Head and neck: Thymus: Thymoma: an overview

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Identity

Note
Introduction
Thymoma is the most frequent primary tumor of the anterior and superior mediastinum with an incidence that ranges between 0.2 and 1.5% from all malignant tumors (Schmidt-Wolf et al., 2003; Koppitz et al., 2012). Usually, it occurs in adults over 40 years and is asymptomatic for long time. When the thymoma becomes invasive, it becomes clinically evident by compression signs or/and respiratory symptoms. Almost half of the cases are detected by routine thoracic X-ray examination and the majority has between 5 and 10 cm in their larger diameter. However, any imagining criterion is specific for the diagnosis of thymoma (Strollo et al., 1997).

The term "thymoma" is restricted to the tumor of the thymus that consists of epithelial cells, independently from the presence and number of lymphocytes. Almost all thymomas occur in the anterior and superior mediastinum of the adult, and occasionally, with other locations (latero-cervical, in the thyroid, in the hilus of the lung). Typical thymoma is a solid, yellow-grayish and lobulated tumor. In approximately 80% of cases the tumor is encapsulated, and when it is large shows many foci of cystic degeneration. Even apparently encapsulated, the rate of recurrences is about 15%. Many "malignant" thymomas have a slow rate of proliferation, and the recurrence is noticed in 2% of the encapsulated and in 20 to 40% of the invasive thymomas. Thymoma is frequently associated with immune-mediated systemic diseases, like neuromuscular syndroms (myasthenia gravis, Lambert-Eaton syndrome, myositis), hypogammaglobulinemia (12%), hematologic diseases (erythroid hypoplasia, erythrocytosis, leukemia), collagen diseases (scleroderma, Sjögren syndrome) or autoimmune endocrine diseases (Boonen et al., 2000).

Epidemiological data showed that in patients with thymoma the incidence of synchronous malignant tumors is higher than in other groups and it was reported in 28% of the cases in a series of 128 patients with thymoma (Welsh et al., 2000).

Myasthenia gravis and thymoma
In patients with myasthenia gravis the thymus shows a marked lymphoid follicle hyperplasia, as unique morphologic abnormality in 65% of cases. In 10% of patients myasthenia gravis is associated with thymoma (with or without follicular hyperplasia) and there are not found abnormalities in the other 25% of cases. This relation is also supported by the fact that 30 to 45% of patients with thymoma develop clinically evident myasthenia gravis (Raica et al., 1999). Until now, there were not demonstrated ultrastructural or immunohistochemical differences between thymomas with and without myasthenia gravis. Recent studies on large series were not able to demonstrate that myasthenia is a prognostic element for the behavior of thymoma. In all cases, myasthenia gravis is associated with a defect of the nicotinic receptor for acetylcholine (AchR). A similar (or even identical) protein was identified in the normal human thymus. This protein is located in a subset of thymic cells that have similar immunphenotype as striated muscle cells (myoglobin and desmin positive), called myoid cells. In the non-neoplastic thymus of patients with myasthenia gravis were identified aggregates of myoid cells, infiltrating the stroma. T cells activate AchR reactive B lymphocytes. Therefore, the process begins in the thymus and than continues in the entire immune system by autoantibodies production. Surgical treatment is more efficient if the thymus has follicular hyperplasia,
and persistence of clinical signs is attributed to resting thymic parenchyma.

