

## Case Report

# Mediastinal type B3 thymoma combined with germ cell tumor: cytologic diagnosis

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**Abstract:** Primary mediastinal thymoma combined with germ cell tumor (GCT) is extremely rare, and is likely to be misdiagnosed. Here we report a case of mediastinal type B3 thymoma combined with seminoma in which the seminoma component was missed by histologic examination and initially diagnosed by using a pleural effusion sample. The patient was a 46 year old male with chest distress, cough, and supraclavicular lymph node enlargement. A large anterior mediastinal mass was revealed by diagnostic imaging. The tumor was completely removed by thoracotomy. Grossly, a solid mass about 10 cm × 8 cm × 5 cm with cystic degeneration was found. Histologic examination revealed Type B3 thymoma accompanying with multiple lymph node metastases. One year later, CT scan found an irregular mass on the right side of anterior-superior mediastinum with a large amount of effusion in the right side pleural cavity. Cytologic examination and immunostains of the pleural effusion sample revealed metastatic seminoma. Then the original surgical sample was reviewed and the seminoma component also was found besides the thymoma. To the best of our knowledge, this is the first description of type B3 thymoma combined with seminoma, diagnosed by histology and pleural effusion together. We also present a literature review.

**Keywords:** Type B3 thymoma, cytology, seminoma

## Introduction

Thymoma is the most common primary epithelial tumor in the anterior mediastinum [10]. However, primary mediastinal germ cell tumors (PMGCTs) are relatively rare, and account for 10% of all mediastinal tumors [1, 2]. Moreover, mediastinal seminomas account for only 1.6% of primary mediastinal tumors [15], and 16% to 37% of PMGCTs [15-17]. Seminomas have also been described in association with other mediastinal tumors or non-neoplastic diseases, such as smooth muscle tumors or multilocular thymic cysts [11, 12, 18]. Simultaneous growth of mediastinal seminoma and thymoma has been reported based on histologic examination [13].

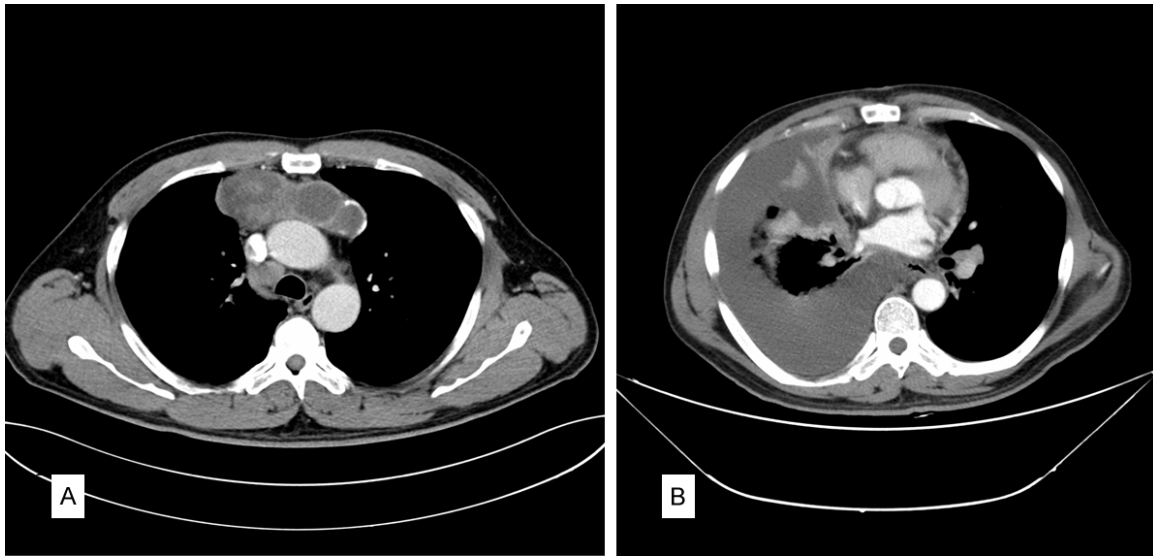
Here we report a case of mediastinal type B3 thymoma combined with seminoma in which the seminoma component was missed by histologic examination of the tissue, and was initially diagnosed on a pleural effusion. This shows that cytologic examination can be helpful to diagnose GCTs. However, cytologic diagnosis of

seminoma is challenging; cell block and immunostains should be applied to establish the correct diagnosis.

