



CASE REPORT

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Adult type granulosa cell tumor of the testis with a heterologous sarcomatous component: case report and review of the literature

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Abstract

Adult testicular granulosa cell tumors are rare sex cord-stromal tumors of which only 45 have been previously reported. As compared with their ovarian counterparts, these tumors may follow a more aggressive course because the proportion of malignant cases is higher. We report here a unique case of a 78-year Caucasian with a left sided adult type granulosa cell tumor with a heterologous sarcomatous tumor component. A heterologous sarcomatous component has occasionally been observed in ovarian tumors but never in testicular granulosa cell tumors. The sarcomatous component showed a higher number of mitotic figures (1/Hpf) and a marked proliferation rate (up to 50% Ki 67 positive cells) compared with the granulosa type tumor component. CD 99 and the progesterone receptor were positive in both tumor components, inhibin and calretinin only in the granulosa cells, and pancytokeratin only in the sarcomatous one. Key words: testis - ovary - granulosa cells - sarcoma - inhibin Running title: testicular sarcomatous granulosa tumor.

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Background

Tumors of sex cord/gonadal stroma are exceedingly rare tumors that, in large series, account for 1.6–6% of adult testicular tumors and occur somewhat more frequently in children [1]. Teilmann [2] used the term gonadal stroma tumor as a histogenetic and morphologic designation for tumors of both genders which derive from an undifferentiated gonadal mesenchyme. They arise in the testis as well as in the ovary. However, the incidence of the single histological types differs widely between genders. Leydig cell tumor is the most common stromal tumor of the testis, followed by Sertoli cell tumors. Whereas granulosa cell tumors are typical for the ovaries.

The adult type of granulosa cell tumor is extremely rare in the testis – 46 cases have been described to date (Table 1) [3-31]. However, in most of the reports these tumors are only the object of immunohistochemical or cytogenetical investigations; thus, the clinical data are utterly or partially missing [12,15,17,31]. In contrast to the adult type, the

juvenile type of granulosa cell tumor is the most frequent testicular tumor in boys younger than six months.

We observed an adult type granulosa cell tumor with a heterologous sarcomatous tumor component, a morphological feature rarely observed in ovarian granulosa cell tumors [32-34], and only in one testicular Leydig-Sertoli cell tumor to date [35].

Case report

A 78-year old Caucasian male was admitted to hospital due to acute neurological symptoms. Upon exploration, he reported a history of hypertension and arrhythmia. Upon physical examination, an enlarged left testicle was noted and an inguinal herniotomy scar on the left side. According to statements by the patient, the testicle had started to enlarge a year earlier. After neurological symptoms had remitted, the patient was transferred to the urological unit for further diagnostic procedures. A painless and solid scrotal mass could be palpated on the left side. The left epididymis was not discernible at palpation. The right testicle and epididymis were unremarkable. Upon ultrasonography, the mass had a diameter of 13 cm and

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Table 1 A synopsis of the morphological and clinical data of the up to now reported cases of the adult type granulosa cell tumor originated in the testis

