



An overview on the differential diagnostics of tumors of the anterior-superior mediastinum: the pathologist's perspective

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Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Abstract: The thymus is the main organ in the (anterior) (pre-vascular) mediastinum, playing a central role in the maintenance of both cellular and humoral immunity. The function of the thymus has been long overlooked due to its involution starting during young adulthood and unawareness regarding its immunological function. A variety of primary tumors and inflammatory/reactive/disreactive processes occur in the mediastinum and may involve the anterior-superior compartment and the thymus. Maldevelopment processes also take place in the pre-vascular compartment mediastinum. Although infective diseases do not currently represent the main processes in western countries, they may represent a diagnostic challenge in developing countries. The purpose of this review is to provide a short overview of the main thymic cellular components, their tumors, pseudotumors, in order to provide insights into their clinical setting and the features which assist pathologists in their differential diagnosis (DD). Specific differential diagnostic points are provided, both for “solid” tumors as well as for haematological malignancies, together with a morphological overview of cases of concern that occur in the anterior mediastinum. The main immunohistochemical characteristics of neoplastic/non-neoplastic pathology and updated specific references are also provided.

Keywords: Mediastinum; pathology; differential diagnosis (DD); immunohistochemistry; thymoma; thymic carcinoma (TC); lymphoma; accessory cell sarcoma; thymic epithelial tumors (TETs)

Received: 30 September 2018; Accepted: 06 December 2018; Published: 22 February 2019.

doi: 10.21037/med.2018.12.01

View this article at: <http://dx.doi.org/10.21037/med.2018.12.01>

An insight into the pathology of the anterior-superior mediastinum

The anterior-superior mediastinum is affected by a variety of tumors, which mainly derive from its main cellular components, the thymus being the only organ solely present in that area, together with lymph nodes of the anterior mediastinum. Ectopic tissues and embryonic remnants are also found, which could give rise to tumors and to endocrine dysfunction. According to Takeda *et al.* (1), the most common location of neoplasia in the mediastinum is the anterior compartment (68%) in adults, whereas the posterior mediastinum is more frequently affected in children

(52%). Neoplasias of pediatric and adult age show different demographical, clinical and histological spectrum (2). In the so-called “prevascular area” of the mediastinum, a variety of tumors derives from stroma, vascular and neural elements. These tissues accompany the parenchymal cells of the thymus. In the review of Carter *et al.*, in this issue (3), the main imaging features of the thymus according to age and functional state and neoplastic transformation are discussed. The occurrence of benign and malignant mediastinal masses and of inflammatory/infectious diseases adds complexity to the diagnostic process of these deep-seated lesions (4).

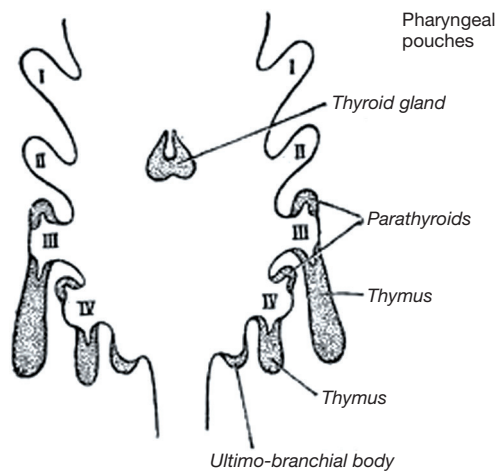


Figure 1 Embryological derivation of the human thymus: scheme showing development of branchial epithelial bodies (https://embryology.med.unsw.edu.au/embryology/index.php/File:Pharyngeal_pouches.jpg). In this historic picture, it was reported that the thymus originates from the third and fourth pharyngeal pouch. However, according to the present knowledge, the human thymus develops from the endoderm of the third branchial cleft. The two thymic endodermal primordia migrate ventrally and caudally and then fuse along the midline. The early thymus anlage initially contained undifferentiated epithelial cells (EC), then cortical and medullary EC develop together with mesenchymal, vascular and lymphoid cells. Complex organogenetic events follow (5-8) and, between 14 and 16 weeks, mature lymphocytes begin to leave the thymus to seed in the peripheral immune system (8).