## Case presentation

### Clinical features

A 46-year-old male patient presented with chest distress, cough, and supraclavicular lymph node enlargement over half a year. CT scan (**Figure 1A**) revealed a huge mass in the anterior mediastinum. Maximum diameter was 10 cm with cystic changes as well as calcification. Fine needle aspiration of supraclavicular lymph node was performed with the diagnosis of metastatic malignant epithelial tumor. Based on these conditions, the patient had thoracotomy and mediastinal tumor resection along with lymphadenectomy. The histologic examination of the surgical specimen showed type B3 thymoma with multiple lymph node metastases. According to the diagnosis, the patient received two courses chemotherapy. One year later, an irregular soft tissue mass about 7.1 cm × 4.3



**Figure 1.** CT scan revealed a huge mass in the anterior mediastinum which showed cystic changes and calcification of the cyst wall (A). Postoperation CT examination one year later showed an irregular soft-tissue mass in the right side of anterior-superior mediastinum with a large amount of effusion in the right pleural cavity (B).

cm in size was found on the right side of anterior-superior mediastinum by CT scan (**Figure 1B**), which was a suspected tumor recurrence. In addition, a large amount of effusion could be seen on the right side pleural cavity. After cytologic examination and immunocytochemistry of the pleural effusion sample, seminoma was considered. Therefore, we reviewed the original surgical sample, the seminoma component also was found beside the main type B3 thymoma.

#### *Macroscopic features*

The tumor was 10 cm × 8 cm × 5 cm in size with medium hardness. The cross-section was gray-red color, accompanying by hemorrhage, necrosis and multiple cystic changes. The resected lymph nodes were firm and the largest one was 3 cm in diameter. The effusion sample was bloody.

