

## OVEREXPRESSION OF CYTOKERATIN 34BETA E12 IN THYMOMA: COULD IT BE A POOR PROGNOSIS FACTOR?

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**Summary.** We included in our study nine normal human thymuses and sixteen thymomas (1 type A, 2 type AB, 3 type B1, 3 type B2, 5 type B3 and 2 type C). The 25 patients were between 7 days and 75 years old, and were submitted to open surgery for correction of congenital heart defects or for mediastinal tumor mass. Biopsies were formalin fixed for 24 hours and then embedded in paraffin using routine procedure. Five micrometers step sections were mounted on silanized slides. Slides from each case were stained with haematoxylin and eosin method for morphological diagnosis. Additional sections were immunostained using monoclonal antibodies against high molecular weight cytokeratin clone 34beta E12. We found a very strong positive reaction for this type of cytokeratin in thymomas type B2, B3 and inconstant in type C comparative with normal thymus. Also, the number and distribution of positive epithelial cells in normal thymus versus thymomas were different. We found positive cells into capsular vessels of thymomas. This could be an invasion marker for apparent encapsulated thymomas. Strong positive reaction in almost all epithelial cells of thymomas types mentioned above was correlated in part with invasion. We concluded that expression of 34beta E12 cytokeratin is a useful marker for thymomas with high grade of malignancy, correlated with vascular and capsular invasion.

**Key words:** CK 34beta E12, thymus, thymomas, epithelial cell, prognosis.

### INTRODUCTION

Thymus is a special organ of the immune system. First, there are controversial opinions regarding origin of epithelial cells. Last data suggest only the endodermal origin of thymus (Blackburn *et al.*, 2004). Second, few data are available about the role of epithelial cells in positive and negative selection of lymphocytes. Also, Hassall bodies remain a mystery concerning their origin and functions in normal human thymus.

Thymomas refer to as tumors of thymus arising from epithelial cells of thymic stroma. First description of thymomas was made by a surgeon, Astley Paston Cooper over 200 hundred years ago.

Despite of this, today is very difficult to classify thymic neoplasia in one type of epithelial tumors and to predict its behaviour. Capsule invasion is the only one accepted marker for outcome of thymomas (Rosai *et al.*, 1999). Tomita *et al.* (2002), found a partial correlation between microvessel density and malignancy of

different types of thymomas; they suggest a partial correlation between microvessel density and malignancy grade of thymic epithelial tumors.

Cytokeratins are markers for epithelial cells. In the normal human thymus and related tumors, positive reaction for such markers might be the first step in immunohistochemical diagnosis of thymomas. Most used cytokeratins are pan-cytokeratin clone MNF 116 and CAM 5.2. High molecular weight cytokeratin clone 34beta E12 was few studied in normal thymus and thymomas (Chilosi *et al.*, 1984).

We proposed to study CK 34beta E12 expression and distribution pattern of epithelial cells of normal human thymus and thymomas and try to make a correlation between this expression and malignancy grade of different types of thymic neoplasia.

#### MATERIAL AND METHODS

Our study included 25 patients. Nine of them were children aged between 3 weeks and 13 years old, with congenital cardiac abnormalities. We also had sixteen adults from 34 to 70 years old with mediastinal mass detected by routine radiological exam or CT scan.

*Tissues.* Human thymus tissues were obtained as tissue discarded at the time of necessary cardiothoracic surgery or after removal of mediastinal tumoral mass. Specimens of each tissue were fixed in 10% neutral buffered formalin for 24 hours, then processed into paraffin blocks using standard histologic procedures. 5 µm step sections were obtained from each case and mounted on silanized slides.

*Morphologic methods.* For every case we performed routine haematoxylin and eosin stain method for morphologic study.

*Immunologic study* for specific highlight of epithelial cells used monoclonal antibodies against high molecular weight cytokeratin, clone 34beta E12. Before immunostaining, sections were pre-treated by microwave heating in citrate buffer, pH6, and 15 minutes for antigen retrieval.

Then, sections were immersed in 3% hydrogen peroxide in distillate water for 5 minutes at room temperature to block endogenous peroxidase activity.

After incubation with primary antibody for 30 minutes, we applied ENVISION working system and bound antibodies was visualized with 3,3'-diaminobenzidine. Nuclei were counterstained with modified Lillie's Haematoxylin. All slides were automatically processed using *Dako Cytomation Autostainer* and then examined in optic microscopy using Nikon Eclipse E600 Microscope.

