- 49% of all RMS
- Variably cellular tumors
- Mucoid/myxoid background
- Spindle, stellate, ovoid cells with variable muscle differentiation
- 5-year survival 66%

Embryonal RMS, NOS
Differential diagnosis

- Embryonal RMS, NOS
  - Botryoid
  - Requires a cambium layer
  - Spindle cell variants
  - Majority of tumor (>80%) must be spindle cell
  - Undifferentiated embryonal sarcoma of the liver
  - Pleuropulmonary blastoma
  - Wilms tumor
  - Skeletal muscle differentiation
  - May even have a cambium layer in tumors abutting the renal pelvis
  - Fetal rhabdomyoma
  - Increased mitoses (≥ 15/50 high power fields), marked hypercellularity, a "cambium layer", and atypical nuclear features are more characteristic of RMS
**Rhabdomyosarcoma Classification Schemes**

**Poor prognosis category**

**Alveolar**
- 31% of all RMS
- Round cell neoplasms
- Conventional and solid variants
- Usually no visible muscle differentiation
- Can be seen as a component of an embryonal RMS

5-year survival 53%
Alveolar RMS
Differential diagnosis

- Malignant small round cell neoplasms
  - Ewing sarcoma/primitive neuroectodermal tumor (ES/PNET)
  - Poorly differentiated or undifferentiated neuroblastoma
  - Desmoplastic small round cell tumor (DSRCT)
  - Lymphoma
- Immunohistochemical stains applied as a panel are helpful
  - vimentin, myogenin, desmin, Myo-D1, cytokeratin, CD99, WT1,
    synaptophysin, chromogranin, leukocyte common antigen and Tdt
  - RT-PCR for PAX3- and PAX7-FKHR fusion gene products can also
    be performed if necessary

Rhabdomyosarcoma
Molecular genetics

- Alveolar RMS often contain chromosomal translocations

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Gene fusion</th>
<th>%</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(2;13) (q35;q14)</td>
<td>PAX3-FKHR</td>
<td>55</td>
<td>Older age, higher tumor invasiveness, increased risk of primary metastases, worse 4-year survival in stage IV patients</td>
</tr>
<tr>
<td>t(1;13)(p36;q14)</td>
<td>PAX7-FKHR</td>
<td>22</td>
<td>Younger age, improved 4-year survival in stage IV patients</td>
</tr>
</tbody>
</table>

- Provide insight into the pathogenesis
- Are useful diagnostically and are detectable by FISH or RT-PCR
  - 23% have neither gene fusion and are a heterogeneous group
- No consistent tumor specific translocation has been identified in embryonal RMS

Rhabdomyosarcoma
Classification Schemes

- Poor prognosis category
- Undifferentiated sarcoma
  - 4% of all tumors submitted to IRSG
  - Heterogeneous group
  - Spindle and/or round cell tumors otherwise unclassifiable
  - Round cell tumors can have a mucoid background
  - Must exclude extra-osseous Ewing/PNET, malignant rhabdoid tumor or ectomesenchymoma
  - Can be vimentin only or polyphenotypic
  - This group of tumors will be treated on a non-rhabdomyosarcomatous strategy in new protocols
  - 5-year survival 40%

Undifferentiated sarcoma
Differential diagnosis

- Differential similar to alveolar RMS
  - ES/PNET
  - Undifferentiated NB
  - Poorly differentiated synovial sarcoma
  - MRT
  - DSRCT
  - Fibrosarcoma

Rhabdomyosarcoma
Classification Schemes

- Indeterminate prognosis category
- RMS with Rhabdoid features
  - Rare type of RMS
  - Large amounts of eosinophilic cytoplasm similar to MRT
  - 27 cases identified in IRS III
    - 22 had a background embryonal histology
    - 5 had an alveolar histology
  - Nuclear chromatin more coarse than MRT
  - Inclusions positive for vimentin and desmin
  - Cytoplasm adjacent to inclusion positive for muscle specific actin
  - No significant survival difference was seen in this group but the numbers were small

Rhabdomyosarcoma
Classification Schemes
Rhabdoid rhabdomyosarcoma

RMS with anaplasia
Anaplastic cells seen in about 3% of RMS
New study suggests this is more common (~13%)
Most commonly associated with embryonal RMS, NOS subtype and common in paratesticular tumors
Diffuse anaplasia (in clusters or large sheets) is indicative of a worse prognosis (50% 5-year survival down from 77%)
Similar to WT, anaplasia is associated with DNA aneuploidy

