ORIGINAL PAPER

Involution of the thymus: a possible diagnostic pitfall

M. RAICA[1)], ANCA MARIA CÎMPEAN[1)],
SVETLANA ENCICĂ[2)], R. CORNEA[3)]

[1)]*Department of Histology and Cytology,*
"Victor Babeş" University of Medicine and Pharmacy, Timisoara
[2)]*Department of Pathology,*
"Niculae Stăncioiu" Heart Institute, Cluj-Napoca
[3)]*Department of Pathology, County Hospital No. 1, Timisoara*

Abstract

There were investigated 22 cases from which the thymic tissue was removed either during surgery for cardiovascular malformations (n = 14), or for myasthenia gravis (n = 8). Histological sections were stained with routine morphologic methods, and immunohistochemistry was performed for cytokeratin, CD20, CD3, and S100 protein. Aspects characteristic for thymus involution were found in 11 cases without myasthenia gravis and in all cases with myasthenia gravis. Morphological changes of the thymus of involution are age-dependent. There were characterized stages of involution, with special reference to cortical – medulla inversion, lymphocyte depletion and sequestration. In advanced-stage of involution, epithelial cells are arranged in cords or compact islands, and could mimic a thymoma or a metastatic carcinoma. The immunohistochemical profile is similar but not identical to the active thymus. We noticed a decreased expression of cytokeratin, and a reduced number of CD3, CD20, and S100 positive cells. Morphologic and immunohistochemical peculiarities of the thymus of involution are discussed in relation with the specific pathology of the organ.

***Keywords*:** thymus, involution, epithelial cells, immunohistochemistry, myasthenia gravis.

Introduction

The thymus plays a crucial role in the development of the immune system of the organism. With ageing, the thymus undergoes involution, the cortex of specific lobules becomes thinner and parenchyma is gradually replaced with adipose tissue. Initially, this process was thought to occur at puberty, but now it is known that the relative decrease in the volume of the thymus is noticed even in the childhood [1, 2].

In the adult human, the thymus consists of a mass of adipose tissue that contains some islands of parenchyma with epithelial cells and few lymphocytes [3, 4].

Involution of the thymus induced by ageing, also known as physiological involution, is a gradual process characterized by changes that lead to atrophy. This process is never complete, because small islands of thymus tissue maybe found even in individuals over 80 years old. The usual morphologic examination reveals the relative decrease in the number of T cells, accompanied by the involution of both cortical and medulla stromal epithelial cells.

In the early stage, it is noticed a significant decrease in the number of T cells of the cortex, without significant changes of epithelial cells that become prominent. Usually, in this stage there are not found significant changes in the medulla.

In advanced-stage there is found a marked decrease in the number of T cells and epithelial cells of the cortex, and the parenchyma is gradually replaced by adipose tissue [5].

Therefore, the remaining thymic parenchyma shows epithelial islands and thin cords that contain only few lymphocytes. In the islands there are found Hassall corpuscles located close each other, some of them with cystic transformation. Over 40 years it is found a significant decrease in the number of S100 protein positive cells, without significant changes in the number of epithelial cells [6].

Consecutive to these changes, the macroscopic parenchyma of the thymus cannot longer be recognized, and only the adipose tissue is visible. Step sections performed from the adipose tissue and microscopic examination may identify remnants of thymus parenchyma. Occasionally, the epithelial component of the involutedly thymus maybe small nodular (probably representing epithelial hyperplasia), and could create the false impression of a microthymoma or even a metastasis of a well-differentiated carcinoma [7]. In this paper, we investigated the peculiar aspects of the involution of the thymus, as a possible pitfall in the histopathologic diagnosis.

Material and methods

There were investigated 22 cases, with age between one day and 45 years, from which the thymus was removed during surgical interventions for cardiovascular malformations (n = 14) or with therapeutic intention in cases with myasthenia gravis (n = 8). Specimens were formalin-fixed paraffin-embedded using the standard histological technique.

