Testicular tumors are rare. It was estimated that there would be approximately 8,000 new cases reported in the United States in 2007 and only 380 cancer-related deaths. Germ cell tumors (GCT) comprise about 98% of all testicular tumors and for most of the deaths. They are the most common malignancy and one of the most common causes of cancer-related death in males between the ages of 15 and 35 years. Because of their relative rarity and diverse morphology, they present a diagnostic challenge to most practicing pathologist. However, pathologists must accept this challenge and become familiar with the cytomorphological features of those entities classified as GCT because the clinical features and treatments vary greatly among them. It is beyond the scope of this presentation to discuss in detail the pathological features of each of these tumor types and I direct your attention to many wonderful chapters and papers available in the pathology literature that do justice to this topic. Alternatively, my goal is to review the immunohistochemical features of each of these lesions and how this technology can be used in the differential diagnosis of “difficult to classify” tumors.

**Intratubular germ cell neoplasia:**
This term refers to the lesion initially described by Skakkebaek as “carcinoma in situ” as well as to other “differentiated” forms of intratubular germ cell neoplasia. Strictly speaking, the lesion originally described by Skakkebaek is now called “Intratubular germ cell neoplasia, unclassified” (IGCNU) by most, at least in the Western Hemisphere. It is characterized morphologically by the presence of enlarged, atypical germ cells located immediately above a usually irregularly thickened basement membrane. In essence, the cytologic features of classic IGCN are those of seminoma. The relationship is supported by the coexpression of a host of histochemical and immunohistochemical markers among both cell types. Tubules whose lumen is filled with these cells may be regarded as “intratubular seminoma”.

Placental-like Alkaline Phosphatase (PLAP) is one of the isoforms of alkaline phosphatase. PLAP antibodies will stain IGCNU as well as the majority of seminomas and embryonal carcinomas as well as a smaller percentage of yolk sac tumors. Immunoreactivity is seen in virtually all cases of IGCN and the staining pattern is usually membranous or cytoplasmic. No other non-neoplastic intratubular cells are immunoreactive for PLAP, but immunoreactivity may be seen in other types of non-germ cell malignancies. C-kit (CD 117) is expressed in a large percentage of IGCN as well as seminomas, but not in other germ cell tumors. Once again, the staining pattern is cytoplasmic/membranous. Despite the overexpression of this antigen, c-kit is rarely mutated in these tumors. Other antibodies which immunoreact with IGCNU but are rarely used in clinical practice include M2A and 43-F. POU5F1 (Oct3/4) is a very interesting marker which was recently described. The gene serves as a transcription factor and its product is expressed in pluripotent mouse and human embryonic stem cells and is down-regulated during
differentiation. Since the gene is also required for self-renewal of embryonic stem cells, knocking out the gene is lethal. Early reports suggest that this antigen is expressed solely in IGCNU, seminoma and embryonal carcinoma, suggesting that these are the types of GCT cells with pluripotency, i.e. with capacity to differentiate.

**Seminoma:**
Seminomas are the most common germ cell tumors arising in the male gonad, whether they arise in a pure state or mixed with other morphologic types. 

“Pure” seminoma account for 27%-30% of testicular GCT and another 15%-18% contain syncytiotrophoblasts. Approximately 1% to 2% are bilateral and bilaterality can occur synchronously or asynchronously. Seminomas reach a peak incidence between the 4th and 5th decade of life, which is approximately one decade later than non-seminomatous germ cell tumors. Microscopically, tumor cells are uniform and have round to vesicular nuclei with clear cytoplasm, prominent cytoplasmic membranes and a centrally-located, round nucleus with a prominent nucleolus. These cells are arranged in sheets or nests separated by thin fibrovascular bands which contain mature lymphoid cells.

Seminoma cells express Placental Alkaline Phosphatase (PLAP) and c-kit (CD-117) by immunohistochemistry but not cytokeratins, CD-30 or inhibin. A minority of seminoma cells may express focal and weak, dot-like or linear immunoreactivity for cytokeratin; however, never diffuse and strong staining throughout the cytoplasm. Like IGCN, seminoma cells express POU5F1 (Oct 3/4) in a nuclear distribution.

Tumors thought to be seminoma but exhibiting atypical histology should trigger consideration of a differential diagnosis of seminoma which includes a) seminoma with “early carcinomatous differentiation”, b) solid variants of embryonal carcinoma or yolk sac tumor, c) lymphoma, d) sex-cord gonadal stromal tumor, and e) metastatic disease, including poorly differentiated carcinoma and melanoma. Other causes of atypical histology in seminoma include poor fixation and faulty processing in the pathology laboratory. “Early carcinomatous differentiation” refers to areas of transition from seminoma to embryonal carcinoma. This concept suggests that seminoma cells are not terminally differentiated but rather, under certain poorly understood circumstances, may differentiate into other germ cell tumor-types.