Case	Age	Testis	Signs duration	Endocrine symptoms	Size cm	Follow up	Source
1	35	R	15 y	gynecomastia	9	8.5 y NED	Laskowski [3]
2	21	L	incidental	gynecomastia	microscopic	autopsy finding	Cohen and Diamond [4]
3	53	R	1 y	gynecomastia	>10	NS	Massachusetts General Hospital [5]
4	52	R	5 y	none	13	NS	Melicow [6]
5	41	L	8 y	gynecomastia	10,1	5 mo DOD	Mostofi et al. [7]
6	53	R	2 y	gynecomastia	10	17 y NED	Marshall et al. [8]
7	44	R	few months	none	3,5	3 y NED	Talermann, 1985
8	41	R	NS	none	1.8	NS	Gaylis et al. [11]
9	83	L	NS	none	NS	DOC	Düe et al. [12]
10	61	R	2 mo	none	5	2 y NED	Nistal et al. [13]
11	26	L	7 mo	gynecomastia	10	14 y NED	Matoska et al. [14]
12	NS	NS	NS	NS	NS	NS	Sasano et al. [15]
13	42	L	NS	none	NS	AWD at surgery	Monobe, Manabe [16]
14	57	R	10 y	none	2,5	3 y DOC	Jimenez-Quintero et al. [17]
15	55	L	not known	none	1,3	NS	Jimenez-Quintero et al. [17]
16	60	L	many years	none	7	11 y 2 mo DOD	Jimenez-Quintero et al. [17]
17	39	L	2 y	none	4	3 y NED	Jimenez-Quintero et al. [17]
18	16	L	incidental	none	1,8	4 mo NED	Jimenez-Quintero et al. 1993
19	29	R	incidental	none	7,5	14 mo AWD	Jimenez-Quintero et al. [17]
20	76	L	incidental	none	0,7	1 mo NED	Jimenez-Quintero et al. [17]
21	NS	NS	NS	NS	NS	NS	Renshaw et al. [18]
22	NS	NS	NS	NS	NS	NS	Renshaw et al. [18]
23	NS	NS	NS	NS	NS	NS	Renshaw et al. [18]
24	NS	NS	NS	NS	NS	NS	Renshaw et al. [18]
25	NS	NS	NS	NS	NS	NS	Renshaw et al. [18]
26	NS	NS	NS	NS	NS	NS	Renshaw et al. [18]
27	51	L	2 mo	incidental	7	13 mo NED	Morgan,Brame [19]
28	48	R	3 y	none	5	7 mo NED	Al-Bozom et al. [20]
29	54	L	incidental	none	NS	NS	Wang et al. [21]
30	33	NS	incidental	none	1	NS	Guzzo et al. [22]
31	51	L	incidental	none	NS	6 y AWD	Suppiah et al. [23]
32	59	L	2 y	none	15	4 y NED	Hisano et al. [24]
33	32	L	incidental	none	1,98	NS	Arzola et al. [25]
34	77	L	incidental	none	4	NS	Lopez, [26]
35	45	R	months	none	6,5	2 y NED	Ditonno et al. [27]
36	12	L	5 y	none	10	NS	Gupta et al. [28]
37	55	NS	lung metastases	none			Hammerich et al. [29]
38	28	L	incidental	none	2,6	NS	Song et al. [30]
39	21	L	incidental	none	1	2 y NED	Hanson, Ambaye [31]
40	77	R	NS	NS	2,5	NS	Lima et al. [36]
41	22	L	NS	NS	1	NS	Lima et al. [36]
42	40	L	NS	NS	2,1	NS	Lima et al. [36]
43	78	L	incidental	none	13	23 mo NOD	recent case

contained cystic spaces surrounded by solid tumor structures (Figure 1). There were no enlarged inguinal or abdominal lymph nodes. Upon computed tomography of the chest and abdomen, a cyst measuring 2.5 cm was found in the right kidney, but masses raising suspicion of metastases were not detected. As a consequence of the clinical findings, inguinal semicastration of the left testis was performed. Twenty three months following surgery, the patient is alive and without signs of progressive disease.

Macroscopically the tumor was composed of solid white or brownish firm masses with multiple cystic spaces.

Microscopically the tumor consisted of rather monomorphous typical granulosa cells with scant cytoplasm and angulated, coffee-bean like, grooved nuclei (Figure 2). The cells grew mainly in solid patterns with some trabecular and gyriform areas and a few cysts lined by tumor cells containing an eosinophilic fluid (Figure 3). Some not well-differentiated microfollicular structures resembling Call-Exner bodies could be found interspersed (Figure 4). Mitotic figures were extremely rare. The tumor showed central necrotic areas. The main part of the tumor was surrounded by fibrotic tissue in which small nests of undifferentiated, spindled cells ordered in sheets were located. These cells did not exhibit any polymorphism and mitotic figures were lacking. At



Figure 1 Ultrasound view of a partly cystic tumor.