Initial sections were stained with Hematoxylin–Eosin and trichrome Masson staining methods. Additional sections were used for immunohistochemistry and stained for the expression of high molecular weight cytokeratin (clone 34betaE12), CD20 (clone L26), CD3 (polyclonal), and S100 protein (polyclonal). Antigen retrieval was performed with microwave in citrate buffer pH6 (15, 5, 25, and 5 minutes, respectively).

The working system was LSAB2 for all antibodies, and the final reaction product was visualized with 3,3'-diaminobenzidine as chromogen in brown. Nuclei were stained with Lillie's modified Hematoxylin. After dehydration and clarification, sections were mounted with permanent medium. Examination was performed with Eclipse600 Nikon microscope, and images were captured with Coolpix950 digital camera.

Results

Characteristic aspects of involution of the thymus parenchyma were found in 11 from 14 cases without myasthenia gravis and in all cases with myasthenia. Microscopically, the involution was characterized by the presence of the thymic tissue organized as islands and cords surrounded by variable amount of adipose tissue (Figure 1a).

The diameter of the islands of thymus parenchyma was largely dependent on the age of the patient. Over 20 years, only small islands consisting of epithelial cells and lymphocytes were noticed. Frequently, the lobular architecture was lost. In these islands, we identified follicular hyperplasia on six from eight patients with myasthenia gravis; the follicles were large, show germinative center, and were located in the remaining medulla (Figure 2a).

We noticed elements that are characteristic for the involution even before puberty, in the perinatal period (the first month of life) and in two children 4 years old. These changes become more evident after 12 years, when the adipose tissue gradually replaces the thymus parenchyma. One of the first signs of involution is the cortical lymphocyte depletion that imparts the aspect of „lymphocyte inversion" (with relative higher density in the medulla). In such cases, the medulla tends to be more intensely stained than the cortex (Figure 1b).

Medulla is the last component of the thymus involved in this process, islands or cords with typical morphology being identified in cases with more than 40 years (Figure 2b). In many cases, the differences from the medulla of the active prepuberal thymus are minimal, excepting for the severe degenerative changes of Hassall corpuscles.

Lymphocyte depletion is often associated with focal concentration of epithelial cells, which may show two aspects. One variant is represented by the arrangement of epithelial cells as anastomosed cords that include smaller or larger groups of remnant lymphocytes (Figure 1c). As the involution progresses, epithelial cells form conglomerates that are oval or irregular in shape and contain just scattered lymphocytes (Figure 1d).

The epithelial cells of these islands are frequently spindle in shape, the nucleus has fine granular chromatin and the cytoplasm is acidophilic. Sometimes, epithelial cells may contain nuclei with coarse chromatin, irregular borders and differences in size from a cell to another, and they are called „pseudoatypical" (Figure 3). Such cells were found as isolated elements in thin cords of involution and they must not be confused with malignant cells.

The immunohistochemical profile of the involutedly thymus is similar, but not identical with that of the prepuberal active thymus. The intensity of the immunohistochemical reaction for high molecular weight cytokeratin was reduced, and restricted to the aggregates of epithelial cells (Figure 4a).

The number of CD20 positive cells was significantly reduced in the remaining medulla, with heterogeneous distribution around Hassall corpuscles (Figure 4b).

The immunoreaction for CD3 was intensely positive (Figure 4c), but the number of T cell from both cortex and medulla was reduced in comparison with the active thymus. S100 protein positive cells were rare in the medulla and Hassall corpuscles (Figure 4d), and absent in cases with age over 40 years.

The immunohistochemical profile was useful to characterize the so-called pseudoatypical cells, which maybe show nuclei with irregular border and shape (Figure 5).

Discussions

Involution of the thymus has not a linear regressive character, because in the neonatal human thymus is noticed a severe and transient $CD4^+$ $CD8^+$ lymphocyte depletion. The depletion is associated with functional changes of the other cell components [8].

