CASE REPORT

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Clinical, morphological and immunohistochemical characterization of a recurrent B1 type thymoma

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Abstract

Type B1 thymoma is widely accepted as a tumor with a non-aggressive behavior even in advanced stage. Most of these tumors are classified as Masaoka stage I or II. They rarely relapse or metastasize and the surgical treatment is considered curative. We have investigated a case of thymoma type B1, which relapsed 13 months after the primary tumor was excised. The patient was diagnosed with a local tumor recurrence after investigations due to the worsening of clinical symptoms of myasthenia gravis (MG). The therapy management of such cases is debatable and protocols not yet approved. For this reason, we have analyzed different clinical, morphological and immunohistochemical characteristics that may be considered as prognostic factors for a more aggressive behavior of such tumors. We have identified some morphologic characteristics rarely seen in this type of thymoma but none considered of prognostic value. In addition, we investigated some possible immunohistochemical markers that are generally associated with a more aggressive clinical outcome in different malignant tumors and thymic epithelial tumors. Among these markers, only p53 was positive and may be useful to predict a more aggressive evolution. In summary, probably the more appropriate approach of the patient is the clinical follow-up together with treatment of the clinical symptoms of myasthenia gravis.

Keywords: thymoma, B1 type, recurrence, immunohistochemistry, prognosis.

Introduction

Thymomas are thymic epithelial tumors characterized by the presence of thymic epithelial stroma and lymphocytes. In the anterior mediastinum are the most common neoplasms, representing approximately 50% from all lesions in this location [1].

Thymomas are histological classified based on microscopic architectural changes, the morphology of epithelial cells, the proportion of epithelial cells/ lymphocytes and the presence of cellular atypia in five types A, AB, B1, B2 and B3 [2].

B1 thymoma is considered a proliferation that mostly resembles the normal cortical area of the thymus with scattered epithelial cells in an abundant lymphocyte background.

Myasthenia gravis is an autoimmune disease commonly associated with thymoma, that may precede or may be clinical apparent after the diagnosis of thymoma. From the histological types, B1 and B2 thymomas are most frequently related with myasthenia gravis [3].

Due to this relation, the patients with thymoma associated MG tend to be diagnosed in lower stages than those without myasthenia.

Although B1 thymoma is considered a tumor with low risk of recurrence, in the literature were reported recurrences, occurring years after primary tumor resection, which showed an increased aggressive behavior compared with primary tumor [4].

We report here a patient with an early thymoma recurrence occurring 13 months after the primary tumor resection.

We have analyzed different clinical, morphological and immunohistochemical features that may influence the more aggressive behavior than usually noticed in such cases.

Patient, Methods and Results

We present the case of H.I., a 60-year-old female, diagnosed with myasthenia gravis in 2006, eye-onset form.

She was treated with corticotherapy, cholinesterase inhibitors and immunosuppressive drugs until 2010.

Due to worsening of the clinical symptoms of myasthenia (Ossermann Class II) a thoracic computed tomography (CT) was performed in March 2010 and a 9/6/4 cm anterior mediastinal mass suspected to be a thymoma was discovered.

The tumor was surgically removed in June 2010 and a R0 resection was achieved. After thymectomy, the patient symptoms have improved.

In September 2011, the patient was admitted in the

Emergency County Hospital from Timişoara with severe dyspnea. A thoracic CT was performed that identified a tumor mass in the prevascular space, with relatively well-defined outline and a density of a parenchymal tissue (42 U.H.) (Figure 1A).

A tumor recurrence was suspected and the patient was transferred to surgery for tumor excision. During surgery, a 5/3 cm well defined, firm mass was found in the anterior mediastinum. A complete resection was performed and the entire tumor was sent to the Pathology Department. Post-surgery the clinical evolution of the patient was not favorable, thus an immunoglobulin treatment was established.

The tissue samples were fixed in 10% formalin, embedded in paraffin, cut into 4-µm thick sections and stained with Hematoxylin–Eosin (HE). The thymoma was histological classified according to recommendations of World Health Organization [2]. Additional 4-µm thick sections were prepared for the immunohistochemical study.

Immunohistochemical study was performed with antibodies against High Molecular Weight Cytokeratin (CKHMW), Ki-67, p53, Bcl-2, Vascular Endothelial Growth Factor (VEGF) and CD117. Their specifications are shown in Table 1.