From the early developmental stages to aging: a survey of thymic cellular components

The human thymus originates as a paired anlage from the third pharyngeal pouch endoderm during the early phases of organogenesis (*Figure 1*), and reaches its final destination in the mediastinum by progressive descent and complex organogenetic events (5-8). However, ectopic thymic tissue may be found along the line of descent as well as in different areas of the mediastinum/thoracic cavity. The papers published by Weissferdt and Moran (9) and by Rashidfarokhi *et al.* (10) list the main sites where ectopic thymic tissue may occur both from pathological and imaging perspectives. Different types of epithelial cells (ECs) develop in the thymus, and cortical and medullary compartments are established. Several detailed electron microscopic and immunohistochemical studies have demonstrated the heterogeneity of EC (11-13).

Medullary TEC (mTEC) and cortical TEC (cTEC) are also functionally heterogeneous (8). In fact, mEC are involved in establishing the central tolerance, and cEC are involved in the positive/negative selection of lymphocytes (14,15). As far as Hassall's corpuscles (HCs) are concerned, their origin and function remain an enigma. HCs resemble other types of stratified squamous epithelia, although they contain different cell types (16) and are believed to represent the terminally differentiated stage of mTEC (17). The concentration of BCL2 positive B-lymphocytes in HC suggested they play a role in the regulation of lymphopoiesis (16). Moreover, the perivascular space (PVS), which constitutes a specific and significant compartment of thymic tissue, develops within the thymic capsule but outside the EC network (18).

Regarding the origin of T lymphocytes in the thymus

According to von Gaudecker and Müller-Hermelink (19), by the 9th week of gestation, prethymic lymphoid precursor cells, derived from the bone marrow (BM), begin to invade the thymus anlage. There, they finally mature to committed post-thymic T cells through a very complex process (20). The T-cell lineage committed BM progenitors enter the thymus via veins in the cortical tissue close to the corticomedullary junction, and then they migrate into the thymic tissue. A specific T-cell transcriptional program is acquired by the T-cell progenitors. Furthermore, specific cell surface antigens are lost and/or acquired. cTEC play a role in the positive selection of lymphocytes, and drive the maturation of T lymphocytes whereas mTEC, through the promiscuous expression of tissue-restricted antigens (TRAs) and direct presentation of these TRAs, play a role as a self-antigen reservoir and as antigen-presenting cells (APCs) (21,22). However, a continuous "cross-talk" is necessary among epithelial and lymphoid components in order to achieve a normal thymic development (23). Thymopoiesis continues to occur in the thymus of human adults late in life despite the age-related thymic involution (18).

Regarding the origin of B lymphocytes in the thymus

The precise B lymphopoiesis which occurs in the thymus has yet to be completely clarified. However, it appears that in the thymus both intrathymic and conventional B lymphopoiesis occurs. B cells reside in the thymic medulla, specifically at the cortico-medullary junction (24). Thymic B cells were first identified in 1987 in human formalin-fixed,

paraffin embedded (FFPE) tissue sections (25). B cells can be seen in the Thymus in early fetal life and show a distinctive phenotype in comparison to other B cell subsets (26), given that it appears they develop independently from BM precursors, and that there is an intrathymic independent pathway for B cell development (14,24). Thymic B cells display unique tolerogenic features and an unclear relationship regarding peripheral B cells (14). Moreover, along with mTEC, they express the autoimmune regulator (*AIRE*) gene, a transcription factor that controls the negative selection of self-reactive T cells and the complex T-cell development (27). Two distinct B cell populations were also described in thymic epithelial tumors (TETs), both in extra-epithelial (in the PVS) and in intra epithelial compartments (28).

