CORRECTION

WITHDRAWAL

WITHDRAWAL FOR "Metastatic Leydig Cell Tumor: A Clinico-pathological Review of Five Cases"

Ahead of Print article withdrawn by the author and internal inquiry committee of Christian Medical College, Vellore, Tamilnadu, India. The Editors were informed that an internal inquiry committee was constituted to look into an allegation of scientific fraud with regard to this article, and it is the recommendation of the committee that this article should be withdrawn.

doi: https://doi.org/10.4132/jptm.2019.05.23

ORIGINAL ARTICLE

Metastatic Leydig Cell Tumor: A Clinico-pathological Review of Five Cases

Mayank Gupta

Department of General Pathology, Christian Medical College, Vellore, India

Corresponding Author

Mayank Gupta

Department of General Pathology, Christian Medical College, 4th floor, ASHA Building, Vellore: 632004, India

Tel: +914162283125

Fax: +914162232035

E-mail: gupta.mayank103@gmail.com

Running title: Metastatic Leydig cell tumour

Abstract

Background: Although Leydig cell tumors (LCTs) account for only 1-3% of all testicular neoplasms, they are the most common sex cord-stromal tumor of the testis. LCT is most common in patients between 10 and 50 years of age, although they can present at any age. Most of them are **clinically** benign, but < 5% of tumors develop metastasis. This study analyzes the clinico-pathological features from five cases of metastatic LCTs. Material and Methods: Routine sections of five cases of LCT were reviewed and the following immunohistochemical markers were assessed: cytokeratin, TTF-1 (thyroid transcription factor), oct3/4, PLAP (placental alkaline phosphatase), CD30, LCA (leukocyte common antigen), synaptophysin, chromogranin, alpha-inhibin, calretinin, CD56, melan-A and androgen receptor (AR). The clinical, biochemical and radiological details were retrieved from the patients' medical records. Results: All five cases were adults who presented with a painless testicular mass. Two out of four cases had hemoptysis. On imaging, the adrenal glands were normal, and all cases had enlarged para-aortic nodes. One case had an enlarged left supraclavicular node without lung involvement, and the rest of the cases had metastatic lung nodules. All the cases were medically unfit for surgery, so the biopsies were taken from the metastatic sites. Histology and immunohistochemical expression of alpha-inhibin and AR confirmed the diagnosis of metastatic LCT. Although all the cases were given chemotherapy, none of them survived.

Conclusion: Metastatic LCTs are rare and have a poor prognosis. The clinico-radiological correlation with histomorphology and a specific immunohistochemical workup provide a definite diagnosis.

Keywords: Leydig cell tumor; Alpha-inhibin; Sertoli cell tumor

Introduction

Leydig cell tumors (LCTs) account for about 3% of the neoplasms of the testis.[1] About 20% of cases occur in children, commonly between 5 and 10 years of age, and about 80% occur in adults, commonly between 20 and 60 years of age.[1–3] Virtually all pediatric cases present with significantly smaller testicular tumors because of early clinical detection of isosexual pseudoprecocity from androgen production. LCT in adults most commonly presents with a testicular mass, and neoplastic androgen production is less readily detected in adults than in children.[1] Bilateral testicular involvement occurs in about 3% of cases, and approximately 30% of cases present with gynecomastia.[1] The majority of these tumors are clinically benign; however, clinically malignant tumors (<5% of LCTs) develop metastasis.[1,4-6] The following study analyzes the clinico-pathological features from five cases of metastatic LCT.

Material and Methods

A retrospective search was done in the pathology database at the Department of General Pathology, Christian Medical College Vellore from January 2001 to December 2018. Five cases of metastatic LCT were retrieved, and slides from all five cases were reviewed. The clinical, biochemical and radiological details from all five cases were retrieved from the patient's medical records. Immunohistochemistry (IHC) was performed in all cases on 5 micron thick tissue sections using an automated Ventana benchmark XT immunostainer with the antibodies listed in Table 1. All procedures performed in the current study were approved by the IRB (number: 561406, date: 26/12/18 **)** in accordance with the 1964 Helsinki Declaration and its later amendments. Informed consent for this study was obtained from the deceased patients' close relatives.

Results

The clinico-pathological features from all five cases are summarized in Table 2. The median age of presentation was 54 years (range, 48-59 years). Three cases presented with a painless right testicular mass, one had a painless left testicular mass, and one case presented with bilateral painless testicular masses. All

five cases had loss of weight and appetite. The second and fourth case had a history of hemoptysis, while the first, third and fifth cases had no symptoms related to the lung. None of the cases had gynecomastia or Cushing's syndrome. Because all cases presented with testicular mass(es), serum tumor markers (alpha-fetoprotein and beta-hCG) were evaluated as germ cell tumor was in the differential diagnosis for each case, despite the patients not belonging to the typical age group. Both alpha-fetoprotein and beta-hCG were normal in all the cases. Serum lactate dehydrogenase (LDH) was mildly elevated in the second and fourth cases.

Serum testosterone, androstenedione and estradiol levels were normal in the three cases who presented with a right testicular mass. The patient with bilateral testicular masses had mildly increased serum testosterone and androstenedione levels while serum estradiol was normal. The patient with the left testicular mass had significantly increased serum testosterone and mildly increased serum androstenedione and estradiol levels.

Radiologically, all cases had enlarged para-aortic lymph nodes. Additionally, the first case had an enlarged left supraclavicular node but no metastatic deposits elsewhere. The remainder of the cases had lung nodules that were suggestive of metastasis. Only the second case had bilateral lung nodules along with liver and mesenteric deposits. The adrenal glands and the rest of the intra-abdominal organs were normal on imaging in all the cases.

As all five cases were medically unfit for an operative procedure, the biopsies were taken from the metastatic sites for exact characterization of the tumors (Table 2). In the first case, the excision biopsy revealed a lymph node infiltrated by a tumor composed of closely packed clusters and nests of cells separated by fibrovascular septae. The cells were polygonal in shape and exhibited moderate to marked nuclear pleomorphism, coarse granular chromatin and clear vacuolated cytoplasm. Brisk mitosis and apoptosis were present (Figures 1-A&B).

Lung biopsies from the rest of the cases showed lung parenchyma infiltrated by tumor with sheets of polygonal cells displaying mild to moderately enlarged nuclei, darkly stained chromatin and eosinophilic cytoplasm. Mitosis or apoptosis were not evident (Figures 1-C&D).

The immunohistochemical results are depicted in Table 3. All cases were diffusely and strongly positive for alpha-inhibin (Figures 2 A&C). The tumor cells showed moderate positivity for CD56 (Figure 2B) in two cases. Androgen receptor (AR) was weakly positive in all cases (Figure 2D), while melan-A showed weak to moderate positivity in two cases only (Figure 2D, inset)**.** All cases were negative for cytokeratin, TTF-1, oct3/4, PLAP, CD30, LCA, synaptophysin, chromogranin and calretinin, except case 2 which was focally positive for cytokeratin. The overall features confirmed the diagnosis of metastatic LCT in all five cases.

The patients in all five cases received palliative chemotherapy comprised of carboplatin and paclitaxel, as all of them were medically unfit for surgery, but none of them survived (Table 2).

Discussion

LCTs are the most common sex cord-stromal neoplasm and account for 1-2% of all testicular tumors.[7] The etiological factors are not known, although some of them have been associated with testicular atrophy, cryptorchidism and infertility.[8] Rarely, these tumors have been associated with Klinefelter syndrome and germ line fumarate hydratase (FH) mutations implicated in hereditary leiomyomatosis and renal cell carcinoma syndrome.[7] None of our cases had features of Klinefelter syndrome, nor did they have a history of testicular atrophy, cryptorchidism, infertility, leiomyomatosis or renal cell carcinoma. Additionally, the activation of hypoxia/angiogenesis pathway has been implicated as one of the factors in the pathogenesis of LCT. [7]

LCT in adults usually presents as a testicular mass without any symptoms, as compared to children who typically present with isosexual pseudoprecocity and are later found to have a testicular mass. About 15%

of adults with LCT present with gynecomastia and rarely with Cushing syndrome. [1,9,10] None of our cases had gynecomastia or Cushing syndrome.

Clinically, most testicular LCTs have been reported as benign.[4,10] However, < 5% of LCTs present with metastasis. Usually, the microscopic features found in testicular LCTs that tend to metastasize have two or more of the following features: infiltrative borders, tumor size > 5 cm, cytological atypia, necrosis, lymphovascular invasion and, > 3 mitosis per 10 high-power fields.[1] Histopathological assessment of the tumor in the testis was not possible as none of the cases in our study underwent orchidectomy. Metastatic tumors in LCT usually involve the regional lymph nodes, lung, liver, bone and kidney.[11]

Previously, only a few studies and several case reports of metastatic LCT involving different sites have been reported (Table 4). All five cases in the present study had para-aortic lymph node involvement, and one case also had a left supraclavicular lymph node. The remainder of the cases had features of metastatic pulmonary nodules in addition to the para-aortic nodes.

Most of the previously reported cases developed metastasis within 2 years of the primary diagnosis, although Bertram KA et al. reported a case in which metastasis developed 8 years later.[15] Similarly, Gulbahce HE et al. reported a case in which metastatic LCT in the lung and perirenal adipose tissue was seen after seventeen years.[16] In this study, four cases died within one year of biopsy diagnosis, and only one case survived 2 years (Table 2).

Although the first case of this study did not belong to the age group wherein germ cell tumors are common, metastatic germ cell tumor was considered as one of the histopathological differential diagnoses. Metastatic carcinoma, including neuroendocrine carcinoma, and high-grade lymphoma were the other possibilities in the first case. Negative expression of oct3/4, PLAP and CD30 ruled out germ cell tumor, while negative expression of LCA ruled out lymphoma. Neuroendocrine carcinoma was ruled out as synaptophysin and chromogranin were negative. The possibility of metastatic carcinoma was unlikely, as cytokeratin was negative in four cases and focally positive in one case. Metastatic Hurthle cell carcinoma of the thyroid and hepatocellular carcinoma were considered in the differential diagnoses in the rest of our cases, but they were excluded by each case's clinical features,

imaging and immunoprofile. A metastatic tumor from the adrenal gland was excluded, as the adrenal glands in all cases were unremarkable on imaging. It is important to note that adrenocortical tumors also express immunohistochemical markers similar to those which were positive in this study, hence clinico-radiological correlation is important. The possibility of Sertoli cell
tumor, not otherwise specified (SCT-NOS) was excluded as there was no definite
evidence of tubular differentiation, and alpha-inhibin was strongly positive (SCT-NOS stains less intensely for inhibin).[21]

It is important to note that variable expression of cytokeratin can be observed in LCT, which can lead to an erroneous diagnosis of carcinoma at metastatic sites.[6,22] Hence, the combination of clinical features, histomorphology, and an extensive immunohistochemical workup, including sex cord-stromal (alpha-inhibin, melan-A and AR) and germ cell tumor (oct3/4, CD30 and PLAP) specific markers, are essential for an accurate diagnosis.

Very few reports on the management of metastatic LCT and the effects of systemic therapy are available. Usually, malignant LCTs are treated with surgery (orchidectomy and retroperitoneal lymph node dissection).[23] Systemic chemotherapy is not that effective, and patients do not typically respond to radiotherapy.[23,24] In the study by Bokemeyer C et al., three out of four cases received chemotherapy in

addition to surgery, and the remaining one case had surgery (orchidectomy and
subsequent radical retroperitoneal lymph node dissection) without adjuvant chemotherapy. Two of the four cases succumbed to the disease, the case in which no adjuvant chemotherapy was given had no disease after a follow up of over 10 months, and there was recurrence in the remaining one case which responded to chemotherapy. [5] All cases in the present report were medically unfit for surgery, received chemotherapy, and did not survive. In the study by Nicolai N, 8 out of 67 cases had metastasis; 5 cases

had only retroperitoneal lymph node involvement, 1 had only visceral involvement, and 2 cases had both retroperitoneal lymph node and visceral involvement. Four of 8 cases received chemotherapy and ultimately succumbed to their disease.[25] In our study, all five cases had retroperitoneal lymph node involvement on imaging, and four cases had additional visceral involvement. Hence, the overall prognosis of metastatic LCT seems to be very poor.

In summary, the present report described the clinicopathological features from five cases of metastatic testicular LCT, which is quite rare. Although most LCT testicular tumors are **clinically** benign, one must keep in mind that LCT can metastasize. Hence, the appropriate immunohistochemical work up as described in addition to clinico-radiological details and histology render a correct diagnosis.

References

1. Kim I, Young RH, Scully RE. Leydig cell tumors of the testis. A clinicopathological analysis of 40 cases and review of the literature. Am J Surg Pathol. 1985 Mar;9(3):177–92.

2. Kaplan GW, Cromie WJ, Kelalis PP, Silber I, Tank ES. Gonadal stromal tumors: a report of the Prepubertal Testicular Tumor Registry. J Urol. 1986 Jul;136(1 Pt 2):300–2.

3. Dilworth JP, Farrow GM, Oesterling JE. Non-germ cell tumors of testis. Urology. 1991 May;37(5):399–417.

4. Heer R, Jackson MJ, El-Sherif A, Thomas DJ. Twenty-nine Leydig cell tumors: histological features, outcomes and implications for management. Int J Urol. 2010 Oct;17(10):886–9.

5. Bokemeyer C, Harstrick A, Gonnermann O, Schober C, Kuczyk M, Poliwoda H, et al. Metastatic leydig-cell tumors of the testis - report of 4 cases and review of the literature. Int J Oncol. 1993 Feb;2(2):241–4.

6. Oliva E, Young RH, Ulbright TM. Sex cord- stromal tumours. In Moch H, Humphrey PA, Ulbright TM, Reuter VE, eds. WHO classification of tumours of the urinary system and male genital organs. 4th ed. Lyon: IARC Press, 2016; 228.

7. Carvajal-Carmona LG, Alam NA, Pollard PJ, Jones AM, Barclay E, Wortham N, et al. Adult leydig cell tumors of the testis caused by germline fumarate hydratase mutations. J Clin Endocrinol Metab. 2006 Aug;91(8):3071–5.

8. Dieckmann KP, Loy V. Metachronous germ cell and Leydig cell tumors of the testis. Do testicular germ cell tumors and Leydig cell tumors share common etiologic factors? Cancer. 1993 Aug 15;72(4):1305–7.

9. Papadimitris C, Alevizaki M, Pantazopoulos D, Nakopoulou L, Athanassiades P, Dimopoulos MA. Cushing syndrome as the presenting feature of metastatic Leydig cell tumor of the testis. Urology. 2000 Jul 1;56(1):153.

10. Suardi N, Strada E, Colombo R, Freschi M, Salonia A, Lania C, et al. Leydig cell tumour of the testis: presentation, therapy, long-term follow-up and the role of organ-sparing surgery in a single-institution experience. BJU Int. 2009 Jan;103(2):197–200.

11. Grem JL, Robins HI, Wilson KS, Gilchrist K, Trump DL. Metastatic Leydig cell tumor of the testis. Report of three cases and review of the literature. Cancer. 1986 Nov 1;58(9):2116–9.

12. Hendry J, Fraser S, White J, Rajan P, Hendry DS. Retroperitoneal lymph node dissection (RPLND) for malignant phenotype Leydig cell tumours of the testis: a 10-year experience. Springerplus. 2015;4:20.

13. Di Tonno F, Tavolini IM, Belmonte P, Bertoldin R, Cossaro E, Curti P, et al. Lessons from 52 patients with leydig cell tumor of the testis: the GUONE (North-Eastern Uro-Oncological Group, Italy) experience. Urol Int. 2009;82(2):152–7.

14. Conkey DS, Howard GCW, Grigor KM, McLaren DB, Kerr GR. Testicular sex cord-stromal tumours: the Edinburgh experience 1988-2002, and a review of the literature. Clin Oncol (R Coll Radiol). 2005 Aug;17(5):322–7.

15. Bertram KA, Bratloff B, Hodges GF, Davidson H. Treatment of malignant Leydig cell tumor. Cancer. 1991 Nov 15;68(10):2324–9.

16. Gulbahce HE, Lindeland AT, Engel W, Lillemoe TJ. Metastatic Leydig cell tumor with sarcomatoid differentiation. Arch Pathol Lab Med. 1999 Nov;123(11):1104–7.

17. Naik R, Upadhyaya K, Pai MR, Baliga PB, Nayak SK. Fine needle aspiration of metastatic malignant Leydig cell tumor of testis: a case report. Indian J Pathol Microbiol. 2007 Jul;50(3):624–5.

