

An In-Depth Look at Leydig Cell Tumor of the Testis

Osama M. Al-Agha, MD; Constantine A. Axiotis, MD

• **Leydig cell tumor (LCT) is a rare tumor of the male testicular interstitium. This article provides an overview of the major pathologic manifestations of LCT of the testis; patient characteristics; clinical, radiologic, and laboratory features; prognosis; and management. LCTs of the testis are frequently hormonally active, leading to either feminizing or virilizing syndromes. The tumor is usually benign, but malignant variants can occur. The pathologic diagnosis of LCT is usually made based on morphologic characteristics of the tumor cells. The significance of Reinke crystals in the diagnosis of LCT both cytologically and histologically is underscored. Pathologists have to be familiar with the diagnostic histopathologic features, immunohistochemical panel of this tumor, and its principal differential diagnoses to prevent tumor misdiagnosis.**

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Testicular neoplasms are classified into 2 major groups: germ cell tumors and sex cord–stromal tumors. Sex cord–stromal tumors are derived from 2 types of somatic cells: the Leydig cells, and the Sertoli cells. Leydig cells are designated by the name of the German anatomist Franz Leydig who first described them in 1870.¹ They are interstitial cells located between the seminiferous tubules and they produce testosterone when stimulated by luteinizing hormone. They are thus involved in the development of secondary male characteristics and maintenance of spermatogenesis.² Tumors of the Leydig cell are rare. They comprise 1% to 3% of all testicular neoplasms but they are the most common interstitial neoplasms of the testis.³ The mechanism of Leydig cell oncogenesis is still poorly understood. The disruption of the hypothalamic–pituitary–testicular axis leading to excessive stimulation of Leydig cells by excess luteinizing hormone is thought to play a role.⁴ However, structural changes of the luteinizing hormone receptors⁵ and G proteins⁶ in Leydig cells have been postulated to induce tumorigenesis. There are no known risk factors for this tumor in the human male. In contrast to germ cell tumors, there does not seem to be a definitive association with cryptorchidism, although the presence or a history of undescended testis in 1 series⁷ suggests that

cryptorchidism may predispose to the development of this tumor, but this requires further elucidation. In addition, rare examples have been reported in patients with Klinefelter syndrome. Leydig cell tumors (LCTs) can be either pure or mixed with germ cell tumors or other sex cord–stromal tumors. They also can occur in extratesticular sites such as the spermatic cord,⁸ adrenal glands,⁹ or the ovaries. This article focuses on pure LCTs of the testis.