#### *Histological and cytological features*

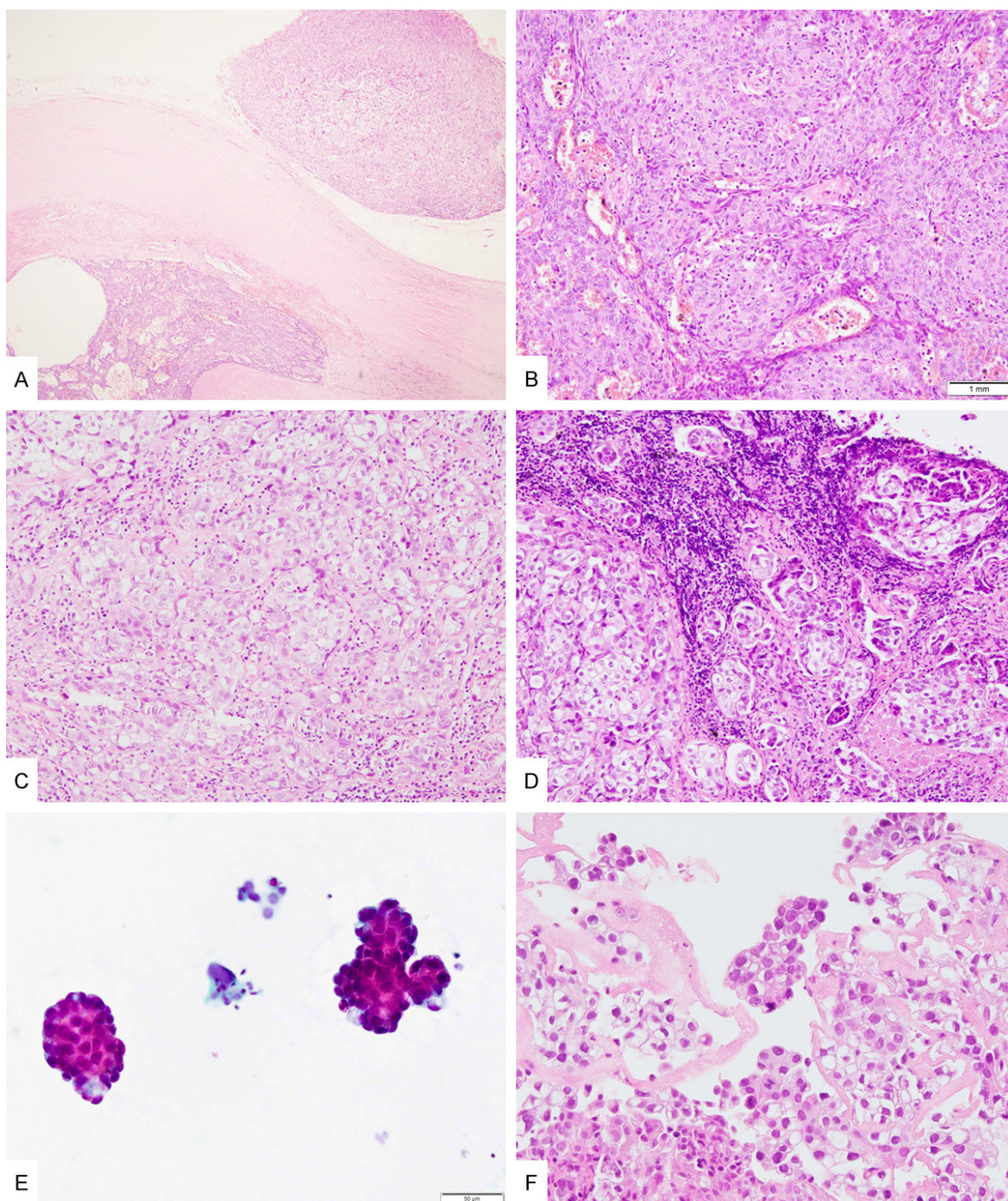
Histologically, the tumor was predominantly made of epithelial cells, with a few small lymphocytes. The epithelial cells were spindle or round shaped with mild to moderate cellular atypia, palisading around a perivascular space (**Figure 2B**). The cytoplasm was abundant and eosinophilic, while the nuclei of these cells were round, spindle, or oval shaped. Mitosis was rare. Type B3 thymoma was diagnosed according to these morphologic features.

One year later, the tumor was recurrent with pleural effusion. 200 ml bloody effusion sample was sent for cytologic examination. After being centrifuged at 2500 revolutions per minute for 10 minutes, the direct smears were prepared with Papanicolaou stain and the cell sediment was fixed in 3 times the volume of 10% neutral-buffered formalin for 1 hour to make cell block in an automatic tissue processor. Many medium to large abnormal, polygonal shape cells were observed in the smears and cell block section (**Figure 2E, 2F**). These cells were arranged in glandular, trabecular, and papillary patterns in the cell block section. The cytoplasm of these cells was abundant and vacuolated. The cytologic features were different from that of type B3 thymoma. Immunocytochemistry showed these cells expressed Placental-like alkaline phosphatase (PLAP) (**Figure 3A**), SALL4 (**Figure 3B**), focally expressed CD117 (**Figure 3C**), D2-40 (**Figure 3D**), while they were negative for TTF-1, Napsin A, TG, P63, CD5, P40, TdT, CD56, CD20, WT-1, CR, CD30, OCT3/4. Combined with cytologic and immunostains analysis of the pleural effusion sample, metastatic seminoma was considered. Then the original surgical sample was reviewed.