We observed distribution, density and immunostaining pattern of epithelial cells for CK 34beta E12 in normal and neoplastic human thymus.

## RESULTS

On routine haematoxylin and eosin stain, all nine normal human thymuses showed well-defined capsule surrounding thymic parenchyma composed of cortex and medulla. The latter presented varying types of Hassall's bodies composed only of epithelial cells or epithelial cells around lymphocyte-rich or degeneration areas.

Positive reaction for CK 34beta E12 was present in epithelial cells of Hassall bodies, with strong, granular pattern. In normal thymus positive reaction for CK 34beta E12 was restricted to the epithelial component and had specific distribution. The most numerous positive cells are found on subcapsular region; also at cortico-medullary junction but with low density.

There are few positive epithelial cells scattered in the cortex of normal thymus. Medulla presented more positive epithelial cells than cortex with reticular pattern and tendency to agglomerate around Hassall bodies. Two of sixteen cases of thymomas showed us spindle or oval shaped epithelial cells, lacking nuclear atypia arranged in whorls-like fashion, accompanied by few or no non-neoplastic lymphocytes.

In type AB thymomas foci having the features of type A thymoma are admixed with foci rich in lymphocytes. B1 thymomas had specific organoid, lymphocyte-rich pattern. Scattered plump epithelial cells with vesicular nuclei and distinct nucleoli among a heavy population of lymphocytes are morphologic features for type B2 thymomas.

Also, perivascular spaces are common and very prominent. We found perivascular arrangement of epithelial cells resulting in a palisade effect. B3 thymomas were composed predominantly of epithelial cells with mild atypia in sheet-like growth of neoplastic area. Type C thymomas lacked immature lymphocytes and exhibited cytologic atypia of epithelial cells admixed with mature lymphocytes.

In all thymomas there was positive reaction for CK 34beta E12. Type A thymomas showed positive cells for CK 34beta E12 with fusiform pattern and high density. For type B1 thymomas, we found moderate to intense reaction for CK 34beta E12 with reticular, homogenous or heterogeneous pattern. In this type of thymoma distribution, morphology and intensity of staining were similar with those found in normal human thymus (Figure 1).

Two from three type B2 thymomas had high density of positive epithelial cells in almost all neoplastic areas (Figure 2). We also found positive cells inside perivascular spaces of such tumors. In one case of B2 thymoma, all neoplastic epithelial cells were positive for CK 34beta E12. Intensity was stronger than type A or AB thymomas, with diffuse, granular pattern.

Also, type B3 thymomas had intense positive reaction for CK 34beta E12 in four of five cases. In the fifth case positive staining was also present but with moderate intensity. Positive cells are found on entire tumoral area with

homogeneous or heterogeneous pattern and diffuse distribution of imunostaining (Figure 4).

Type C thymomas had an inconstant reaction for antibodies against high molecular weight cytokeratin. One of them presented positive stain of restant epithelial cells zone included in a negative tumoral area. Second case showed strong positive reaction on entire tumoral area. Also we found positive cells inside capsular vessels (Figure 3) and perineural.

### DISSCUSIONS

Cytokeratins are markers for epithelial cells. Staining for such markers might be the first step in immunohistochemical diagnosis of thymomas. Kuo (2001) immunophenotyped epithelial tumors of thymus for different types of cytokeratins, excepting CK 34beta E12. The most used cytokeratins in thymic tumors are CK MNF116 and CAM 5.2. There are few studies about CK 34beta E12 in thymomas (Chilosi *et al.*, 1984).

We did not found any study about expression of this type of cytokeratin in normal human thymus, neither any correlation between this and its expression in different types of thymomas. We found a preferential expression of high molecular weight cytokeratins in subcapsular and medullary epithelial cells of normal human thymus, also in epithelial cells of Hassall bodies.

These findings suggest that neoplastic changes of such epithelial cells might be origin for some thymomas especially type B2 and B3 because they have a greater number of positive epithelial cells than other thymomas. Hiroaki *et al.* (1999) noticed that minimally invasive thymoma could metastasise by hematogenic route. So, positive cells for CK 34beta E12 inside capsular blood vessels and perivascular spaces might be a prognostic marker for invasion in thymic tumors apparent well encapsulated.

