



CASE-BASED FOCUSED LITERATURE REVIEW

Fine needle aspiration diagnosis of metastatic Leydig cell tumor. Report of a case and review of the literature

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Leydig cell tumors are rare sex cord-stromal tumors that account for less than 1% of all testicular tumors. Less than 10% of these tumors show metastatic malignant behavior.

Herein we present a case of metastatic malignant Leydig cell tumor in an iliac lymph node diagnosed on fine-needle aspiration (FNA) in a 70-year-old man. The patient was referred from an outside institution with lymphadenopathy and had a past medical history of lymphoplasmacytic lymphoma/Waldenström's macroglobulinemia and past surgical history of orchiectomy. An iliac lymph node FNA was performed demonstrating large discohesive plasmacytoid cells with indistinct cell borders; abundant and finely granular cytoplasm; round, eccentric nuclei with evenly distributed chromatin; and prominent nucleoli. The tumor cells were positive for inhibin and negative for calretinin and keratin leading to the diagnosis of metastatic malignant Leydig cell tumor. Review of the patient's history and of previous pathologic material, careful evaluation of cytomorphologic features, and the judicious use of immunohistochemistry can allow an accurate diagnosis of metastatic Leydig cell tumor.

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Introduction

Leydig cell tumor (LCT) is a rare sex cord stromal tumor that comprises less than 1% of all testicular neoplasms¹⁻³ LCTs can occur at any age but they are common in prepubertal boys and men aged 30-60 years. While the majority of these tumors,

especially those occurring in the prepubertal children have a benign behavior, 5-10% behave in a malignant manner.^{2,4,5} LCT are hormonally active and produce androgens, mainly testosterone, but can also produce estrogen by either direct production of estradiol or by peripheral aromatization of the testosterone. In androgen-secreting tumors, most adult patients are asymptomatic as the excess androgen rarely causes notable effects. In estrogen-secreting tumors, adults generally present with gynecomastia associated with loss of libido, erectile dysfunction, impotence, and infertility.³ The etiology and biology of LCT are still unknown; rarely this tumor may be seen

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in association with the hereditary leiomyomatosis renal cell carcinoma (HLRCC) syndrome caused by germline mutations of fumarate hydratase (FH) gene.⁶

This tumor is rarely encountered in cytology practice because FNAs, like biopsies, are contraindicated in testicular tumors to prevent the possibility of seeding the tumor. Herein we present the case of a 70-year-old male with an enlarged iliac lymph node that was diagnosed as metastatic Leydig cell tumor by FNA.

Case report

A 70-year-old male presented for a check-up visit to his primary care physician. His past medical history included lymphoplasmacytic lymphoma/Waldenström macroglobulinemia first diagnosed 12 years previously, malignant Leydig cell tumor diagnosed 3 years previously, and benign prostatic hypertrophy. A surveillance CT of the abdominopelvic region showed an enlarged iliac lymph node on the left side, which had increased in size from a previous CT, and measured 3.1×3.1 cm. His complete blood count and IgM levels, which were monitored due to his Waldenström's macroglobulinemia history, were within normal limits. A CT-guided fine needle aspiration (FNA) of the enlarged iliac lymph node was performed.

The aspirate smears were cellular with large discohesive cells with indistinct cell borders situated in close proximity to branching capillaries (Fig. 1). The cells were large, rounded or polygonal with abundant finely granular cytoplasm. With Diff-Quik, the cytoplasm was blue (Fig. 2); no vacuoles were

seen. In Papanicolaou-stained smears, the cytoplasm was scant and poorly defined with reddish granules (Fig. 3, Fig. 4). Similar granules were seen in the smear background and were accompanied by naked nuclei. The nuclei were round to oval and were eccentrically located conferring the tumor cells a plasmacytoid appearance, reminiscent of neuroendocrine tumors. They were mostly single, although occasional binucleated and multinucleated cells were present. The chromatin was fine and distributed evenly. Single central nucleoli were visible in most cells and were occasionally prominent. Some nuclei demonstrated grooves, but no inclusions or mitotic figures were identified.

The immunohistochemical stains performed on cell block sections showed that the tumor cells were positive for inhibin (Fig. 5) and negative for calretinin, CD45, pancytokeratin, and CD56.