Table 1 – ***Characteristics of the antibodies used in the present study***

Antigen	Host	Clone	Antigen retrieval	Visualization system	Manufacturer
CKHMW	Mouse	34βE12	Boiling in citrate buffer, pH 6	EnVision	Dako
Ki-67	Mouse	MIB-1	Boiling in citrate buffer, pH 6	EnVision	Dako
P53	Mouse	DO-7	Boiling in citrate buffer, pH 9	EnVision	Dako
Bcl-2	Mouse	124	Boiling in citrate buffer, pH 9	EnVision	Dako
VEGF	Mouse	VG1	Boiling in citrate buffer, pH 9	EnVision	Dako
C-kit	Rabbit	CD117	Boiling in citrate buffer, pH 9	EnVision	Dako

After dewaxing and rehydration, sections were pre-treated by microwave heating in citrate buffer, pH 6/pH 9, and 20 minutes for antigen retrieval. After incubation with primary antibody for 30 minutes, we applied EnVision (K5007, Dako, Glostrup, Denmark) working system, visualized with 3,3'-diaminobenzidine as brown staining, nuclei being counterstained with Hematoxylin.

A positive reaction of the immunohistochemical staining was considered if more than 10% of the tumor cells were positive. The reaction was considered weak if <25% cells were positive, medium (25–50% positive cells) and intense (>50% positive cells).

The expression of Ki-67, p53 and Bcl-2 was quantified as a score obtained by summing up the product between staining intensity (0 – negative, 1 – weak, 2 – moderate, and 3 – strong) and the percentage of stained cells within the tumor as previously described [5]. A positive reaction was considered a score >30.

The study protocol was approved by the local research ethic committee and informed consent of the patient was obtained according to the *World Medical Association (WMA) Declaration of Helsinki*.

Histopathological assessment

Macroscopically, the tumor was fleshy in consistency and surrounded by adipose tissue. The tumor measured 6/4/2 cm and appeared like a grey-white, well-circumscribed, lobulated mass. On cut section the tumor was homogenous, had a lobulated appearance with lobules separated by fibrous strands, a white-tan color, and some cystic degenerative changes (Figure 1B). On serial sections, some areas with penetration of the tumor capsule were identified (Figure 1B – inset).

Microscopically a lobular appearance was observed, proliferating lobules being separated by thick fibrous septa. The tumor resembled the cortical area of the normal thymus, but these areas were predominant throughout the tumor. Small medullar-like areas were seen. The proliferation consisted of inconspicuous epithelial cells in a lymphocyte rich background. The epithelial cells were seen isolated, generally small, with vesicular nuclei and small nucleoli. Rare atypical cells with large, irregular nucleus and prominent nucleolus were identified (Figure 1C).

Numerous perivascular spaces were observed some of them with large dimensions. The tumor was encapsulated but areas with pericapsular fat tissue invasion were seen. Zones with perivascular invasion were also identified (Figure 1D).

Immunohistochemical findings

The tumor epithelial cells reacted moderate/intense (2+/3+) with anti-CKHMW antibody. The positive reaction had a reticular heterogeneous appearance, positive cells having a granular cytoplasmic staining pattern. Positive reaction in this type of thymoma is similar to that observed in normal thymus. Some tumoral areas containing epithelial cells positive for CKHMW were identified in the pericapsular fat (Figure 1E).

The antibody anti-VEGF stained only isolated tumor cells, the reaction being considered negative (<10% tumor cells positive) after counting five fields with the highest density of marked cells.

P53 was found to be positive in the nuclei of tumor cells. The intensity of staining was weak to moderate. Isolated, intense stained nuclei were observed with atypical morphology – large nuclei, irregular nuclear membrane contour (Figure 1F).

The immunohistochemical expression of Ki-67, Bcl-2, CD117, was negative in the tumor epithelial cells of the B1 thymoma. The immunohistochemical reaction was positive for Ki-67 and Bcl-2 in non-neoplastic lymphocytes of the tumor.

