THYMUS AND THYMOMA: WHAT'S NEW?

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Summary. The thymus is the prototype of lymphoid and epithelial organ that consists of lymphoid and epithelial cells. In spite of remarkable progresses made in the field of the immunohistochemical characterisation of the thymus parenchyma, the diagnosis of thymoma largely depends on the interpretation of conventional morphologic aspects. Histogenesis of this organ is a multi-step process, and many stages reproduce lesions and changes found in the adult thymus. The normal structure and its variants are extremely helpful to differentiate normal from pathologic aspects. Particular aspects of the thymus structures were shown in myasthenia gravis, despite the behaviour of thymoma in these patients is not clearly understood. Authors performed a detailed description of the conventional pathology of the thymoma, based on the new classifications, recently adopted. The immunohistochemical profile could be helpful in the diagnosis of many cases, and also seems to be useful in prediction of invasion that is the most important criterion in prognosis.

Key words: thymus, thymoma, histogenesis, pathology grade, immunohistochemical profile.

INTRODUCTION

The thymus is the prototype of lymphoid and epithelial organ that consists of lymphoid and epithelial cells. In addition, it contains a relatively large spectrum of cells that are mesenchymal in origin, neuroendocrine cells, and probably, germ cells. In spite of remarkable progresses made in the field of the immunohistochemical characterization of the thymus parenchyma, the diagnosis of thymoma largely depends on the interpretation of conventional morphologic aspects. The immunohistochemical diagnosis is important nowadays specially in classification of thymoma, characterization of other specific tumors of the thymus, and in research.

HISTOGENESIS AND DEVELOPMENT

The thymus derives from the third brachial pouch and possible there is a minor contribution of the fourth. In the 6th week of pregnancy, the endoderm of the

third pharyngeal pouch form a pronounced invagination that finally is detached from the wall of the primitive pharynx and forms the thymic primordia. There are proofs to support the involvement of an ectodermic component in the development of the thymus. The thymic primordia migrate caudal and medial together with inferior parathyroid glands. In the 8th week the primordium growths, forming two epithelial structures that fuse on the median line and occupies the final position in the anterior and superior mediastinum.

The cranial extremity becomes thin during migration, it is then fragmented and, in normal conditions, these fragments are resorbed. In some cases islands of thymic tissue may persist in soft tissues of the neck, around parathyroids, or even in the thyroid parenchyma. As the migration came to an end, epithelial cells become stellate and arranged as a network. The surrounding mesenchymal tissue is condensed to form the capsule, and trabeculae that impart the parenchyma into lobules. In the 10th week, fetal liver-born and bone marrow-born small lymphocytes populate the thymic parenchyma that differentiate into cortex and medulla (Hale, 2004). Approximately at the same time occur small tubular structures lined by cuboidal epithelial cells that form Hassall's corpuscles. Classic data showed that the thymus growths until puberty, and after that involutes and persists as an "atrophic" organ in the adult.

SELECTION OF THYMOCYTES

First precursors of thymic lymphocytes were demonstrated to be triple negative cortical lymphocytes (CD3, CD4, CD8) that express a marker of pluripotent human stem cells (CD34). These cells proliferate and differentiate under the influence of the endodermic epithelium. The large majority of newly formed cortical lymphocytes degenerate by apoptosis. During histogenesis, T cells are permanently submitted to positive and negative selection. By negative selection degenerate auto reactive T cells, with damaged DNA or with metabolic changes. Disturbances of apoptosis might be responsible for the presence in the bloodstream of auto reactive lymphocytes. T cell receptor (TCR/CD3) plays an important role in selection. During differentiation, rearrangement of TCR genes induces the unique, clonal expression of receptors. Potential auto reactive T cells that express TCR with high affinity for self-degenerate by negative selection. T cells that express TCR with low affinity survive, and the process is known as positive selection. Experimental data demonstrated that activation of the CD3/TCR complex induces the preferential elimination of immature CD4+/CD8+ thymocytes.

