WORLD HEALTH ORGANIZATION (WHO) HISTO-MORPHOLOGIC CLASSIFICATION OF THYMOMAS. PROS AND CONS

Remus Cornea¹, Anca Maria Cimpean², Marius Raica²

REZUMAT
Timomul este o tumoră care se dezvoltă din celulele epiteliale timice și care poate fi sau nu infiltrată extensiv de limfocite non-neoplazice, însă termenul este utilizat indiferent de prezența sau numărul limfocitelor. Clasificarea morfologică a acestor tumori a născut numeroase controverse și deși au fost propuse numeroase forme de-a lungul timpului, de abia recent s-a ajuns la un consens de clasificare (OMS 1999, revizuit în 2004). Totuși lucrurile sunt departe de a se fi simplificat și deși această clasificare este în uz, persistă anumite dificultăți și, pe măsură ce este analizată în diverse studii, întrebările la care trebuie să răspundă sunt tot mai frecvente. Timoamele sunt tumori rare ca incidență în populația generală, studiile populaționale largi sunt, deasemenea, greu de realizat. Pentru că există o mare variabilitate a aspectelor morfologice, ce se pot întâlni chiar la aceleași cazuri, dar și o marcată heterogenitate genetică, rezultatele sunt contradictorii în cea ce privește utilitatea clasificării histologice, dar și corelația cu factorii de prognostic, management-ul clinic și terapeutic, precum și identificarea unor markeri moleculari specifi cu rol prognostic și terapeutic. Acest review urmărește principalele întrebări ridicate de introducerea clasificării histo-morfologice OMS 1999 și revizuită în 2004 a timoamelor, prin evaluarea principalelor studii realizate în domeniul. Această evaluare se face prin prisma factorilor de reproducibilitate a acestei clasificări, corelația cu stadiul clinic, prognosticul și supraviețuirea, și dacă această clasificare poate fi utul în prezicerea comportamentului clinic și a unui tratament corespunzător.

Cuvinte cheie: timom, clasificare, diagnostic, prognostic

ABSTRACT
The thymoma is a tumor which develops from the thymic epithelial cells and which may or may not be extensively infiltrated by non-neoplastic lymphocytes. Nevertheless this term is used regardless of the presence or the number of lymphocytes. The morphologic classification of these tumors has born a lot of controversies, and although numerous variants have been proposed in time, only recently a kind of consensus for this classification has been reached (WHO 1999, revised in 2004). Furthermore, these issues are far from being simple and even if this classification is largely used nowadays, there are some difficulties which persist and many questions that need to be answered are more frequent as a result of new analytical studies. Because of the low incidence of thymomas, large populational studies are rare. Moreover, due to high variability of the morphologic aspects of these tumors- which may appear different even in the same cases- and a marked genetic heterogeneity, the results regarding the utility of histologic classification are contradictory. In addition, the correlation with prognostic factors, the clinical and therapeutical management and the identification of the specific molecular markers with prognostic and therapeutical values need to be improved. Since the acceptance of the WHO histo-morphologic classification of thymomas in 1999 many studies have investigated the reproducibility factors, correlation with the clinical stage, the prognostic factors, in order to provide valuable information regarding prediction of the clinical behavior and a proper treatment. However the questions to be answered are more numerous and represent the aim of the present review.

Key Words: thymoma, classification, diagnosis, prognosis

INTRODUCTION
The term thymoma is restricted for tumors developed from the thymic epithelial cells, with or without lymphocytic component. Although they are generally rare, the significance of these tumors derive from the fact that they are frequent among the lesions occurring in the anterior mediastinum, representing 50% of the anterior mediastinal lesions, and about 20% of all mediastinal tumors.
Thymomas are also relevant due to the clinical association with autoimmune diseases, especially myasthenia gravis. The bidirectional relation between the myasthenia gravis with thymoma is important because of the former’s concomitant or later association in the evolution of a thymoma (present in 10-20% of the cases).\textsuperscript{2,4,5}

On the other hand, in patients with thymoma the myasthenia is present or appears in 30-50% of the cases. Currently the thymus is surgically resected in patients with myasthenia gravis because at least an improvement of clinical symptoms due to myasthenia gravis can be obtained, even if there is no associated thymoma.