This phenomenon is confirmed on preparations stained with conventional methods, which reveal a thin cortex with few $Cd1^+$ cells. Early precursors of T cells express CD34, but in newborn the majority (75%) is CD1 negative. The increase in the number of CD34 positive precursors signal out regeneration of T cells toward the end of the first month of life [9–11].

The involution of the thymus may be noticed even in the first day of the postnatal life and lasts for 20 to 30 days. The proportion of T cells found in apoptosis is 3 to 4 times higher in the newborn than in the child, and the majority are CD3 negative cells. Changes in the number of T cells are not accompanied by significant phenotypic changes of epithelial cells. At present time, the transient neonatal involution of the thymus is thought to be physiological, and occurs as a result of the prolonged exposure of the fetal thymus to maternal corticosteroids. From the second month of postnatal life, the number and immunophenotype of T cells are normal for the immunological active thymus [12].

Another involutive model, unrelated with ageing, is called accidental or stress induced involution. This model is frequently found in the case of a severe stress with direct effect on the thymus, and it is based on the release of large amounts of corticosteroids [13].

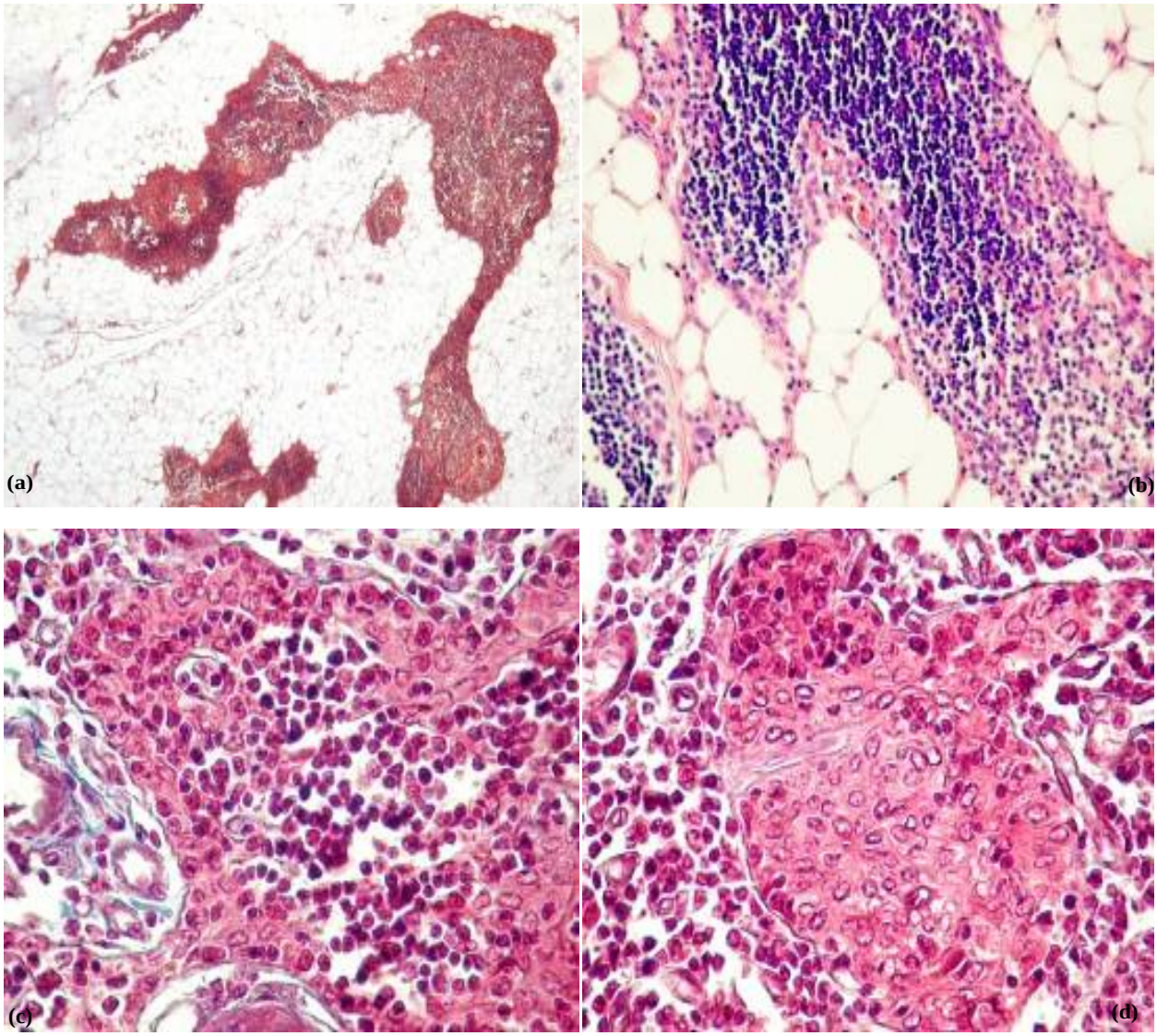

Figure 1 – ***Island of involuted thymic tissue surrounded by adipose tissue (Masson stain, ×400 – a). Inversion between cortex and medullar, with epithelial cells prominent in the periphery of the lobule (HE stain, ×200 – b). Lymphocytes sequestrated between cords of epithelial cells (Masson stain, ×400 – c). Island of epithelial cells surrounded by residual lymphocytes (Masson stain, ×400 –d)***

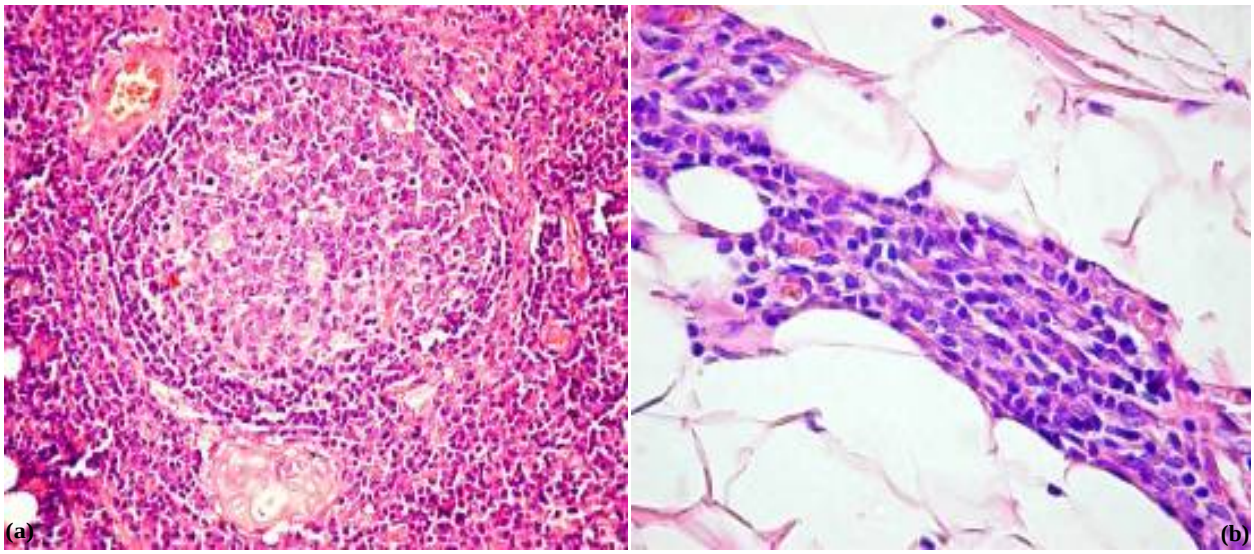

Figure 2 – ***Secondary lymphoid follicle in the thymus medulla, myasthenia gravis (HE stain, ×200 – a). Island of involuted thymic tissue, with arrangement characteristic for the medulla (HE stain, ×400 – b)***