DIFFERENTIATION AND NORMAL PROLIFERATION OF EPITHELIAL CELLS

After the formation of the supporting network, epithelial cells continue to growth. The expansion of epithelial cells requires the continuous presence of the

mesenchyme. In contrast, differentiation of immature thymic epithelial cells, including acquisition of markers of mature cortical and medullary epithelium, occurs in the absence of ongoing mesenchymal support (Jenkinson *et al.*, 2003). Recently, it was demonstrated the existence of thymic epithelial stem cells (TESC), but only in some rodents.

Until now, the presence of TESC in the normal human thymus has not been documented yet. Epithelial progenitor cells constitute 30% of the cell population of the thymic primordium and seem to be enough to generate the thymus *in vivo*. This cell population is identified in mice by two antibodies: MTS20 and MTS24 (murine thymus). MTS20 and MTS24 positive cells may differentiate in all types of thymic epithelial cells and attract lymphoid progenitors. TESC persist in the adult thymus and it is thought that they are necessary for the maintenance and regeneration of the organ (Blackburn and Manley, 2004).

Present data are supported by observation on cell lines from thymoma that spontaneously differentiate into both cortical and medullary epithelial cells. Nowadays, there are no data on the existence of TESC, their immunocytochemical profile, ultrastructure and distribution in the adult normal thymus, neither on their involvement in the histogenesis of malignant tumors of the thymus. On the other hand, many malignant lesions of the thymus recapitulate developmental stages, and this is why the knowledge of development is so important in the routine diagnostic. Also, there are important the histological variants, mainly in the case of involution.

HISTOLOGY

The thymus is an encapsulated and lobulated organ. Trabeculae consist of dense irregular connective tissue that support blood and lymphatic vessels, and nerve fibers. The basic unit of the thymus parenchyma is the lobule with an outer cortex and inner medulla. Both consist of T cells and epithelial cells, but the rate between them is different. The thymus is the only organ that has an epithelial stroma, and depending their location, epithelial cells are of cortical and medullary type. Epithelial cells send many cytoplasmic processes that form desmosomes with neighboring cells to form a diffuse network. The epithelial nature of these cells is best demonstrated by immunohistochemistry, because all express intensely cytokeratin. Moreover, some subtypes co-express markers that are characteristic for neuroendocrine cells (chromogranin A, thymosin, thymopoietin, a/o). Perivascular and subcapsular epithelial cells form the blood-thymus barrier that is restricted to the cortex (Suster and Rosai, 1997). The barrier maintains T cells in "innocent" stage along their differentiation. A subset of epithelial cells located deep in the cortex is called "nurse" cells. They have abundant cytoplasm that includes many mature lymphocytes. Nurse cells realize a specialized medium for maturation, differentiation, and selection of lymphocytes.

Hassall's corpuscles are found only in the medulla, as round or oval, intensely acidophilic structures. Epithelial cells are concentrically arranged and contain variable amount of keratin with high molecular weight. Frequently, in the central area epithelial cells are degenerated, and so, the Hassall's bodies may be cystic, calcified or necrotic. A marked cystic transformation is often found in congenital syphilis and this lesion is known as Dubois abscess. Many multilocular thymic cysts are the result of cystic dilation of Hassall's bodies, due to inflammatory conditions. Sometimes in the corpuscle may be noticed ciliated or goblet cells, as a result of their endodermic origin. Thymic lymphocytes form the largest part of the cell population of the cortex. Mitotic active lymphoblasts (15%) are more numerous close to the capsule. Small lymphocytes with reduced mitotic activity are found in the cortex and they are rarely found in the medulla.

In the thymus of children the incidence of CD4+ CD8+ T cells is 80 to 85%, but their number dramatically decreases. In the deep cortex lymphocytes degenerate and residual bodies are found in the cytoplasm of macrophages ("starry sky" aspect). Lymphocytes are characterized by marked immunophenotypical heterogeneity. Depending the degree of maturation, lymphocytes are classified as subcapsular, cortical, and medullary, and sequences of their maturation are shown in Table 1.