The thymic tumors represent a major concern for the pathologists, when broad spectrum knowledge about thymic histomorphologic aspects is necessary, for the clinicians, due to the frequent association of paraneoplastic syndromes, and for the surgeons, taking into account the fact that both the clinical stage, and a complete resection of these tumors are important distinct prognostic factors.\textsuperscript{5-15}

Other serious issues are the histogenetic and morphologic variability of the thymic epithelial tumors, the difficulties regarding the histomorphologic classification in forms that are relevant to the prognosis, the behavior of the tumor and therapy, and the inconsistency of the prognostic factors. All these specified aspects make these tumors a challenge for the pathologist, but also for the clinician, surgeon and oncologist, because of their contribution to the therapeutic decision.

### Classification of thymic epithelial tumors

The classification of thymic epithelial tumors has provoked numerous debates over the time and experienced many changes; those that have had the greatest impact and are used predominantly are summarized in Table 1.

#### The WHO (2004) classification of the thymic epithelial tumors

After years of debates, WHO has established a consensus regarding the classification of thymic epithelial tumors, by introducing a system of six categories, to ensure standardization of the previous systems, to facilitate the reporting of the tumors and to provide the clinicians and the researchers a general analysis model based on the unification of the various proposed classifications. Later, in 2004, WHO offered the final form, which is currently widely used in the world, even if the controversies still persist.\textsuperscript{16-21}

This classification is based on the epithelial cell morphology and the report between the epithelial and lymphocythic component. (Table 2) There are some principles of this classification, as follows: there are two main categories of thymoma depending on the shape of the epithelial neoplastic cell, spindle (type A thymomas) and round/ polygonal/ epithelioid (type B thymomas). The type B thymomas are further divided according to the number of lymphocytes and the cellular atypia in three categories: B1 (lymphocytic rich), B2 and B3 (epithelial rich). The thymomas that combine the features of the type A and the B1-like (rarely B2- like) are defined as AB thymomas.\textsuperscript{1} The thymic carcinomas

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Table 1. The correlation between the most important proposed histo-morphologic classifications for thymomas.

<table>
<thead>
<tr>
<th>Bernatz et al 1961\textsuperscript{16}</th>
<th>Muller-Hermelink 1989\textsuperscript{17}</th>
<th>Suster and Moran 1999\textsuperscript{18}</th>
<th>WHO (1999)\textsuperscript{1}</th>
<th>Kuo 2000\textsuperscript{19}</th>
<th>WHO 2004\textsuperscript{20}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spindle cell thymoma</td>
<td>Medullary thymoma</td>
<td>Thymoma</td>
<td>Thymoma type A</td>
<td>Spindle cell thymoma</td>
<td>Type A</td>
</tr>
<tr>
<td>Mixed lymphoepithelial thymoma</td>
<td>Mixed</td>
<td>---</td>
<td>Type AB</td>
<td>Mixed thymoma</td>
<td>Type AB</td>
</tr>
<tr>
<td>-</td>
<td>---</td>
<td>---</td>
<td>----</td>
<td>Small polygonal cell</td>
<td>----</td>
</tr>
<tr>
<td>Lymphocyte-rich</td>
<td>Predominantly cortical</td>
<td>---</td>
<td>Type B1</td>
<td>Organoid thymoma</td>
<td>Type B1</td>
</tr>
<tr>
<td>-</td>
<td>Cortical</td>
<td>---</td>
<td>Type B2</td>
<td>Large polygonal cell</td>
<td>Type B2</td>
</tr>
<tr>
<td>Epithelial-rich</td>
<td>Well differentiated thymic carcinoma</td>
<td>Atypical thymoma</td>
<td>Type B3</td>
<td>Squamoid</td>
<td>Type B3</td>
</tr>
<tr>
<td>-</td>
<td>Other thymic carcinoma</td>
<td>Thymic carcinoma</td>
<td>C thymoma</td>
<td>Thymic carcinoma</td>
<td>Thymic carcinoma</td>
</tr>
</tbody>
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are defined depending on their differentiation (squamos, mucoepidermoid, etc.). The term type C thymoma from the 1999's WHO classification was removed because all the malignant non-organotypical thymic tumors, other than germ cells tumors, were placed in the thymic carcinomas category.²

Table 2. Morphological aspects of thymomas (after WHO 2004).