**Classification**

**Note**

**Thymoma: general features**

The large majority of thymomas consist of a mixture of neoplastic epithelial cells and non-neoplastic lymphocytes. The rate between these two cell populations is variable from a case to another, or even in different areas of the same tumor. Neoplastic epithelial cells may be polygonal, round, oval, and stellate or spindle in shape. Nuclei have a fine granular chromatin, smooth outline, and nucleolus is often prominent (especially in round and polygonal shaped cells). Thymomas with epithelial predominance contain prominent perivascular spaces with lymphocytes, proteinaceous fluid, red blood cells, and foamy macrophages. More rare are found rosette-like (without evident lumen) or glandular-like aspects or even abortive Hassall corpuscles. The presence of rosettes with well-defined lumen does not correlate with the diagnosis of thymoma, and usually is a character of a carcinoid tumor of the thymus. In some cases may be noticed micro-cystic, tubular and pseudo-papillary zones. In lymphocyte-rich thymoma areas with medullary differentiation are frequently noticed. The presence of rosettes with well-defined lumen does not correlate with the diagnosis of thymoma, and usually is a character of a carcinoid tumor of the thymus. In some cases may be noticed micro-cystic, tubular and pseudo-papillary zones. In lymphocyte-rich thymoma areas with medullary differentiation are frequently noticed. The capsule of the tumor is thick, fibrous, and often calcified. It gives rise to fibrous septa that form angulated lobules into the parenchyma of the tumor. It is thought that extensive fibrosis in some cases is the result of spontaneous tumor regression, but there are no proofs to support this hypothesis.

Ultrastructurally, neoplastic epithelial cells contain branched tonofilaments, form desmosomes, and have long cytoplasmic processes and a distinct basal lamina. Epithelial cells are arranged in sheets and cords that contain lymphocytes, and the aspect is mimicry of the medulla of the normal thymus. These characters are useful to differentiate thymoma from other tumors of the mediastinum (carcinoid, malignant lymphoma, germ cell tumor or solitary fibrous tumor). The presence of epithelial cells on ultrastructural examination does not necessary mean thymoma. We must be aware especially on malignant lymphoma that included islands of normal thymus.

The tumor microenvironment was significantly less investigated than neoplastic cells of thymoma. In part, tumor progression may be stimulated of inhibited by changes in the tumor stroma, and it was found a significant increase in the number and spatial distribution of mast cells and high values for immature and intermediate blood vessels, which are characteristics of tumor-associated angiogenesis (Raica et al., 2007; Raica et al., 2009; Raica et al., 2010).

**Classification**

**Classification of thymoma**

Classification of thymoma is one of the most elusive in pathology, based on one hand on the reduced number of cases and on the difficulty to interpret histological findings on the other. The majority of the tumors of the thymus are thymomas and the term is restricted to the epithelial proliferations of the thymic stroma.

The present classification of thymoma includes the type A (“A”, atrophic), type B (“B”, bioactive), and C (“C”, carcinoma).

Over the years, there were proposed many systems, but only two or three remained in use. Perhaps the most simple, but at the same time, the most subjective, is the classification proposed by Lattes and Bernatz that take into account the predominant cell type, as follows: spindle thymoma, thymoma with lymphocyte predominance (over 66% lymphocytes), with epithelial predominance (over 66% epithelial cells), and predominantly mix (epithelial cells between 34 and 66%). Examination is extremely subjective, because there are estimated two different cell types, as demonstrated by immunohistochemistry. Moreover, this classification brings nothing in the field of prognosis.

In last years, the classification proposed by Muller-Hermelink had a strong impact, because it is histogenetic and correlates with prognosis. The major criticism is related to the "well differentiated thymic carcinoma", included in the group of thymoma by all other publications (Rosai, 1999; Suster and Moran, 1999; Kuo, 2001). This is the reason why many pathologists prefer the classification proposed by Juan Rosai (Rosai, 1999).

Malignancy of thymoma is defined in terms of natural evolution.

Completely encapsulated thymomas were considered to be benign, and invasive thymomas were considered malignant. On this basis, Masaoka (1981) introduced the "clinical" classification that found many adepts: I - encapsulated at macroscopy, without microscopic invasion; II1 - macroscopic invasion in the adipose tissue or pleura; II2 - microscopic invasion of the capsule; III - macroscopic invasion in surrounding organs; IVa - pleural or pericardial spread; IVb - distant metastasis. With few changes, this classification was incorporated in the TNM system, proposed by Yamakawa.

