The Role of the Surgical Pathologist in Evaluation and Management of Germ Cell Tumors of the Testis

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GERM CELL TUMORS OF TESTIS

- Occur in young adults (20 - 50s)
- May present with metastasis or develop extensive metastasis
- Most tumors are curable by modern therapies
- Histology complex: multiple types each with numerous patterns
- Pathologic diagnosis corner stone of contemporary management

Pathologic Features that matter in Clinical Practice

GERM CELL TUMORS OF THE TESTIS

Objectives:
- Pathologic Features that matter in clinical practice
- Criteria for diagnosis of different components
- IHC work up
- Reporting (primary and metastatic sites)
TUMORS OF THE TESTIS

- Germ cell tumors (90 - 95%)
- Sex-cord stromal tumors (3 - 4%)
- Malignant lymphoma (<1%)
- Metastasis (<1%)

GERM CELL TUMOR

- Classic seminoma
- Spermatocytic seminoma
- Pure or Mixed (non-seminomatous) tumor
  - Embryonal carcinoma
  - Yolk sac tumor
  - Choriocarcinoma
  - Teratoma

HISTOGENESIS
CHROMOSOMAL CONSTITUTION OF GERM CELL TUMORS

- Gain in sequences of 12 p (most often i (12p))
- Tumors including ITGCN - aneuploid
- Seen in >95% of germ cell tumors
- Gain chromosome 9 in spermatocytic seminoma

INTRATUBULAR GERM CELL NEOPLASIA

- Edge of invasive tumor
- Infertility (1% - bxs) – approximately 50% progress in 5 years
- Cryptorchid testis (2-8%)
  - Contralateral testis cancer (5%)
  - Not seen in: Pediatric yolk sac tumor
  - Pediatric teratoma
  - Spermatocytic seminoma

GERM CELL TUMORS – *Important issues before you look at slides*

- Know the serum AFP & HCG levels
- Carefully review the gross findings to target sampling
- Take a section of the cord margin
- LET THE BISECTED SPECIMEN FIX AFTER MAKING ADDITIONAL CUTS – architectural and cytologic features are key to the dx of germ cell tumor components
ITGCN

• Basilar proliferation of clear cells
• Large nuclei
• Vesicular chromatin
• Prominent nucleoli
  - Seminoma -like
ITGCN

ITGCN - Immunomarkers

c-kit
Oct 3/4
Podoplanin
PLAP
INTRATUBULAR GERM CELL NEOPLASIA

- Situations where the diagnosis is clinically relevant
  - Seminoma vs sex cord stromal tumor
  - Seminoma vs Spermatocytic seminoma
  - Pure teratoma vs. Epidermoid cyst
  - Biopsies for infertility
  - Orchietomies for cryptorchidism

*Not everything that is abnormal and intratubular is ITGCN*

Pitfall: normal spermatogonia in adult testis
Pitfall: normal spermatogonia in adult testis

C KIT false (+)

Oct 3 more specific for ITGCN

ITGCN
Intratubular embryonal carcinoma
Intratubular seminoma
Intratubular spermatocytic seminoma
Intratubular B cell Lymphoma
Intratubular Prostate CA
SEMINOMA

Immunohistochemistry

(+) c-kit
   Oct-3/4
   Podoplanin
   PLAP

(-) Cytokeratin
   CD30
   AFP
   βHCG (syncytiotrophoblasts only +)

- c-kit
SPERMATOCYTIC SEMINOMA

Unique
- Older age group (average age 52 yrs)
- Not associated with ITGCN
- Not associated with 12p abnormalities
- PLAP, keratin, AFP, HCG (-)
- Not associated with other germ cell components
- No ovarian counterpart or extragonadal location
- Clinically benign except if associated with sarcomatous transformation
SPERMATOCYTIC SEMINOMA

Gross
- Circumscribed
- Mucoid appearance
- Cystic degeneration

Microscopy
- Sheets, nests, lobules
- Variable loose edematous stroma
- 3 cell types:
  - Small (6-8/µ)
  - Medium (15-20/µ)
  - Giant (15-100/µ)
SPERMATOCTIC SEMINOMA – INTRATUBULAR GROWTH

- 12 cases reported
- Most cases result in death

SPERMATOCTIC SEMINOMA – SARCOMATOUS TRANSFORMATION

- 12 cases reported
- Most cases result in death

TESTIS - IHC

Screening panel
- Germ cell tumor: PLAP (+), EMA (-)
- Sex-cord stromal tumor: Calretinin, Inhibin, CD99, melan-A (+)
- Lymphoma: CD45
- Visceral malignancy: EMA (+)
**GERM CELL TUMORS - IHC**