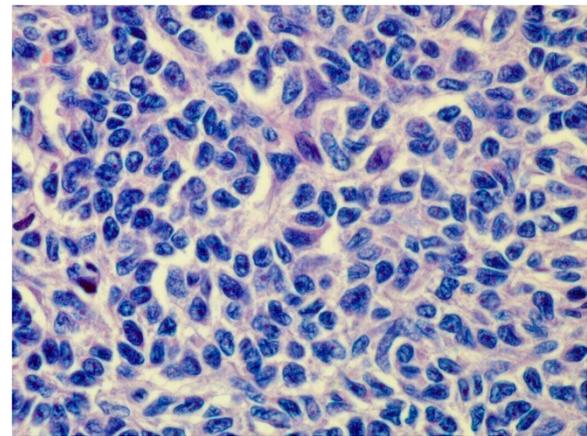


Figure 2 Tumor forms nest and cord composed of small cells with scant cytoplasm and typical grooved, coffee bean-like nuclei, resembling its ovarian counterpart. (H&E, orig.magni.x40).

the border of a hyalinized area a proliferation of large, polygonal and spindled cells with many tumor giant cells were present (Figures 5 and 6). The cells had a clear or slightly eosinophilic cytoplasm and markedly polymorphic nuclei with large nucleoli. Almost all mitotic figures (1/hpf) were atypical. Accordingly, the proliferation rate of these sarcomatous cells, assessed by MIB-1 antibodies, was consistently higher (focally > 50%) than that of the typical granulosa cells (<1%). Very large, spindled sarcomatous cells were also found scattered among fibrous collagen rich tissue. The sarcomatous area was infiltrated by a startling number of eosinophilic leucocytes.

Immunohistochemically, single typical granulosa cells showed a weak reactivity for inhibin and a strong but patchy distributed reactivity for calretinin. Vimentin and CD 99 (Figure 7) as well as progesterone receptor (PR) were strongly expressed in the typical granulosa

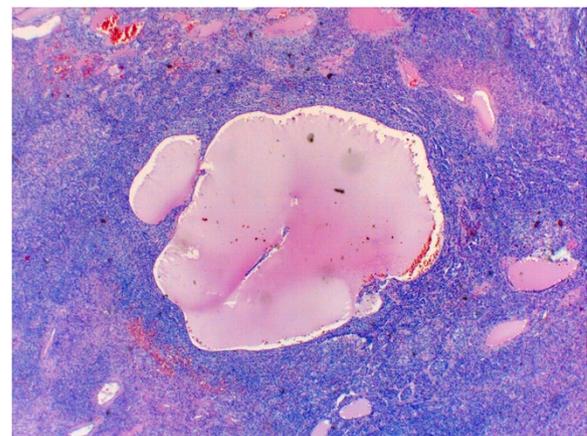


Figure 3 Granulosa cell tumor with cysts resembling the ovarian follicle cysts (H&E, orig.magni.x4).

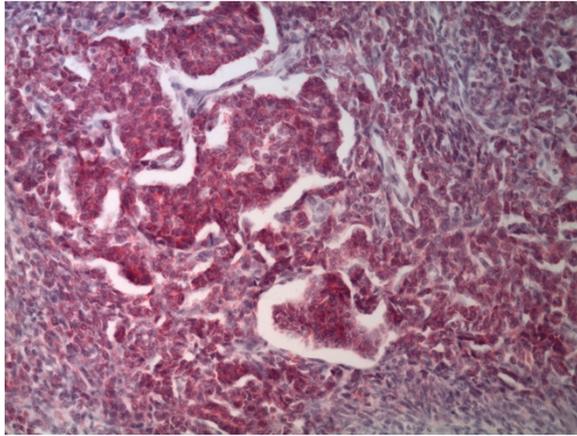


Figure 4 Higher magnification of the tumor shows cords and follicular nesting of cells similar to those observed in granulosa cell tumor of the ovary. The tumor cells are inhibin positive (Inhibin immunohistochemistry, orig.magni.x20).

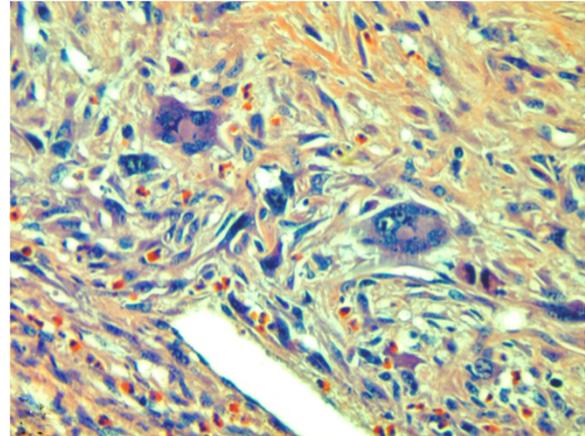


Figure 6 Sarcomatous area with bizarre multinucleated giant cells embedded in a collagenous stroma and a marked infiltration with eosinophils (H&E, orig.magni.x40).

cells (>70%) as well in the sarcomatous one, whereas androgen- (AR) and estrogen- (ER) receptor antibodies did not show any reactivity. Interestingly, the sarcomatous tumor cells reacted strongly with pancytokeratin antibodies (AE1:AE3) (Figure 5); by way of contrast, the typical granulosa cells were completely unreactive.

Since the FOXL2 402C → G (C134W) mutation is also present in adult-type granulosa cell tumors occurring in men, although in a smaller proportion when compared with the rates reported in women [36], we also performed this mutation analysis. To this end, DNA of the tumor cells was extracted from serial sections (FFPE tissue) after manual microdissection using the Maxwell 16 System (Promega GmbH, Mannheim, Germany) in accordance with manufacturer's instructions.

Mutation hotspot region containing codon 134 was amplified by PCR (primer sense: 5'-CAACTACTGGACGCTGGACC-3'; primer antisense: 5'-GTTGCCCTTCTCGAACATGTCT-3'). Sequencing analysis was performed using the PyroMark Q24 system (Qiagen, Hilden, Germany) in accordance with manufacturer's instructions (sequencing primer: 5'-CGCTGGACCCGGCCT-3'). Detailed information about PCR and sequencing conditions is available on request. A FOXL2p.C134W mutation was nonetheless not detectable.

Discussion

Adult type testicular granulosa cell tumors (GCT) are rare sex cord-stromal tumors. However, subsequent to a thorough study of the literature we were able to find

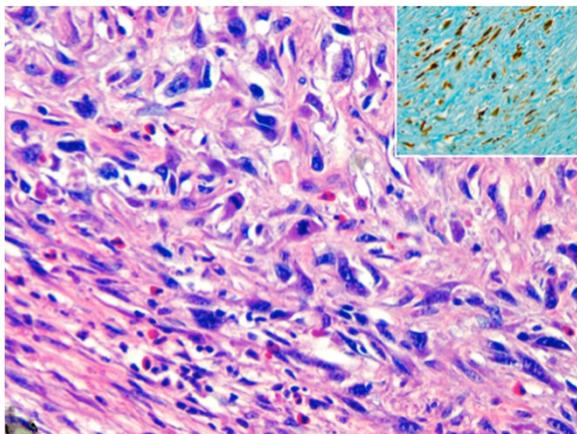


Figure 5 Sarcomatous area with spindled and stellate cells with atypical mitoses. The insert shows pancytokeratin (AE1/AE3) positive spindle cells of the sarcomatous area. (H&E and immunohistochemistry, orig.magni.x40).

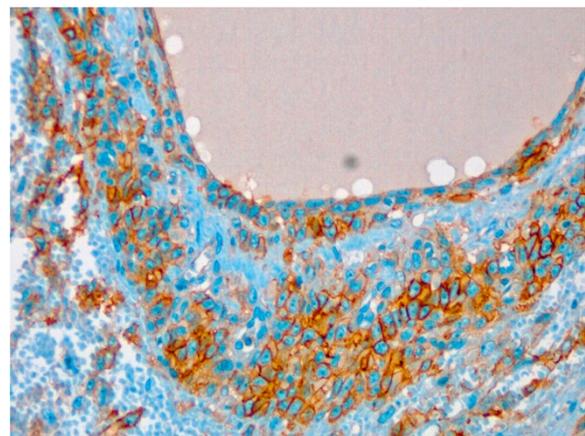


Figure 7 A follicular structure with surrounding granulosa cells strongly positive for CD 99. (CD 99 immunohistochemistry, orig.magni.x20) orig.magni.x20).