A few cells similar to the tumor cells in the pleural effusion were found beside the main type B3 thymoma (**Figure 2A, 2C**) and in some of the enlarged lymph nodes (**Figure 2D**). These cells



## Mediastinal type B3 thymoma combined with germ cell tumor



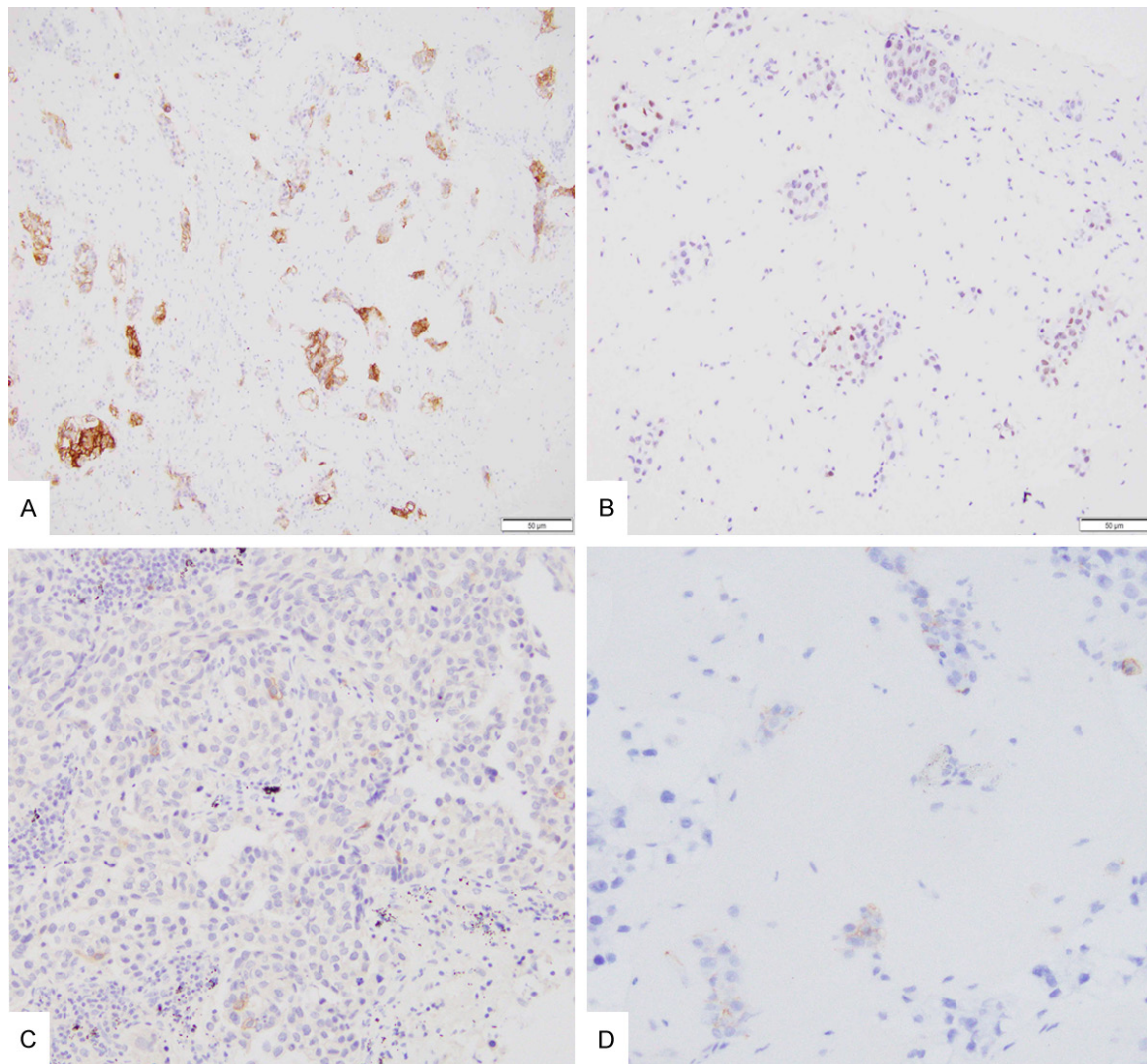
**Figure 2.** (A) Two distinct components-thymoma (in the lower left) and seminoma (in the upper right) were observed. H&E  $\times 40$ . (B) The higher magnification of the type B3 thymoma component. H&E  $\times 200$ . (C) The high magnification of the seminoma component H&E  $\times 400$ . (D) Metastatic seminoma in lymph nodes. H&E  $\times 200$ . (E) The tumor cells of seminoma in the cytologic smear of pleural effusion. Papanicolaou  $\times 400$ . (F) The tumor cells of seminoma in the cell block section (E) H&E stain  $\times 400$ .