The grade of thymoma is another unsolved problem and a permanent subject of dispute. It was shown that thymoma without overt cytological atypia have better prognosis (Yoneda et al., 2000), but exceptions from this rule were also reported. The grading system largely accepted now is in fact a compromise: we have not a better one! Therefore, the grade was in part overlapped on the pathologic diagnosis. Actually, the histologic grade of thymoma in its present form has any impact on prognosis or therapy.
Clinics and pathology

Pathology

Pathologic diagnosis
- Thymoma type A (spindle cell, medullary) consists of cells that are spindle or oval in shape, without atypia, and lymphocytes are rare or even absent. The pattern of proliferation is focally storiform, and may contain rosette-like structures and pseudo-glands close to the capsule. Hassall's corpuscles are rarely found. Nuclei of tumor cells have fine granular chromatin with small nucleoli. Not all cells are spindles in shape. Some of them may be oval and form the large majority of the cell population. More than 95% of thymoma type A are encapsulated,
but some of them may invade the capsule, or vary rare, the lung. Exceptionally were reported cases with such a thymoma with atypical cells, mitotic activity and necrosis. It is often difficult to classify such a case: undifferentiated thymoma type A? Spindle cell variant of thymoma type B3? Or sarcomatoid carcinoma? Until now, there are no data to help us. The presence of a reticular fiber network around individual cells and its absence around perivascular spaces would be in the favor of thymoma type A, but this observation was not enough investigated to be used. There were described some "unusual" forms of thymoma type A: with pseudo-sarcomatous stroma, haemangiopericytic, with some "unusual" forms of thymoma type A: with spindle cell variant.

- **Thymoma type AB** (mix) is characterized by type A areas and lymphocyte-rich areas. Between the two different patterns of the tumor may be a clear-cut edge or the transition between them is gradual. Frequently, type A area is reduced, but it has the same aspects as described above. The term "mix" is used to draw attention on the dual cell population: neoplastic epithelial and non-neoplastic lymphocytes.

- **Thymoma type B1** (lymphocyte-rich, lymphocytic, cortical) is similar to the normal functional thymus and consists of areas that are close in structure with the cortex (that predominate) and medulla. Differentiation from the normal thymus may be impossible at low power magnification. Medullary differentiated areas are usually round and may be erroneously interpreted as germ center; on occasion, they may contain aggregates of epithelial cells or Hassall bodies. Perivascular spaces are rarely found and are less prominent than in other forms of thymoma.

- **Thymoma type B2** (cortical) consists of isolated or in small groups arranged epithelial cells and many lymphocytes. Usually, perivascular spaces are numerous and large. Occasionally, epithelial cells are arranged in palisade around perivascular spaces. Foci of medullary differentiation are less evident (with or without Hassall corpuscles) or they are absent. Epithelial cells are polygonal in shape (thymoma with large polygonal cells), more numerous than in thymoma type B1, they have nuclei with fine granular chromatin, prominent nucleoli, and rich cytoplasm. Lymphocytes may be immature, with large nuclei, visible cytoplasm and high mitotic activity (Ki67 index over 80%). Opposite to thymoma B1, this tumor does not recapitulate the differentiation of the normal thymus.

- **Thymoma type B3** (epithelial, atypical, well differentiated thymic carcinoma) consists of epithelial cells that are round or polygonal in shape. Atypical elements are mild or absent, but the epithelial component proliferates in large sheets and lymphocytes are reduced in number. Epithelial cells have small nucleoli and mitotic figures are rare. The tumor preserves some characters of thymic differentiation: lobulation, dual cell population, and perivascular spaces. The perivascular arrangement of epithelial cells and squamous differentiated areas are frequently found. This tumor is more frequently invasive than "conventional" thymoma, but it may co-exist with thymoma B1, B2 or C.