*Intratubular germ cell neoplasia*
- PLAP (+)
- c-kit (+)
- Oct 3/4 (+)
- Podoplanin (+)

*Classic seminoma*
- AE1/AE3, CAM 5.2: usually (-) (or focal)
- c-kit, Oct 3/4 & Podoplanin (+)
- CD30 (Ber-H2): usually (-) (or focal)
- AFP: (-)

*Spermatocytic seminoma*
- Negative for most markers except c-kit

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**GERM CELL TUMORS - IHC**

*Embryonal carcinoma*
- AE1/AE3, CAM 5.2: (+)
- CD30 (Ber-H2): (+)
- Oct 3/4 (+)
- AFP: (+)
- c-kit: (-)

*Endodermal sinus tumor (YST)*
- AE1/AE3, CAM 5.2: (+)
- AFP: (+)
- Glypican 3 (+)
- CD30 (Ber-H2): (-)
- c-kit: (-)
- Oct 4 (-)

*Choriocarcinoma*
- AE1/AE3, CAM 5.2: (+)
- ßHCG: (+)
- Glypican (+) – mainly syncytiotrophoblasts
- Human placental lactogen: (+)
- Oct 4 (-)

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**EMBRYONAL CARCINOMA**

**Gross**
- Usually small (average 4 cm)
- Variegated - hemorrhage, necrosis
- Extension into paratestis (20%)
EMBRYONAL CARCINOMA

Patterns
- Solid sheets
- Gland formation
- Papillary structures

Cells
- Marked cellular pleomorphism
- Nuclear overlap “syncytia”
- Irregular chromatin distribution
- Prominent nucleoli
- Increased mitoses
EMBRYONAL CARCINOMA

- AE1/AE3 (+)
- CD30 (+)
- Oct 4 (+)
- C kit (-)
- AFP (-)
- Glypican (-)

YOLK SAC TUMOR - MICROSCOPY

Patterns
- Endodermal sinus
- Reticular
- Microcystic
- Papillary
- Glandular
- Etc. (+/- 11 patterns)

Cells
- Cuboidal - in between seminoma and embryonal carcinoma
- Spindled
YST - microcystic pattern

YST - myxomatous pattern

YST - Enteric/endometrioid pattern
YST - combination of patterns

Immunohistochemistry

(+)
- Keratin
- AFP
- PLAP

(-)
- c-kit, Oct3/4
- CD30 (BerH2)
- βHCG (in syncytiotrophoblasts only)
Glypican-3 expression in testicular germ cell tumors

- Yolk sac tumor 100%
- Embryonal carcinoma 0-8%
- Choriocarcinoma 30 - 100% (syncytiotrophoblasts)
- Teratoma 0-40% (immature elements)
- Seminoma 0%
- ITGCN 0%
CHORIOCARCINOMA

- Very rare in pure form (<1%)
- Frequently admixed with other germ cell tumor components (7%)
- Frequently present with metastases (hemoptysis, hepatomegaly, CNS dysfunction)
- Symptoms of ↑ βHCG production (gynecomastia, thyrotoxicosis)

CHORIOCARCINOMA

- Usually small hemorrhagic nodule
- Scar
- Clinically/grossly inapparent
CHORIOCARCINOMA

Microscopy
• Hemorrhage
• Necrosis
• Vague/ill-formed villiform arrangement
• Biphasic appearance:
  - Syncytiotrophoblasts – multinucleated cells WRAPPING AROUND
  - Cytotrophoblasts – nests, round to polygonal cells
CHORIOCARCINOMA

EMBRYONAL CARCINOMA WITH “APPLIQUE FORMATIONS”
SEMINOMA WITH EXTENSIVE SYNCYTIOTROPHOBLASTS

CHORIOCARCINOMA

Immunohistochemistry

- Keratin (+)
- βHCG (+)
- HPL (+)

TERATOMA

- Tumor composed of several tissue types (endoderm, ectoderm, mesoderm)
- Mature – histologically adult tissue type; OR Immature – fetal/embryonic tissue type
- Age important
  - Children: Benign (no matter how immature)
  - Post-pubertal: Malignant (no matter how mature)
- 65% occur in first 2 yrs of life
- Post-pubertal: young adults, 24% present with metastasis
Teratoma, predominantly mature