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td>A tumor composed of neoplastic epithelial cells, spindle or oval in shape, inconspicuous nucleoli, without nuclear atypia, and few or no lymphocytes.</td>
</tr>
<tr>
<td>Type AB</td>
<td>A tumor which consists of areas similar to those from A thymoma but mixed with areas lymphocyte-rich, the border between being sharp or less distinct.</td>
</tr>
<tr>
<td>Type B1</td>
<td>The tumor resembling the typical thymus appearance, associating areas similar to the thymic cortex with foci with medullary differentiation. The cortical areas are prevalent and in excess compare to the small medullary areas. The neoplastic epithelial cells are scant, small and dispersed in the lymphocytic component.</td>
</tr>
<tr>
<td>Type B2</td>
<td>A tumor composed of large plump/polygonal neoplastic epithelial cells, with vesicular nuclei and distinct nucleoli, the tumoral cells are usually outnumbered by the nonneoplastic lymphocytes. The perivascular spaces are common.</td>
</tr>
<tr>
<td>Type B3</td>
<td>Tumor composed predominantly of round/polygonal neoplastic epithelial cells; the nucleoli are less prominent, with mild nuclear atypia and with a poor lymphocytic component. The perivascular spaces and squamous metaplasia are common.</td>
</tr>
<tr>
<td>Thymic carcinoma</td>
<td>Thymic tumor with loss of organotypical differentiation of the organ and with clear cytological atypia, generally similar with that encountered in other organs. There is a lymphocyte population which is mature.</td>
</tr>
</tbody>
</table>

Problems and difficulties in thymomas diagnosis (and unusual variants), clinical integration, prognosis, evolution and therapeutical approach using the WHO classification

With all the improvements made by the WHO classification in this complex field of thymomas, many unresolved issues still persist. Some of these are referring to the clinical usefulness, the prognostic value of the different thymomas subtypes, the existence of some histopathological variants which cannot be included in any of the categories, and the existence of variability in the diagnosis among the pathologists.

Arguments on the thymomas morphologic diagnosis (including unusual variants)

There are some discussions concerning the difficulties in the standardization of some histo-pathologic criteria for the thymoma subtypes, especially for the type B subgroup. The difference in size of the epithelial cells (bigger and more numerous in type B2 thymoma compared to the B1) and the amount of the lymphocytes that are used to differentiate the different subtypes are useful but questionable.

Taking into account the significant heterogeneity of the thymomas, the proportion between the two components - epithelial and lymphocytic - varies widely from case to case or even in the same case in different tumoral lobules, and by the existence in the same tumor of areas that look different in morphology, is necessary to include all the excisional and resectional fragments in paraffin-embedded blocks and to cut many sections of these, reasons that may lead to difficulties in making a diagnosis.²,22 On the other hand, the size of the epithelial tumoral cells in B3 thymomas is smaller and the nucleoli are less conspicuous compared to those in type B2 thymomas.² Furthermore, if we consider that the atypia of the tumoral epithelial cells may not be more obvious in B3 thymoma than in the B2 subtype, and the large cells are frequently encountered also in B3 thymoma associated with atypical cells, some difficulties may occur for the pathologists to classify in one of this two subtypes.

Suster and Moran (2008) suggest that the type AB thymoma is in fact a spindle cell thymoma (type A) lymphocytic-rich corresponding to the morphology of the epithelial cells from the type B component and this supplementary category is not justified.²⁰ In addition, from the histogenetical point of view, the origin of the type A component from AB thymoma derives from the medullar epithelial cells, but the origin of the type B component is not certain, the variants are: the epithelial cells from cortico-medullar border or the subcapsular epithelial cells.² All these aspects suggest that these criteria cannot be used unitary and make it more difficult to assess the histo-morphologic diagnosis.

Another issue is the lack of reproducibility of the diagnosis. Although Park and Chen obtained a rate of reproducibility of 95% and more than 90% respectively, it may be possible that the analysis has not been achieved strictly independent.⁶,¹¹,²¹ On the other hand, Rieker, by reevaluation of 218 cases of thymomas, found a weak agreement between pathologists for those “unfamiliarized” (who are not well acquainted with) with this kind of tumors, especially in B subgroup thymomas (B1, B2, B3) and an obvious improvement when two groups have been created A - B2, respectively B3 - thymic carcinoma.¹⁵ In another study made by Vergheze, which consisted of 95 cases of thymic epithelial tumors diagnosed by each member of a group of 17 pathologists, using the WHO 2004 classification, the results were disappointing from the interobservers agreement.
point of view and the diagnosis of a given thymoma subtype. Probably the results variability is also due to this diagnosis and its fitting inside this classification. When two subgroups were created, first including the type A, AB, B1 and B2 thymomas and the second with B3 thymomas and thymic carcinomas, the agreement was improved, but the better agreements results, according to the statistic analysis, were obtained when the subgroups were A-AB-B1 on one hand, and B2-B3-thymic carcinoma on the other hand. A reason for these discrepancies may be the presence of combined thymomas, which have an incidence between 3-21% of all thymomas, especially in the group of B2-B3-thymic carcinomas. Individually, the A thymoma and AB respectively were accurately characterized by the majority of the pathologists and the main difficulties were in differentiating the B subtypes one from the other, and the B3 thymoma from the thymic carcinoma, where were the biggest disagreements. Considering also the morphologic variability, a better defined morphological, immunohistochemical or genetical criteria for each specific subtype is required, or we need to redefine, recombine and simplify the classification especially in the B group thymomas.