There were described some unusual variants of thymoma with polygonal and round cells: microcystic, cystic, cribriform, with clear cells, rich in plasma cells, with myoid cells and starry sky. The classification of these forms is often based on the expression of monoclonal cytokeratin to differentiate them from thymic carcinoma with similar features.

- **Pathology of thymoma after the preoperative treatment with corticosteroids.** Corticosteroids are well-known inducers of thymic involution. It was noticed that preoperative administration of corticosteroids significantly reduces the diameter of the tumor. There are few studies on this subject, but data are extremely useful for pathologist. There are significant microscopic differences between the biopsies before and after the treatment with corticosteroids. Tateyama et al. (2001) noticed reduction of the tumor mass between 5 to 70%, depending the dose and length of the treatment. The predominance of proliferating epithelial cells may change. In some cases the dominant cell population becomes spindle in shape, and in others were noticed glandular-like or haemangiopericytoma-like structures. Atypical cells were rare or absent and many epithelial cells had acidophilic cytoplasm and condensed nuclei. The number of lymphocytes dramatically decreased, some of them showing fragmented nuclei. In tumors with massive regression extensive fibrosis, foamy macrophages, and necrosis are frequently noticed. Perivascular palisade of epithelial cells, cystic structures, and bizarre multinucleated giant cells, or giant cells with lobulated nucleus may be noticed in some cases. In all cases published until now it was observed the transformation in thymoma with epithelial predominance.

The presence of degenerative lesions does not necessary means regression of the tumor. It is thought that epithelial cells degeneration is a consequence of depletion in immature T lymphocytes.
In these conditions, the knowledge of preoperative medication is essential to avoid a possible confusion with well-differentiated carcinoma (as defined in Marino-Muller-Hermelink classification).

- **Atypical thymoma: a distinct entity?** Based on the difficulties in the classification of thymoma, Suster and Moran (1999) proposed the term "atypical thymoma". In this category were included all the cases that preserve some organo-typical characters of thymic differentiation, but associated with a given grade of cell atypia. The cell population is predominant epithelial, with the tendency to squamous transformation at the level of both architecture and individual tumor cells. Frequently, tumor cells are arranged around perivascular spaces, mimicking glandular differentiation. This lesion is equivalent to the "well differentiated thymic carcinoma" from Marino-Muller-Hermelink classification. Atypical thymoma is more frequently invasive than the conventional thymoma and can be found in association with typical areas of thymoma or thymic carcinoma. Although the existence of this subtype is supported by some data, until now atypical thymoma was not included in WHO's classification.

- **Thymic carcinoma (former thymoma type C)** is a rare tumor with poor prognosis in comparison with thymoma (Tomita et al., 2002), and is defined as an epithelial proliferation whose individual cells show clear characters of malignancy. Opposite to conventional thymoma, it is not associated with myasthenia gravis. The diagnosis is usually by exclusion, because there are not clear criteria to differentiate this tumor from carcinoma developed in other organs. The thymic carcinoma shows no character of thymoma (perivascular spaces, abortive Hassall bodies, a/o). The only major difference is the positive immunoreaction for CD5 in epithelial cells (this immunoreaction is negative in typical thymoma and non-thymic carcinoma). Lymphocytes may be present, even in large number, but always they are mature T (or rarely B) in type. The most common form is the squamous cell carcinoma (90% of cases), but there were reported many other variants: lymphoepithelioma-like, sarcomatoid, clear cell, basaloïd, papillary, and anaplastic (Snover et al., 1982; Iezzoni and Nass, 1996; Shimosato and Mukai, 1997; Matsuno et al., 1998). The prognosis of thymic carcinoma largely depends of the pathologic variant (better in cornified squamous cell carcinoma).

CD70, a member of the TNF family, is expressed by many cases with thymic carcinoma and usually is negative in thymoma type B3, and therefore, the method is useful for the differential diagnosis (Rosai, 2004). Tumor cells also express Ki67 and p53, but the prognostic relevance of these markers is not yet clarified.

