CASE REPORT

Classical testicular seminoma in young man

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Abstract

A 29 year old man was admitted in Urology Department of County Hospital of Constanta for left testicular increased size in last six months, without other symptoms. Testicular tumor was palpated and visualized by ultrasound. Computed tomography did not revealed metastatic lymph nodes. Beta-human chorionic gonadotropin and alpha-fetoprotein were within the normal range. We diagnosed the case as a left testicular tumor and performed high orchiectomy. Histological examination revealed typical seminoma. Subsequently, the patient was given two courses of systemic chemotherapy (bleomycin, etoposide, cisplatin) as an adjuvant therapy. The patient has remained free of disease six months after discharge.

Key words: typical seminoma, placental alkaline phosphatase (PLAP), immunohistochemistry.

⌑ Introduction

Testicular germ cell tumors are the most common malignancy in males between 15 and 34 years of age, and represent a major cause of death attributable to cancer in this age group [1, 2].

Every year, approximately 7400 new cases of testicular tumors are diagnosed in the U.S.A. The incidence of this type of cancer has increased progressively throughout the twentieth century [3–5].

Ninety percent of testicular tumors arise from germ cells. It is generally accepted that testicular germ cell tumors (with the exception of spermatocytic seminomas) originate from a common precursor lesion known as intratubular germ cell neoplasia [6–8].

Germ cell tumors can be subdivided into seminoma and non-seminomatous germ cell tumor (NSGCTs), which consist of embryonal cell carcinoma, choriocarcinoma, yolk sac tumor, and teratoma. Neoplasms that contain more than one tumor cell components, *e.g.* seminoma and embryonal cell carcinoma, are referred to as mixed germ cell tumors.

Seminoma and NSGCTs not only present with distinctive clinical features, they also differ with respect to therapy and prognosis.

Neoplastic disease progression, from normal to premalignant to malignant phenotypes, is associated with genetic instability manifested by alteration of gene expression that is often associated with characteristic morphologic phenotypes [9].

It is generally well accepted that most but not all testicular germ cell tumors arise from a common neoplastic precursor lesion, intratubular germ cell neoplasia. According to this model seminomas evolve directly from intratubular germ cell neoplasia and embryonal carcinomas could arise directly from intratubular germ cell neoplasia or through an intermediate stage corresponding to seminoma.

The other types of testicular germ cell tumors, teratomas, yolk sac tumors, and choriocarcinomas, may evolve directly from seminoma or embryonal carcinoma [8, 10–12].

⌑ Patient and methods

A 29-year-old man was admitted in Urology Department of County Hospital of Constanţa for left testicular increased size in last 6 months, without other symptoms. Clinical examination revealed swollen left hemiscrotum and enlarged non-tender testicular mass with movability on superficial and deep grounds, without local inflammation signs. No inguinal or other lymphadenopathy was palpated. Serum β-HCG and α-pheto-protein level were within normal limits: 0.75 ng/ml (β-HCG normal values: 0–1 ng/ml), respectively 14 ng/ml (α-pheto-protein normal values: 1–16 ng/ml). Additional blood investigations were not performed. Ultrasonographic examination of the left testis showed a mass (8×6.5 cm in diameters) with a homogeneous structure, surrounded by a thin liquidian film. Computed tomography did not reveal a possible deep adenopathy (Figure 1).

The patient underwent a left orchidectomy.

Pathological findings

The left orchidectomy specimen weighed 27 g, comprising a 7×5×5 cm testis with attached 3.5×1.1 cm spermatic cord.
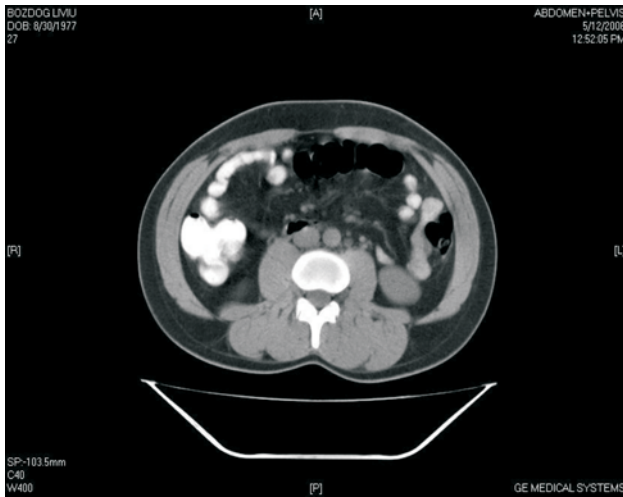
Figure 1 – *CT scan lower lombar section not detect metastases on this level*