CLINICAL PROFILES

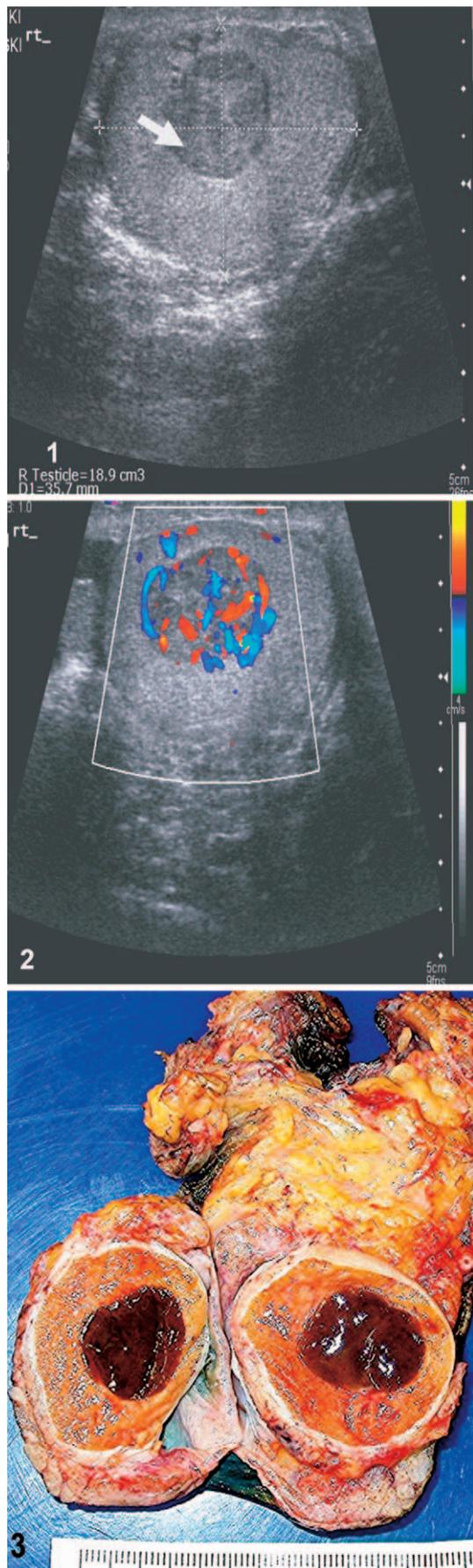
Testicular LCTs can occur at any age but they are common in prepubertal boys (most often between 5 and 10 years of age) and in men aged 30 to 60 years.^{7,10} Leydig cell tumors are always benign in children, whereas in adults they are malignant in 10% of cases. Leydig cell tumors are hormonally active and considered as one of the steroid-secreting tumors. They produce androgens, mainly testosterone, but can also produce estrogen by either direct production of estradiol or by peripheral aromatization of the testosterone moiety. In androgen-secreting tumors, boys usually present with symptoms of precocious puberty (growth of the penis, pubic hair, accelerated skeletal and muscle growth, advancement of bone age, and skin changes), whereas in adults, most patients are asymptomatic as the excess androgen rarely causes notable effects. In estrogen-secreting tumors, feminizing stigmata predominate: Boys usually present with gynecomastia and breast tenderness associated with feminine hair distribution and gonadogenital underdevelopment, whereas adults generally present with gynecomastia associated with loss of libido, erectile dysfunction, impotence, and infertility. Physical examination of the testes usually reveals an intratesticular mass on palpation. If the tumor is impalpable, ultrasonography is the investigative procedure of choice. Radiologically, LCT of the testis typically has a homogeneous hypoechoic sonographic appearance (Figure 1, arrow) (a heterogeneous pattern should alert one to the possibility of a malignancy). Color Doppler imaging, which detects vascular blood flow, often shows hypervascularity of the tumor with a prominent peripheral and circumferential blood flow¹¹ (Figure 2). It should be emphasized that when patients present with either feminizing or virilizing symptoms, clinicians should consider LCT in the differential diagnosis and examination of the testis is warranted. Clinicians should be alert to the fact that an LCT should not be excluded if a mass is not palpable as the tumor can be clinically occult¹² and testicular ultrasonography should follow. Laboratory studies in LCTs are nonspecific. The steroid secretion pattern in LCTs is variable. Although serum testosterone level is usually elevated, serum estradiol measurement is in-

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From the Department of Pathology, State University of New York, Downstate Medical Center at Brooklyn.

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Reprints: Osama M. Al-Agha, MD, Department of Pathology, State University of New York Downstate Medical Center, 450 Clarkson Ave, Box 25, Brooklyn, NY 11203 (e-mail: osama.al-gha@downstate.edu, droalagha@yahoo.com).



creased in cases with feminization stigmata. Because hyperandrogenism and hyperestrogenism can also be caused by adrenal hyperplasia or tumor, laboratory evaluation for testicular and adrenal steroids, as well as pituitary gonadotropins and adrenocorticotrophic hormone (ACTH), is important to determine a diagnosis. Importantly, serum tumor markers testing for α -fetoprotein, β -human chorionic gonadotropin, and placental alkaline phosphatase should be within normal ranges. These markers are often elevated in certain germ cell tumors but not in pure LCTs.

PATHOLOGIC FEATURES

Gross Features

Benign LCT classically presents as a small (3–5 cm in diameter), sharply delineated, solid mass embedded within the testis. The tumor color usually ranges from brown to yellow to gray-white (depending on the lipid content of the tumor). The golden brown appearance (which is usually imparted by the abundant lipofuscin pigment of tumor cells) is very characteristic (Figure 3). Malignant LCTs are usually larger (more than 5 cm in diameter), have infiltrative margins, show areas of hemorrhage and necrosis, replace the testis, and/or extend beyond testicular parenchyma. Leydig cell tumors is usually unilateral. Bilateral tumors are rarely observed (only in 3% of cases).⁷

Microscopic Features

Growth Pattern.—The most common growth pattern of tumor cells is nests or sheets separated by delicate fibrovascular septa (Figure 4). Other growth patterns include small clusters or trabecula separated by a variable amount of fibrous or myxoid stroma.¹³ A microcystic growth pattern has also been described.¹⁴ Rare cases of LCT with unusual features such as adipocyte differentiation, ossification, and calcification have been reported.¹⁵

Cell Types.—Four types of cells in LCTs have been described: (1) large polygonal cells with abundant, finely granular, eosinophilic cytoplasm, indistinct cell borders, and regular, round to oval nuclei, some with a prominent nucleolus; (2) same as in (1) but distinct cell borders and smaller nuclei; (3) small cells with scant, densely eosinophilic cytoplasm and grooved nuclei; and (4) spindle-shaped (sarcomatoid) cells.¹⁶ The tumor can consist predominantly of 1 type or a mixture of the 4 types of cells.