Differential diagnosis of thymoma and related tumors may represent itself the topic of a review: it includes malignant lymphomas, carcinoid, neuroendocrine carcinoma, germ cell tumors, stromal tumor, and cervical tumors with thymic derivates. Involvement of the thymus should be also considered, particularly in the case of small specimens taken by endoscopic procedures. In this condition, aggregates of epithelial cells can be over-interpreted as thymoma (Raica et al., 2007). Actually, an extensive immunophenotyping is frequently needed. It is important to mention here especially cervical tumors with thymic derivate that include cervical ectopic thymoma, hamartomatous thymoma, spindle epithelial tumor with thymus like elements (SETTLE) and carcinoma with thymus like elements (CASTLE).

The ectopic cervical thymoma has a clear preference for female, a benign behavior and the same features as the mediastinal counterpart. Ectopic hamartomatous thymoma occurs mainly in males, supraclavicular or suprasternal, and consists of spindle shaped cells with mesenchymal aspect. The tumor lacks atypical aspects, necrosis and mitotic activity. One component of the tumor that may be
noticed only focal consists of solid squamous nests, anastomosed cords and cysts lined by epithelium. Small aggregates of adipose cells are found between neoplastic cells. Lymphocytes are scant, but present in all cases. Some authors reported a concentration of myoid cells, but their significance is unknown. Despite such a tumor can rich large diameter, it has not a corresponding tumor in the mediastinum and is benign (Zhao et al., 2000).

Spindle epithelial tumor with thymus like elements (SETTLE) is rare, occurs in younger and develops around the thyroid. It is a biphasic tumor, one component is spindle and shows mitotic activity, and the other is cystic glandular. The natural evolution is slow, and metastasis was reported after years or even decades.

Carcinoma with thymus like elements (CASTLE) also has the tendency to be present around or even within the thyroid. On the basis of conventional pathologic diagnosis, it cannot be differentiated from thymic carcinoma. Despite local recurrences are frequent, the long-term prognosis is good.

**Genes**

**The molecular profile of thymoma**

The immunohistochemical profile of epithelial cells is wide, and best studied are, of course, cytokeratins. They also express the epithelial membrane antigen and carcino-embryonic antigen. Many data from the literature revealed the value of cytokeratin 8/cytokeratin 18 and high molecular weight cytokeratin for the diagnosis of thymoma (Encina et al., 2004).

The immunohistochemical expression of cytokeratin is heterogeneous, excepting for cytokeratin 19 and high molecular weight. On the other hand, cytokeratin 7 is positive in many cases, but it stains only few neoplastic epithelial cells. This is why it is strongly recommended to use a pan-cytokeratin that contains clones 7, 8, 18 and 19. In such an instance, the immunoreaction is intense and homogeneous, excepting for spindle cell thymoma. The immunohistochemical method is useful not only in the diagnosis, but also in the classification of thymoma. The epithelial membrane antigen is usually expressed only in pseudo-glandular area. Collagen type IV and laminin are found in large amount around individual spindle cells. It is well known that thymoma-associated lymphocytes are non-neoplastic, and specific immunophenotyping is not necessary. Many T cells have not the characteristic profile for mature lymphocytes and express Ki67 antigen. Thymoma also contains a S100 protein positive cell population that consists of interdigitate, and with lesser extent, Langerhans cells. Their number and distribution correlates with the microscopic variant, but their presence is not predictive for invasion. A subset of interdigitate cells that co-express CD20 are called “asteroid”, and their significance is unknown. Collagen IV and laminin are found in large amount around individual spindle tumor cells but only a slight expression of these molecules is noticed in thymoma with polygonal or round tumor cells. Somehow strange, epithelial cells from thymoma and thymic carcinoma express high levels of somatostatin receptor, but on this finding is based the scintigraphic detection of mediastinal tumors or thymoma recurrence.