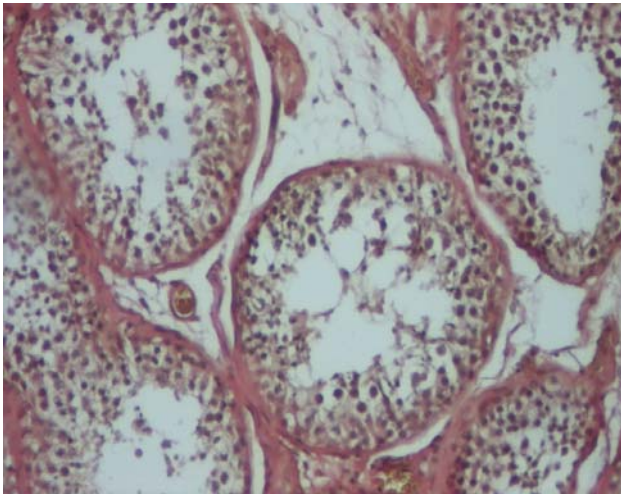**Figure 2 – *Gross appearance of testicular seminoma; solid unencapsulated tumor mass with lobular aspect on cut surface***

Figure 3 – *The presence in tumor periphery by the normal seminiferous tubules; note lower interstitial edema (×200, van Gieson stain)*
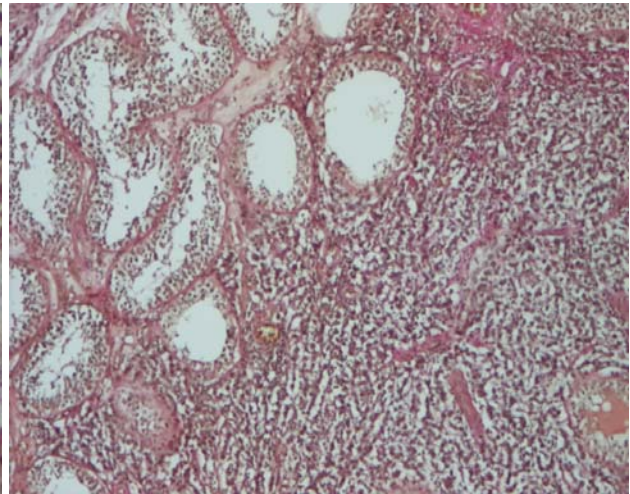

Figure 4 – *The microscopic aspect of classical testicular seminoma (on the right side); note the absence of conjunctive capsule; on the left side, normal seminiferous tubules (×40, van Gieson stain)*
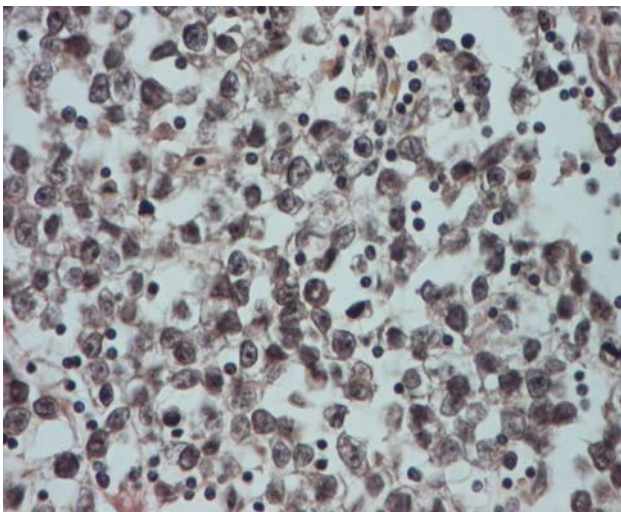

Figure 5 – *Relatively uniform malignant germ cells with large nuclei and prominent nucleoli; note a fewer mitosis (×400, van Gieson stain)*

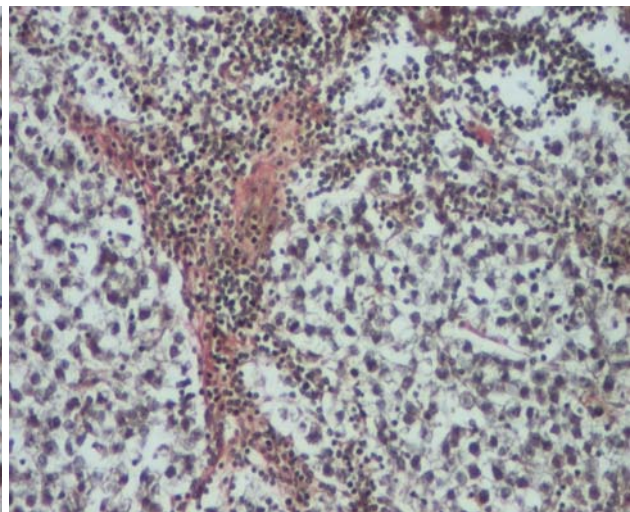**Figure 6 – *The tumor cells are arranged in nests outline by fibrous bands infiltrated by lymphocytes (×200, van Gieson stain)***