ITGCN

MATURE TERATOMA

• Adult type
• Organoid
• Histologically recognizable as adult tissue
• Ectoderm – Epidermis, neuronal tissue
• Endoderm – GI, respiratory muscosa, mucous glands, etc.
• Mesoderm – Bone, cartilage, muscle, fat, etc.
MATURE TERATOMA

TERATOMA - EPIDERMOID CYST - BENIGN

Lacks ITGCN

Look for ITGCN
TERATOMA - DERMOID CYST

Lacks ITGCN

Teratoma, predominantly immature

IMMATURE TERATOMA

- Fetal type
- Embryonic tissue
- Immature mesenchyme – cellular spindled
- Immature skeletal muscle – rhabdomyoblasts
- Immature neuroepithelial structures
IMMATURE TERATOMA

- Somatic/visceral malignancy in teratoma, i.e.:
  - Adenocarcinoma
  - Squamous cell carcinoma
  - Rhabdomyosarcoma
  - PNET
- Occurs mostly after chemotherapy – “chemo-selection”
- Size criterion of >4X field
MIXED GERM CELL TUMOR

Embryonal Ca + Teratoma - 25-30%
Embryonal Ca + Seminoma - 16%
Embryonal Ca + YST + Teratoma - 11%
Other combinations…
TESTIS TUMOR - Rule 1
Axiomatic to perform an orchiectomy for a testicular mass

• Broad category:
  - Germ cell tumor (ITGCN)
  - Sex-cord stromal tumor (rare)
  - Lymphoma (interstitial growth)
  - Metastasis
    - Looks different
    - Vascular-lymphatic invasion
    - Interstitial growth

TESTIS IHC: Screening panels

• Germ cell tumors
  - PLAP
  - SAL4
  - Oct 3/4
  - EMA(-)
  - Vimentin (-)

• Sex cord tumors
  - SF1
  - Melan A
  - Inhibin
  - Calretinin
  - CD99
  - Synaptophysin

• Lymphoma: CD-45, CD3, L26
• Visceral malignancy: EMA (+), vimentin (-)
LEYDIG CELL TUMOR

INHIBIN

SERTOLI CELL TUMOR

CALRETININ

TESTIS TUMOR – Rule 2

Seminoma or Non-Seminomatous

• Seminoma
  - Surveillance or radiation (Stage I, II)

• Non-Seminomatous (Mixed)
  - Chemotherapy ± RPLND or surveillance (Stage I, II)
TESTIS TUMOR – Rule 3

Is there vascular-lymphatic invasion?

- If present: pT2 tumor
- Determines stage & therapy
- Excludes most pts from “surveillance”

Use strict criteria
- e.g. as in thyroid follicular neoplasms
- Preferably peritumoral
- Usually more than one focus
Are serum HCG or AFP levels increased?

**HCG:** Syncytiotrophoblasts or choriocarcinoma

**AFP:** Yolk sac tumor

- **DETERMINES SAMPLING**
  - more extensive & from different appearing areas
  - ? entire tumor (in case of pure seminoma and elevated AFP levels)
- **MAY INFLUENCE TYPE OF THERAPY**
IHC IN GERM CELL TUMORS - RULE 5

- ITGCN: c-kit, Oct3/4, PLAP, Podoplanin
- Seminoma: c-kit, Oct3/4, Podoplanin
- E Ca: CD-30 (Ber H2), Keratin, Oct3/4
- YST: AFP, Keratin, Glypican 3
- CC: βHCG, HPL

**Cytokeratin AE1/AE3:** E Ca, YST, T, CC
**Oct 3/4:** Seminoma, E Ca
**PLAP:** Minimal / no value – except in ITGCN
CD30 (Ber H2)

AFP

DIFFUSE EMBRYOMA

GERM CELL TUMOR – Rule 6

Pure seminoma is a diagnosis of exclusion

• Sample liberally, # cms of size of tumor + 2-3 sections – confirm “pure” histology
• Check AFP, HCG levels
• Check for ITGCN (rule out Sertoli cell tumor & other mimics)

GERM CELL TUMORS – RULE 6

Know problems associated with seminoma diagnosis

• Differential diagnosis
• Seminoma with glandular architecture
• Seminoma with atypia
• Seminoma cells obscured
• Absence of mass lesion – exclusive intertubular growth
CLASSIC SEMINOMA

Differential diagnosis

• Spermatocytic seminoma
  - Older males
  - Polymorphic cellular population
  - Oct 4, Podoplanin (-)