The patient's prior pathology material from the orchiectomy, performed 3 years earlier, and the retroperitoneal lymphadenectomy performed 2 years earlier, was reviewed. The diagnosis of malignant Leydig cell tumor with marked nuclear atypia, foci of brisk mitotic activity and focal necrosis was confirmed as well as metastases to 2 out of 35 lymph nodes. The histopathologic features seen in the lymph node metastases were similar to those seen in the fine needle aspiration specimen and a diagnosis of metastatic Leydig cell tumor was rendered.

Two months after the iliac lymph node FNA, the patient underwent resection of a $6.8 \times 5.5 \times 4.0$ cm pelvic tumor mass that was diagnosed as metastatic Leydig cell tumor. After another 5 months, a liver biopsy was also was

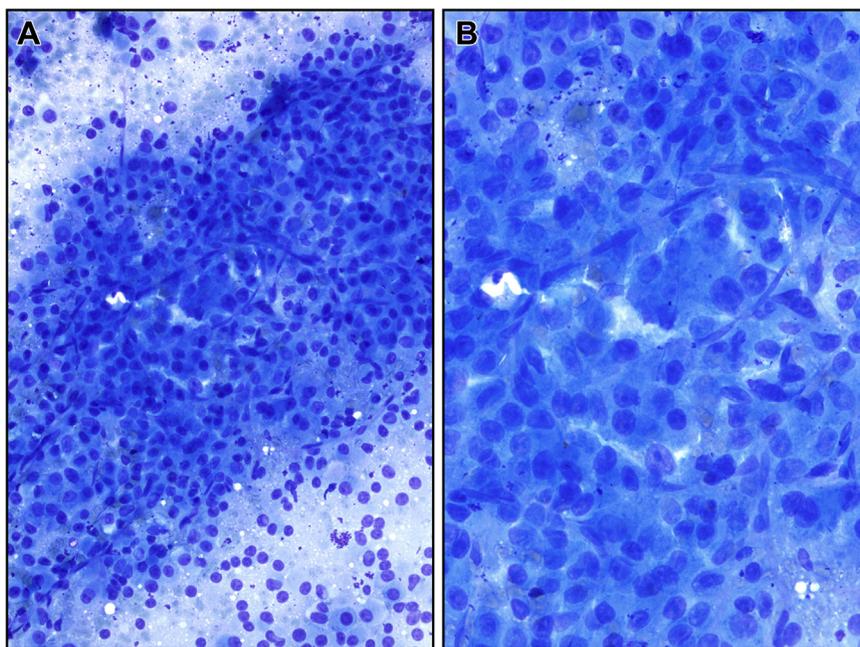


Figure 1 Fine needle aspiration, iliac lymph node. A. Relatively uniform neoplastic cells arranged around branching capillaries, single plasmacytoid cells and naked nuclei against a granular bluish background. B. Higher power showing endothelial lining of the capillaries and mild anisonucleosis and nuclear pleomorphism as well as occasional prominent nucleoli. (Diff-Quik stain, A $\times 100$, B $\times 400$).

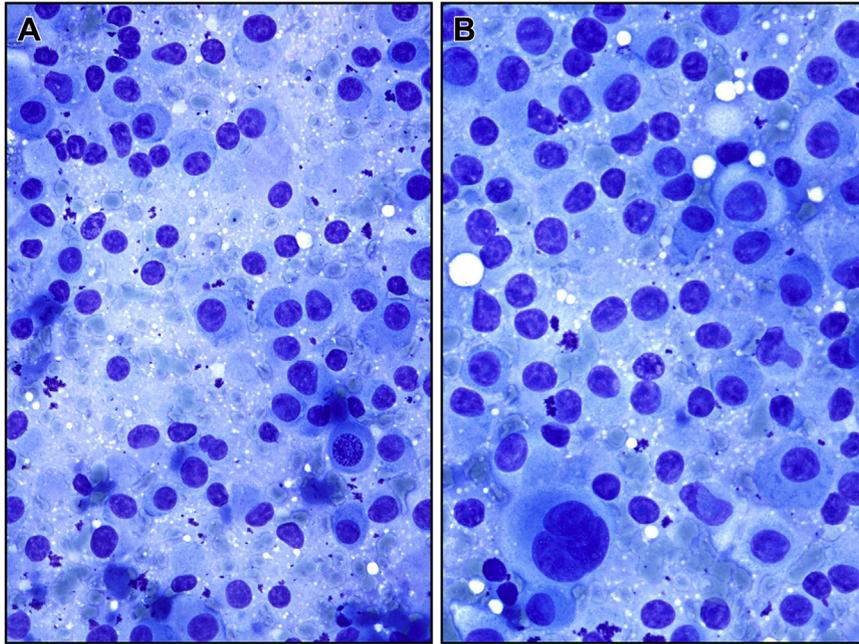


Figure 2 A and B. Fine needle aspiration, iliac lymph node. High power appearance of the neoplastic cells showing large, discohesive cells with eccentric, round to oval nuclei and abundant cytoplasm. Note the occasional nuclear grooves and multinucleation. (Diff-Quik stain, $\times 1000$).

