

Seminoma's Architectural Variants, Immunophenotype and Differential Diagnostic

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Introduction: Although rare, representing only 1–2% of all tumours in man, testicular germ cell tumours (TGCT) are the overwhelming majority (98%) of testicular neoplasms among male patients between 15 to 40 years of age. Due to their increasing incidence and the characteristically young targeted population, they become a problem of public health in some developed countries. Classically, TGCTs are classified in three main groups: classical seminoma, non-seminomatous germ cell tumours (pure or mixed) and the spermatocytic seminoma. As SS is a very rare tumour, with a benign evolution, in practice the main differential diagnosis to be made is between seminoma and non-seminomatous tumours. Distinguishing these two categories is essential as the prognostic and the therapeutic approach is very different: if radiotherapy is the main treatment for seminoma, for non-seminomatous tumour a cisplatin based chemotherapy will be proposed.

Material and methods: This study proposes a morphologic and immunohistochemical evaluation of an important number of seminomas emphasising their unusual architectural features.

Results: The majority of the seminomas (46 cases), either pure or as a component of non-seminomatous germ cell tumours, had a solid architecture. We identified syncytiotrophoblasts cells in only one case in conventional stain and 11 cases were associated with a scattered intertubular spread. Eighteen cases showed unusual patterns: tubular-trabecular (9 cases), microcystic areas (5 cases) and 4 seminomas had focal nuclear pleomorphism. Areas of focal or extensive fibrosis and hemosiderin laden macrophages were identified in 4 cases. IGCNU, conventional seminomas and all the unusual architectural variants of seminoma had the same immunoprofile: positivity for PLAP and negativity for AFP and CD30.

Conclusions: Our study confirms the high architectural variability of seminomas, with unusual histological patterns like intertubular, tubular-trabecular, microcystic and pleomorphic. In the great majority of cases, the diagnosis of seminoma relies on the histological pattern in conventional stain. Only few cases may be prone to be diagnostically challenging, including tumours with unusual patterns. In these circumstances, the use of a panel of antibodies is mandatory for a correct diagnosis.

Keywords: seminoma, non-seminomatous germ cell tumours, testis

Introduction

Although rare, representing only 1–2% of all tumours in man [1], testicular germ cell tumours (TGCTs) represent the overwhelming majority (98%) of testicular tumours among male patients of 15 to 40 years [2]. Over the past four decades, without a certain explanation, the number of new cases is continuously rising worldwide [3] with an annual increase of about 4% [4,5]. They maintain their highest rates in Nordic European countries while it is the lowest in the Middle East and Asia [6]. Due to their increasing incidence and the characteristically young targeted population, TGCTs have become a problem of public health in some developed countries.

Classically, TGCTs are classified in three main groups: classical seminoma, non-seminomatous germ cell tumours (pure or mixed) and the spermatocytic seminoma (SS), a special type of TGCT generally affecting elderly male patients. As SS is a very rare tumour, with a benign evolution, and surgical resection alone is considered to be curable [7,8], in practice the main differential diagnosis to be made is between seminoma and non-seminomatous tumours. Distinguishing these two categories is essential as the prognostic and the therapeutic approach is very different: if radiotherapy is the main treatment for seminoma, for non-seminomatous tumour a cisplatin based chemotherapy will be proposed.

Seminomas represent up to 50% of these tumours and usually the morphological pattern is very suggestive for

this type of tumour. But in some instances seminoma may adopt unusual morphological aspects that can mimic non-seminomatous tumours.

This study proposes a morphologic and immunohistochemical evaluation of an important number of seminomas emphasising their unusual architectural features.

Material and methods

A total of 93 routine and consultation cases of TGCTs from the archives of the Pathology Departments of both University Hospitals of Tîrgu Mureş, Romania and San Cecilio, Granada, Spain were selected for this study. All the sections were obtained from orchidectomy specimens that were fixed overnight in 10% buffered formalin. For each case a mean number of 8 slides were available in H&E stain.