Rhabdomyosarcoma
Classification Scheme

• Sclerosing rhabdomyosarcoma
  – Not included in the current ICR classification
  – Newly described pattern that differs from current categories and can be seen in both children and adults
  – Differential diagnosis often includes angiosarcoma, osteosarcoma, chondrosarcoma, malignant small cell tumors and sclerosing epithelioid fibrosarcoma
  – Typically shows only focal positivity for desmin and myogenin but strong diffuse positivity for MyoD1


Sclerosing Rhabdomyosarcoma
**Rhabdomyosarcoma**

**Classification Scheme**

- Pleomorphic rhabdomyosarcoma
  - Uncommon in general, most cases in adults
  - Not to be confused with anaplasia in a childhood RMS
  - Not included in the ICR

**Post-therapy changes**

- Studies evaluating the prognostic significance of this cytologic response is limited by
  - Small numbers of tumors with both pre- and post-chemotherapy samples
  - Small size of the specimens

- Morphologic changes following chemotherapy
  - Skeletal muscle differentiation
  - Decreased proliferation
  - More noticeable in the botryoid and embryonal, NOS RMS subtypes

- The available data suggests that residual proliferative activity and lack of differentiation in a treated RMS are usually associated with poor outcome

- While some patients with tumors showing cytodifferentiation and a lack of proliferative activity do well, the predictive value of these features is poor

- There are no good guidelines as to how to best characterize the biologic potential of differentiated tumor following treatment
**Rhabdomyosarcoma**

**Clinical Grouping and Staging**

- Clinical grouping
  - surgical – pathological classification
  - divides tumors into 4 groups based on amount and extent of original tumor after initial surgical procedure
  - Used to guide radiation therapy for local control
- Grouping does not take into account tumor site and size
- Staging = Tumor-node-metastasis system applied to RMS
  - Site
  - Invasiveness
  - Size
  - Lymph node involvement
  - Metastatic disease
  - Used to assign risk-based chemotherapy

**Group I**  Localized disease
- Completely resected grossly and microscopically
  - Confined to muscle or organ of origin
  - Infiltration into adjacent tissues (like along fascial planes) but grossly and microscopically completely excised

**Group II**  Regional spread
- Completely resected grossly
  - May have involved lymph nodes resected
  - a. Grossly resected tumor with microscopic residual disease
  - b. Infiltration of the distal most regional node

**Group III**  Incomplete resection
- Gross residual
  - Biopsy only
  - After major resection

**Group IV**  Distant metastatic disease present at onset
- Includes positive fluid cytology (CSF, ascites, pleural, etc...)

**Group V**  Distant metastatic disease present at onset
- Includes positive fluid cytology (CSF, ascites, pleural, etc...)

**Stage 1**
- Valid sites
  - Orbit
  - Head and neck, non-parameningeal
  - Genitourinary tract, non-bladder, non-prostate
  - Any T
  - Any N
  - M0
- 3-year FFS Stage 1 86%

**Stage 2**
- Valid sites
  - Bladder/prostate
  - Extremity
  - Cranial parameningeal
  - Other
    - Trunk, retroperitoneum, perineum, biliary, infrathoracic
  - T1 or T2
  - Size less than 5 cm
  - N0 or N1
  - No metastases
- 3-year FFS Stage 2 80%

**Stage 3**
- Valid sites
  - Bladder/prostate
  - Extremity
  - Cranial parameningeal
  - Other
    - Trunk, retroperitoneum, perineum, biliary, infrathoracic
  - T3 or T4
  - Size greater than 5 cm
  - N0 or N1
  - No metastases
- 3-year FFS Stage 3 68%

**Stage 4**
- Any site
  - Any T
  - Any N
  - Any lymph node
  - Positive metastases
- 3-year FFS Stage 4 25%

### Case 4

**Clinical presentation**

- 5 month old white female presented to her pediatrician
  - fever
  - tachypnea
  - labored respiration