Cytologic Features.—In all 4 cell types, the cytoplasm is often eosinophilic but can also be pale to clear because

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Figure 1. Ultrasound of a right testis with Leydig cell tumor in a 75-year-old patient. The tumor typically appears as a hypoechoic homogeneous mass (arrow) within the testicular parenchyma.

Figure 2. Color Doppler sonography of the same testis as in Figure 1 showing high vascularity of Leydig cell tumor with a prominent peripheral and circumferential blood flow. The hyperperfusion is focal as it appears within the tumor only. This pattern of vascularity is one of the characteristic radiologic features of Leydig cell tumor. (The brighter the color, the faster the blood flow; red and blue indicate the different directions of the blood flow; blue indicates flow toward, and red away from, the transducer.)

Figure 3. Gross appearance of Leydig cell tumor of the same testis. Leydig cell tumor usually presents grossly as a small, well-circumscribed, solid mass embedded within the testis. The golden brown color of the tumor is one of its characteristic gross features.

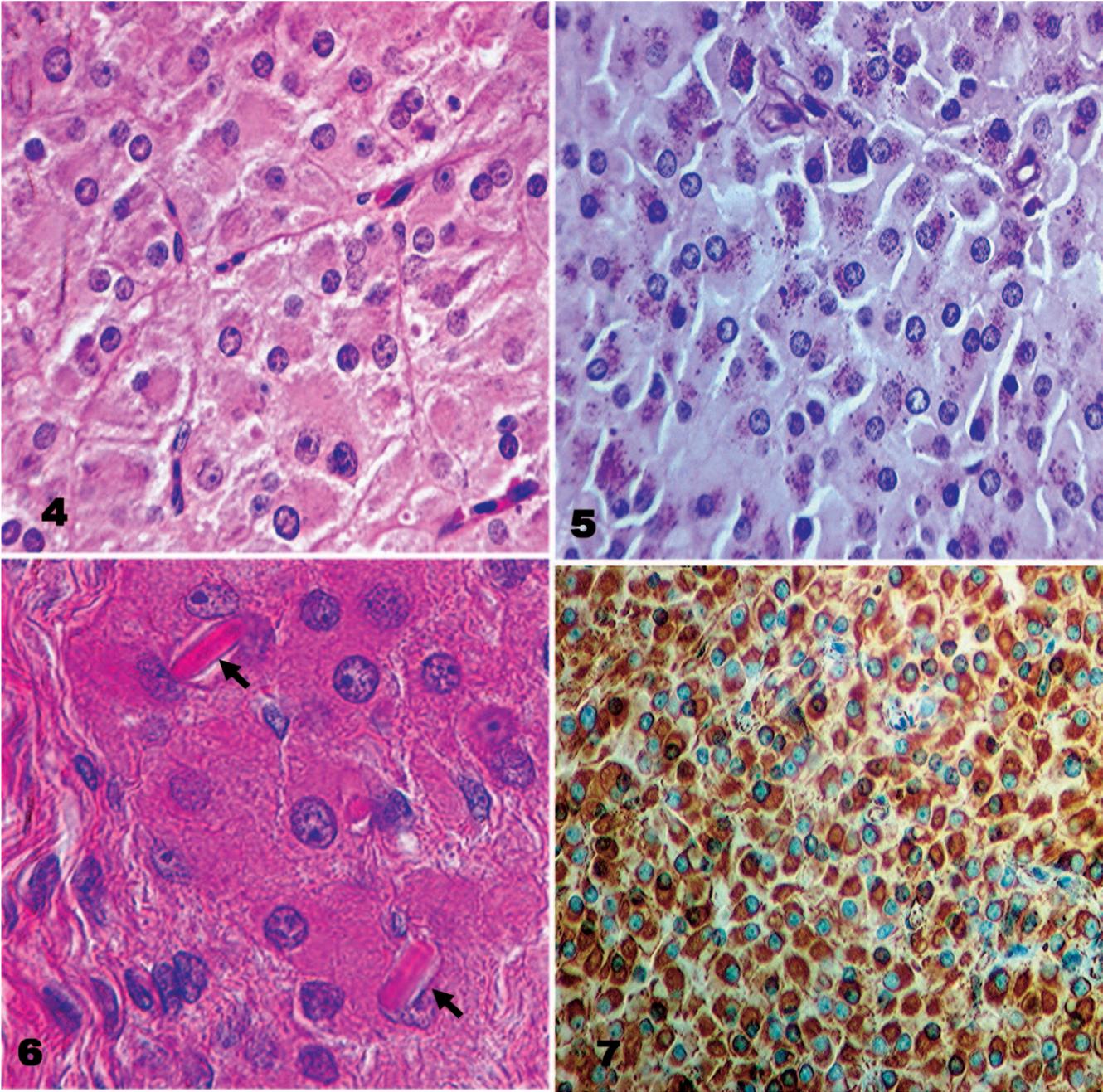


Figure 4. Histologic section of Leydig cell tumor. The tumor cells have abundant, eosinophilic, finely granular cytoplasm and regular round nuclei, some with visible, single or multiple nucleoli. The nuclei are either centrally or eccentrically placed. Note the distinct cell borders and the delicate fibrovascular septa between the tumor cells. The cytoplasm clearing is because of the lipidized contents (hematoxylin-eosin, original magnification $\times 400$).

Figure 5. Periodic acid-Schiff stain can easily highlight the cytoplasmic lipofuscin granules of tumor cells, one of the diagnostic clues of Leydig cell tumor (original magnification $\times 400$).

Figure 6. Intracytoplasmic Reinke crystals in Leydig cell tumor. The crystals appear as refractile, cylindrical, rectangular or rhomboid structures arranged in a linear fashion (arrows). Identification of Reinke crystals is very helpful for the distinction of Leydig cell tumor from other similar lesions (hematoxylin-eosin, original magnification $\times 800$).

Figure 7. Leydig cell tumor typically shows strong, diffuse cytoplasmic reactivity for α -inhibin. α -Inhibin is a sensitive and specific marker that serves to separate testicular sex cord-stromal tumors including Leydig cell tumor from germ cell tumors (α -inhibin, original magnification $\times 400$).