• Sertoli cell tumors: Particularly malignant
  - Similarity
    - Nested, sheet-like growth, occasional hollow tubules
    - May have stromal fibrosis and inflammation
    - Clear cells
  - Helpful
    - Nuclei less pleomorphic; mitoses rare
    - ITGCN absent
    - Keratin (+), inhibin, melan-A, calretinin (+)

SERTOLI CELL TUMOR – Solid pattern
MALIGNANT SERTOLI CELL TUMOR

Keratin AE1/AE3

SEMINOMA WITH “GLANDULAR ARCHITECTURE”
- Seminoma involving rete testis
- “Tubular” seminoma
- Seminoma with “early carcinomatous transformation”

(Sample image of glandular architecture)
SEMINOMA WITH ATYPIA
• Poor fixation and processing – commonest cause
• Seminoma with increased mitoses and atypia
  • “Anaplastic seminoma”
  • D/D embryonal ca, YST, lymphoma, Sex cord tumor
• Serum with early carcinomatous transformation
  • Criteria: Strong keratin or true gland formation
  • Significance: Depends on institution; some may treat as non-seminoma

POORLY FIXED SEMINOMA
SEMINOMA WITH ECD

STRONG AND DISTINCT KERATIN EXPRESSION
STRONG AND DISTINCT KERATIN EXPRESSION

AE1/AE3

SEMINOMA CELLS OBSCURED

- Crush artifact
- Fibrotic areas
- Inflammation, granulomas
- Regression
- Cells obscured within inflammation

*Associated ITGCN helpful*
Regressed Germ cell tumor

Intratubular germ cell neoplasia, unclassified
Intertubular growth pattern of seminoma

Intratubular embryonal carcinoma

Lymphoplasmacytic infiltrate
SEMINOMA WITH ABSENCE OF MASS LESION

- “Interstitial seminoma”
  - Seminoma obscured by hyperplastic Leydig cells
- ITGCN with focal invasive seminoma

INTERSTITIAL PATTERN OF SEMINOMA
TERATOMA: WHAT REALLY MATTERS? - Rule 7

**Mature:**
- Normal/adult type/organoid elements

**Immature:**
- Embryonic/fetal/undifferentiated/primitive

**Age of the patient:**
- **Adult:** Malignant, irrespective of maturity
- **Pediatric:** Favorable outcome, in spite of marked immaturity

SECONDARY MALIGNANCY IN TERATOMA - Rule 8

“Teratoma with malignant transformation”
- Rhabdomyosarcoma, PNET, Wilms’, etc.
- Squamous cell ca, adenocarcinoma, etc.

**Criteria:**
- “Overgrowth”: 1/2 to entire, 4X objective

**Significance:**
- **Primary tumor:** No known impact
- **Secondary tumor:** Recurrence, “resistance” to chemotherapy, aggressive course

GERM CELL TUMOR – Rule 9

**What really matters?**

- **Seminoma**
  - Pathologic stage
  - Size
  - >99% of stage I, IIA, IIB eventually cured

- **Mixed germ cell tumor**
  - >80% embryonal carcinoma (↑)
  - >50% teratoma (↑)
  - Advanced tumors: size and number of metastases
GERM CELL TUMOR
Histopathologic prognostic factors – primary tumor
• Pathologic stage
• Vascular-lymphatic invasion (pT2 stage)
• Pure or >80% embryonal carcinoma histology – adverse
• >50% mature teratoma – favorable
• Cord margin status
• Tumor size

GERM CELL TUMOR – Rule 10
Post-therapy: What really matters?
• Necrosis, foam cells, fibrosis
  - Often present
  - No significance
• Any tumor – S, E Ca, YST, CC, immature teratoma
  - Chemotherapy
  - Hence sample all tissue
• Residual mature teratoma - Controversial
GERM CELL TUMOR

Histopathologic prognostic factors – RPLND specimen

• Number of positive lymph nodes
• Size of positive lymph nodes
• Presence of embryonal carcinoma, yolk sac tumor, choriocarcinoma, seminoma
• Presence of somatic malignancy (sarcoma, carcinoma, etc.)

Metastatic colorectal cancer to testis
METASTATIC TUMORS TO TESTIS

Malignant MELANOMA - the big mimic!

METASTATIC TUMORS – D.DX

<table>
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<tr>
<th>SEMINOMA</th>
<th>RENAL CELL CA</th>
<th>MELANOMA</th>
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CDX2