- A nasal swab was positive for Influenza B

- She was admitted to the hospital

---

**Risk stratification scheme for RMS**

<table>
<thead>
<tr>
<th>Risk (Protocol)</th>
<th>Stage</th>
<th>Group</th>
<th>Age</th>
<th>Size</th>
<th>Mitochondria</th>
<th>Myocytes</th>
<th>Nodes</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low, subgroup A</td>
<td>0 (cSRS)</td>
<td>1</td>
<td>Favorable</td>
<td>≤10</td>
<td>EBVB</td>
<td>MB</td>
<td>N0</td>
<td>VAC x 3MT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Favorable</td>
<td>≥11</td>
<td>EBVB</td>
<td>MB</td>
<td>N0</td>
<td>VAC x 3MT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Favorable</td>
<td>≥11</td>
<td>EBVB</td>
<td>MB</td>
<td>N0</td>
<td>VAC x 3MT</td>
</tr>
<tr>
<td>Intermediate</td>
<td></td>
<td>4</td>
<td>Favorable</td>
<td>≥11</td>
<td>EBVB</td>
<td>MB</td>
<td>N0 % N1</td>
<td>VAC x 3MT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>Favorable</td>
<td>≥11</td>
<td>EBVB</td>
<td>MB</td>
<td>N0 % N1</td>
<td>VAC x 3MT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>Favorable</td>
<td>≥11</td>
<td>EBVB</td>
<td>MB</td>
<td>N0 % N1</td>
<td>VAC x 3MT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>Favorable</td>
<td>≤10</td>
<td>≥100</td>
<td>MB</td>
<td>N0 % N1</td>
<td>VAC x 3MT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>Favorable</td>
<td>≤10</td>
<td>≥100</td>
<td>MB</td>
<td>N0 % N1</td>
<td>VAC x 3MT</td>
</tr>
</tbody>
</table>

**Clinical presentation**

- 5 month old white female presented to her pediatrician
  - fever
  - tachypnea
  - labored respiration

- A nasal swab was positive for Influenza B

- She was admitted to the hospital

---

**Differential diagnosis**

Congenital lobar emphysema  
vs.  
Cystic pulmonary airway malformation (also known as CCAM)
Left upper lobectomy 11 x 7 x 1.3 cm
Case 4
Diagnosis
Pleuropulmonary blastoma, Type I (PPB)

Case 4
Staging evaluation
• Staging evaluation consisted of
  – Full-body CT scan
  – Head MR
• There was no evidence of metastatic disease
• A small left renal cyst was seen
• A few small intestinal polyps were seen
• Family history notable for B-cell leukemia in the mother and a maternal cousin with neuroblastoma

Case 4
Follow-up evaluation
4 months post surgery
3 cysts in left lung
2 cysts in left kidney
Case 4

Follow-up evaluation

6 months post surgery

4 cysts in left lung
2 cysts in right lung
3 cysts in left kidney

Prompting

Partial nephrectomy and wedge resections of left lower lobe cysts

Case 4

Partial nephrectomy

• Gross examination
  1.5 cm subcapsular multiloculated cyst

• Microscopic features
  Cystic structure lined by bland cuboidal and flattened epithelium
  Walls showed mesenchymal cells evenly dispersed in loose myxoid stroma

Case 4

Diagnosis #2

Cystic nephroma
(Multilocular cyst)

Left lower lobe wedge resection
Case 4
Diagnosis #3

Pleuropulmonary blastoma, Type I, (Multifocal)

Case 4
Management

• Patient was started on
  – Vincristine
  – Actinomycin D
  – Cyclophosphamide
• Treatment complicated by veno-occlusive disease
• Regimen changed to
  – Ifosphamide
  – Carboplatin
  – Etoposide

Case 4
Clinical course

Patient has tolerated 4 courses of chemotherapy (of 4 planned)
Radiographic studies show persistent right lung cysts and new left kidney cysts

Pleuropulmonary blastoma (PPB)

• This case highlights a number of interesting aspects of PPB
  – Subtle diagnostic features in the Type I variant
  – Association of other dysplastic/neoplastic conditions
    • Patient herself
    • Close family members
  – Difficult management decisions that arise in patients with bilateral and multifocal disease

Pleuropulmonary blastoma (PPB)