To confirm the diagnosis, the slides were all reviewed based on the latest World Health Organization (WHO) Testicular Germ Cell Tumours Classification 2004, and only one representative slide for each case was selected for the immunohistochemical study.

Four- μ m-thick serial sections were taken on charged slides from each representative paraffin block and heated overnight in a 56°C oven. After a heat-induced epitope retrieval (HIER) accomplished using EDTA, pH 9.0 (Dako, Glostrup, Denmark) for 20 minutes at 95°C, the slides were incubated for 1 hour with the primary antibodies against alkaline placental phosphatase (PLAP, prediluted,

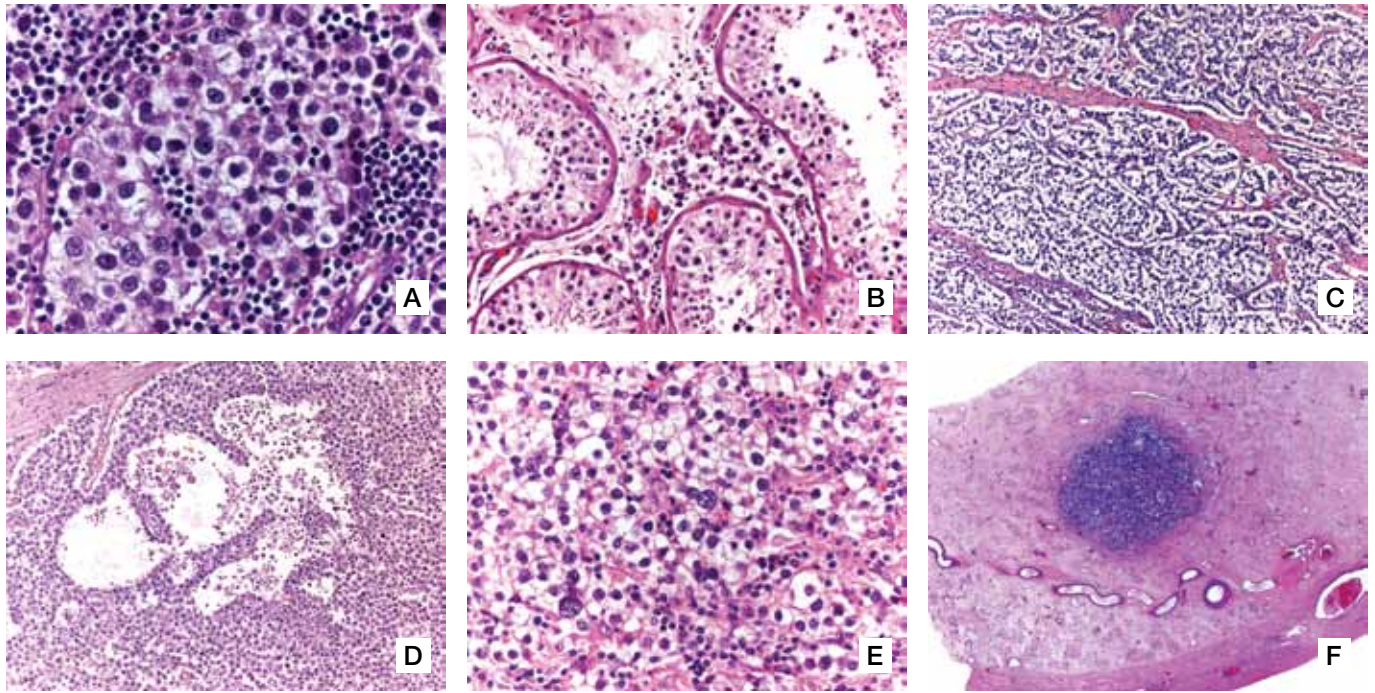


Fig. 1. Architectural variants of seminoma. **A:** Representative morphology of seminoma cells admixed with an abundant lymphocytic infiltrate; **B:** Isolated or small groups of cells infiltrating between the atrophic tubules some with IGCNU lesions; **C:** Tubular arrangement in an otherwise classic seminoma; **D:** Pseudocysts with an eosinophilic proteinaceous content; **E:** Subcapsular minimal cellular pleomorphism; **F:** Fibrotic areas of spontaneous regression, surrounding a remaining seminoma nodule.