of abundant lipid accumulation. It also contains abundant lipofuscin (also called lipochrome) pigment. The lipofuscin pigment appears on hematoxylin-eosin-stained sections as golden yellow to brown cytoplasmic (mainly perinuclear) granules. It also can be highlighted by periodic acid-Schiff stain with or without diastase (Figure 5). Abundant cytoplasmic lipofuscin pigment is considered as one of the distinctive features of LCT, although it is nonspecific as it also occurs in other steroid-secreting tumors as well as in aging cells. Reinke crystals are also distinctive for LCT. These are pale-staining, refractile, cylindrical, rectangular or rhomboid structures (or inclusions) arranged in a linear fashion (Figure 6, arrows). Both intracytoplasmic and intranuclear Reinke crystals have been described.¹⁷ The significance of these crystals remains obscure. The crystals can be picked up on hematoxylin-eosin-stained sections. They can be highlighted by Giemsa or Masson trichrome stains. Reinke crystals are identified in less than half of all LCTs. Mitoses in benign LCT are generally rare and nuclear atypia is absent or minimal. Microscopic features that indicate malignancy include marked cytologic and nuclear atypia, necrosis, lymphovascular invasion, increased mitotic rate (more than 3 to 5 per 10 high-power fields) with atypical mitotic figures, DNA aneuploidy, and increased expression of proliferative markers (Ki-67/MIB-1 and p53 protein).¹⁸

Cytology

Leydig cells on smears can occur singly or in clusters. They can be identified with their aforementioned characteristic histomorphologic features. All reports on cytodiagnosis of LCTs describe the diagnostic significance of Reinke crystals. Identification of the crystals on cytologic examination serves to clinch the diagnosis. The crystals are best appreciated on air-dried, Giemsa-stained smears than in fixed smears or tissue sections on light microscopy. Gupta et al¹⁹ suggest that some of the crystals dissolve during fixation and processing used for histopathologic preparation and staining, making them more difficult to identify in paraffin sections than cytologic smears. Interestingly, scrape cytologic preparation was found by Hribar et al²⁰ to be helpful for revealing the crystals. This was explained by the fact that scraping disrupts the cytoplasm of the Leydig cells and releases the crystals causing them to assume an extracellular location, thus aiding in their identification. The scrape cytologic testing can thus be a useful adjunct for intraoperative diagnosis of LCT.