• First described as a distinct entity in 1988
  Manivel JC, Priest JR, Watterson J, Steiner M, Woods WG, Wick MR, Dehner LP
  Pleuropulmonary blastoma: The so-called pulmonary blastoma of childhood
  Cancer 62:1516-26, 1988

• Unique pulmonary or pleural based neoplasm
  – Earliest form presents as a bland appearing multiloculated cyst with small foci of tumor cells
  – More advanced cases as a solid heterogeneous overtly malignant neoplasm containing primitive cells
Study Methods

PPB Type

Type I
Early form
Purely cystic

Type II
Intermediate form
Mixed cystic and solid

Type III
Late form
Purely Solid

PPB Clinicopathologic Subtypes

- Two primary clinical presentations
  - CXR with cysts +/- dyspnea
  - Pneumothorax (recurrent)
  - Fever, pain and respiratory distress
  - Weight loss and malaise
  - CXR = pneumonia
  - No antibiotic response
  - CT scans \rightarrow Mass

PPB Type I

Potentially deceptive lesion grossly
Benign appearing peripherally
located thin walled cysts
No grossly identifiable solid,
nodular or plaque-like thickenings

PPB Clinicopathologic Subtypes

Type I PPB
Microscopic Pathology
The low power appearance
of the lesion is bland
Variably cellular cyst walls
Lined by benign epithelium

PPB Type I PPB Subtypes

Type I PPB
Microscopic Pathology
The neoplastic nature of
the lesion may be
appreciable only focally
Sometimes appearing as a
distinct "cambium layer"
Sometimes very subtle
with small subepithelial
buds of primitive cells

PPB Clinicopathologic Subtypes

Type I PPB
Microscopic Pathology
Or as a septal spindle
cell proliferation
Or as small nodules of
primitive cartilage
Clinicopathologic Subtypes

Type I PPB

Microscopic Pathology
- Rhabdomyoblastic differentiation by light microscopy and immunohistochemistry
- The absence of muscle differentiation does not preclude a diagnosis of PPB

Differential diagnosis
- Benign cysts
  - The most problematic differential is with CPAM 4 (so-called distal airway CCAM-CPAM)
  - The descriptions and Illustrations of CPAM 4 show significant overlap with Type I PPB
  - Uncertain at this time if these two entities, if they are unique, can be clearly separated
  - Use caution when applying CPAM 4 to a multilocular cyst in a young child

Management of cysts
- The existence of the purely cystic PPB complicates the non-operative management of asymptomatic pulmonary cysts in young children
- If observation is the management choice, close radiographic follow-up is recommended with a low threshold for surgical removal in the event of interim development of septal thickening or nodularity
- If the patient or close family members have history of pediatric neoplasms or thyroid disease, if the patient has more than one cyst, or if the patient presents with pneumothorax, we strongly recommend excision of the lesion

Pathologic handling of cystic lung disease in young children

- It is our recommendation for peripheral thin-walled cysts in young children (< 3 years), the pathologist needs to presume that the lesion is a PPB and submit the entire lesion if necessary to prove otherwise

Pleuropulmonary blastoma

Differential diagnosis
Type I PPB
- Benign cysts
  - Main differential is with CPAM 1 (large cyst CCAM-CPAM)
  - CPAM 1 has features suggesting an origination from proximal airways compared with Type I PPB
  - Distinguishing features of Type I PPB
    - Characteristic multilocular architecture
    - Well-circumscribed border with adjacent lung
    - Presence of collections or layers of small primitive cells within the septa
    - Septal lining that is predominantly flattened or “alveolar type”
    - Frequently multifocal
    - Frequently present with pneumothorax

Management of cysts

Progression of Cysts to PPB
(21 Registry + Literature cases)

Presymptomatic
Cyst observation
PPB

Outcome unknown

Pathologic handling of cystic lung disease in young children

- It is our recommendation for peripheral thin-walled cysts in young children (< 3 years), the pathologist needs to presume that the lesion is a PPB and submit the entire lesion if necessary to prove otherwise
Type I PPB in 2-day-old infant
Born at 31 wk gestation

Type I PPB in 2-day-old term infant
Seen at SLCH

Type I PPB in 10-day-old infant

Features of Progression in Type I PPB
Features of Regression in Type I PPB

Local Regression in Type I PPB

- Important for diagnosis
  - May explain the variability in cellularity in some cases
  - Means that you must sample widely to find the tumor cells (or exclude their presence)

- Important for understanding natural history
  - Perhaps not all Type I are destined to progress to Type II…?
Proposed pathogenesis of Type I PPB

1. Regression
2. Maturation
3. Progression

The mechanisms determining the fate of a Type I PPB are not known but with a better understanding of this process we may be able to better predict who would most benefit from adjuvant chemotherapy.