were also arranged in glandular, trabecular, and papillary patterns with abundant cytoplasm and a few small, infiltrating lymphocytes. Mitosis could be found (average 4 mitotic figures per high-powered field) among the tumor

cells of lymph node accompanied by necrosis. Immunohistochemical staining showed these cells were diffusely positive for PLAP, partially positive for SALL4, CD117, and D2-40 and negative for CK19 and P40. In contrast, the epithe-



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**Figure 3.** The tumor cells of seminoma were positive for PLAP (A, cell block) and SALL4 (B, cell block), focal expression of CD117 (C, lymph node) and D2-40 (D, cell block), EnVision × 400.

**Table 1.** Immunohistochemical stains for mediastinal tumor

Immunohistochemical Marker	Thymoma	Seminoma
CK19	+	-
P40	+	-
CD5	-	-
TdT	lymphocytes+	Lymphocytes-
CD117	-	+
D2-40	-	+
OCT3/4	-	-
PLAP	-	+
SALL4	-	+

lial cells of the thymoma showed diffuse positivity for CK19, and P40 and were negative for

PLAP, CD5, and CD117. TdT was positive for the small lymphocytes in the thymoma while it was negative in the seminoma. The immunostains of the mediastinal tumor are summarized in the **Table 1**. Based on the comprehensive analysis of the clinical features, imaging findings, histologic characteristics of the surgical specimen, cytologic morphology of the pleural effusion and immunophenotyping, mediastinal type B3 thymoma combined with seminoma was diagnosed.

### Discussion

Primary mediastinal thymoma combined with seminoma is extremely rare, and is likely to be

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**Table 2.** Immunophenotypic features of GCTs, thymoma and carcinoma

	Immunoreactivity
Seminoma	PLAP(+), CD117(+), D2-40(+), AFP(-), SALL4 (+), OCT3/4(+), SOX17(+)
Embryonal carcinoma	CK(+), CD30(+), SALL(+), PLAP (approximately+), AFP(-), LCA(-), OCT3/4(+), SOX2(+)
Yolk sac tumor	CK(+), AFP (+), SALL4(+), LCA(-), PLAP (focally+), GPC3(+), GATA-3(+), ZBTB16(+)
Thymoma	CK19(+), P40(+) CD5(-), CD30(-), PLAP(-), LCA(-), SALL4(-)
Metastatic carcinoma	CK(+), LCA(-), SALL4(-)

misdiagnosed. As far as we know, there are only 3 cases reported that were diagnosed histopathologically [3, 13]. In 2014, Weissferdt et al. described two cases of male patients aged 32 and 34 years old, respectively who had both thymoma and seminoma in a single mediastinal mass. In 2017, Lee et al reported a 35-year-old patient with multiple thymic masses that included one of type B2 thymoma and two seminomas occurring as separated tumors. Now we report a case of mediastinal type B3 thymoma combined with seminoma in a single mass in which the seminoma component was first diagnosed by using pleural effusion sample. To the best of our knowledge, this has not been reported before.