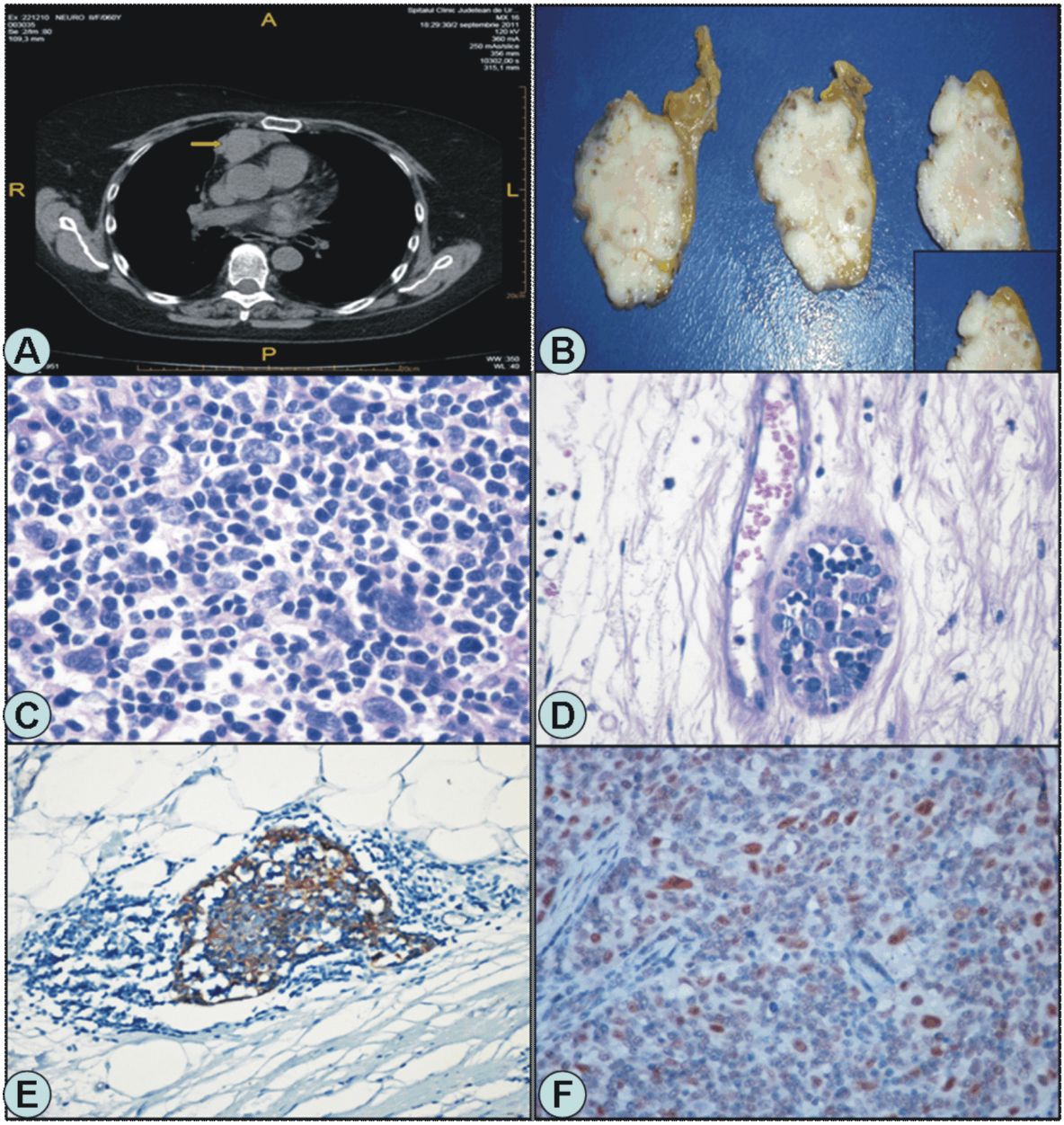

Figure 1 – ***(A) Well-defined tumor mass in the anterior mediastinum. (B) Cut sections of an encapsulated thymoma with areas of capsular invasion (inset). (C) Atypical epithelial cells in B1 thymoma (HE stain, ob. ×10). (D) Perivascular invasion by isolated group of tumor cells (HE stain, ob. ×40). (E) Tumor area with pericapsular fat invasion (Anti-CKHMW, ob. ×10). (F) Positive epithelial cells for p53 in B1 thymoma (Anti-p53, ob. ×20).***

Discussion

Thymomas are relatively unique among tumors because the prognosis appears to be related to invasion characteristics observed during surgery than the histological type and grade of tumor. Recurrence of a thymoma after complete primary resection is uncommon although thymomas recurrence is considered an important factor to predict the outcome for thymic malignancies [6, 7]. The recurrence rate for B1 thymoma varies between 0–9% [6, 8–12] and the time for relapse is between 30 months–25 years [8, 11, 12]. Our case with relapse after 13 months from primary tumor excision is the earliest recurrence of a B1 thymoma published in literature so far.

In patients with recurrences, the rate associated with MG is very high (~93%) [11]. Often the recurrence is clinically asymptomatic although the common presentation in these patients is the worsening MG symptoms [11]. Accordingly, in our case the recurrence was brought to attention due to unresponsive MG symptoms of the patient to clinical treatment. Presence of the clinical symptoms at the time of operation does not affect the long-term outcome [6], however patients with thymoma-MG association do not respond to thymectomy for improving the myasthenia treatment as well as the patients without thymoma [13]. For this reason, it is possible that the same unknown mechanism that induces tumor development could be responsible for relapse, therefore the patients who are not clinically responsive after thymectomy need to be further follow up.

Predictors for recurrence in patients with thymic epithelial tumors are considered Masaoka stage, complete resection, histology and size [7, 14]. In a large study

Gupta R *et al.* (2008) [15], confirmed the idea that thymic capsule invasion cannot be considered a significant prognostic factor, so only capsular invasion does not influence survival, but spreading of the disease to surrounding organs. Therefore, the invasion in perivascular fat seen in our case does not carry a risk for outcome. Nevertheless is not known if the perivascular invasion identified may be considered an aggressive behavior factor as in tumor with other locations.