Vascular endothelial growth factor (VEGF) was less investigated in the normal thymus and thymoma. The immunohistochemical expression of VEGF is strong in the prenatal thymus and in the majority of cases with thymoma (Cimpean et al., 2008). Virtually, all thymomas type B are positive, with the strongest intensity in type B3 and thymic carcinoma. In experimental model it was shown that gene targeting of VEGF-A in thymus epithelium disrupts the blood vessel architecture (Muller et al., 2005). Extensive studies on large series of patients could support the introduction of antiangiogenic therapy in cases with aggressive tumors of the thymus. Besides VEGF, other growth factors involved in tumor-associated angiogenesis were detected in thymoma neoplastic cells, like platelet-derived growth factor and its cognate receptors (Cimpean et al., 2011). Their clinical significance for tumor progression and metastasis of thymoma is still uncertain.

Epidermal growth factor receptor (EGFR) is expressed in over 60 to 70% of thymomas (Henley et al., 2002), but from these, only half showed correlation with gene analysis. EGFR gene mutations were not detected in thymoma or thymic carcinoma (Suzuki et al., 2006). EGFR immunohistochemical expression did not correlate with conventional prognostic factor, but by detection by fluorescent in situ hybridization it was found a significant correlation with the type, invasion and clinical stage. EGFR immunoreactivity was associated with more aggressive thymoma types B2 and B3, but a definite correlation with thymic carcinoma was not established (Aisner et al., 2010). Based on this data, EGFR expression may be considered as an individual prognostic factor and a possible target for therapy.

Other molecular markers were also investigated to predict the outcome of thymoma, like c-kit or HER2 protein. It was found that c-kit is expressed by tumor cells in 38% of the cases with thymoma type B and the negative reaction is associated with better prognosis (Aisner et al., 2010). C-kit is expressed by 80% of the cases with thymic carcinoma, and together with CD5 positive reaction, it is a useful diagnostic tool (Nakagawa et al., 2005). The expression of c-kit in thymic carcinoma and invasive thymoma deserves further investigations to establish a target-based therapy.

Recently, a molecular analysis of 34 patients with thymoma showed a differential expression of the genes related to both metastasis and stage and there were identified some potential candidates for the therapeutic strategy (Badve et al., 2012).
Prognosis

On one hand, the microscopic diagnosis of thymoma is based on the architecture of the tumor (fibrous bands, perivascular spaces, lack of lobulation, lack of differentiation between cortex and medulla), morphology of epithelial cells, and the presence of a dual cell population. On the other hand, regarding the prognosis, the morphologic diagnosis recognizes invasive and non-invasive thymoma. It is well known the long-term prognosis in thymoma type A and AB, and the malignant behavior of thymoma type C. In the case of thymoma type B the border between benign and malignant is far to be fully characterized. Actually, the invasion of the capsule is the only accepted microscopic marker of an aggressive tumor. The invasion of the capsule has two stages: invasion without penetration and invasion with penetration and invasion of the adipose tissue around the thymus (Hiroaki et al., 1999). Invasion must be checked on many step sections, because there were described cases with apparent intact capsule but containing neoplastic epithelial cells in capsular veins. Thus could be explained 15% of local recurrences in apparent encapsulated thymoma.

Taken separately, anyone of these markers (excepting for the invasion of the capsule) may define the prognostic status in tumors of the thymus. This is why there are necessary additional studies on large series of patients with long-term follow-up.

The overall survival of patients with thymoma for all stages is 67% after 5-years follow-up (stage I and II 95-100% at 5 years and stages III and IV 33% at 3 years) (Schmidt-Wolf et al., 2003). Studies performed on large series of patients showed no differences in overall survival between stages I and II, and it is suggested that capsular invasion should not be used to define the stage (Asamura et al., 2004). Another critic of the actual stage III is given by the lack in defining the degree of extension. The solution could be the aggregation of stage I and II, and splitting the stage III based on the number of invaded organs. Therefore, the prognosis should be better reflected in survival and in accord with long-term overall survival.

References


This article should be referenced as such: