COMPARATIVE MORPHOLOGICAL AND MORPHOMETRICAL ANALYSIS OF TWO THYMOMA CASES

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COMPARATIVE MORPHOLOGICAL AND MORPHOMETRICAL ANALYSIS OF TWO THYMOMA CASES (Abstract): Thymoma is a primary tumor of the thymus epithelial cells and one of the most common neoplasms in the anterosuperior mediastinum, especially in middle-aged or older adults. There were some difficulties in thymoma diagnosis due to high variability of the morphological thymoma aspects. WHO 2004 morphologic classification reached a kind of consensus of the morphological thymoma types and established a correlation with prognostic factors. Aim: The aim of this study was to assess morphologically and morphometrically the two thymoma specimens for searching prognosis according with atypical features and variable invasive character. Material and methods: The study included 2 thymoma cases, one AB and the other B1 type, both in adults, operated for mediastinal tumoral masses. Surgery specimens were processed for histological examination. Qualitative and quantitative assessment of thymoma specimens was applied. Results: The study reveals various histological aspects and cytological measurement results explaining different invasiveness features in the two thymomas. The two thymoma types show a close relationship to blood vessels. Conclusions: Usually, the current state of the art in thymoma diagnostic defines invasion, both clinically and histologically. The morphometrical analysis, rarely used in clinical practice, could be a useful tool in prognosis assessment and offer an evolutive behavior of the tumor. Keywords: THYMUS, THYMOMA, MEDIASTINUM.

Thymic epithelial neoplasms are usually seen in the fourth and fifth decades of life (1, 2). Thymoma is a primary mediastinal neoplasm arising from or exhibiting differentiation toward thymic epithelial cells, typically with the presence of non-neoplastic lymphocytes (3, 4). Thymomas can be classified as benign or malignant (5). Cytologically benign thymomas are of three major types, such as lymphocyte rich, epithelial cell rich, and spindle cell type or according with their architecture as cortical, medullary, and mixed. Thymomas that are cytologically malignant are uncommon, having as a hallmark a significant cytological atypia. The local spreading feature characterizes the invasive tumors (6). We hereby present two thymoma cases with different morphological and morphometrical features. In this article, we describe pathologic features of thymomas and discuss these disease entities in terms of classification and staging evalua-
tion, in which radiology plays a crucial role. The prognosis assessment for thymic masses is necessary, as well. Morphologically, atypia, mitotic activity and invasiveness are histological features used in tumor prognosis assessment.

**MATERIAL AND METHODS**

We present two patients, who underwent tumor excision to treat surgically the thymomas, one in 2008 and the other one in 2010, both having myasthenia gravis. We described and assessed the histological characteristics of the two thymomas, and between thymoma types and cell variables.

Morphology assessment was made with an optical microscope (Olympus CX41). The measurements were analyzed with a color image analysis system: Quick PHOTO MICRO 3.0. We studied the tumor histological structure and cytology by using usual and specific (hematoxilin eosin, elastic von Gieson, and Gordon Sweet for reticulin) stains.

Quantitative morphometry was performed by appreciation of the ratio between lymphocytes and epithelial cells, micro vessel density and nuclear area of the epithelial cells. The measurements were expressed in mean values and frequencies.

**RESULTS**

Two biopsies from mediastinal masses were performed. Histopathological examination revealed features of thymoma. The results of histological and cytological analysis varied in few points.

In AB thymoma, surgical biopsy showed a nodular encapsulated tumor having 2 points of capsular infiltration (fig.1, a, b). Cytologically, we found a mixture of variable proportions of lymphocyte-poor (fig.1, c), lymphocyte rich areas (fig.1, d) and discrete intermixed foci. Lymphocyte deprived areas were composed from spindle epithelial cells with few lymphocytes. Epithelial spindled to oval cells were essentially the same as those of type A, having bland nuclei, medium sized to large and round to oval.

Morphometrically, tumoral area was composed from a dual population of cells with a lymphocyte predominance of 71, 52%. Lymphocyte to epithelial cell ratio was of 1.8. Tumoral epithelial cells had an average nuclear area of 45.3 µm². All these morphological features are common in AB thymoma. Angiogenesis was another morphological feature of the tumor. We found an average of 3 micro vessels on HPF (HE, x400).