clone 8A9, DAKO, Glostrup, Denmark), CD30 (prediluted, clone Ber-H2, DAKO, Glostrup, Denmark) and alpha-fetoprotein (AFP, prediluted, polyclonal, DAKO, Glostrup, Denmark). A biotin-free polymer-enzyme secondary antibody [Dako EnVision™ FLEX /HRP (SM802), Dako, Glostrup, Denmark] was used as part of the Dako EnVision™ FLEX Visualization Systems that also includes DAB as a chromogene.

The accepted patterns of positivity were: membranous and/or cytoplasmic for PLAP and AFP, while only the membranous stain was considered for CD30. The staining intensity was graded as weak (1) moderate (2) and strong (3). The slides were evaluated by two pathologists (L.A. and P.O.) and a common score was recorded in specially designed charts after a consensus was reached.

Results

Tumour type and architecture

Of the 93 cases, 40 (43%) were pure seminomas and 53 (57%) non-seminomatous germ cell tumours (GCTs). Of the latter group 36 tumours had a mixed histology, 12 of them containing a seminomatous component. The most frequent combinations of tumours in non-seminomatous GCTs were: embryonal carcinoma (EC) with yolk sac tumour (YST), seminoma with EC or YST, choriocarcinoma (CHOR) and teratoma. Seventeen cases were excluded from the study as they had pure non-seminomatous germ cell histology: 14 ECs, 1 YST and 2 SSs (Table I).

Intratubular germ cell neoplasia unclassified type (IGCNU) was obvious in 73 cases adjacent to 35 semino-

mas and 38 non-seminomatous GCTs. The lesions were generally diffuse but sometimes they had a lobular distribution. In 20 cases IGCNU lesions could not be identified due to tumour extension, fibrosis or oedema.

The majority of the seminomas (46 cases), either pure or as a component of non-seminomatous GSTs, were conventional seminomas and had a solid architecture (Figure 1A). We identified syncytiotrophoblasts cells in only one case in conventional stain and 11 cases were associated with a scattered intertubular spread (Figure 1B). Eighteen cases showed unusual patterns: tubular-trabecular in 9 cases (Figure 1C), microcystic areas in 5 (Figure 1D) and 4 seminomas had focal nuclear pleomorphism (Figure 1E). All these cases were included for immunohistochemical study. Areas of focal or extensive fibrosis and hemosiderin laden macrophages were identified in 4 cases (Figure 1F).

The other components of GCTs were recognised based on the morphology as it was described in the WHO classification. ECs consisted of a mixture of papillary solid and glandular areas, while the majority of the YST areas

Table I. The histology of the cases included in this study

	PURE TGCTs	The number of cases of mixed non-seminomatous GCTs containing different components
Seminomas	40	12
Embryonal carcinomas	14	34
Yolk sac tumour	1	27
Choriocarcinoma	-	11
Teratomas	-	22
Spermatocytic seminoma	2	0