Table 1
Sequences of T cell maturation and immunophenotype

Stage	Positive markers	Significance
I	CD5, CD8, CD4, CD3, CD2, CD1, CD7	Large thymic blasts (0.5–5%)
II	CD5, CD8, CD4, CD3, CD2, CD1, CD7, CD14, CD38	Common cortical thymocytes (60–80%)
III	CD5, CD3, CD2, CD7	Mature medullary thymocytes

OTHER CELL TYPES IN THE PARENCHYMA OF THE THYMUS

Besides epithelial cells and lymphocytes, the parenchyma of the normal thymus contains a large variety of cells, including neuroendocrine and germ cells. Their distribution and significance (if known) is shown in Table 2.

Table 2
Distribution and significance of non-epithelial non-T cells of the thymus

Cell	Distribution	Significance
B lymphocyte	Medulla, perivascular space	Lymphoid follicles may be present
Asteroid cells	Medulla	Aggregates of B an T lymphocytes
Macrophages	Predominant in the cortex	Phagocytosis
Interdigitate	Medulla	Antigen presenting cells
Langerhans	Medulla	Antigen presenting cells
Eosinophilic granulocyte	Medulla, connective septa, Hassall body on occasion	Frequently found in children, rare in adult

Table 2   (continued)

Mast cell	Perivascular space, septa	Unknown
Plasma cell	Medulla	More numerous in involution
Endocrine	Medulla	Rare in the normal thymus
Myoid	Medulla and junction with the cortex	Characters of striated muscle cells, desmin positive
Germ cell	Medulla?	Not yet demonstrated in human. Unknown significance*

*Despite that germ cells were not demonstrated in the normal human thymus, all germ cell tumors of the mediastinum arise in the thymus. We must look better to the normal!

INVOLUTION OF THE THYMUS

The thymus involutes with aging, the cortex becomes thinner and parenchyma is partially replaced by adipose tissue. It was thought that this process begins at puberty, but it is known now that the relative volume of the thymus decreases even in the mid-childhood. In the adult are found large masses of adipose tissue with scattered islands of thymic tissue that consist of epithelial cells and few lymphocytes.

Early involution is characterized by the decrease of cortical T cells without significant changes in the medulla. In late involution there is a marked regression of cortical T cell, epithelial cells and they are replaced with adipose cells (Figure 1). Therefore, the remnant thymic parenchyma consists of islands or cords of epithelial cells that occasionally include lymphocytes. Hassall's corpuscles (mainly with cystic transformation) are usually present.

A transient involution of the thymus is noticed in the newborn and lasts for 20 to 30 days. It is believed that such a physiologic involution is the effect of the prolonged exposure of the fetal thymus to maternal corticosteroids (Varas *et al.,* 2000; Wick, 2001). The same mechanism, but associated with the presence of progesterone receptor in the epithelial cells of the thymus, seems to be responsible for the involution induced by stress.

As a matter of fact, it is very important to differentiate between the late phase of involution, with islands of spindle or cuboidal epithelial cells arranged in small islands or cords, from a metastasis of a carcinoma. Sometimes the differential diagnosis may be difficult because of the presence of tubular or pseudoglandular structures that reproduce some stages of the development.

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. 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 immunophenotype 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 then 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 remnant thymic parenchyma.

THYMOMA

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 thymoma 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%.

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 (specially 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 is against 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.

In electron microscopy, 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 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 8/18 and high molecular weight cytokeratin for the diagnosis of thymoma. The cytokeratin profile of thymoma is shown in Table 3.

Table 3
Cytokeratin profile of thymoma

Thymoma cell type/CK	7	8	10	13	14	18	19	20	34βE12
Spindle	+	+	-	-	+	+	+	-	+/-
Small polygonal	-	-	-	-	+	-	+	-	+
Mix	+	+	-	-	+	+	+	-	+
Organoid	+	+	+	+	+	+	+	-	+
Large polygonal	+	-	-	-	-	-	+	-	+
Squamoid	+	-	+	+	-	-	+	-	+

CK – Cytokeratin (after Kuo, 2001, modified)

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 in 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.