In B1 thymoma (fig.2, a), morphological analysis showed a multinodular tumor with a thick capsule and an area of infiltration with involvement of the adjacent fat tissue. The septa had different thicknesses.

Morphologically, we emphasized zones resembling with cortical areas composed from lymphocytes with inconspicuous epithelial cells (fig.2, b). In lymphocyte, rich-areas we saw densely packed (fig.2, c), small lymphocytes. Epithelial cells had nuclei ranging from medium sized to large, with nucleoli varying from inconspicuous to prominent. Epithelial cells ranged from infrequent to occasional Hassall corpuscles. Medullary foci appeared as circumscribed round pale zones due to lose packing of lymphocytes (fig.2, d).

Morphometrically, we identified the same dual cell population, with two thirds (2/3) or more small lymphocytes from entire cell mass. The lymphocyte to epithelial cell ratio was of 2.53. The most epithelial cells were small with polygonal shape and an average nuclear area of 21 µm². Angiogenesis was greater than in AB thymoma, represented by 4 vessels on HPF (HE, x400).
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**Fig. 1.** Thymoma AB. a, b-foci of capsular infiltration (HE, x4); c-lymphocyte-poor area (HE, x40); d-lymphocyte-rich area (HE, x40).

**Fig. 2.** Thymoma B1. a-area of fat tissue infiltration (HE, x4); b-lymphocyte rich-area (HE, x40); c-densely packed, small lymphocytes (HE, x40); d-loose packing of lymphocytes (HE, x40).
Morphometrically, we found different results between the two thymomas, according with epithelial cell sizes and shapes and their proportions reported to lymphocytes, all these distinctive features explaining differences in tumor invasiveness.

Despite angiogenesis study results are not too different; it may explain different degrees of malignancy among thymic epithelial tumors.

According with pathological stage (i.e., as defined after resection), both encapsulated tumors were in II\textsuperscript{nd} stage of evolution, the first with microscopical invasion through the capsule (IIa stage for AB thymoma) and the second with invasion into surrounding fat (IIb stage for B1 thymoma).

**DISCUSSION**

Thymoma is a rare primary tumor of the epithelial cells of thymus and the most frequent tumor of the anterosuperior mediastinum. Its incidence increases with age (70\% of cases are observed in patients past the age of 40) (7).

Distinctive features reminiscent of the normal thymus make the pathological diagnosis of thymoma easy in most cases. A fibrous capsule surrounds the tumor and sends thick, fibrous septa, dividing the tumor into well-demarcated lobules.

Microscopically, there is a distinctive dual cell population, including lymphocytes, small with dark nuclei, and epithelial cells, larger and lighter (1).

Quantitatively, the ratio between lymphocyte and epithelial cell varies widely. Thymomas are often classified according to the relative ratio of cells between predominantly lymphocytic, mixed epithelial–lymphocytic, and predominantly epithelial. In 40\% of cases the tumors are predominantly lymphocytic, in 20\% they are mixed and the rest are predominantly of epithelial type. The mean nuclear area increased significantly in the increasing order of noninvasive thymoma and invasive thymoma. The morphological and morphometric results demonstrated a significant difference in degree of malignancy between non-invasive and invasive thymomas. Cornea R shows a significant increase in the number of micro vessels in the temporal area, regardless the histological type of the tumor. So, in patients with thymic epithelial tumors, there appears to be a significant correlation between tumor angiogenesis and invasiveness (8).

The epithelial cells represent the neoplastic component of thymomas. They are typically round or oval, but sometimes have a spindle-shaped nucleus, which may represent the predominant cell in about 5 to 12\% of thymomas, justifying the term “spindle cell thymoma,” which tends to grow more slowly. The lymphocytes are not considered neoplastic and, as in the normal thymus, are constituted mainly of T cells in various stages of maturation (1).