Accessory cells of the thymus

In addition to the main immune cell types, the thymus contains classical dendritic cells (cDCs) of intrathymically and peripherally derived subsets, with antigen presenting cell (APC) capability (29). Moreover stromal cells such as macrophages, which have a distinctive role in the removal of apoptotic lymphocytes, can also be found (30). Plasmacytoid DC with a presumptive role in presenting peripherally acquired antigens for central tolerance induction and interdigitating dendritic cells (IDCs) are also present in the thymus (29,31,32). However, thymic dendritic cells subsets and functions are still ill-defined (33).

The PVS

The PVS constitute an interface between the central thymus and the periphery. In the PVS, both peripheral T and B cells occur, similar to lymph nodes (18,26). The T lymphocytes have a phenotype of cytotoxic or memory T cells, so evidently they immigrate from the periphery. A subset of B cells in the PVS derives from germinal centers. In fact, they express somatic mutations in immunoglobulin genes (18,34). Thymic B cells from the thymic medulla or PVS could give rise to B cell thymic lymphoma (35).

On the supposed origin of germ cells in the thymus

Along the midline, germ cell tumors (GCTs) also develop, the anterior mediastinum constituting the main site of extragenital GCT development, alongside with retroperitoneum. It has been suggested that during

embryogenesis ectopic germ cells diffuse in several sites including thymus (36-38) and that these cells could give rise to GCT (39). Benign teratomas could arise from cells derived from the branchial clefts (3rd and 4th) which give origin to the thymic gland according to some studies. As referred from Busch *et al.* (40) extragonadal GCT could also derive from germ cells that have spread throughout the body during embryogenesis to contribute to important regulatory, hematological or immunological processes.

Mediastinal GCT and their differential diagnoses have been extensively discussed by Kalhor and Moran in a paper of this issue (41) and will not be discussed further here.

All the above-mentioned cell types may develop tumours. However, age and clinical/imaging contexts vary greatly and, in this paper, we propose some examples of difficult or paradigmatic diagnoses.

Diagnostic approach to anterior mediastinal tumors

Section 1: the main epithelial and lymphoid tumor types

TETs

TETs are a distinctive group of rare tumors with unique properties. The WHO classification (42), in widespread use, lists EC tumors with organotypic and morphologic features and immunological properties, the thymoma (THY) (*Figure 2*), whereas thymic carcinoma (TC) do not show organotypic and functional properties of differentiated EC. TC of different subtypes reproduce specific morphologic features of carcinomas that arise in other organs. However, some specific immunophenotypic features can assist in the pathological diagnosis. Moreover, the WHO classification includes among TET the neuroendocrine tumors. *Table 1* lists the main immunohistochemical markers useful to characterize TET and other mediastinal tumors.

Thymoma and TC

Several classification schema developed in the 20th century, reported and discussed (53) in this issue. In recent years, the World Health Classification (WHO) (54) proposed a compromise following a long debate among pathologists from several countries. In this setting, thymoma was defined as deriving or from the “atrophic”, effete EC of adult thymus, or from the “bioactive” EC of the young thymus. With time, this view provided the basis for “classifying” thymoma. According to the schema proposed by Moran and Suster, however, thymoma could be classified according to the presence of atypia in EC (55,56). In a recent paper (57),

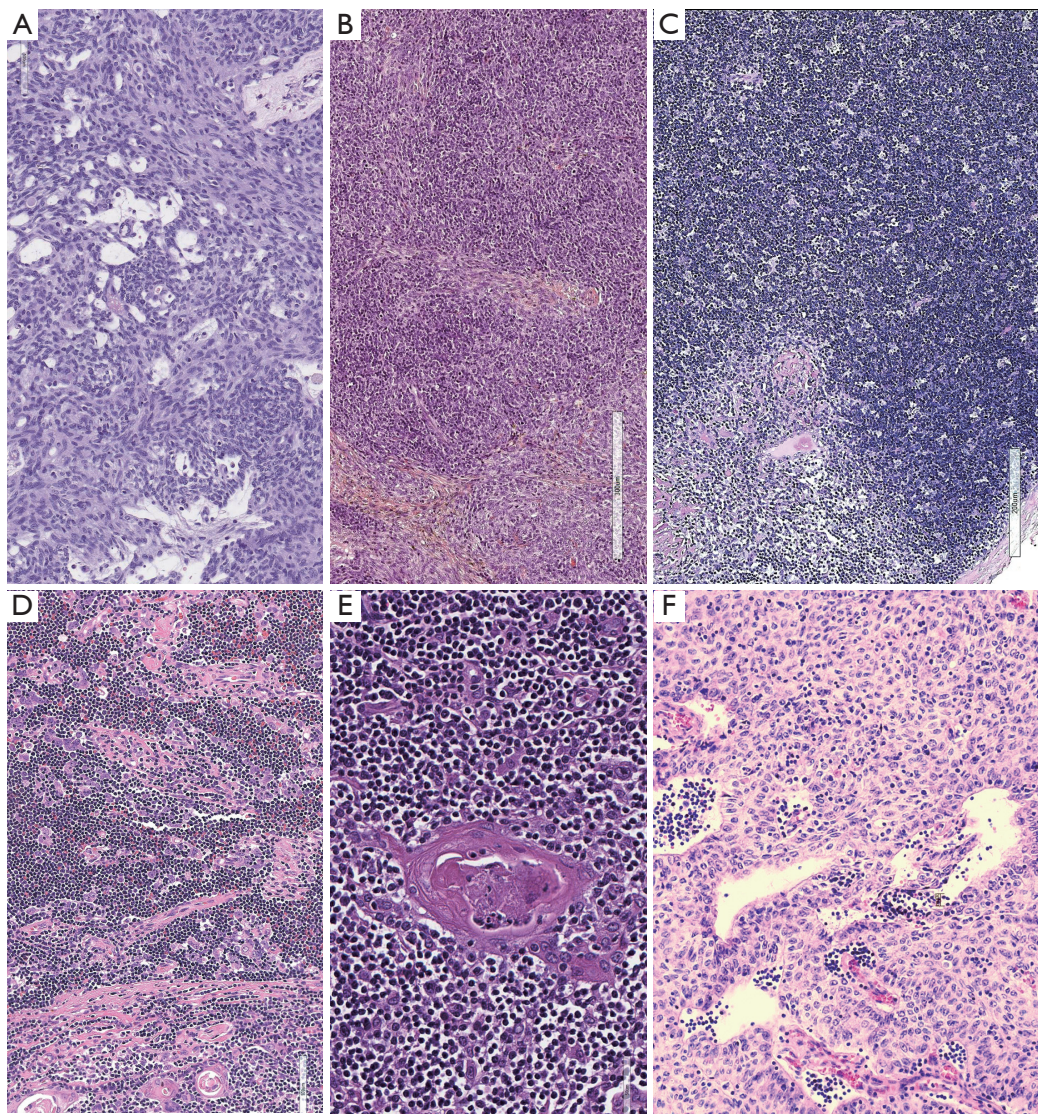


Figure 2 Overview of thymoma subtypes and cells. (A) Type A thymoma, H&E 200 \times , prevalence of spindle epithelial cells (EC); (B) type AB thymoma, H&E 100 \times , areas lymphocyte-rich alternate with areas spindle EC rich; (C) type B1 thymoma, 100 \times , a medullary (clear) island is present in the background of immature dark blue thymocytes; EC are not seen at low magnification; (D) type B2 thymoma, H&E, 100 \times , nests and ribbons of EC in the background of dark thymocytes; (E) Hassall's corpuscle in thymus and in thymoma, H&E, 200 \times : these structures are composed of different types of EC, appear most frequently in type B1 thymoma and their functions are still largely unknown; (F) type B3 thymoma: sheets of EC forming perivascular palisades; a limited number of lymphocytes is seen and perivascular spaces (PVS) are seen as clear spaces surrounding small vessels.

cases from different sources were classified according to this schema and staged according a recently proposed system (58); the authors provided outcome data supporting their view.

Thymoma

In the WHO classification, both in the 2004 (59) and 2015 (42) editions, the morphology, immunoarchitecture, imaging and clinical findings of mediastinal masses were

integrated in THY diagnosis. Furthermore, in the review by Marx and co-workers in this issue (43), the pathology of myasthenia gravis (MG) associated thymoma is explained in detail; a description of the WHO histotypes and principles of differential diagnosis (DD) is reported. MG is usually associated with the AB or B type thymoma, i.e., the THY with a cortical component. Filosso *et al.* (60) reported

Table 1 Main immunohistochemical markers useful to characterize TET and other mediastinal tumors in their differential diagnosis (42-50)

Markers of thymic epithelial tumors (epithelial component)

Pan-cytokeratin (such as AE1/3): cTEC, mTEC

Low mol. weight cytokeratin (such as CAM5.2)

High mol. weight cytokeratin (such as 34βE12): cTEC; mTEC; thymoma EC

CK19: cTEC, mTEC, neoplastic EC in all thymoma subtypes

CK20: negative in normal and neoplastic TEC; + in rare thymic adenocarcinoma

CK 5/6

p63: nuclear + in TEC and TET; cross reacts with PMBL

Pax8: reactive with thymomas, TC and with carcinoid

Glut1: useful in the DD B3 thymoma/TC

CD5: T cells, epithelial cells of many TCs

CD117: epithelial cells of most TC

CD20: aberrant expression in “medullary” type EC in TET (A and AB type)

Desmin: myoid cells in thymomas and TC

Ki-67: proliferating cells

Calretinin: negative in TET; positive in mesothelioma

Epstein Barr virus (EBV): positive in 50% of lymphoepithelioma-like TC

Neuroendocrine thymic tumors markers

Cytokeratins (CKs): cytoplasmic dot-like positivity

Synaptophysin

CD56

Neuron specific enolase (NSE)

Napsin A negativity

Thyroid transcription factor-1 (TTF1) positivity in a limited % of cases

Markers of lymphoid component in TET/markers of mediastinal lymphoma

Immature T-cell markers: CD1a, CD10, CD99, terminal-deoxy-nucleotidyl-transferase (TdT)

Other T-cell markers: CD3, CD5, CD4/CD8

CD45

Table 1 (continued)

Table 1 (continued)

LMO2 (LIM domain only 2): positivity in neoplastic lymphoblasts

CD30

CD15

CD20

PAX5

CD79a

CD56

OCT-2/BOB.1

Mal (myelin and lymphocyte protein)

MUM1

Epstein Barr virus (EBV), EBV-encoded RNA-1 (EBER) by in situ hybridization

Epstein Barr virus, late membrane protein 1 (LMP1) by immunohistochemistry

Markers associated to hematological diseases/neoplasms/pseudotumors

CD34

Myeloperoxidase (MPO)

Glycophorin

CD61

Fact VIII

CD68/PG-M1

CD68/KP1

Human herpes virus 8 (HHV8) [latency-associated nuclear antigen 1 (LANA-1)]

CD138

IgG4

Some of the epithelial-directed antibodies cross react with lymphoid or haematological cells (as an example CD5, CD20, CD117, CD34). For GCT the reader is referred to the review from Kahlor and Moran in the same issue (41). Markers for DD with other spindle cell tumors and with malignant peripheral nerve sheet tumors (MPNST): the diagnosis of these tumors is also discussed by den Bakker *et al.* (51) in the same issue, by Marx *et al.* in recent contributions (42,44,52) and by different authors in a recent book (50). TEC, thymic epithelial cells; TET, thymic epithelial tumors; TC, thymic carcinomas; cTEC, cortical thymic epithelial cells; mTEC, medullary TEC; GCT, germ cell tumors; DD, differential diagnosis; PMBL, primary mediastinal B cell lymphoma.