Expression of CK 34beta E12 is strong in type B2 and B3 thymomas but inconstant for type C. In a comparative study between expression of CK MNF 116 and CK 34beta E12 (Encică *et al.*, 2004) we found that latter cytokeratin is more sensitive and stains a great number of normal and neoplastic epithelial cells than CK MNF 116. Also, CK 34beta E12 stains epithelial cells inside blood vessels and perivascular spaces but CK MNF116 not.

### CONCLUSIONS

CK 34beta E12 stains subcapsular and medullary epithelial cells including those of Hassall bodies in normal human thymus. Number, distribution and intensity of stained cells are not correlated with age. CK 34beta E12 is more sensitive than others cytokeratins used for thymomas diagnosis, except type C

thymomas where reaction was inconstant and probably the useful marker remain CAM 5.2. Antibodies against CK 34beta E12 cytokeratin stain intravascular epithelial cells; perineural and those of perivascular spaces might be considered a marker for invasion. Also, great density and intensity of positive reaction in thymomas type B2 and B3 suggest that high molecular weight cytokeratin might be considered a specific marker and prognosis factor for outcome of thymomas.

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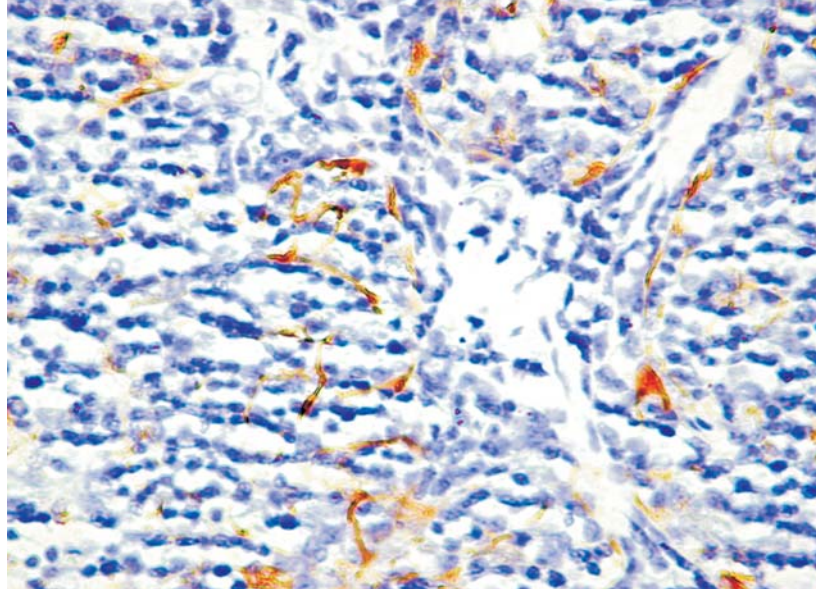


Figure 1 – Type B1 thymoma stain with CK 34beta E12.  
Imunoreaction had a reticular pattern

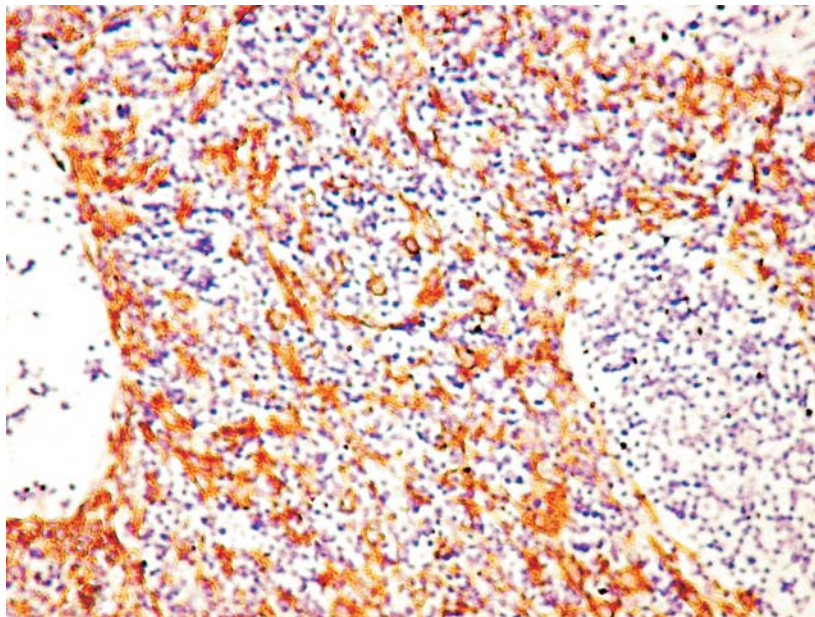


Figure 2 – Immunoexpression of CK 34beta E12 in type B2 thymoma. The number of positive cells is greater comparative with type B1 thymoma