The testis was smooth surfaced with a tan, mostly homogeneous cut appearance. The testis contained a 6×5.5×4.5 cm, white/yellow, hard, nodular tumor with only a thin rim of testicular tissue. No other discrete nodules were observed macroscopically (Figure 2).

No gross involvement of the tunica albuginea or adnexa was evident. Sampling comprised a total of 15 tissue blocks.

Histological evaluation

Light microscopy revealed partially preserved testicular architecture with lower interstitial edema and patchy chronic inflammatory cell infiltrates comprising lymphocytes, plasma cells, and aggregates of histiocytes (Figure 3).

Sections from the yellowish brown area reveal a malignant germ cell proliferation. The tumoral cells are large, relatively uniform, round, with sharply outlined boundaries. The tumor edges invade the surrounding tissue, without a circumscription conjunctive capsule (Figure 4).

The tumor cells have an abundant clear cytoplasm due to amounts of glycogen. The nuclei are large, centrally located and have a clumped chromatin pattern with prominent centered nucleoli and irregular contours (Figure 5).

Atypical mitosis is few. The tumor cells are typically arranged in nests outline by fibrous bands; these bands are infiltrated by lymphocytes (Figure 6).

The final pathological diagnosis was classical testicular seminoma.

Immunohistochemistry

Placental alkaline phosphatase (PLAP) immunostaining was performed using a mouse monoclonal antibody (clone 8B6, DAKO; 1 : 50 dilution). The slides were deparaffinized and subjected to heat-induced epitope retrieval using a steamer and DAKO Target retrieval solution (pH 6.0–6.2, DAKO). The slides were steamed for 20 minutes, cooled at room temperature for 20 minutes, rinsed with deionized water, and then placed in Tris-buffered saline for 5 minutes.

Endogenous peroxidase was blocked by placing the slides in 3% hydrogen peroxide for 10 minutes. Immunohistochemical staining was performed manually according to the protocol in the manufacturer's guide. A standard streptavidin–biotin complex technique with diaminobenzidine as chromogen was used. The sections were counterstained with Mayer Hematoxylin, dehydrated, cleared in xylene, and mounted. The normal testicular tissues adjacent to the tumor areas served as an internal control. Negative controls were obtained by staining protocols omitting the first antibody, or by using non-immune mouse sera in place of the first antibody.

Expression of PLAP was classified as follows: absent/weak (cytoplasmic staining less intense than in adjacent normal germ cells) and strong (staining of similar intensity to that of normal germ cells). The extent of positive staining was divided arbitrary into the following categories: focal (<10% positive tumor cells), moderate (10–50% positive tumor cells), and diffuse (>50% positive tumor cells).

Immunohistochemistry for PLAP (*Placental Alkaline Phosphatase*) showed a positive reaction on cell membranes (++ in >50% tumoral cells) (Figure 7).
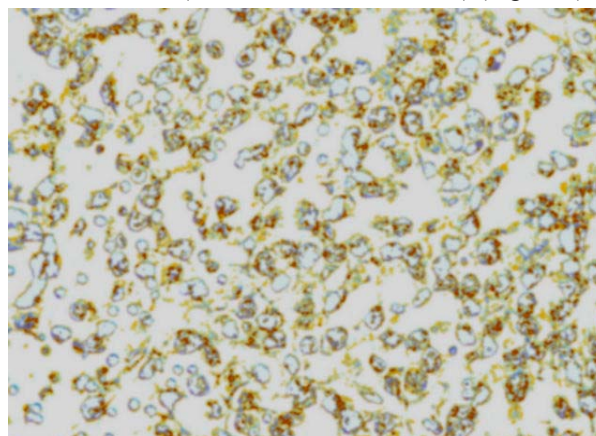

Figure 7 – *PLAP-positive cells showed a brown membrane signal confirmed seminoma diagnosis (×400)*

⚏ Discussions

The classic pattern of germinoma is distinctive and readily recognized based on its overall sheet-like arrangement of clear cells with well-defined cytoplasmic borders (in well-fixed specimens) and flattened, "squared-off" nuclear membranes that is subdivided into variably sized, smaller groups of cells (alveolar aggregates, nests, clusters) by lymphocyte-bearing, fibrovascular septa. There are, however, some unusual patterns that are prone to misinterpretation. Occasional germinomas have a microcystic or cribriform arrangement that may suggest yolk sac tumor [9, 13].