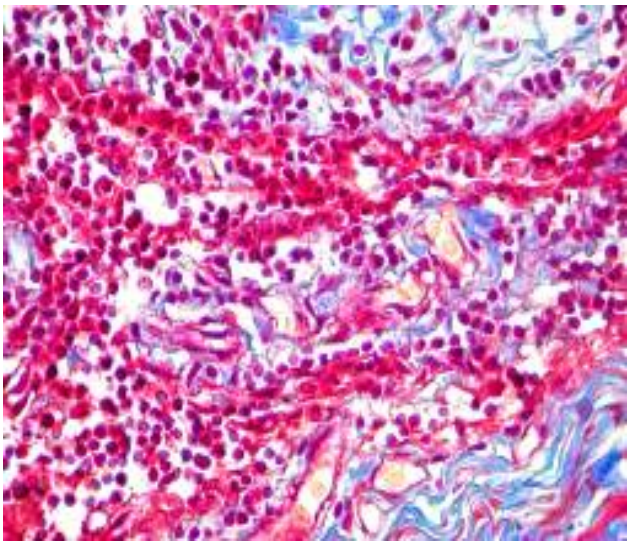

Figure 3 – ***Perivascular epithelial thymic cells (Masson stain, ×200)***

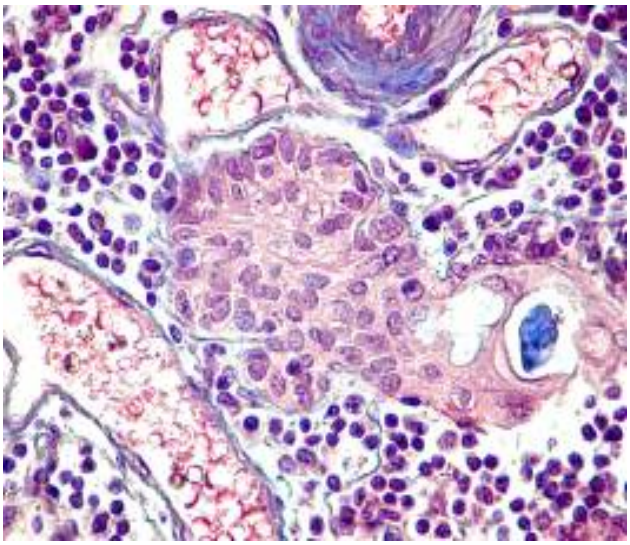

Figure 5 – ***Aggregate of epithelial cells without lymphocytes between them. Note the irregular borders of nuclei, the weak cytoplasm acidophilia (Masson stain, ×400)***