CLASSIFICATION OF THYMOMA

It is one of the most "elusive" in general pathology. 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, it is the classification proposed by Lattes and Bernatz that takes 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 why many pathologists prefer the classification proposed by Juan Rosai. A comparison between these two classifications is shown in Table 4.

Table 4

Comparison between classifications proposed by Rosai and Muller-Hermelink

Rosai (adopted by WHO) (2004)	Muller-Hermelink (1999)
Type A thymoma	Medullary thymoma
Type AB thymoma	Mix thymoma
Type B1 thymoma	Predominantly cortical thymoma
Type B2 thymoma	Cortical thymoma
Type B3 thymoma	Well differentiated thymoma
Type C thymoma	Thymic carcinoma

Legend: A from atrophic, B from benign, C from carcinoma

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. 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, as we can notice in Table 5. Actually, the histologic grade of thymoma in its present form has any impact on prognosis or therapy.

Table 5
The grading system of thymoma

Tumor type	Grade	Histologic criteria
Thymoma (typical)	Well differentiated	Differentiation similar to normal parenchyma: preserved Without atypical cells
Atypical thymoma	Moderate–well differentiated	Differentiation similar to normal parenchyma: preserved Slight-moderate atypical cells
Thymic carcinoma	Undifferentiated	Differentiation similar to normal thymus: lost Presence of atypical cells and other criteria of malignancy

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 (Figure 2). 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 rosettes, micronodular,

basaloid, with papillar and glandular structures. Anyway, we do not know yet what means "usual" thymoma type A.

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 is 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 (Figure 3, a and b).

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 (Figure 4, a and b). 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 (Figure 5). 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 Muller-Hermelink classification).

Thymic carcinoma (*thymoma type C*) 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 (Figure 6).

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, basaloid, 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).

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.

Actually, an extensive immunophenotyping is frequently needed. It is important to mention here especially cervical tumors with thymic derivates that include cervical ectopic thymoma, hamartomatous thymoma, SETTLE and 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 concentration of myoid cells, but their significance is unknown. Despite such a tumor may 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.

PROGNOSIS

On one hand, the microscopic diagnosis of thymoma is based on the architecture of the tumor (fibrous bands, perivascular spaces, lost 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. Other markers, suspected to be useful in the prognosis of thymoma, are summarized in Table 6.

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.

Table 6
Markers with potential value in the prognosis of thymoma

Author	Marker	Comments
Encică *et al.*, 2004	34βE12	Strong expression in invasive thymoma, weak positive or negative in thymoma type A and AB
Chen *et al.*, 1999	p53	10 to 20% noninvasive thymoma are weak positive >50% of invasive thymoma are moderate positive
Chen *et al.*, 1999	Bcl-2	Non-invasive thymoma are negative Invasive thymoma and thymic carcinoma are positive
Hiroshima *et al.*, 2002	Ki67	High index in thymic carcinoma, positive correlation with p53 in thymoma type B3
Tomita *et al.*, 2002	MVD	Few blood vessels in thymoma type A and AB; high MVD in the periphery of thymoma type B and thymic carcinoma predicts invasion
Kuo, 2000	ChrA	Many positive cells predict unfavorable prognosis of thymic carcinoma; usually absent in conventional thymoma. No enough data to be introduced in routine practice