17 cases above and beyond those referred to in one of the recent literature reviews (28 cases) [31]. Fifteen out of the 45 (33%) reported cases were published after the year 2000, which does not imply that incidence actually increased, but rather, that the tumor is more frequently being recognized.

Morphological diagnosis is based primarily on the typical morphology of the granulosa cells with their coffee-bean like, angulated and grooved nuclei. Macrofollicles ought to be present, the presence of the Call Exner bodies makes correct diagnosis easier; however, they are not always found and thus, are not indispensable to diagnosis [12]. Young and Scully [37] require that “all or almost all” of the tumor should be composed of granulosa cells. In fact, undifferentiated Sertoli cell tumors can have a very similar cytomorphological features but form predominantly ill-defined tubular structures resembling primitive testicular tubules and do not have follicular structures. If the morphological features are ambiguous, the term “incompletely differentiated” (WHO) is preferable. The very rare fibrothecoma of the testis can be also mistaken for undifferentiated sex cord tumor since it manifests the same immunoreactivity as all other stromal tumors. This tumor, however, is composed of highly monotone spindle cells which are embedded in an acellular fibrous stroma [38].

To the best of our knowledge, this is the first case of GCT with heterologous sarcomatous changes observed in the testis. The sarcomatous cells did not show any differentiation, they were negative for desmin and SMA. As in many soft tissue sarcomas, the cells were also positive for pancytokeratin antibodies (AE1:AE3) in our case. With the exception of one case which showed “some evidence of rhabdomyosarcomatous differentiation [32]”, the sarcoma cells in all other cases described in the ovaries did not show any special differentiation [33-35].

One could argue that the observed sarcomatous component originates from a concomitant germ cell tumor growing simultaneously with the granulosa tumor. Quite apart from the fact that the tumor has been entirely processed and no germ cell tumor remnant could be found, a smooth transition from the differentiated to the sarcomatous component could be observed. Moreover, the simultaneous occurrence of germ cell and stromal tumors in the unilateral testis is extremely rare without any common genetic or oncogenetic background [1]. In contrast to the germ cells, the cells of the gonadal stroma tumors are differentiated and not pluripotent; can therefore not give rise to somatic type malignancies (carcinomas, differentiated sarcomas) as the cells of teratomas do. The somatic type malignancies arising in teratomas are well differentiated, completely identical to the tumors originally developing in other organs e.g. soft and nervous tissue, intestine etc. [39], whereas the sarcomas described to date in stromal tumors [32-35] were all composed of undifferentiated

spindle cells. It can be assumed that these tumors develop from undifferentiated gonadal stroma.

A molecular characterization of sarcomatous changes in ovarian GCT showed a down-regulation of the GCT-specific genes such as inhibin, estrogen receptor and FSH receptor [35]. A significant up-regulation of genes with an inflammatory response was consistent with the presence “of a marked inflammatory infiltrate”, which was a striking morphological feature in our case as well. However, contrary to these findings of McNeilage et al. [34], the PR receptor was not down-regulated in our case, but immunohistochemically strongly expressed.

Some authors claim that testicular GCT can be mistaken for lymphoma, metastatic carcinoma or even for melanoma [12,35,36], but lymphoma and metastatic carcinoma cells tend to spread in the interstitium between testicular tubules and usually do not form solid tumor masses [1].