18. Lam JS, Borczuk AC, Franklin JR. Metastatic Leydig cell tumor of the testicle in a young African American male. Can J Urol. 2003 Dec;10(6):2074–6.

19. Powari M, Kakkar N, Singh SK, Rai RS, Jogai S. Malignant Leydig cell tumour of the testis. Urol Int. 2002;68(1):63–5.

20. Soria JC, Durdux C, Chrétien Y, Sibony M, Damotte D, Housset M. Malignant Leydig cell tumor of the testis associated with Klinefelter's syndrome. Anticancer Res. 1999 Oct;19(5C):4491–4.

21. Guo CC. Neoplasms of the testis. In Zhou M, Magi- Galluzzi C, eds. Genitourinary Pathology: Foundations in diagnostic pathology. 2nd ed. Philadelphia, Elsevier,2015:649.

22. Emerson RE, Ulbright TM. Neoplasms of the testis. In Cheng L, MacLennan GT, Bostwick DG, eds. Urologic Surgical Pathology. 4th edition. Maryland Heights, MO: Mosby Elsevier, 2020; 802.

23. Acar C, Gurocak S, Sozen S. Current treatment of testicular sex cord-stromal tumors: critical review. Urology. 2009 Jun;73(6):1165–71.

24. Cost NG, Maroni P, Flaig TW. Metastatic relapse after initial clinical stage I testicular Leydig cell tumor. Oncology (Williston Park, NY). 2014 Mar;28(3):211, 214.

25. Nicolai N, Necchi A, Raggi D, Biasoni D, Catanzaro M, Piva L, et al. Clinical outcome in testicular sex cord stromal tumors: testis sparing vs. radical orchiectomy and management of advanced disease. Urology. 2015 Feb;85(2):402–6.

Conflict of interest and acknowledgement-None declared.

Table 1: Antibodies with their respective clones, dilution and antibodies.

Antibody	Clone	Dilution	Company
Cytokeratin	AE1/AE3	1:50	DAKO
TTF-1	8G7-G31	1:200	DAKO
oct3/4	NINK	1:50	DAKO
PLAP	8A9	1:25	DAKO
CD30	BER-H2	1:50	DAKO
LCA	2B11+P27/26	1:50	DAKO
Synaptophysin	SP11	RTU	VENTANA
Chromogranin	LK2H10	RTU	VENTANA
Alpha-inhibin	(R1)	RTU	Pathnsitu
Calretinin	DAK-calret 1	1:100	DAKO
CD56	123-C3	1:50	DAKO
Melan-A	A103	1:50	DAKO
Androgen receptor	SP107	RTU	CELL-MARQUE

TTF-1- Thyroid transcription factor -1, PLAP- Placental alkaline phosphatase, LCA- Leucocyte common antigen, RTU- Ready to use.

Table 2: Clinicopathological features, biopsy site & duration of survival after biopsy diagnosis of 5 cases.

Case no:	Age	Testicular involvement	Serum testosterone	Serum androstenedione	Serum estradiol	Biopsy site	Duration of survival after biopsy diagnosis.
1	51y	Right Testicular mass	Normal	Normal	Normal	Left supraclavicular lymph node	2 years
2	59y	Bilateral testicular masses	Mildly increased	Mildly increased	Normal	Bilateral lungs	4 months
3	48y	Left testicular mass	Significantly increased	Mildly increased	Mildly increased	Left lung	7 months
4	54y	Right testicular mass	Normal	Normal	Normal	Right lung	10 months
5	57y	Right testicular mass	Normal	Normal	Normal	Right lung	9 months

Table 3: Immunohistochemical results of 5 cases

Antibody	Immunohistochemical results				
	Case 1	Case 2	Case 3	Case 4	Case 5
Cytokeratin	-	focal +	-	-	-
TTF-1	-	-	-	-	-
Oct3/4	-	-	-	-	-
PLAP	-	-	-	-	-
CD30	-	-	-	-	-
LCA	-	-	-	-	-
Synaptophysin	-	-	-	-	-
Chromogranin	-	-	-	-	-
Alpha-inhibin	++/+++	+++	+++	+++	+++
Calretinin	-	-	-	-	-
CD56	++	++	-	-	-
Melan-A	-	+/++	+/++	+	+
Androgen receptor	+	+/++	+	+	+