Ultrastructural Features

Electron microscopy of LCTs reveals ultrastructural features of steroid-secreting cells. These features are (1) abundance of smooth endoplasmic reticulum, (2) mitochondria with tubulovesicular cristae, and (3) numerous lipid droplets. Collectively, these features distinguish tumors of steroid-secreting cells, including LCT, from all others. In addition, electron microscopy of LCT shows numerous lipofuscin granules. They appear as rounded or irregularly shaped electron-dense bodies, which consist of lipid droplets accumulating in lysosomes. Reinke crystals can appear in various patterns including prismatic, hexagonal lattices, or hexagonal microtubules with parallel lines.²¹

Immunohistochemistry

The immunohistochemical markers that have been proved to be of the greatest utility for the evaluation of LCT are α -inhibin, calretinin, and Melan-A. α -Inhibin is a characteristic marker for sex cord-stromal tumors including LCT²² and, therefore, it is considered as the best marker to differentiate sex cord-stromal tumors from germ cell tumors (Figure 7). Calretinin is reported to be a valuable marker for LCT.²³ Melan-A (Mart-1) is also considered as a good marker for steroid-secreting tumors including LCTs²⁴ (once melanoma is excluded). Tumor cells, in most reported studies, show positive reactivity for vimentin but negative reactivity for cytokeratins.²⁵ Occasional reports about positive reactivity to S100 protein²⁶ and synaptophysin²⁷ are also available. Of note, the positive staining with vimentin and negative staining with cytokeratin combined with the presence of spindle cell morphology (as mentioned earlier) supports the mesenchymal origin of LCT. This immunophenotype of LCT is identical to that of adrenocortical tumors. This finding supports the postulation that both Leydig cells and adrenocortical cells share the same mesodermal origin. The existence of Leydig cells in the adrenal glands and the existence of adrenal rests in the testicles provide additional evidence for the common origin of the two.

DIFFERENTIAL DIAGNOSIS

To facilitate the discussion of its differential diagnosis, LCT can be divided into 2 major types depending on the histologic morphology: (1) conventional LCT and (2) unconventional LCT. The distinction should be between these 2 types and their mimickers.

Differential Diagnosis of Conventional LCT

Conventional LCT (ie, tumors in which the cells are round to polygonal with eosinophilic cytoplasm, arranged in sheets, nests, or trabecula) must be differentiated from Leydig cell hyperplasia, granular cell tumor, malakoplakia, the testicular nodules of adrenogenital syndrome, and other tumors of the testis (Table). Leydig cell tumor differs from Leydig cell hyperplasia by being solitary, whereas nodular Leydig cell hyperplasia is characteristically multifocal. In addition, LCT is larger (more than 0.5 cm in diameter), whereas the foci of Leydig cell hyperplasia are smaller (less than 0.5 cm in diameter). Granular cell tumor enters the differential diagnosis by having sheets of cells with abundant eosinophilic cytoplasm and central small nuclei. Because S100 protein can be positive in LCT, it might not be helpful in this situation. Using the distinctive immunopanel of LCT (α -inhibin, calretinin, and Melan-A) with the identification of Reinke crystals and lipofuscin pigment helps secure the diagnosis for LCT. Excessive secretion of ACTH in some hypothalamic-pituitary-adrenal axis conditions (such as ACTH-secreting pituitary adenoma after bilateral adrenalectomy) may lead to hyperplasia of the interstitial ACTH-dependent adrenal rests that exist in the testis. These hyperplastic adrenal rests are referred to as testicular "tumors" of adrenogenital syndrome (TTAGS). These cells are hormonally active in secreting androgens. They can be confused macroscopically with LCT because of their brown color (as they contain abundant lipofuscin pigment similar to Leydig cells), but they

Distinction Between Leydig Cell Tumor of the Testis and Other Testicular Neoplasms*