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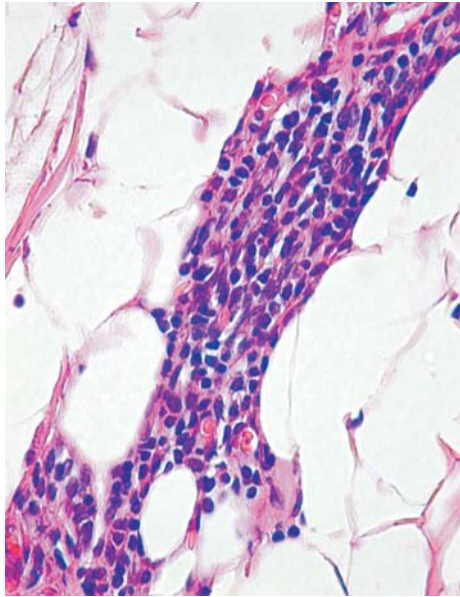
Figure 1 – Thymus of involution. Island with epithelial cells and lymphocytes included in adipose tissue (H&E staining method)
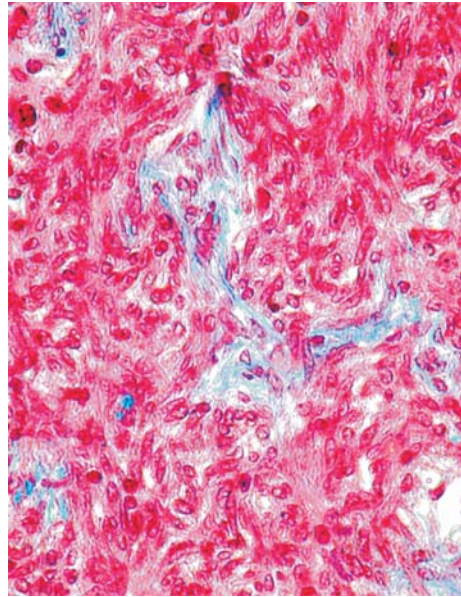

Figure 2 – Thymoma type A. Predominance of spindle-shaped cells (Masson trichrome staining method)
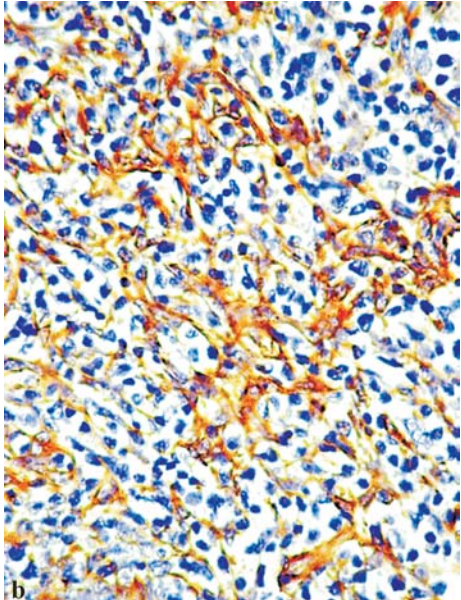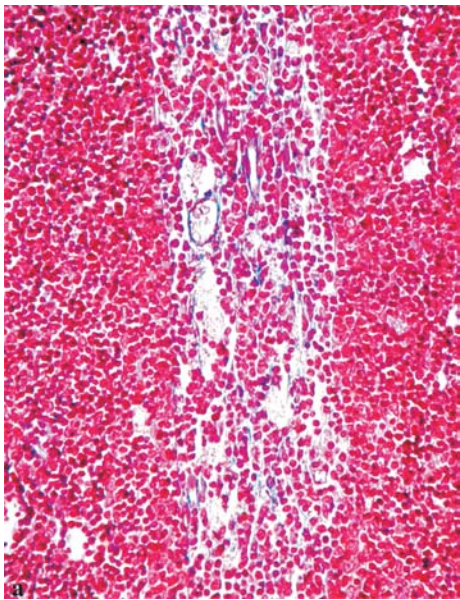