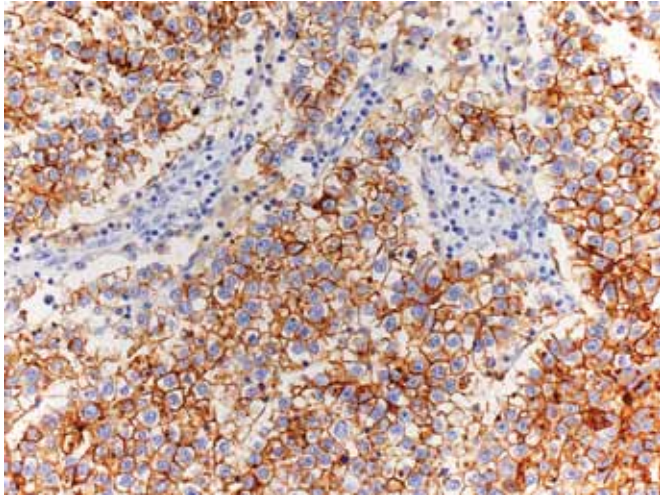


Fig. 2. PLAP positivity in seminoma

had the classic microcystic architecture. The teratomatous somatic tissues were represented by colonic-type mucin-secreting glands, immature glands, neuroepithelium, stromal elements, cartilage, and squamous and respiratory type epithelium.

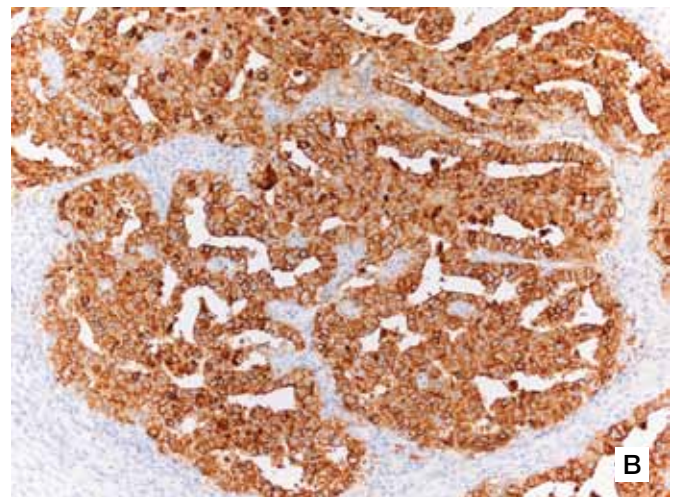
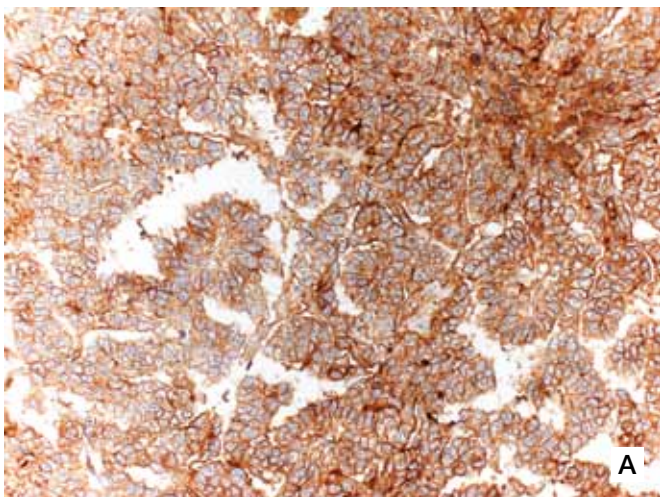


Fig. 3. Glandular and papillary areas of embryonal carcinoma positive for CD30 (A) and PLAP (B).