	Seminoma	Yolk Sac Tumor	Testicular Tumor of Adrenogenital Syndrome	Leydig Cell Tumor	Sertoli Cell Tumor
Average age range, y	30–40	Pure: Infants/children <3 Mixed: 20–30	Any age. Usually occurs in conditions with ACTH elevation.	Any age. Peaks in age range of 5–10 and 30–50	15–85, peaks in age of 45
Characteristic gross features	Homogenous, grey-white (tan or cream colored), devoid of hemorrhage or necrosis (if pure seminoma). usually replaces the entire testicular parenchyma.	Nonencapsulated, homogenous, tan, yellow-white mass. Usually smaller than seminoma. If mixed, areas of hemorrhage and necrosis can be present.	Nodules with brown cut surface. Bilateral and multifocal.	Homogenous, well-circumscribed mass (3–5 cm) with white to yellow to brown cut surface. Unilateral and solitary.	Homogenous, well-circumscribed, mass with grey-white to yellow cut surface.
Histopathology	Uniformly round cells, clear abundant cytoplasm with "squaring," large central nucleus with single or multiple nucleoli. Cells are arranged in sheet or multinodular growth pattern with intervening fibrous septa infiltrated by lymphocytes. Syncytiotrophoblasts can be present.	Cuboidal or elongated cells in reticular, microcystic, papillary patterns. Schiller-Duval bodies are pathognomonic.	Polygonal cells in nest or sheet growth pattern with eosinophilic, finely granular, abundant cytoplasm and central round nuclei with prominent nucleoli. Tumor nodules are separated by fibrous bands. Cells contain abundant lipofuscin pigment. Reinke crystals are characteristically absent.	Polygonal cells with eosinophilic cytoplasm, round nuclei with prominent nucleoli. Cells can be sarcomatoid. Intracytoplasmic lipofuscin pigment and Reinke crystals. The cells are arranged in nests, sheets, or trabecula. Microcystic growth pattern had been described.	Tubules or cords in nested or diffuse arrangement lined by monolayer of low columnar cells with oval nuclei. Can be either pure or intermixed with Leydig cell tumor (Sertoli-Leydig cell tumor).
Laboratory tests	Serum PLAP can be elevated. Serum hCG can be elevated (if trophoblasts are present).	Serum AFP is elevated.	Serum ACTH and androgens are elevated.	Androgens OR estrogens are elevated. Serum ACTH is low.	Androgens OR estrogens are elevated.
Immunopanel					
Positive	PLAP CD117 (c-Kit) OCT-4 hCG (if trophoblastic component is present)	Cytokeratin AFP	Inhibin† Calretinin† Melan-A† Vimentin†	Inhibin Calretinin Melan-A Vimentin S100 (+/–) Synaptophysin (+/–)	Inhibin Vimentin EMA Cytokeratin (+/–)
Negative	Cytokeratin EMA AFP CD30 Inhibin Calretinin Melan-A	EMA CD30 Inhibin Melan-A Calretinin	Cytokeratin† AFP† PLAP† hCG†	Cytokeratin AFP PLAP hCG	AFP PLAP hCG

* ACTH indicates adrenocorticotrophic hormone; PLAP, placental alkaline phosphatase; hCG, human chorionic gonadotropin; AFP, α-fetoprotein; and EMA, epithelial membrane antigen.

† Similar to the immunopanel of Leydig cell tumor. Immunohistochemistry is not helpful in the distinction.

are almost invariably multifocal and bilateral, whereas LCT is usually solitary and unilateral. Microscopically, cells of TTAGS have abundant eosinophilic and finely granular cytoplasm and centrally located nuclei with single prominent nucleoli and thus closely mimic Leydig cells. Identification of Reinke crystals, which is characteristic of LCT (but not TTAGS), is very helpful. Clinically, serum ACTH is elevated in TTAGS but normal to low in LCT (the steroid hormones secreted by LCT exert negative feedback on ACTH secretion from the pituitary). The importance of the distinction between LCT and TTAGS relies on the fact that metastases have never been reported in TTAGS and orchiectomy is unnecessary with this diagnosis as regression of TTAGS with glucocorticoid therapy is possible.²⁸ Malakoplakia of the testis can also look sim-