Figure 3 – a) Thymoma type B1. The tumor reproduces the architecture of the normal thymus (Masson staining); b) Epithelial cells are arranged in network that contains many lymphocytes. Immunohistochemistry for high molecular weight cytokeratin
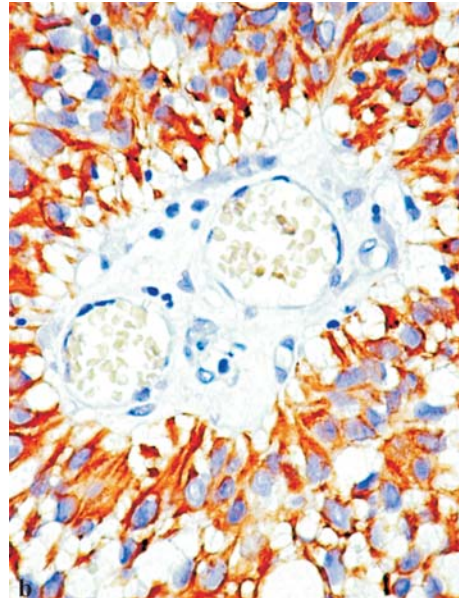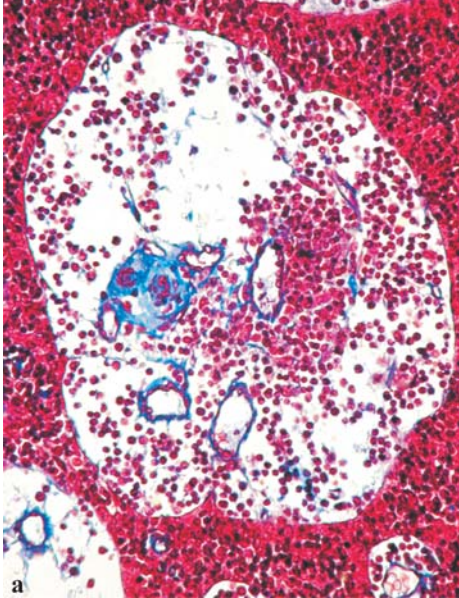
Figure 4 – a) Thymoma type B2, with large perivascular space containing lymphocytes,
red blood cells and macrophages; b) Palisade arrangement of epithelial cells
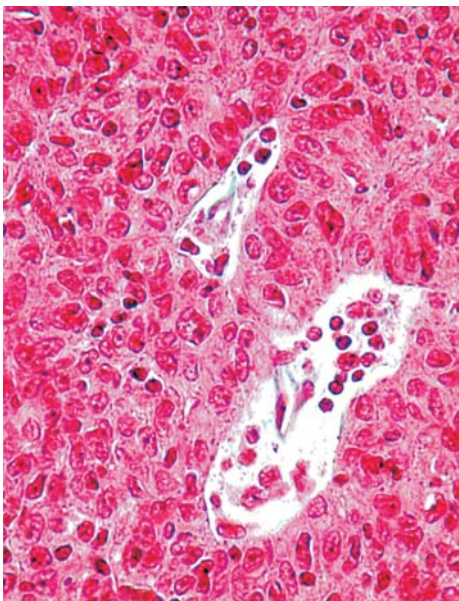around the perivascular space, high molecular weight cytokeratin

Figure 5 – Thymoma type B3. The proliferation
includes small perivascular spaces, and
epithelial cells show mild atypical
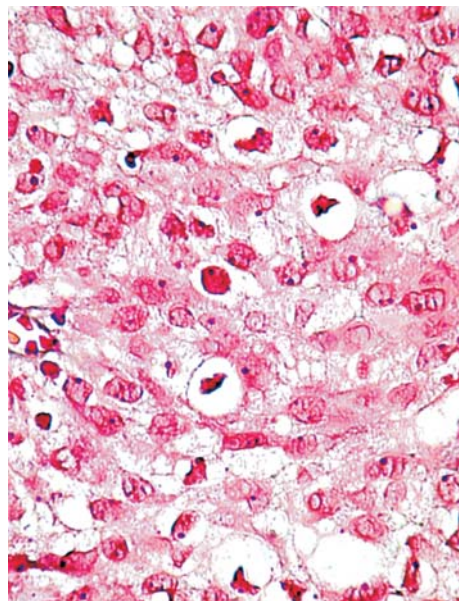features (Masson trichrome method)

Figure 6 – Thymic carcinoma with clearly
atypical proliferating cells (Masson
trichrome staining method)