diagnosed as metastatic Leydig cell tumor. The patient passed away shortly after that.

Discussion

Leydig cells, the interstitial cells between seminiferous tubules of the testicles, were first described in 1870 by the German anatomist Franz von Leydig (1821-1908).⁷ They produce testosterone when stimulated by luteinizing hormone and are involved in developing secondary male

characteristics and maintaining spermatogenesis. The mechanism of oncogenesis of Leydig cell tumors is not well-understood. There are no known risk factors for Leydig cell tumors and they are not known to be associated with cryptorchidism as germ cell tumors are.

On histology, Leydig cell tumors most commonly demonstrate nests or sheets separated by delicate fibrovascular septa. They also can have small clusters or trabecula separated by a variable amount of fibrous or myxoid stroma. Abundant cytoplasmic lipofuscin pigment, appearing as golden yellow to brown cytoplasmic granules is a distinctive feature, though nonspecific. On immunohistochemical staining, inhibin and steroidogenic factor 1 (SF1) positivity is characteristic of sex cord-stromal tumors and the best way to differentiate them from germ cell tumors. Of note, SF1 is also positive in adrenal cortical neoplasms, and it may be differentiated from LCT based on morphologic and clinicoradiological features. The differentiation of LCT from other testicular sex-cord stromal tumors is made morphologically, although SOX9 and FOXL2 may be used as evidence supporting Sertoli cell and respectively granulosa cell differentiation. Calretinin, Melan-A and vimentin also are typically positive, while EMA is negative and cytokeratins are typically negative, as in the current case.

The presence of metastases is the only definite evidence of malignancy; however large tumor size, infiltrative borders, angiolymphatic invasion, cytologic atypia, necrosis and high mitotic activity are suggestive of malignant behavior.⁸ Metastases are present at diagnosis in up to 2-3% of patients; they involve retroperitoneal lymph nodes (LN),

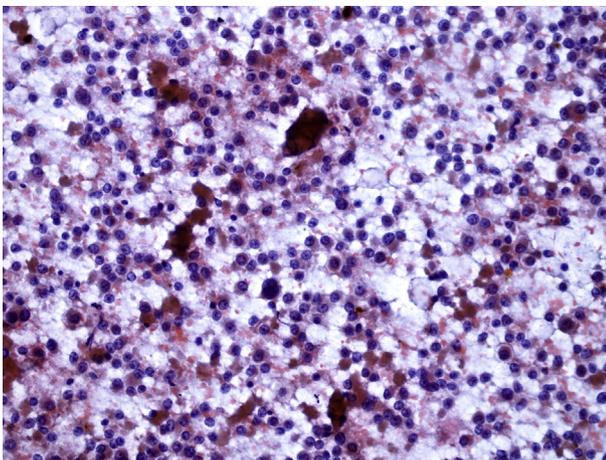


Figure 3 Fine needle aspiration, iliac lymph node. Single cell pattern of neoplastic cells with monotonous appearance. (Papanicolaou stain, $\times 1000$).