+++: strongly positive; ++: moderately positive; +: weakly positive; -: negative

Table 4: Previous studies and case reports on metastatic testicular LCT

Study/ case report	Authors	No. of cases that developed metastasis out of total no. of testicular LCT cases (for studies)	Site of metastasis
Study	Kim I et al [1]	5/30	flat bones, para-aortic lymph nodes, pleura, peritoneum, liver, pre-sacral and inguinal region, lung, around left kidney, retroperitoneum & retroperitoneal lymph nodes
Study	Hendry et al [12]	2/5	retroperitoneal lymph nodes
Study	Di Tonno F et al [13]	1/52	supraclavicular lymph node
Study	Conkey DS et al [14]	2/16	para-aortic & mediastinal lymph nodes
Case report	Bertram KA et al [15]	-	retroperitoneum, liver, ribs, spine & mesentery
Case report	*Papadimitris C et al [9]	-	retroperitoneal lymph nodes
Case report	** Gulbahce HE et al [16]	-	lung & perirenal adipose tissue without involving renal parenchyma
Case report	Naik R et al [17]	-	right inguinal region
Case report	Lam JS et al [18]	-	lung
Case report	Powari M et al [19]	-	lung and liver
Case report	+Soria JC et al [20]	-	lung & iliac lymph nodes

*Incidentally, this patient also had Cushing syndrome due to ectopic cortisol production by the tumour, resulting in hypokalemia.

** Tumour in perirenal adipose tissue also showed sarcomatoid features.

+ This patient also had Klinefelter's syndrome.

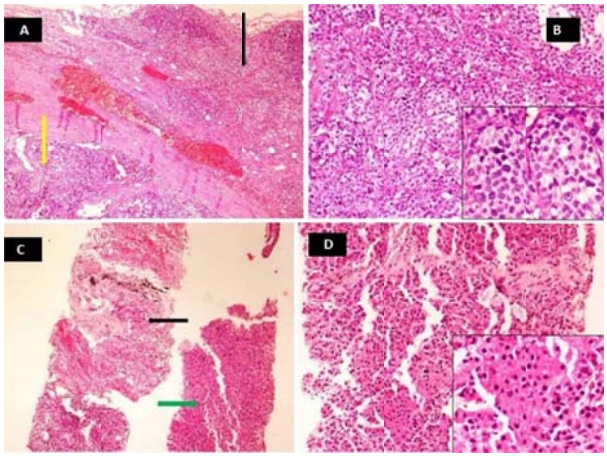

Figure 1. H&E stain: A) Lymph node (black arrow) and adjacent metastatic tumor (yellow arrow) from case 1, 4x magnification; B) Tumor at 10x magnification and inset in B shows tumor at 40x magnification from case 1; C) Metastatic tumor in the lung (normal lung parenchyma-black arrow and metastatic tumor–green arrow) from case 2, 4x magnification; D) Tumor at 10x magnification and inset in D shows tumor at 40x magnification from case 2.

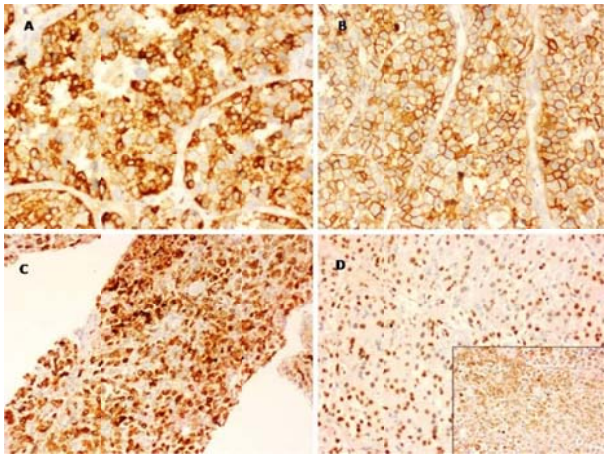

Figure 2. Immunohistochemistry: A) Tumor cells positive for alpha-inhibin in case 1, 20x magnification; B) Tumor cells positive for CD56 in case 1, 20x magnification; C) Tumor cells positive for alpha-inhibin in case 2, 10x magnification; D) Tumor cells positive for AR in case 2, 20x magnification, inset in D shows tumor cells positive for melan-A in case 2, 20x magnification.