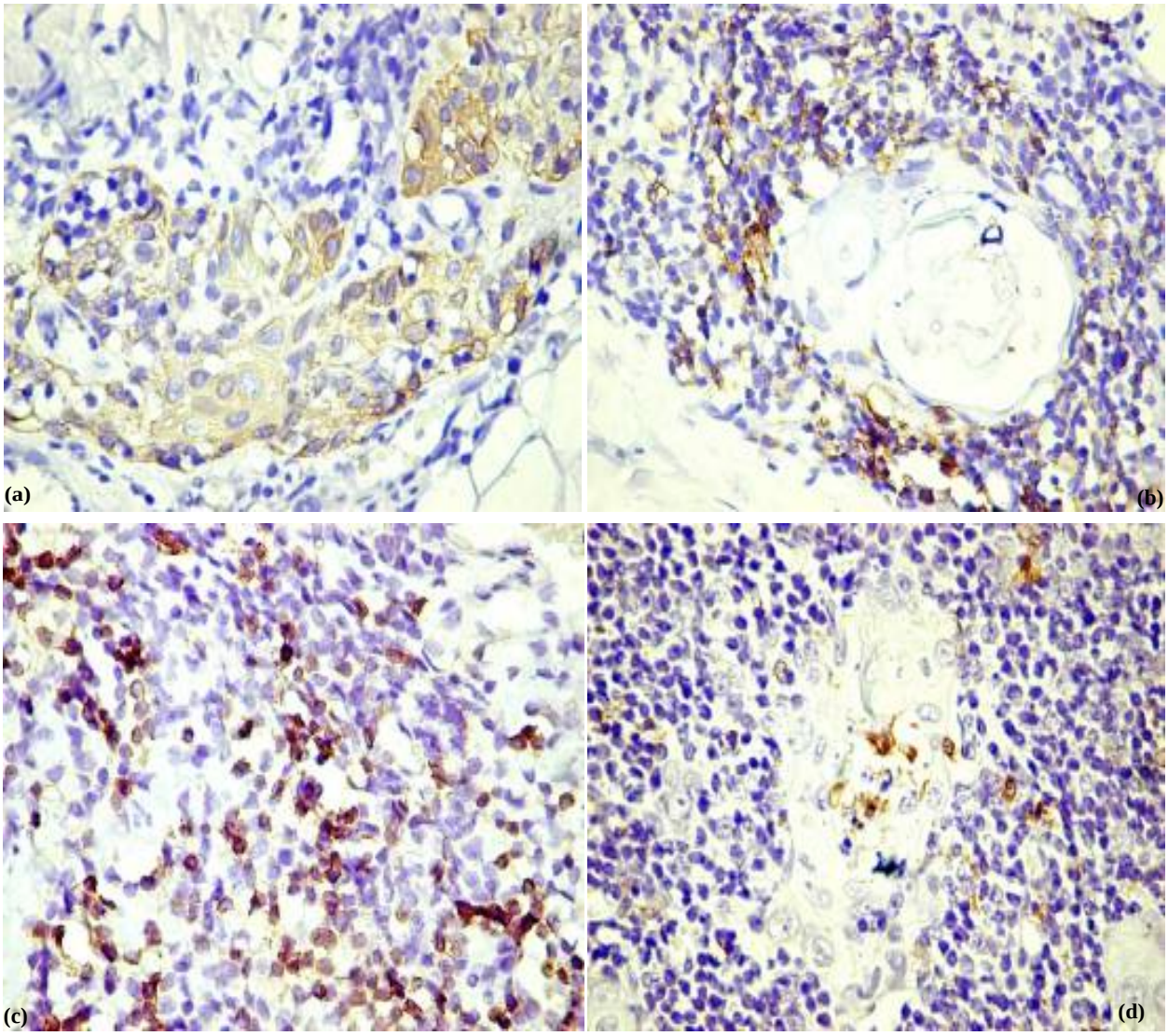

Figure 4 – ***Weak expression of cytokeratin in epithelial cords of the involuted thymus. Immunoreaction for high molecular weight cytokeratin 34betaE12, ×400 (a). Heterogeneous distribution of CD20 positive cells around Hassall corpuscle, ×400 (b). CD3 positive cells with heterogeneous distribution, ×400 (c). Rare antigen presenting cells within and around Hassall corpuscle. Immunoreaction for S100 protein S100, ×400 (d)***

Moreover, it was demonstrated that receptors for progesterone from epithelial cells of the thymus are involved in stress-induced involution, but not in the physiologic one.

As a consequence of high levels of corticosteroids, there is a massive decrease in the number of cortical T cells. Microscopic examination reveals many degenerated cells that ultimately are engulfed by macrophages. The relative high number of macrophages gives the well-known aspect called „starry sky". If the stimulus persists long enough, the border between cortex and medulla is obscured, and the epithelial component is focally disorganized. In addition, there is a cystic transformation of Hassall corpuscles and occur some elongated structures with cystic aspect, similar with those found in early stages of development of Hassall corpuscle [14].

The process of involution may have a rapid rate of progression, with complete lymphocyte depletion in only 7 days. Blood vessels have large diameter in comparison with the diameter of the lobules, containing fibro-hyaline area. Finally, as T cells were lost, is found fibrous changes of the remnant parenchyma and its replacement with adipose tissue. What do it remains are only some islands of parenchyma with few lymphocytes, aspect similar with that found in the physiologic involution. Immunohistochemically, it is reduced the expression of cytokeratin, also confirmed by our investigations. Plasma cells are present, sometimes in high number, and CD4 and CD8 are focally positive. CD1 is negative and CD2 is focally positive [15, 16].