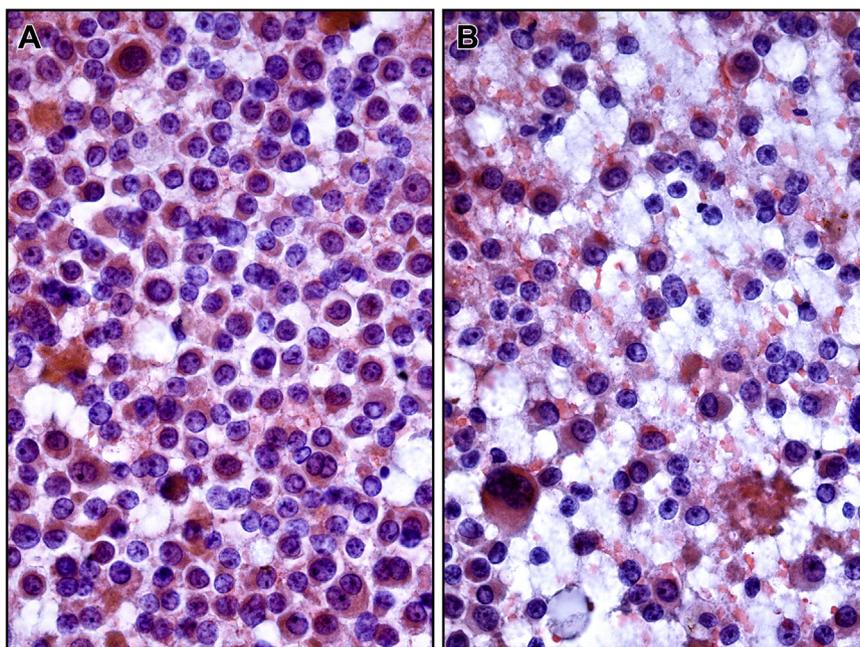


Figure 4 A and B. Fine needle aspiration, iliac lymph node. High power appearance of the neoplastic cells showing discohesive, focally binucleated cells with slightly irregular round to oval nuclei and granular eosinophilic cytoplasm. Note the fine, evenly distributed chromatin, occasional nuclear grooves and prominent nucleoli. (Papanicolaou stain, $\times 1000$).

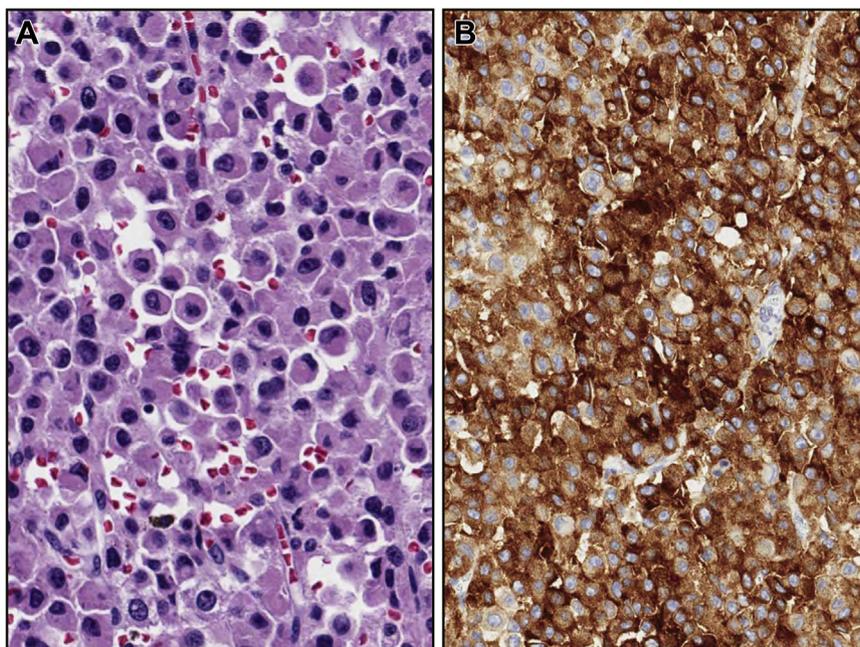


Figure 5 Fine Needle aspiration, iliac lymph node. Cell block demonstrates plasmacytoid cells with nuclear pleomorphism. B. Inhibin immunostain is diffusely positive. (Cell block, A. hematoxylin and eosin stain, $\times 400$; B. Immunostain $\times 400$).

Table 1 Previously reported fine needle aspiration cytology findings of Leydig cell tumors.