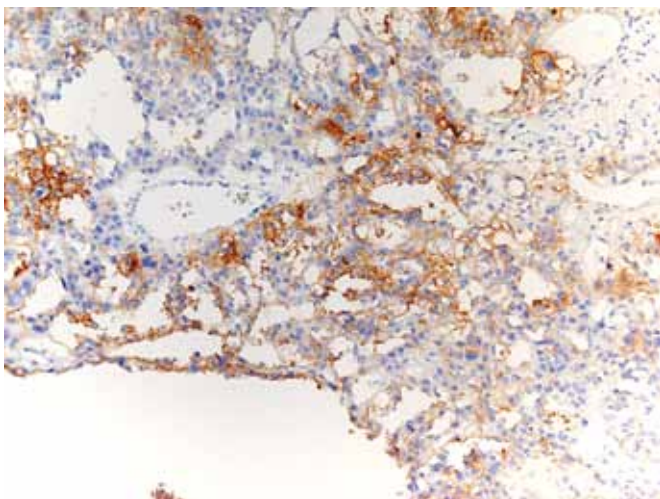


Fig. 4. PLAP positivity in a microcystic area of yolk sac tumour

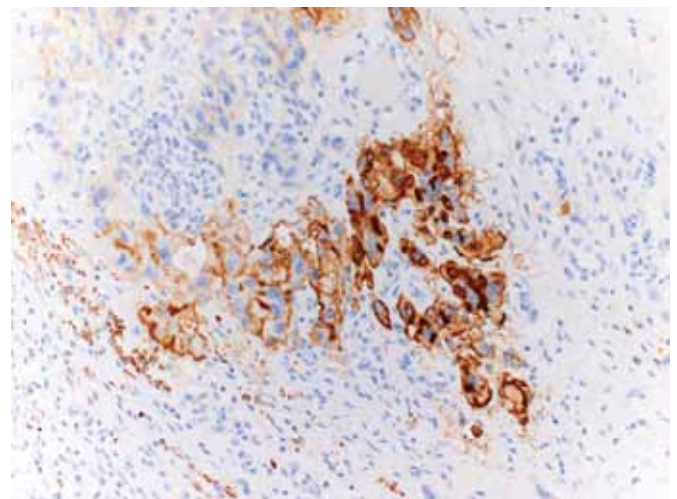


Fig. 5. Syncytiotrophoblasts with strong positivity for PLAP

Typical CHORs have never been found as a pure tumour, but as a component of non-seminomatous GCTs.

Immunohistochemical stains

IGCNU and conventional seminomas had the same immunoprofile. Of the 52 cases of pure seminoma or seminomatous component of a non-seminomatous GCT, 50 expressed PLAP: more than 90% of the neoplastic cells were positive for this antibody, the intensity of the staining being moderate or strong (Figure 2). All the unusual architectural variants of seminoma had the same immunoprofile as well. This positivity, whether weak or moderate, was noticed also in the seminomas with focal cellular pleomorphism.

However, 2 seminomas with solid architecture and with very characteristic morphological features were negative for this antibody.

All the 73 cases that associated IGCNU had a moderate PLAP positivity.

AFP and CD30 were not expressed by all seminomas and IGCNU lesions, CD30 being positive only in the activated cells of the accompanying lymphoid follicles.

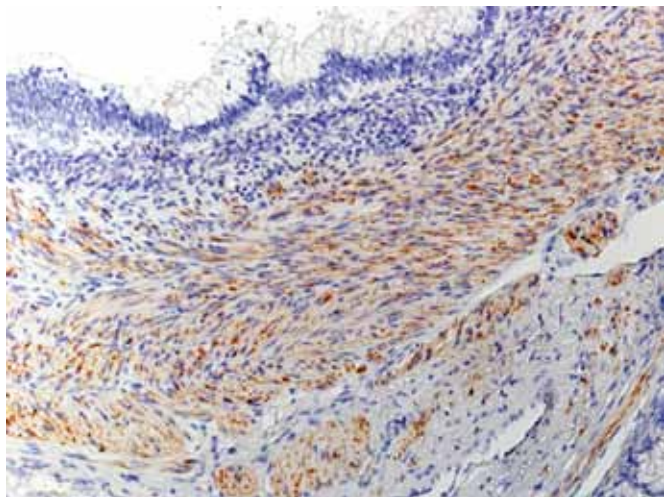


Fig. 6. Periglandular PLAP positive stroma in teratoma

The other components of mixed non-seminomatous GCTs had a specific immunohistochemical profile. All the cases of EC showed a strong membranous and a moderate cytoplasmic positivity for CD30 (Figure 3A). 95% of EC had a moderate or strong membranous positivity for PLAP (Figure 3B), and AFP was constantly negative in morphological areas of EC.