Type I PPB Natural history

- Early Type I PPBs have uniform distribution of primitive cells
- Features of Regression (necrosis, hemorrhage, dystrophic calcification) are common
- Regression be responsible for the variability in tumor cell burden in later Type I PPB

Type I PPB Treatment

- Appears to be a recurrence free survival advantage in patients receiving adjuvant chemotherapy (p<0.05)
- Trend toward overall survival advantage (p=0.08)
- If the treatment decision is watch and wait, the “watching” needs to be with q 3–4 month CT scans until age 5
- Given the regression phenomenon, it seems likely that some children can be safely followed with close radiographic surveillance. We do not know yet how to predict which children will most benefit from chemotherapy.

PPB Type II

- Similar to Type I PPB in that it has a cystic component with layers of primitive cells beneath a benign epithelium/mesothelium
- Solid components characterized by an overgrowth of malignant sarcomatous elements

PPB Type III

- Purely solid malignant neoplasm
- High grade, multi-patterned sarcoma

PPB Clinicopathologic Subtypes

Type II and III PPB

- Microscopic Pathology
  - Solid tumor components in Type II and Type III PPBs have overtly malignant features
  - Composed of one or more of four basic histologic patterns: blastemal, rhabdomyosarcomatous, spindle cell sarcoma, and cartilaginous
  - These patterns often intermixed with a primitive mesenchymal component
PPB Clinicopathologic Subtypes
Type II and III PPB
Microscopic Pathology
Anaplastic cells commonly seen, especially in purely solid tumors
Pleuropulmonary blastoma
Differential diagnosis
Type II and III tumors

- Rhabdomyosarcoma
  - If there is a cystic component
    - probably a PPB
  - Most reports of RMS arising in CCAM are probably PPB
  - If "heterologous" elements are present
    - probably a PPB
  - If it is based in the lung and not chest wall, it is probably a PPB
  - Both are treated with a similar chemotherapy strategy but a PPB diagnosis will also direct
    - Head MR or contrast CT because of high propensity for brain mets
    - Detailed family history

Pleuropulmonary blastoma
Differential diagnosis- Type II and III PPB

- Synovial sarcoma
  - Primary pleural and pulmonary synovial sarcomas can be cystic
  - The spindle cell component of a PPB can look remarkably similar to monophasic synovial sarcoma

However
- Synovial sarcoma usually occurs in older children and adolescents and young adults
  - With rare exception, PPB is a tumor of young children (most less than 5 years of age)
  - The spindle cell pattern in a PPB is usually associated with other patterns and immature mesenchyme and more monophasic in synovial sarcoma
  - CK, EMA and CD99 do not stain the spindle cell component of a PPB, SYT SK fusions are not seen in PPB

Pleuropulmonary blastoma
Differential diagnosis
Type II and III tumors

- Teratoma or Yolk sac tumor
- Metastatic Wilms tumor
- Congenital fibrosarcoma
- Cartilaginous neoplasms
  - mesenchymal chondrosarcoma

Pleuropulmonary blastoma
Special studies

- Immunohistochemistry
  - Generally adds little except in those cases (usually limited sampling) where it is necessary to distinguish from synovial sarcoma, metastatic Wilms tumor, etc...
    - Only the epithelial lining or entrapped bronchial structures will be positive for cytokeratin
    - Staining for muscle markers is helpful when positive but is not seen in all cases
    - S100 staining is seen in cartilaginous nodules
Pleuropulmonary blastoma
Special studies

- Molecular genetics
  - Type II and III tumors have multiple chromosomal abnormalities by karyotyping
  - Trisomy 8 appears to be common
    - Present in 60-80% of cases
    - Can be detected by interphase FISH on paraffin tissue
    - May aid in separation from benign cysts keeping in mind that
      in cases where you need it tumor cells might be sparse
  - Trisomy 2 also described
  - p53 mutations