#	Author, year	Country	Patient age	Presentation	Tumor size (cm)	FNA site	Cytologic findings	FNA diagnosis
1	Crucioli & Fulciniti, 1987	England	30	Painless mass	1	L testis	Medium-sized, round/oval cells with anisonucleosis, cytoplasmic granules and vacuoles, Reinke crystals identified on review, naked nuclei and lacy, pinkish-grey background.	Seminoma
2	Vielh et al, 1991	France	43	Painless mass	3	L testis	Highly cellular smears with cells present singly and in small groups; pericapillary arrangements, large cells with abundant grey-blue cytoplasm with small violet granules and microvacuolation (ROM). Rare binucleated cells. Rare intranuclear inclusions. Rare mitoses. No Reinke crystals.	LCT
3	Gupta et al, 1994	Kuwait	40	Painless mass	5	L testis	Large oval/polygonal cells with abundant pale cytoplasm, bluish-violet cytoplasmic granules and vacuoles. Central or eccentric nuclei with occasional inclusions and prominent nucleoli. Numerous cytoplasmic and intranuclear Reinke crystals.	Suspicious for LCT
4	Assi et al, 1997	Italy	26	Painless mass	2	L testis	Cellular smears with cells present singly and in small groups; large polygonal cells with abundant gray-blue cytoplasm (ROM) and round or oval nuclei with fine reticular chromatin and rare nuclear grooves. No intranuclear inclusions. Rare Reinke crystals.	LCT
5	Elmberger & Collins, 1999	Sweden	32	Enlarged LN, liver mass on CT, 2 years postorchietomy	NA	Retroperitoneal LN, paraaortic LN and liver	Cellular smears with cells present singly and in small groups; large polygonal cells with well-delineated abundant granular cytoplasm (ROM). Round or oval occasionally eccentric mildly pleomorphic nuclei with fine chromatin and distinct central nucleoli. No nuclear grooves or intranuclear inclusions. Rare Reinke crystals.	Metastatic LCT

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Table 1 (continued)

#	Author, year	Country	Patient age	Presentation	Tumor size (cm)	FNA site	Cytologic findings	FNA diagnosis
6	Ortiz et al, 1999	Spain	25	Gynecomastia	NR	Testis, side unspecified	Uniform cells, present singly and in small groups; large polygonal cells with poorly defined abundant acidophilic cytoplasm (H&E). Round nuclei with fine chromatin and visible but not prominent nucleoli. No nuclear grooves, intranuclear inclusions or Reinke crystals.	Not mentioned
7	Jain et al, 2001	India	32	Painless mass	4	L testis	Cellular smears with cells present singly and in small groups; large round to polygonal cells with abundant pale-blue cytoplasm (ROM) containing coarse, dark lipofuscin granules. Eccentrically placed round or oval nuclei with evenly distributed chromatin and moderate pleomorphism. Occasional binucleation. Occasional prominent nucleoli. No grooves or intranuclear inclusions. Nuclear and cytoplasmic Reinke crystals.	LCT
8	Naik et al, 2007	India	53	Painless mass	NR	R inguinal	Cellular smears with cells present singly and in small groups; large round to polygonal cells with moderate anisonucleosis. Occasional binucleation. Occasional prominent nucleoli. Occasional intranuclear inclusions. Numerous naked nuclei. No Reinke crystals.	"metastasis probably of testicular tumor"
9	Handa et al, 2010	India	70	Painless mass	NR	R testis	Cellular smears with cells present singly and in small groups; large round to polygonal cells with ill-defined abundant pale cytoplasm (ROM) with granules and vacuoles. Round or oval occasionally eccentric mildly pleomorphic nuclei with evenly distributed chromatin and distinct central nucleoli. Rare intranuclear inclusions. Rare Reinke crystals.	LCT

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Table 1 (continued)

#	Author, year	Country	Patient age	Presentation	Tumor size (cm)	FNA site	Cytologic findings	FNA diagnosis
10	Valeri et al, 2011	Greece	49	Painless mass, gynecomastia	10	R testis	Cellular smears with cells present singly and in small groups; pericapillary arrangements. Small to medium-sized round to polygonal cells with well-defined abundant eosinophilic cytoplasm (Pap) with granules and microvacuolation. Round or oval occasionally eccentric markedly pleomorphic nuclei with fine chromatin and variably conspicuous, focally prominent nucleoli. Rare nuclear grooves but no intranuclear inclusions. "Dirty" background, mitoses and naked nuclei. No Reinke crystals.	"LCT with atypical features"
11	Damodaran et al, 2014	India	42	Painless mass, gynecomastia, azoospermia	NR	Testis, side unspecified	Cellular smears with cells present singly and in small groups; cells arranged around fibromuscular stroma. Large round to polygonal cells with abundant cytoplasm showing vacuoles. No mention of nuclear grooves, intranuclear inclusions or Reinke crystals.	LCT
12	Biemer et al, 2019, Current case	USA	70	Enlarged iliac LN on CT, 3 years after LCT diagnosis	NA	L common iliac LN	Cellular smears with cells present singly and in small groups; cells arranged around branching capillaries. Large round to polygonal cells with abundant finely granular non-vacuolated cytoplasm. Eccentric mildly pleomorphic nuclei with evenly distributed chromatin and distinct central nucleoli. Focal binucleation and naked nuclei. Occasional nuclear grooves, but no intranuclear inclusions or Reinke crystals.	Metastatic LCT