There are some rare and unusual morphologic variants, like micronodular thymoma with B cell hyperplasia, metaplastic thymoma, and sclerosing thymoma, which cannot be included in defined subtypes of thymoma and do not have applicable prognostic criteria.

The results of the molecular research regarding the thymic epithelial tumors pathogenesis, even though they are generally similar to those described in other epithelial tumors, are limited and variable, generally due to the low number of cases, and without statistic significance, and so far do not affect the therapeutic decision. Referring to this, there is no single immunohistochemical marker to differentiate between the thymomas subtypes and their expression in different thymoma subtypes are very controversial. However, there are several markers that might make the difference between a B3 thymoma and a thymic carcinoma. Although the expression of bcl-2 was considered to be positive in the majority of the thymomas, some recent studies support that only B3 thymoma have a variable positivity, while others showed that bcl-2 expression is negative in B3 thymoma. The same is true for the c-kit expression, considered to be positive in 0-5% of thymomas and 73-86% from thymic carcinomas. CD5 (a receptor that signals tumor growth) was classically considered positive in the thymic carcinoma but not in the conventional thymoma, but this assumption was contradicted by Alexiev, who demonstrated that the B3 thymoma may contain CD5 positive tumoral epithelial cells and therefore, CD5 cannot be used as reliable marker for the thymic carcinoma.

Arguments on clinical integration aspects of thymomas

While in other epithelial tumors the border between benign and malignant can be established with certainty, in the case of thymoma, due to structural and cytologic variability, this is a controversial subject. There is an increase of clinical aggressiveness from type A thymoma to the thymic carcinoma, but the results are variable, and almost all the studies (even if some of them enrolled a large number of patients) have not managed to standardize the results. Okumura sustains the existence of correlation between the histological type of thymoma and the possibility of invasion and that is: A < AB < B1 < B2 < B3 < thymic carcinoma. This is also supported by the fact that most A and AB thymomas are found in the I and II stage, while in B group thymomas there is an increasing number of thymomas in stage III and IV for B2 and B3 types. Although some subtypes are considered “benign” (A, AB and B1), all the histologic thymomas categories have the potential of invasion, recurrence and metastatic dissemination, features specific to malignant tumors. Some of the thymomas with a benign histological appearance may behave clinically aggressive and develop a quick evolution.

At this moment, the influence of this classification on the clinical management is rather ambiguous. There is no unitary approach regarding the subsequent treatment when, after the surgical resection, an A or AB type thymoma is found -which are classical considered “unaggressive”- but who are in stage III or IV in Masaoka clinical classification, as well a B3 thymoma or a thymic carcinoma, which are minimally invasive. Also, the therapeutical approach after an incisional biopsy dealing with a type B3 thymoma or thymic carcinoma is uncertain, if is necessary to use chemotherapy or radiotherapy or both before the surgery, without knowledge about the clinical stage. Therefore, it seems that the clinical value of the histologic WHO classification depends on the clinical stage of the tumor and it has value only in the clinical context.

Arguments on prognosis and the evolution of thymomas

The correlation between thymomas subtypes defined by the WHO classification and their prognostic suggested by some authors, is disputed by other studies. Chalabreysse demonstrated
minimal differences of survival between the B1-B2-B3 thymomas, and that the type A and AB thymomas may have a more aggressive behavior than B thymomas.\textsuperscript{13} Rieker showed in his study that AB and B1 thymomas had the most favorable prognosis, while the A and B2 thymomas behaved more aggressively.\textsuperscript{13} Generally, the type A thymoma was regarded as a benign tumor without the risk of relapse if it can be completely removed surgically, but if these thymomas associate genetic modification with chromosomal imbalances they may have a more aggressive clinical behavior.\textsuperscript{42,43} There are also known cases with local recurrences and metastasis, or even a malignant conversion to a thymic carcinoma.\textsuperscript{44,45} To be certain that these histomorphologic thymoma subtypes have a prognostic value, they should be investigated independently and all the other prognostic factors that can influence the results must be excluded.