The marked decrease in the intensity of the immune processes in the involution is also revealed by the low number of S100 protein positive cells, and their absence in patients over 40 years old that we reported above, which come to demonstrate previous findings [17].

A third modality of thymus involution, called „early", is secondary to an injury of the epithelial cells, and occurs in patients with acquired immunodeficiency syndrome. Infection with the human immunodeficiency virus affects both epithelial and T cells. In histological section it is noticed a marked involution, with T cell depletion in the cortex and medulla, abnormal cortical matrix, rich in fibronectin, the difference between cortex and medulla is obscured, mononuclear infiltrates, giant multinucleated cells, and cystic transformation of Hassall corpuscles or event their absence [18, 19].

Late involution and final disorganization of the thymus may be observed in old individuals. One autopsy study performed on cases with age between 63 and 91 years revealed the presence of rests of thymic tissue in all cases [20].

Aspects were characterized by branched cords of non-cornified epithelial cells, containing Hassall corpuscles and surrounded by adipose tissue. B and T cells, demonstrated by immunohistochemistry, were located predominantly outside of the epithelial cords.

The evaluation of the involutive character of the thymus parenchyma maybe sometimes difficult and this aspect is found especially in 20 to 25 years old patients. In the table below we summarize some useful criteria in order to make the difference between the „normal mature" thymus as it is found in the child and teenager, and the thymus of involution, as it is found in the adult.

Table 1 – ***Differences between mature, active thymus and involution (after Suster S and Moran CA, 1999 [21, 22] modified)***

Normal mature thymus („active")	Normal involutedly thymus (adult)
▪ Lobulation: present; ▪ Cell population: epithelial and lymphocytes; ▪ Well-defined cortex and medulla; ▪ Perivascular spaces; ▪ Area of medulla differentiation; ▪ Absence of cytological criteria of malignancy.	▪ Spindle or oval in shape epithelial cells, arranged in small groups or cords included in adipose tissue; ▪ Cystic degeneration; ▪ Glandular structures; ▪ Rosette-like epithelial structures.

These criteria are important in the pathologic diagnosis of thymoma, because cases that show these elements are thought to be well differentiated. All tumors that lost the organotypical characters are less differentiated.

Conclusions

The morphological and immunohistochemical investigation of the thymus from 22 patients, aged between 1 day and 45 years revealed the following aspects: involution of the thymus is not linear, and transient involution maybe noticed in the first month after birth and around 4 years; initial stages of the postpuberal involution begin in the cortex and is characterized by lymphocyte depletion; immunohistochemically, it was found a marked reduction of CD3, CD20 and S100 protein positive cells; in some cases, epithelial cells aggregate and form compact islands that maybe mistaken with a metastasis or a thymic carcinoma.

References

[1] BLACKBURN C. C., MANLEY N. R., *Developing a new paradigm for thymus organogenesis*, Nat Immunol, 2004, 4(4):278–289.

[2] HALE L. P., *Histology and molecular assessment of the thymus*, Ann Diagn Pathol, 2004, 8(1):50–60.

[3] FLORES K. G., LI J., SEMPOWSKI G. D., HAYNES B. F., HALE L. P., *Analysis of the human thymic perivascular space during aging*, J Clin Invest, 1999, 104(8):1031–1039.

[4] HALE L. P., MARKERT M. L., *Corticosteroids regulate epithelial cell differentiation and Hassall body formation in the human thymus*, J Immunol, 2004, 172(1):617–624.

[5] GILL J., MALIN M., SUTHERLAND J., GRAY D., HOLLANDER G., BOYD R., *Thymic generation and regeneration*, Immunol Rev, 2003, 195:28–50.

[6] NAKAHAMA M., MOHRI N., MORI S., SHINDO G., YOKOI Y., MACHINAMI R., *Immunohistochemical and histometrical studies of the human thymus with special emphasis on age related changes in medullary epithelial and dendritic cells*, Virchows Archiv B Cell Pathol, 1990, 58(3):245–251.