Abbreviations: L, Left; R, Right; ROM, Romanowsky-type stain; H&E, hematoxylin and eosin; Pap, Papanicolaou stain; NA, Not applicable; NR, Not reported; LN, lymph node; LCT, Leydig cell tumor; CT, computed tomography.

lung, liver, mediastinal LN, kidney and brain, while an additional 3-6% of patients develop metastases to the same sites after a median follow-up of 2 years. Treatment of LCT consists of radical orchiectomy, with enucleation or testis-sparing surgery reserved to selected pediatric cases, which have a benign behavior, and small (less than 2.5 cm) tumors.^{8,9} Malignant LCT are treated by orchiectomy with retroperitoneal lymph node dissection; radiation therapy can be used for palliation, but chemotherapy is ineffective, since LCT are chemoresistant, and no targeted therapy is currently available.

There are a few previous case reports of Leydig cell tumors diagnosed on cytology in the literature,¹⁰⁻²⁰ however, to our knowledge there have been only 2 other reports of metastatic malignant Leydig cell tumor diagnosed on FNA^{18,19} (Table 1). In previous cytopathology reports, Leydig cell tumors were described as cellular and showing tumor cells singly and in discohesive clusters, frequently attached to capillaries. The tumor cells were large, round to polygonal, with pale abundant, granular and vacuolated cytoplasm, and nuclei that were round to oval, central to paracentral, with evenly distributed chromatin and a single prominent nucleolus.¹² Naked nuclei and granular or dirty background were occasionally seen, as were mitoses, nuclear grooves and intranuclear inclusions. Five of the eleven reported cases showed variable numbers of Reinke's crystals, although they were sometimes only identified retrospectively after a careful search. Reinke's crystals, named after the German anatomist Friedrich Berthold Reinke (1862-1919) who described them in 1896,²¹ are rod-shaped or hexagonal cytoplasmic (rarely nuclear) inclusions, pathognomonic for Leydig cells and their tumors. However, Reinke's crystals are only found in about 30% of LCT, and even less frequently in malignant ones. Identifying Reinke's crystals and intracytoplasmic lipofuscin pigment can aid the diagnosis,¹⁴ although neither were found in our case.

In the patient presented herein, due to the history of lymphoplasmacytic lymphoma, a major differential diagnostic consideration was a hematolymphoid malignancy, in particular one with plasma cell differentiation. However, despite their plasmacytoid appearance, the neoplastic cells were too large, had granular cytoplasm, nuclear grooves, and showed pericapillary arrangement, all cytomorphologic features that did not support a hematolymphoid neoplasm.

The differential diagnosis of these cells, especially when encountered in lymph node aspirate, is very wide and includes all tumors composed of loosely cohesive or discohesive large cells with granular cytoplasm (Table 2). Such tumors include, but are not restricted to, metastases from hepatocellular carcinoma (HCC), renal cell carcinoma (RCC), oncocytic and apocrine neoplasms (including oncocytic carcinoma, Hürthle cell carcinoma, apocrine carcinoma and salivary duct carcinoma), neuroendocrine tumors (including medullary carcinoma), paraganglioma, adrenal cortical carcinoma, malignant granular cell tumor, alveolar soft part sarcoma and malignant melanoma. Given the vast differential diagnosis of tumors with this morphological pattern, a broad immunohistochemical panel including cytokeratins, EMA, Calretinin, Melan-A, inhibin and SF-1, is recommended to arrive to a definitive diagnosis.

Review of the patient's history and of previous pathologic material, careful evaluation of cytomorphologic features, and the judicious use of immunohistochemistry can allow an accurate diagnosis of metastatic Leydig cell tumor.

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Table 2 FNA Differential diagnosis of Leydig cell tumors.