Some authors have suggested that the B2 and B3 thymomas have a more aggressive behavior compared to A, AB and B1 subtypes, this second group being considered with a low risk.\textsuperscript{8,11,12} This assumption has been confirmed by a meta-analysis (which included 15 retrospective studies) made by Marchevsky in 2008, suggesting the structuring of three categories A-B1/B2/B3, other than the thymic carcinoma.\textsuperscript{46,47} Kim includes the B2 type in the first category, the subgroups proposed by him being A-B2/B3/C, considering that these categories correlate more specific with the prognosis and survival.\textsuperscript{7} Similarly, Sonobe put emphasis on the discovery of a type B3 thymoma, this having an unfavorable prognosis and clinical behavior.\textsuperscript{48}

The intermediate survival for the type B3 thymoma, suggested also by other authors, compared to the A-B2 thymomas group on one hand, and the thymic carcinoma on the other hand, may prove right the group led by Suster and Moran, who recommended splitting the thymomas in 3 categories: thymoma, atypical thymoma and thymic carcinoma, at least on the further prognosis and therapeutic approach.\textsuperscript{7,9,18,20,21,25,49} The authors underline support the continuous spectrum from well differentiated (conventional thymoma) to poorly differentiated tumors (thymic carcinoma) with the intermediate category of atypical thymomas (B3 subtype from WHO classification). These categories are supported in part by a genetical correlation, the most frequent genetic imbalances of the B3 thymoma being gains on chromosome 1q and recurrent losses on chromosome 6, changes that are also frequent in the thymic carcinoma.\textsuperscript{43,50} Thus, the B3 thymoma and the thymic carcinoma can be considered a distinct prognosis group compared to A-B2 thymomas.

Arguments refering to the therapeutical approach

The primary standard therapy for thymomas is the surgical resection, with complete resection of the invasive tumors when possible.\textsuperscript{51,54} Depending on the tumoral stage, the multimodal treatment includes radio- and chemotherapy, with or without surgical treatment.\textsuperscript{51,53} There are very few studies that correlate the therapeutic action required with the histological subtype, being suggested that the A, AB and B1 thymomas do not require adjuvant therapy, while in B2, B3 thymomas and in thymic carcinoma this is necessary and useful, an improvement being observed at 5 year-survival in these cases.\textsuperscript{11,55}

The thymic carcinomas, such as epidermoid type or lymphoepithelial-like, are treated by surgical resection and radiotherapy, chemotherapy being added in the large tumors extended locally or with distance metastasis.\textsuperscript{51} This aggressive multimodal therapy is also suggested by Greene.\textsuperscript{56} Still the adjuvant therapy has inconstant results, and taking into account the increased risk of recurrence, we must rely on the clinical staging proposed by Masaoka, and on the complete or incomplete tumoral resection, these factors being actually essential for the future therapeutical approach.\textsuperscript{54,57}

CONCLUSIONS

The purpose of any morphologic classification is to be simple, effective, to offer clinical suggestions to the treatment and to the evolutive behavior of the tumor, but the most important is to ensure reproducibility of the diagnosis between the pathologists.

Considering the high variability of these tumors and because every of the thymoma subtypes may be associated with local invasion or distant metastasis, it is not recommended to predict the prognosis of a thymoma only according to the histological subtype without taking into account the clinical stage. Therefore, the tumoral stage (the tumoral invasion) and the histological criteria should be assessed independently to predict the behavior of a tumor.

On the other hand, most surgeons rely on the Masaoka clinical stage and on the assessment if the tumor can be totally removed, therefore further therapeutic decision must integrate all these factors.

In view of these issues, the natural question is: do we need a complex histological classification? Probably yes, because the epithelial thymic tumors have a great genetic, morphological and clinical diversity, but a careful evaluation of the subtypes is necessary. More accurate criteria for defining these tumors are required,
in order to ensure a better reproducibility and a more effective clinical, prognostic, and therapeutic integration.

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