In our experience, this finding is more common in seminoma than dysgerminoma. This change may, in part, be secondary to edema because some examples of this phenomenon have faintly eosinophilic fluid within the cystic spaces, but in other examples this feature is lacking. Some cases have few lymphocytes, further complicating the interpretation since a lymphocytic infiltrate is a usual feature of typical germinoma, occurring in almost all of them, [14] and its presence is a helpful criterion for the diagnosis of germinoma, whereas yolk sac tumor generally lacks lymphocytes.

Helpful differential features include that the cystic spaces in these unusual cases of germinoma frequently contain exfoliated tumor cells or inflammatory cells and are lined by polygonal tumor cells rather than the flattened lining cells with compressed nuclei of microcystic yolk sac tumor.

The key differential findings, however, are retention of the usual cytological features of germinoma cells and absence of other patterns that would be expected in yolk sac tumor. Immunostains for cytokeratin (AE1/AE3), alpha-fetoprotein (AFP), and OCT3/4 [15] are helpful, typically staining negatively (AE1/AE3 and AFP) and positively (OCT3/4) in germinoma and showing opposite reactivities in yolk sac tumor.

Undifferentiated germ cells can be detected by their positive immunoreactivity to placental alkaline phosphatase (PLAP). This marker is normally expressed in primordial germ cells during embryogenesis and in gonocytes, *i.e.*, the fetal germ cell [16–18]. PLAP is known to be expressed in testicular tissue only in germ cell tumors or in CIS cells in children and adults [19]. The postnatal presence of PLAP-immunopositive germ cells that are similar in appearance to gonocytes is considered pathologic [18, 20].

Occasional germinomas have a solid tubular pattern characterized by elongated nests with a somewhat palisaded arrangement of tumor cells at their periphery [21, 22]. This may cause confusion with Sertoli cell tumor, but adjacent intratubular germ cell neoplasia of the unclassified type (IGCNU) may be helpful, if present, and more reliably the nuclei have the typical appearance of germinoma nuclei. When the light microscopic features are ambiguous, immunostains for placental alkaline phosphatase and OCT3/4 (positive in germinoma and negative in Sertoli cell tumor) and inhibin (negative in germinoma and often positive in Sertoli cell tumor) [23–25] are helpful.

Focal intertubular growth is common in seminoma, and rarely characterizes most of, or the entire, tumor. Seminomas with purely intertubular growth may not produce a clinical mass, and the tumor is often heralded by metastases or found during the investigation of infertility. The neoplastic infiltrate in these cases may be deceptively subtle and potentially overlooked at scanning magnification. The seminiferous tubules remain intact with, in some cases, expansion of the space between them if the tumor cells are sufficiently numerous. In some cases, however, the seminoma cells subtly infiltrate the interstitium as individual cells or small clusters without appreciable intertubular expansion. They may be admixed with clusters of Leydig cells, which may further mask their presence. Clues to this process include the common associated lymphocytic infiltrate in the interstitium (although this may be misinterpreted as a manifestation of viral orchitis), the usual presence of IGCNU and the frequently associated testicular atrophy and Leydig cell hyperplasia [26]. Because lymphomas and metastases may show prominent intertubular growth, it is important to identify the cytological features of seminoma cells in these cases to rule out other neoplastic infiltrates. Appropriate immunostains can easily resolve the differential if necessary.

Germinomas may have areas with cells that have denser, sometimes eosinophilic or amphophilic cytoplasm and increased nuclear crowding and irregularity; this appearance must be distinguished from embryonal carcinoma. Frequently, these findings relate to suboptimal fixation of germinoma, resulting in poorly defined cell membranes, cytoplasmic autolysis and secondary nuclear crowding, but occasionally they occur as real phenomenon in well-processed material. Some germinomas may have these features diffusely, yielding an overall plasmacytoid appearance. In the past, many such testicular cases were considered "anaplastic" seminomas, a term that often causes confusion in the minds of pathologists and oncologists alike and which is not recommended. Tickoo SK *et al* identified a number of seminomas that had these histological features and that also tended to stain more prominently for cytokeratin and CD30 than the usual seminoma. They found that these "atypical" seminomas tended to present at a higher tumor stage and advocated that they may merit more aggressive treatment. Others, me included, however continue to place these cases into the seminoma category without separately designating them as atypical since it is not clear that different treatment is indicated. On most occasions the diagnosis of embryonal carcinoma is straightforward, but small foci within a background of germinoma may be challenging to identify. Any distinct epithelial differentiation, gland or papilla formation should be regarded as evidence of embryonal carcinoma. In the absence of distinct epithelial features, a combination of findings (nuclear pleomorphism, nuclear crowding and irregularity, dense cytoplasm, indistinct cell borders) will cause concern for transformation of germinoma to embryonal carcinoma. For those borderline cases where there is ambiguity at the routine light microscopic level, immunostains for CD30 and cytokeratin (AE1/AE3) may be useful [27].