Thymomas occur most frequently in adults between the ages of 55 and 65 years, and are exceedingly rare in children and adolescents while primary mediastinal seminomas predominantly occur in young males. However, some adult patients with seminomatous tumors have been reported, ranging from 21 to 46 years old [19]. Mediastinal germ cell tumors are uncommon and seminomas are less frequent than non-seminomas [20, 22]. Due to the slow growth of seminomas, most of them have been bulky at the time of diagnosis. Symptoms of mediastinal seminoma are usually not characteristic and some cases are diagnosed incidentally [20, 21, 23]. 60% to 70% of patients with mediastinal seminoma tumors have metastasis or infiltration at the time of diagnosis. The lungs and other thoracic organs are the most common sites involved [24]. Pleural or pericardial effusion caused by germ cell tumor that was found by CT scan had been reported. However, cytologic examination of these cases was not available [34]. So, this is the first description of seminoma diagnosed by pleural effusion.

Microscopically, classical seminoma is composed of round or polygonal tumor cells and

reactive lymphocytes with a nest-like distribution pattern. The cytoplasm of tumor cells is abundant and vacuolated. There is an anaplastic variant of seminoma. The tumor cells of anaplastic seminoma (AS) have overall morphologic features of classical seminoma, but they are usually arranged in pseudoadenoid/acinar or cribriform glands with obvious cellular irregularity accompanied by focal necrosis. The mitotic figures of AS are more than three per high-powered fields (HPF) [26, 27]. Kademian et al., in their 1977 study, and Bobba et al., in their 1988 study, demonstrated a worse prognosis and higher relapse rate of AS compared to CS [28, 29]. According to the cytologic morphology of this case, it was consistent with anaplastic seminoma.

Cytologic diagnosis of seminoma in the serous effusion can be challenging, especially when no history or histologic diagnosis is available. Based on the history and cytomorphology of this case, the differential diagnoses include: metastatic carcinoma, thymoma, and other types of germ cell tumor, such as embryonal carcinoma or yolk sac tumor. The cytologic features of these tumors usually overlap, and it is hard to differentiate them just by cytomorphology. Immunocytochemical staining should be employed for differential diagnosis. The immunophenotypic features of these tumors are listed in **Table 2**. The GCTs markers include PLAP, AFP, CD30, hCG, GPC3, CD117, D240, OCT3/4 and SALL4 [8]. It was reported recently that PLAP and SALL4 were broad spectrum markers of GCTs, and the diagnosis of GCT could be established if these two markers were both positive [14]. Nevertheless, it takes more markers to make clear the subtypes of GCTs. CD117 and D2-40 were described as specific markers for extragonadal seminoma [8, 30]; CD30 is specific for embryonal carcinoma while AFP and GPC3 are specific for yolk sac tumor. Besides these, Weissferdt et al reported that SOX17 had been identified as a novel marker for semi-

noma [30]. The positive rate of SOX17 in mediastinal seminoma was 97%, which was similar to that in testicular seminoma (94%-100%) [31-33]. However, this antibody is not available in our lab. In this case, D2-40 and CD117 were both expressed, while AFP, GPC3, CD30 and OCT3/4 were all negative, so seminoma was diagnosed.

Cytologic examination is a minimally-invasive, rapid and reliable diagnostic method. The cytologic diagnosis of GCTs is difficult, and should be based on the comprehensive analysis of clinical manifestations, imaging findings, and immunophenotyping. It has been proven that GCTs can be diagnosed by fine-needle aspiration [4-7, 25]. However, cytologic diagnosis of GCTs by using pleural effusion has not been described. In this rare case, the seminoma component was ignored histologically and it was primarily diagnosed by using the pleural effusion sample, which was also identified beside the thymoma in the surgical sample after the serous effusion cytologic diagnosis.

Although both type B3 thymoma and seminoma tumors can occur in the mediastinum, the presence of these two tumors in a single mass simultaneously is rare. Sufficient sampling is necessary for patients with huge mediastinal mass to avoid missed diagnosis. Cytologic examination can be helpful to diagnose GCTs, but cytologic diagnosis of these tumors can be challenging; clinical information, imaging findings, cell blocks and immunostains should be combined to establish the correct diagnosis.

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## Disclosure of conflict of interest

None.

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