The microcystic, glandular and solid areas of yolk sac tumour component showed moderate or strong positivity for AFP. In almost one quarter of YSTs we noticed scattered areas with weak or moderate PLAP positivity (Figure 4). CD30 showed no positivity for YST areas.

Choriocarcinomas were negative for PLAP, AFP and CD30, however PLAP showing moderate to strong positivity in the syncytiotrophoblasts (Figure 5). The syncytiotrophoblasts of the pure seminoma were negative for all antibodies.

Teratomatous components did not express PLAP, AFP and CD30. In 3 cases PLAP highlighted only the mature stromal elements surrounding the glands (Figure 6).

Discussion

Seminomas are considered undifferentiated neoplasms with cells resembling embryonic germ cells and according to the Ulbright's theory on TGCTs histogenesis, it possesses the capacity to differentiate into all types of GCTs. It is the most frequent infiltrating germ cell neoplasia of the testis, representing up to 50% of the cases [9]. In our series of 93 cases, 40 were pure seminomas, representing 43% and a seminomatous component was present in 12 mixed germ cell tumours.

The classical microscopic feature of seminoma is usually easily recognised and is no matter of confusion. Tumour architecture is usually solid, but thin lymphocyte-rich fibrous septa may divide the lesion into clusters, interconnecting nests or columns.

A lymphocytic infiltrate is a usual and typical feature of seminoma and was present in all the cases, even being associated with epithelioid granulomas and lymphoid follicles in four cases. The majority of cytotoxic T lymphocytes were

referred to as possibly responsible for the regressive burn-out changes encountered most frequently in seminomas [10]. This lymphocytic infiltrate seems to accompany the tumour even in metastatic sites [11] and we might suppose that it is responsible for the regressive changes even in these locations.

The tumour cells were of moderate size, with uniform, round to oval nuclei, a pale to clear cytoplasm, and well-defined cell borders. Mitotic figures were prominent, but their number had no prognostic significance. Syncytiotrophoblast cells were present in only one case of pure seminoma: they were typical syncytiotrophoblasts with multinucleation and expressed β HCG.

Areas of fibrosis, haematoxylin bodies and hemosiderin-laden macrophages were present together with recognisable areas of seminoma in 3 cases. In another case, the testicular parenchyma was almost completely replaced by a dense fibrotic tissue. In these cases a meticulous sampling of the testicle should include areas of the remaining parenchyma in order to identify IGCNU [12]. Our case was an exception to the rule as evaluation of multiple slides did not highlight the presence of the pre-invasive lesion. Still, a nodular seminoma of 2 mm diameter was finally identified in only one section [13].

However, even if this classical feature is the rule, sometimes seminomas may show unusual patterns that are prone to misinterpretation.

In 2 cases the typical solid architecture of seminoma raised problems of differential diagnosis with SS because these cases displayed focal cellular atypia with less defined cytoplasmic borders, dark cytoplasm and enlarged, crowded nuclei. SS was excluded due to the young age of the patients, the presence of IGCNU in the surrounding seminiferous tubules, the presence of lymphocytic infiltrate and positivity of the tumour cells for PLAP.

By contrast, the differential diagnosis with EC and the solid pattern of YST entailed only the immunohistochemical profile of the tumour: lack of expression of CD30 and AFP (a characteristic antibody for endodermal differentiation) and the strong positivity for PLAP confirmed the diagnosis of seminoma [9].

Isolated seminoma cells, characteristic for the intertubular pattern, were identified at the periphery of 11 solid seminomas lying within the interstitium of the testis. They can rarely be found as pure pattern and do not pose problems of differential diagnosis with other TGCTs but might be confused with primary or secondary lymphoma or plasmacytoma [14] or other metastatic carcinoma or sarcoma. These tumours lack IGCNU changes and exhibit a different immunohistochemical profile (LCA positivity and other specific markers for lymphoma and plasmacytoma) [15].