PPB Centrally-Reviewed Set
Patient characteristics

- 125 cases
  - Includes 50 from original series
  - 46% Female
- 0 – 236 months

<table>
<thead>
<tr>
<th>Type</th>
<th>N</th>
<th>%</th>
<th>Year 1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>38</td>
<td>30%</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>II</td>
<td>52</td>
<td>41%</td>
<td>24 (48%)</td>
</tr>
<tr>
<td>III</td>
<td>33</td>
<td>26%</td>
<td>19 (38%)</td>
</tr>
</tbody>
</table>

Solid, NOS = 2

(Prial et al. Cancer 197:80:147)

PPB Pathologic Types

Age Distribution for Type I PPB
- Median = 9 months
- Range 0 to 32 months

Age Distribution for Type II PPB
- Median = 36 months
- Range 6-236 months

Age Distribution for Type III PPB
- Median = 39 months
- Range 18-64 months

PPB Pathologic Types – All Types

The difference in median age at diagnosis for each PPB type is statistically significant

(P<0.01) Kruskal Wallis method
**Pleuropulmonary blastoma**

**Treatment**

- Treatment is based on a pediatric sarcoma multimodality approach
  - Complete resection if feasible
  - Neo/adjuvant/adjuvant chemotherapy
    - Vincristine, Actinomycin D and Cyclophosphamide for Type I tumors (4 courses)
      - Not all Type I tumors are treated with chemotherapy
    - VAC alternating with Cisplatin and Doxorubicin for Type II and III tumors (6 courses)
  - Radiation therapy in select cases

**Event Free Survival Analysis at 5 years**

<table>
<thead>
<tr>
<th>Type</th>
<th>Percent Survival at 5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, N=33</td>
<td>78.3</td>
</tr>
<tr>
<td>II, N=49</td>
<td>62.6</td>
</tr>
<tr>
<td>III, N=31</td>
<td>30.7</td>
</tr>
</tbody>
</table>

*P<0.001, Kaplan-Meier; log-rank*

**PPB Patterns of Spread**

- Direct extension
- Hematogenous
  - Brain: 26/172 (15%)
  - Bone: 10/172 (6%)
  - Liver: 7/172 (4%)
  - Ipsilateral lung, ovary, spinal cord, adrenal, pancreas, eye

CNS metastases are not uniformly fatal

Unpublished data PPB registry

**PPB Tumor Predisposition Syndrome**

- Approximately 40% of patients with PPB have multifocal disease (lung and/or other organs) and/or relatives with cancer/dysplasia
- 25% have multifocal lung tumors/cysts
  - 25% of these also have tumors in organs other than the lung (cystic nephroma or rhabdomyosarcoma)
  - 20% of these have relatives with tumors in spectrum
- 15% have unifocal disease and a positive family history
  - (about 1 in 5 families appear to be “multiplex”)

**PPB Familial disease**

- Diseases seen in association with PPB’s
  - Other PPB’s
  - Cystic nephromas 9.2% of PPB Families
  - Nephrogenic rests
  - Wilms tumor
  - Thyroid hyperplasia and neoplasia
  - Rhabdomyosarcoma
  - Medulloblastoma
  - Gonadal tumors
  - Hodgkin disease
  - Leukemia
  - Langerhans cell histiocytosis
  - Intestinal juvenile type polyps

Cystic nephroma photograph courtesy of Francoise Boman, Lille, France

Unpublished data PPB registry

**PPB Familial disease**

- Familial cases
  - Important clues to the pathogenesis waiting to be discovered
  - Ongoing genetic study of PPB sponsored by Washington University
- Fresh tissue limited
  - Some studies amenable to paraffin tissue
    - LOH studies
    - 1p analysis
    - CGH analysis
    - PPB Registry has a protocol for receiving and distributing material for research
  - [http://www.ppbregistry.org](http://www.ppbregistry.org)
  - [http://ppbstudy.wustl.edu](http://ppbstudy.wustl.edu)
PPB
Key points
• Be careful with benign appearing cystic lung disease in young children
• Consider Type I PPB in cases resembling the description for Type 4 CPAM
• Understand the relationship between the three types of PPB and clinicopathologic features
• Register your cases with the PPB Registry

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