Tumor type	Leydig cell tumor	Renal cell carcinoma	Hepatocellular carcinoma	Oncocytic carcinoma
Architecture				
Cellularity	High	Variable	High	High
Smear pattern	Predominantly dispersed, small groups, pericapillary arrangements, naked nuclei	Loosely cohesive sheets, transgressing capillaries	Cohesive, trabeculae, pseudo-acinar groups, endothelial rimming, transgressing capillaries, single cells, naked nuclei	Dispersed cells, loosely cohesive
Cells Cytoplasm Nuclei				
Cell size and shape	Large rounded to polygonal Mild anisocytosis	Large polygonal Mild anisocytosis	Large polygonal Mild to marked anisocytosis	Medium-sized, rounded to polygonal Mild anisocytosis
Cell borders	Ill-defined	Well-defined	Well-defined	Well-defined
Cytoplasm	Moderate to abundant, with fine to coarse granules (lipofuscin) and small vacuoles, Reinke's crystals	Abundant, vacuolated, may be granular; may contain hemosiderin, hyaline globules	Abundant, granular, may be vacuolated; may contain bile pigment or hemosiderin, hyaline globules	Abundant, granular; may be vacuolated
Nuclei	Predominantly eccentric rounded nuclei with fine evenly dispersed chromatin and visible to prominent nucleoli May show grooves, INI, binucleation, multinucleation	Central large nuclei with mild to marked pleomorphism, hypo or hyperchromasia, prominent nucleoli, binucleation, multinucleation	Central large nuclei with mild to marked pleomorphism, hypo or hyperchromasia, prominent nucleoli, INI, binucleation, multinucleation	Central rounded nuclei with mild to marked pleomorphism, hyperchromasia, prominent nucleoli
Background	Granular	Bloody, may show necrosis	May show necrosis	May show necrosis
IHC				
Epithelial markers	—/+	+	+	+
Neuroendocrine markers	—	—	—	—
S100	—	—	—	—
Inhibin, Calretinin, MelanA	+	—	—	—
Other	SF1	PAX8	HepPar1, arginase	BSND
Molecular				

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Apocrine carcinoma	Granular cell tumor	Alveolar soft part sarcoma	Neuroendocrine tumors	Paraganglioma, pheochromocytoma	Adrenal cortical carcinoma
Variable Dispersed cells, loosely cohesive	Variable Dispersed cells, syncytial groups naked nuclei	Moderate to high Dispersed cells, syncytial groups naked nuclei	High Dispersed cells, small groups, transgressing capillaries	High Dispersed cells, small groups, syncytia	High Dispersed cells, small groups
Large, polygonal Mild to marked anisocytosis	Medium-sized to large, polygonal Mild anisocytosis	Medium-sized polygonal Mild anisocytosis	Small to medium-sized, round Mild anisocytosis, focal "endocrine atypia"	Small to medium-sized, round to polygonal Mild to marked anisocytosis	Small to medium-sized, indistinct cytoplasm Mild anisocytosis
Well-defined Abundant, large granules	Ill-defined Abundant, coarsely granular	Ill-defined Abundant, coarsely granular and variably vacuolated, rhomboid crystals	Well-defined Moderate amounts of cytoplasm, fine granules, variable vacuoles	Ill-defined Moderate amounts of cytoplasm, fine granules, variable vacuoles	Ill-defined Moderate amounts of cytoplasm, fine granules, small vacuoles. Intracytoplasmic hyaline globules may be seen
Central rounded nuclei, pleomorphism, prominent nucleoli May show INI	Central medium-sized pleomorphic round to spindle shaped nuclei, prominent nucleoli	Central uniform large round nuclei with single central macronucleoli	Eccentric, small round nuclei, salt-and-pepper chromatin, May show INI, binucleation, multinucleation	Central or eccentric medium-sized round to oval pleomorphic nuclei, coarse chromatin, May show INI	Central or eccentric small- round to oval nuclei, coarse chromatin, irregular nuclear membranes, and occasional prominent nucleoli
May show necrosis	Granular	Granular	Bloody	Bloody	Bloody or bubbly
+	–	–	–	–	–
–	–	–	+	+	–/+ variable
–	+	–	–	(+) sustentacular	+/- variable
–	–	–	–	+	+
AR, mammaglobin, GCDFP15	S100	TFE3 ASPL-TFE3	TTF1, CDX2, Islet1 (site determination)		SF1

with histologic, immunohistologic, and electron microscopic analysis. *Diagn Cytopathol.* 1991;7:601–605.

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