**Spermatocytic Seminoma:**
Spermatocytic seminomas are rare, comprising less than 2% of testicular neoplasms. The peak incidence is in the sixth decade of life; however, occurrence in younger patients as early as the third decade of life is reported. This tumor occurs only in the male gonad, may be unilateral or bilateral, and is not associated with cryptorchidism. Microscopically, tumor cells are arranged in solid sheets or nests of round cells. Occasionally the tumor cells may be arranged in nests or pseudoglandular arrangements within an edematous or mucoid stroma. Cytologically, it is possible to identify three distinct cell types; small, medium and large although cells of intermediate size predominate. Immunohistochemical stains for PLAP are negative, although occasional cells may be weakly immunoreactive. Cytokeratins are negative, although occasional cells may exhibit dot-like cytoplasmic staining. CD-30 is negative, while some investigators have reported immunoreactivity for CD-117 (c-kit)
**Embryonal Carcinoma:**
Embryonal carcinomas comprise up to 3% of pure GCT, although it is a common component of mixed germ cell tumors. It rarely presents as pathologic stage I disease and is not associated with elevation of HCG or alfaetoprotein (AFP). Microscopically, tumor cells are large, irregular, and epithelioid. They exhibit scanty cytoplasm, large pleomorphic nuclei with coarse chromatin, and multiple irregular nucleoli. Common findings include nuclear overlap, individual cell necrosis, and apoptotic bodies. The pattern of growth is quite variable: gland-like, papillary, syncytial, and solid areas are commonly encountered. The solid variant of EC may be confused with “atypical” forms of seminoma, although the latter does not exhibit the same degree of cytologic anaplasia as embryonal carcinoma. EC may also have overlapping morphologic features with yolk sac tumor (YST) but, once again, close attention to subtle cytomorphologic differences and immunohistochemistry will resolve the majority of cases. Most ECs are immunoreactive for PLAP, low molecular weight cytokeratins, CD-30 and POU5F (Oct 3/4). They do not express CD-117, AFP or HCG.

**Yolk Sac (Endodermal Sinus) Tumor:**
Yolk sac tumors (YSTs) are characterized by multiple patterns of growth that recapitulate the yolk sac, allantois, and extra embryonic mesenchyme. It has a bimodal age distribution; infants and young children and postpubertal males. In the latter group, it rarely presents in a pure form but is present in almost half of mixed germ cell tumors. In children it commonly presents in its pure form, usually within the first two years of life. These tumors are associated with serum elevation of AFP in the overwhelming majority of cases. Microscopically these tumors are quite variable due to the multiple subtypes which are usually intermixed. Tumor cells of YST are usually immunoreactive for AFP, and low molecular weight cytokeratins. PLAP staining is variable and may be absent. CD117 (c-kit) and CD-30 (focal, weak staining may be present) are usually negative as is Oct 3/4.

**Choriocarcinoma:**
Choriocarcinoma is composed of syncytiotrophoblastic, cytotrophoblastic, and other trophoblastic cells. It comprises less than 1% of testicular GCT in its pure form; however, may be encountered as a component of a mixed GCT in up to 10% of cases. In its pure form, these tumors occur in the second and third decades of life, are commonly associated with very high levels of serum HCG, and exhibit metastatic disease at the time of initial presentation. Small foci of choriocarcinoma within a mixed germ cell tumor do not alter the prognosis. Most cases will have syncytiotrophoblasts and cytotrophoblasts with occasional intermediate trophoblastic cells present. Syncytiotrophoblasts are immunoreactive with HCG as well as inhibin, epithelial membrane antigen, and low molecular weight cytokeratins. PLAP may be positive but staining is variable.

**Teratoma:**
The term teratoma refers to neoplasms composed of tissues that have differentiated along any of the three somatic pathways: ectoderm, mesoderm, or endoderm. Tumors composed of only one of these components are regarded as monodermal teratomas. Teratomas may be composed of mature tissues, embryonal-type tissues, or a mixture of both. Historically they were subclassified as immature and mature forms based on their degree of differentiation. The World Health Organization now recommends that these morphologies be considered as a single entity.
based on their overlapping genetic features. The immunohistochemical profile will be consistent with the degree of differentiation. The epithelial component will express cytokeratins while the mesenchymal components will express vimentin. Few epithelial cells lining cysts may express oncofetal proteins such as CEA, AFP and the levels of these proteins in cyst fluid may markedly elevated. However, these findings rarely are associated with significant serum elevation of the corresponding oncofetal protein.

Recently novel markers have been developed that may be very useful in classifying GCT. As with Oct 3/4, markers such as Sox 2, GDF-3, Lefty, NANOG (and others), have the added value of helping us better understand the stem cell origin of this disease and how “somatic” differentiation translates into predictable patterns of antigenic expression. Whether these markers will take hold in clinical practice remains to be seen.
REFERENCES