The clear majority of thymomas do not present considerable atypia or anaplasia. There are no reliable histologic features of “malignancy” for thymomas. The malignant behavior of a thymoma is indicated by microscopical or macroscopical invasion of the tumor capsule or of surrounding organs or by the presence of metastasis. In 90\% of cases, they are well encapsulated and multilobulated, while in 10\% of cases, the rupture of the capsule and invasion to the adjacent tissues are observed. About 30 to 40\% of thymomas are invasive. On the other hand, a well-encapsulated thymoma may, on occasion, recur years after surgical resection (3).
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It was recently postulated that thymomas may be further subdivided into those with cortical, medullary, and mixed differentiation, reflecting the anatomy of the normal thymus (2).

Cortical thymomas contain large epithelial cells with vesicular chromatin and prominent nucleoli, whereas medullary thymomas show oval to spindle-shaped epithelial cells with dispersed chromatin and inconspicuous nucleoli. Mixed thymomas contain both cortical and medullary components. Medullary and mixed thymomas appear to follow a more benign course than cortical ones. They show a lower incidence of invasiveness, lower recurrence rate, and better survival, even when capsular invasion is present. This classification has also been correlated with the size of epithelial cell nuclei using morphometrical analysis (3).

The thymomas behavior is unpredictable. The stage of the tumor at the time of diagnosis and the adequacy of the surgical excision are among the factors that influence the outcome of these tumors. The presence of clinical symptoms, large tumor size, local invasion or metastases at the time of the surgery, and predominant epithelial features are poor prognostic factors (9).

A multimodality approach that includes surgery, chemotherapy, and radiation therapy is suggested for treatment. Surgery is the mainstay of the treatment. However, complete resection is generally impossible or not feasible at diagnosis because of local invasion of important structures and metastasis (3).

The new WHO classification seems to correlate with invasiveness. The proportion of invasive thymomas was higher in B1 cases than in AB cases as in literature reports (Type B1, 69.1% comparing with Type AB, 47.3%) (10).

CONCLUSIONS

In our study, both thymoma types presented in a low stage of evolution. AB thymoma very rarely recurs while B1 thymoma may be sometimes aggressive. We noted both thymoma types show a close relationship to blood vessels. In our opinion, the micro vessel density is associated with thymoma invasiveness, and the higher radiotracer fixation may be due to increased angiogenesis. The histological prognosis assessment of these enlarged mediastinal masses is necessary, because thymomas could be sometimes aggressive tumors.

REFERENCES


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**DIFFERENT VANCOMYCIN-INTERMEDIATE STAPHYLOCOCCUS AUREUS PHENOTYPES SELECTED FROM THE SAME ST100-hVISA PARENTAL STRAIN**

The aim of this study is to characterize the factors related to peptidoglycan metabolism in isogenic hVISA/VISA ST100 strains. Recently, we reported the increase in IS256 transposition in invasive hVISA ST100 clinical strains isolated from the same patient (D1 and D2) before and after vancomycin treatment and two laboratory VISA mutants (D23C9 and D2P11) selected from D2 in independent experiments. High performance liquid chromatography-mass spectrometry (HPLC-MS) analysis of peptidoglycan muropeptides showed increased proportion of monomeric muropeptides and a concomitant decrease in the proportion of tetrameric muropeptide in D2 and derived mutants when compared to the original strain D1. In addition, strain D2 and its derived mutants showed an increase in cell wall thickness with increased pbp2 gene expression. The VISA phenotype was not stable in D2P11 and showed a reduced autolysis profile. On the other hand, the mutant D23C9 differentiates from D2 and D2P11 in the autolysis profile, and pbp4 transcription profile. D2-derived mutants exhibited differences in the susceptibility to other antimicrobials. Our results highlight the possibility of selection of different VISA phenotypes from a single hVISA-ST100 genetic background (Sabrina Di Gregorio, Silvina Fernandez, Arabela Cuirolo, Olivier Verlaine, Ana Amoroso, Dominique Mengin-Lecreulx, Angela Famiglietti, Bernard Joris and Marta Mollerach. Different Vancomycin-Intermediate *Staphylococcus aureus* Phenotypes Selected from the Same ST100-hVISA Parental Strain, Microbial Drug Resistance 2017; Vol. 23, Issue 1: 44-50).

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