B1 type thymoma is a tumor with more favorable outcome than AB, B2 and B3 thymomas [6, 9]. Although considered a tumor with low risk of recurrence, have been reported remote recurrences after primary tumor resection, which showed an increased aggressiveness compared with primary tumors [4]. We have seen in our case areas with B2 morphology but these were <10% from tumor areas. We have also identified numerous perivascular spaces, some very large and atypical tumor cell, which are more common in B2 and B3 types. If these morphologic features may influence the outcome, remain to be further established.

Increasing tumor size is associated with recurrence and death. The primary tumor in the current case had 9 cm in length and statistically has a great risk for recurrence according to some studies [14, 16]. Even so, if the size alone is a reliable factor to predict the recurrences remains debatable at least from the size point of view.

Identification of CK34βE12-positive neoplastic cells in vessels of the tumor capsule or in the perithymic fat helps to classify apparently encapsulated thymomas and indirectly suggests prognostic role of this marker. Our case showed a fine network of epithelial cells moderate/intensely stained for HMWCK that is diagnostic for B1 thymoma. Furthermore, we have seen areas of tumor invasion into pericapsular fat in which the epithelial cells where positively stained. Therefore, CK34βE12 can be used as a marker to detect microscopic tumor capsular invasion and vascular invasion as a potential indication for a less good behavior.

VEGF is involved in the development and progression of malignant tumors and we have described a correlation between VEGF expression and the aggressive behavior potential of thymomas [17]. The same as previously published results for B1 thymomas [18], we have not found a positive reaction in our case, result that may be associated with a good prognosis for the patient.

P53 expression is increasing in intensity from noninvasive thymomas towards thymic carcinomas, correlates with more advanced stages [19] and shows inverse correlation with tumor resection [20], as well as with overall survival and survival without disease symptoms [21]. Khoury T *et al.* (2009) [5], has found positive expression for p53 protein in all cases with recurrent thymomas and suggests that p53 positivity could predict tumor recurrence regardless of tumor type. Correspondingly, in our case we observed a positive expression for this marker in the nuclei of epithelial tumor cells that may suggest a possibility of a greater risk for recurrences. Moreover, we observed also a particular aspect, nuclei with morphological atypical features were intensely stained and this correlation morphology–p53 positivity may be an evidence for a less good behavior. Coexpression of Bcl-2 and p53 protein is observed in the majority of the thymomas and appears to correlate with clinical aggressiveness [5]. Nevertheless, we found a positive expression in the proliferated epithelial cells only for p53 but not for Bcl-2.

C-kit (CD117), a tyrosine kinase receptor, expression correlates with aggressiveness and may be a target in selected cases for monoclonal therapy with Kit-inhibitors in thymomas [22]. We did not find a positive expression in our case.

Ki-67 proliferation index correlates negatively with survival in thymomas and thymic carcinomas [23]. The Ki-67 expression found in our case was negative, even Khoury T *et al.* (2009) [5] found a positive expression in 75% of analyzed B1 type thymoma cases. Thus, Ki-67 may not be a reliable marker to analyze the outcome of such recurrent tumors.

Surgical resection represents the standard therapy for B1 thymomas treatment and currently the most beneficial. Tumor resection has proven important for recurrence, survival being higher in patients with re-excision than in patients treated only with radio- or chemotherapy [11]. Best survival outcomes are found to depend on the degree of completeness of the repeat resection. Therefore, the members of the *European Society of Thoracic Surgeons* (ESTS) agree that recurrence should be removed whenever possible [24]. According to *NCCN 2010 Guidelines* [25], high-risk patients with R0 resection can benefit of postoperative radiotherapy (category 2B of evidence). Our case is not a high-risk patient (not by clinical stage, histological type, incomplete resection or size) however it was early recurrent and clinically the myasthenia gravis symptoms were not improved. The postoperative management for recurrent thymoma is debatable and the same for this case. Probably, the patient would not benefit from postoperative radiotherapy as are current evidences for stage II thymomas [8, 26] and may avoid radiation, the more appropriate follow-up being annual chest CT together with treatment for clinical symptoms of myasthenia gravis.

Conclusions

We report the case of an early relapse (13 months) of a B1 type thymoma published to date. We have identified some morphological aggressive factors (primary tumor size, tissue or perivascular invasion, atypical cells) rarely seen in this type of thymomas. The expression of p53 may have a role in predicting the future evolution of cases with increasing risk of recurrences. We consider that all the patients with thymoma, even those with low histological and clinical risk, need to be carefully evaluated in time after primary resection. Furthermore, taking into account that the prognosis is not known in these cases and therapy is currently not standardized, we believe that improving the therapeutic management is related not only to the morphological characteristics of the tumor but also to the field of molecular markers, their value remains to be discovered.

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