[7] ROSAI J., *Rosai and Ackerman's Surgical pathology*, Mosby, St. Louis, 2004, 462–513.

[8] MARX A., WILLISCH A., SCHULTZ A., *Pathogenesis of myasthenia gravis*, Virchows Arch, 1997, 430(5):355–364.

[9] MATSUNAGA T, RAHMAN A., *In search of the origin of the thymus: the thymus and GALT may be evolutionarily related*, Scand J Immunol, 2001, 53(1):1–6.

[10] REVEST J. M., SUNIARA R. K., KERR K., OWEN J. J., DICKSON C., *Development of the thymus requires signaling through the fibroblast growth factor receptor R2-IIIb*, J Immunol, 2001, 167(4):1954–1961.

[11] MILLER J. F., *The discovery of thymus function and of thymus-derived lymphocytes*, Immunol Rev, 2002, 185:7–16.
[12] VARAS A., JIMÉNEZ E., SACEDÓN R., RODRÍGUEZ-MAHOU M., MAROTO E., ZAPATA A. G., VICENTE A., *Analysis of the human neonatal thymus: evidence for a transient thymic involution*, J Immunol, 2000, 164(12):6260–6267.
[13] TATEYAMA H., TAKAHASHI E., SAITO Y., FUKAI I., FUJII Y., NIWA H., EIMOTO T., *Histopathologic changes of thymoma preoperatively treated with corticosteroids*, Virchows Arch, 2001, 438(3):238–247.
[14] RAICA M., ENCICA S., MOTOC A., CIMPEAN A. M., SCRIDON T., BARSAN M., *Structural heterogeneity and immunohistochemical profile of Hassall corpuscles in the normal human thymus*, Ann Anat, 2006, 188(4):345–352.
[15] ASHOUR M., *Prevalence of ectopic thymic tissue in myasthenia gravis and its clinical significance*, J Thorac Cardiovasc Surg, 1995, 109(4):632–635.
[16] TAUB D. D., LONGO D. L., *Insights into thymic aging and regeneration*, Immunol Rev, 2005, 205:72–93.
[17] VANDENABEELE S., HOCHREIN H., MAVADDAT N., WINKEL K., SHORTMAN K., *Human thymus contains 2 distinct dendritic cell populations*, Blood, 2001, 97(6):1733–1741.
[18] SEEMAYER T. A., LAROCHE A. C., RUSSO P., MALEBRANCHE R., ARNOUX E., GUÉRIN J. M., PIERRE G., DUPUY J. M., GARTNER J. G., LAPP W. S., *Precocious thymic involution manifest by epithelial injury in the acquired immune deficiency syndrome*, Hum Pathol, 1984, 15(5):469–474.
[19] KNUTSEN A. P., ROODMAN S. T., FREEMAN J. J., MUELLER K. R., BOUHASIN J. D., *Inhibition of thymopoiesis of CD34+ cell maturation by HIV-1 in an in vitro CD34+ cell and thymic epithelial organ culture model*, Stem Cells, 1999, 17(6):327–338.
[20] KRAFT R., FRANKHAUSER G., GERBER H., *Age-related and terminal disorganization of the human thymus*, J Radiation Biol, 1988, 53(1):169–176.
[21] SUSTER S., MORAN C. A., *Primary thymic epithelial neoplasms: spectrum of differentiation and histological features*, Semin Diagn Pathol, 1999, 16(1):2–17.
[22] SUSTER S., MORAN C. A., *Malignant thymic neoplasms that may mimic benign conditions*, Semin Diagn Pathol, 1995, 12(1):98–104.

Corresponding author
Marius Raica, Professor, MD, PhD, Department of Histology & Cytology, "Victor Babeş" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300 041 Timişoara, Romania; Phone/Fax +40256–200 256, E-mail: raica@umft.ro

Received: January 20th, 2007

Accepted: March 15th, 2007