Seminoma with syncytiotrophoblasts cells is considered a variant of seminoma in the latest WHO classification [16]. Even if literature reports up to 20% of such cases [17] in our study syncytiotrophoblasts cells were identified in only one pure seminoma. As the presence of these cells in seminomas does not impart an adverse prognosis [18] they

should be immunohistochemically identified with antibodies against β HCG or cytokeratins only if the β HCG elevated serum levels does not normalise after orchidectomy, in order to exclude a possible CHOR, responsible for still very high levels of β HCG.

In seminoma, the cells are generally uniform with bland atypia. However, slight cellular pleomorphism might create confusion with areas of EC as encountered in 4 of our cases. These cells were considered by some authors as areas of transition to EC [19] but if this is not confirmed by immunohistochemistry, they should be considered seminomas, as they share the same clinical outcome [16]. In our cases, these areas were present at the periphery of the tumour, either under the tunica albuginea or close to areas of necrosis. We might speculate that these changes might be influenced by fixation or even represent ischemic changes induced by the large size of the seminomas. Their immunoprofile was characterised by CD30 negativity and discarded the possibility of an incipient EC.

Areas with tubular architecture surrounded by an abundant fibrous stroma were sometimes simulating trabeculae, especially in a subcapsular location of the tumour. The cells in these cases had clear cytoplasm and the inflammation was scarce. Similar features have been described in isolated cases of seminoma since 1980, in tumours with cribriform or solid architecture [20,21]. They represent rare instances and are important to be recognised as they can mimic various non-seminomatous (i.e. YST with glandular architecture) or even non GCTs (i.e. Sertoli cell tumour) [22]. In our cases, AFP was never positive in these areas, excluding a YST.

The microcystic or cribriform architecture may suggest a YST. This is a less frequent variant of seminoma [22] and it was identified in 5 of our cases as isolated foci on the background of the classic solid architecture. They appeared as isolated microcystic spaces with a poorly outlined contour, sometimes dilated and filled with an eosinophilic acellular material, suggesting stasis and oedema [9]. The typical polygonal cells of seminoma in microcystic areas contrast with the flattened cellular profiles and more variable nuclear features of YST cells lining microcystic spaces. The lack of hyaline globules and intercellular basement membrane and the presence of delicate fibrous septa with lymphoid infiltrate favour a diagnosis of seminoma. The characteristic IHC profile confirmed this diagnosis, even if PLAP was positive in a few YSTs. This fact highlighted that characteristic microcystic architecture should be carefully corroborated with the morphology of the entire tumour before diagnosis. SS also may develop cystitic areas and extensive oedema but the presence of the 3 cell types and the absence of IGCNU, corroborated with the age of the patient and a different immunohistochemical profile, avoided the confusion.

Conclusions

Our study confirms the architectural variability of seminomas, with unusual histological patterns, like intertubular, tubular-trabecular, microcystic and pleomorphic. In the

great majority of cases, the diagnosis of seminoma relies on the histological pattern in conventional stains. Only few cases may be prone to be diagnostically challenging, including tumours with unusual patterns. In these circumstances, the use of a panel of antibodies is mandatory for a correct diagnosis. The seminomatous components showed, in a great majority of cases, a strong PLAP positivity, but unfortunately this antibody is not specific for seminoma, as it was also positive in an important number of ECs and also in a few cases of YSTs. CD30 and AFP, specific markers for ECs and YSTs were always negative in all the seminomatous components. This immunoprofile showed us that the use of only one antibody can lead to misdiagnosis and that the use of a panel of antibodies is mandatory. Furthermore, the relative lack of specificity of PLAP might be enhanced by using new antibodies described in the recent literature but the value of which is yet to be confirmed.

Acknowledgement

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