ilar to LCT grossly as it may form a single, homogeneous, yellow or brown mass. The eosinophilic histiocytes of malakoplakia may be misconstrued as Leydig cells, but they typically involve tubules as well as interstitium in the background of xanthogranulomatous inflammation. Michaelis-Gutmann bodies (rounded, lamellar, basophilic, calcific bodies inside and outside histiocytes) are pathognomonic of malakoplakia. LCT can be easily distinguished from Sertoli cell tumor and other testicular tumors in the sex cord-stromal category with the exception of the large cell calcifying variant of Sertoli cell tumor, which resembles LCT by having sheets of cells with large eosinophilic cytoplasm. However, areas of calcification discriminate this tumor from LCT (except LCT with unusual calcification). Testicular germ cell tumors can be easily dis-

tinguished from conventional LCT by their characteristic histomorphologic features. Other differential diagnoses include malignant melanoma and metastatic carcinoma (particularly from the prostate). Bilaterality; common involvement of the epididymis and spermatic cord; intertubular infiltration of tumor cells with invasion of the tubules; and the malignant cytologic features such as nuclear and cellular pleomorphism, high mitotic activity, and necrosis can differentiate these tumors from LCT. Appropriate immunostains will also help exclude these diagnoses.

Differential Diagnosis of Unconventional LCT

LCTs lacking the microscopic features of conventional LCT, as described earlier, should be distinguished from their mimicking lesions.

LCT With Sarcomatoid Pattern.—Sarcomas enter the differential diagnosis of sarcomatoid LCT. Because sarcomatoid LCT is usually associated with areas displaying the conventional patterns of LCT, careful search for this conventional component is very helpful to settle the diagnosis. Therefore, thorough sampling of the tumor can be necessary to identify these components and resolve this differential. Additionally, the eosinophilic cytoplasm and the typical nuclear appearance of Leydig cells as well as their Reinke crystals are maintained in many cases of sarcomatoid LCT. Immunoreactivity for LCT markers (α -inhibin, calretinin, and Melan-A) may not be helpful in the differential because these markers are all reported to be negative in the sarcomatoid LCT.¹⁶

LCT With Microcystic Pattern.—The primary differential diagnostic consideration for this variant is yolk sac tumor (YST). The key to the correct interpretation is finding a component of conventional LCT. Other features can also be of help. The lack of α -fetoprotein elevation is an important clinical indicator against YST because nearly all testicular YSTs are accompanied by such elevations. In addition, pure YSTs are rare in adults, so the absence of other types of germ cell tumor components provides additional evidence against YSTs. Lack of cytologic and nuclear atypia and low mitotic activity favor a diagnosis of LCT over YST. Lipofuscin pigment and Reinke crystals may prove helpful in the differential. Immunohistochemically, YSTs are positive for α -fetoprotein and cytokeratin but negative for α -inhibin. Conversely, LCTs are positive for α -inhibin but negative for α -fetoprotein and cytokeratin.¹⁴

PROGNOSIS AND MANAGEMENT

Benign LCTs are classically treated by orchiectomy, although testis-sparing surgery by tumor enucleation with clear margins has recently been considered especially in boys and young men, to maintain their fertility.²⁹ Malignant LCTs are treated by orchiectomy with retroperitoneal lymphadenectomy as metastasis frequently involves retroperitoneal nodes. Other metastatic sites are liver (45%), lungs (40%), and bone (25%).⁷ Malignant LCT does not respond favorably to chemotherapy and irradiation. Survival time has ranged from 2 months to 17 years (median, 2 years).³⁰ Although benign LCTs can be cured by resection, testicular dysfunction and infertility can be a serious sequel in patients with estrogen-secreting LCTs. Long-term excess of estrogen can cause impairment of spermatogenesis, most likely because of hypothalamic-pituitary inhibition as well as direct blockade at the testicular

level.³¹ This calls for early detection and management of these tumors to preserve the reproductive capacity.

SUMMARY

Leydig cell tumor of the testis is a rare, benign, interstitial tumor of the sex cord–stromal category. Awareness of the constellation of its clinical, radiographic, and pathologic features aids in establishing the correct diagnosis and distinguishing this neoplasm from other lesions of the testis. Leydig cell tumor is a steroid-secreting tumor. Clinicians have to consider LCT in either virilizing or feminizing syndromes. Testicular ultrasound is a useful means for tumor detection, especially if the tumor is not palpable. A careful search for the characteristic cytologic features of tumor cells is very important for diagnosis. LCTs may infrequently have unusual features. Immunohistochemistry can help confirm the diagnosis in challenging cases. The diagnostic immunopanel of LCT is diffuse cytoplasmic positivity for α -inhibin, calretinin, Melan-A, and vimentin and negative immunostaining with cytokeratin.

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