Immunohistochemistry is obviously helpful for achieving a correct diagnosis. One must keep in mind, however, that with few exceptions all types of sex cord/gonadal stroma tumor cells show reactivity for the same antibodies. By and large, but not in all cases, the tumor cells of GCT are positive for inhibin, calretinin, vimentin and CD 99 [20,31]. According to Renshaw et al. [18] SMA and S-100 are constantly expressed in all testicular sex cord/gonadal stroma tumors, but other authors cannot confirm these findings [20,30]. CD 99 is consistently expressed, whereas EMA has never been detected in the different types of sex cord/gonadal stroma tumors of the testis; these antibodies are thus useful for the differential diagnosis stromal tumor vs. carcinoma metastasis [20,31,40]. Pancytokeratin and desmin are often but not constantly detected [12,20,31]. Also very useful to the diagnosis of these tumors is the detection of estrogen (ER) and progesterone receptors (PR), and in this connection PR are detected more often than ER (75% vs. 50%) [12]. PlAP immunohistochemistry is obviously the best method to distinguish the stromal from the germ cell tumors of the testes. The PR positivity in the sarcomatous part of the tumor presented here is the only evidence that these cells derive from the granulosa cells and not from the supporting stroma.

A more difficult task is the correct classification of poorly differentiated sex cord/stroma tumors, since the granulosa and Sertoli cells can have a similar appearance and can also express all of the immunohistochemical markers discussed above. Almost all Leydig and granulosa-cell tumors, but only about half of Sertoli-cell tumors, react with inhibin and calretinin antibodies [1]. A valuable marker could be the melan-A/MART-1 (A 103) melanoma antibody, which is positive in all Leydig and in many Sertoli-cell tumors, but negative in GCTs [41]. CD 56 is constantly expressed in GCT, but has never been tested in the diagnostics of

the testicular GCTs. CD56 is a regulator of growth and differentiation in ovarian folliculogenesis [42].

One of the major problems of histological diagnosis of testicular sex cord/stroma tumors is the prediction of malignancy. Atypical mitotic figures occur more frequently and the mitotic index is significantly higher in malignant tumors; vascular invasion, infiltrative margins, and necrosis are also associated with malignancy, but are not very reliable [1] when used as a single criterion. The only reliable predictor of malignancy in GCT as in all other stromal tumors is a tumor diameter > 5 cm [1,17,31].

Due to the few cases reported, our knowledge of the biological behavior and the clinical features of this tumor is still fragmentary. The average age of the diseased men is 45 years (Range 12 – 83 years) with an accumulation (45%) of cases in the 5th and 6th decade (Table 1). In 65.5% of cases (21/32) the tumors were smaller than 2 cm (1.7 cm in median, range: 0.7 – 15 cm). Endocrine symptoms (gynecomastia, loss of libido and potency) were observed only in six cases, 26 cases did not have such symptoms and in the other cases this clinical information is missing (for references, see Table 1). In the known cases the duration of clinical signs ranged from a few months to 10 years! Nineteen case reports included a follow up: 2 patients died of disease, one of them 11 years after diagnosis [7,17], 3 patients died of unrelated diseases [12,20], 3 patients were alive with disease, one of them 6 years after surgery [16,17,23]. The longest follow-up of one of the 13 patients who were alive without evidence of disease was 14 years [14].

Even this small series of cases shows that testicular adult type GCTs behave in an aggressive manner in one quarter of cases. The clinical problem is that there is no specific therapy for these tumors. Various chemotherapeutic agents and regimens have been used for patients with metastatic disease with limited or no success. The “prophylactic” retroperitoneal lymphadenectomy is an option which is very successful in stage I diseases, but not in the higher stages when the lymph nodes are already affected [43].

In summary, we present a unique case of a testicular granulosa cell tumor of adult type with a sarcomatous component whose impact on the clinical course is unknown.

Conclusion

The correct classification of poorly differentiated sex cord/stroma tumors can be difficult. Adult type testicular granulosa cell tumors (GCT) are rare sex cord–stromal tumors challenging the diagnostic skills of surgical pathologists. Rarely, these tumors can display a sarcomatous component as described in the reported case.

Consent

Written informed consent was obtained from the patient for publication of this case report.

Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

GM and TS carried out histological and immunohistochemical analysis of the tumor. GM wrote the pathological part of the manuscript and TS helped to draft the manuscript. RS and AH performed molecular analysis of tumor tissue and took part in drafting the molecular part of the manuscript. ML and SS carried out clinical diagnostics and wrote the clinical part of the manuscript. All authors read and approved the final manuscript.

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