Positivity in the problematic areas for these markers that contrasts with negativity in the typical germinoma areas provides support for an embryonal carcinoma component. If the borderline findings are diffuse, impressive AE1/AE3 and CD30 reactivity should be required, rather than focally prominent positivity for one of these markers, before accepting the tumor as embryonal carcinoma.

About 5% of germinomas have distinct admixed syncytiotrophoblast cells [14, 28], although additional cases with inconspicuous syncytiotrophoblast cells can be identified using immunohistochemical stains for human chorionic gonadotropin (hCG) [29].

When these cells are widely dispersed, as is typically the case, this phenomenon does not usually create diagnostic confusion. However, when they occur in sizable clusters, there may be concern for choriocarcinoma. Unlike choriocarcinoma, however, these tumors lack the plexiform admixture with cytotrophoblast cells, which show a greater degree of pleomorphism than the uniform, germinoma cells associated with admixed syncytiotrophoblast cells. Additionally, other areas of the tumor have the typical findings of germinoma. Syncytiotrophoblast cells in germinoma, just as in choriocarcinoma and other types of germ cell tumor, synthesize and secrete hCG, which may, therefore, cause a number of hormonal manifestations [30].

These include androgenic ones from secondary hyperplasia of Leydig cells in the testis or stimulation of the ovarian stroma; estrogenic ones, either because of direct stimulation of ovarian stroma and/or non-neoplastic follicles or peripheral conversion of androgen to estrogen, thereby resulting in abnormal uterine bleeding or, in men, gynecomastia; and hyperthyroidism because of the thyroid-stimulating hormone-like activity of hCG [31].

A number of other interesting, paraneoplastic manifestations may be seen in association with germ cell tumors, especially germinomas, including hypercalcemia [32], hypoglycemia [33], exophthalmos [34, 35], autoimmune hemolytic anemia (usually ovarian dermoids), ataxia telangiectasia [31], and limbic encephalopathy [36, 37].

Malignant germ cell tumors of the testis frequently metastasize to lymph nodes in the retroperitoneum and mediastinum as well as other locations. Most often these lesions resemble the original testicular primary tumor or have a pattern of a germ cell tumor type that was not seen in the primary tumor. Rarely, these metastatic lesions will present as somatic-type malignancies, taking the form of a carcinoma or sarcoma. In such cases, the diagnosis of a germ cell origin of the metastatic lesion is made based on the known history and lack of any other primary malignancy which could account for the metastatic lesion. Careful examination of the metastatic lesion in these tumors may occasionally show a small component resembling germ cell tumor. However, in very rare instances, these somatic-type malignancies of germ cell origin may occur as primary mediastinal lesions, or in the absence of a known testicular primary because of spontaneous regression ("burned-out" germ cell tumor).

Germ cell tumors represent a heterogeneous group of malignant cell lines with a variety of frequently overlapping histological pictures or with mixed components suggesting a common "precursor" embryonic cell dysfunction. Histological conversion to a more mature subtype is theoretically possible in a metastatic location with or without therapeutic intervention, as well as synchronous or metachronous development of two different primary germ cell tumours as a result of a common pathogenetic mechanism concerning genetic instability or abnormalities during the pluripotent embryonic germ cell differentiation and maturation.

Subsequently, the patient was given two courses of systemic chemotherapy (bleomycin, etoposide, cisplatin) as an adjuvant therapy. The patient has remained free of disease six months after discharge.

⏚ Conclusions

Patients with a history of testicular germ cell tumor require careful long-term monitoring of the contralateral testicle due to the risk of bilateral disease and potentially long latent period between the first and second tumors. Overall the clinical outcome is good in these patients when they are treated appropriately for histology and stage. In patients with metachronous tumors treatment of the contralateral tumor is rarely altered by prior treatment of the initial tumor.

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