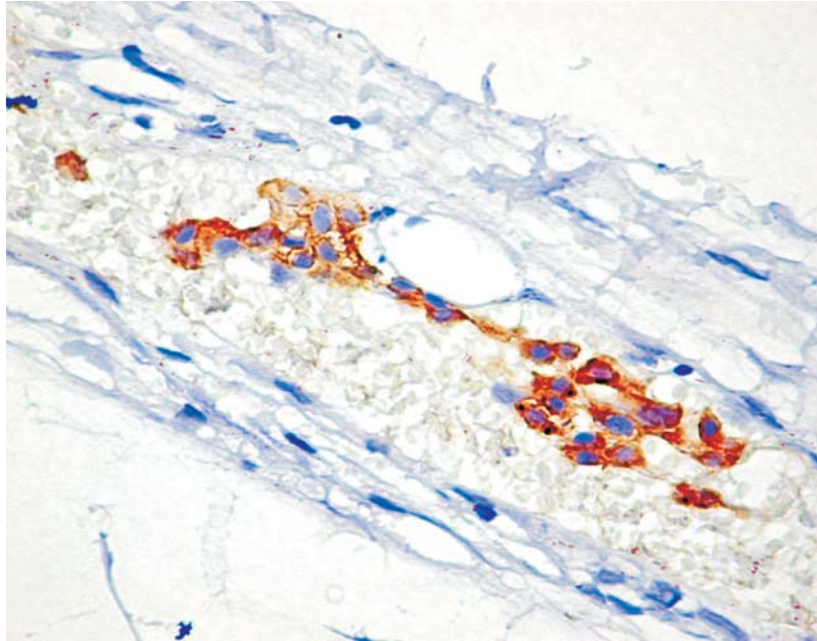


Figure 3 – Positive cells for CK 34beta E12 inside capsular vessel of type C thymoma

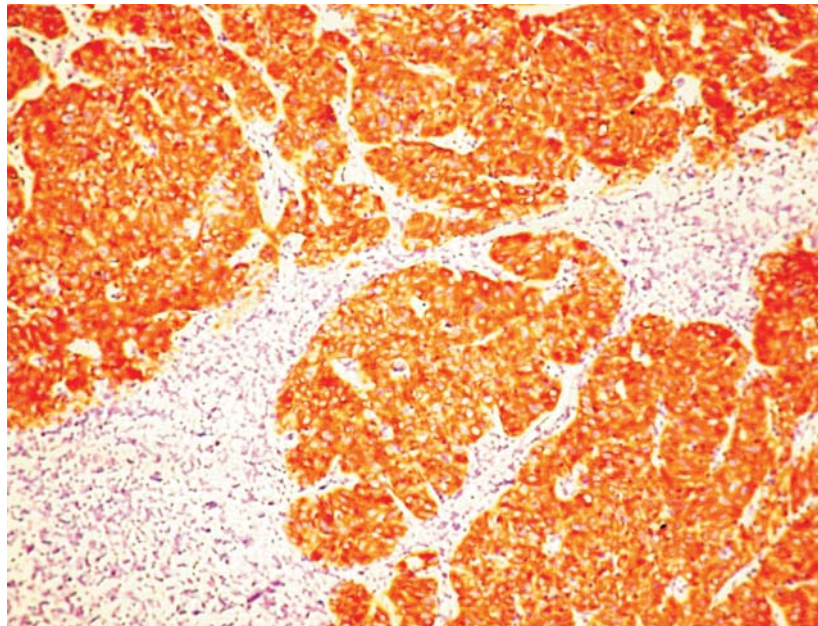


Figure 4 – Type B3 thymoma. Strong positive reaction for CK 34beta E12 in almost all neoplastic cells