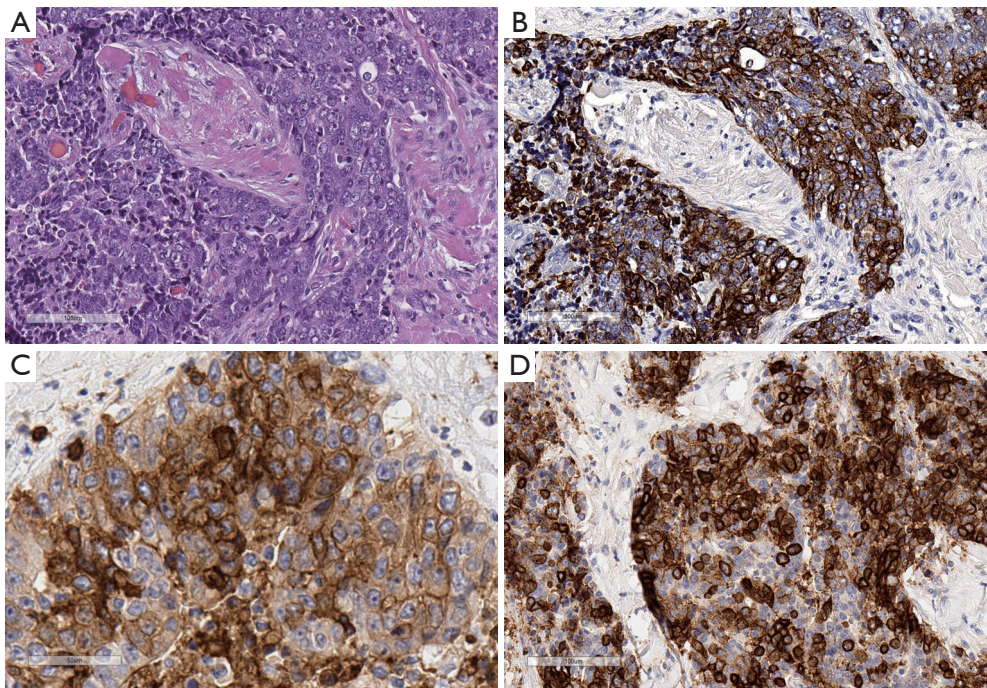


Figure 3 Thymic poorly differentiated squamous cell carcinoma (SQCC): (A) H&E, 200 \times , sheets of poorly differentiated EC without lymphocytes in between; (B) CKMNF116, 200 \times : most EC are stained; (C) CD5, 400 \times , EC aberrantly express CD5, a marker of T lymphocytes; (D) CD117, 200 \times , most EC reacts with CD117 in thymic carcinoma (TC).

a positive correlation between MG and B-component containing THY. Weis *et al.* (61), in a series of 4,221 thymoma from the International Thymic Malignancy Interest group (ITMIG) database (62), reported that MG is more frequent in type B1–3 thymomas (35–49%) than in type A and AB (25–26%).

The TC

TC of different subtypes such as the ones listed in the WHO classification (42) occurs in the anterior superior mediastinum, the squamous cell carcinoma (SQCC) being the most frequent among the several rare subtypes (Figure 3A,B). TCs also rarely develop in thymoma (63), requiring specific attention and reporting rules, such as discussed by Marx *et al.* (44). The micronodular TC and the malignant counterpart of the micronodular thymoma (64,65) merits attention here due to the rarity of this histotype, which is yet to be listed in the WHO classification. Relevant immunohistochemical markers can support diagnosis because the expression of CD5, CD117 is useful in distinguishing TC from carcinomas that arise in other organs (45,46,66–72) (Figure 3C,D), whereas GLUT1 and MUC1 appears to be relevant regarding the DD of TC with B3 thymoma (45,73,74). PAX8, a transcription factor

involved in the embryonic development of a limited number of organs, and found to be expressed in TET (47,48) and a few other tumor types (75,76), should also be considered in DD with other non thymic tumors.

Thymic adenocarcinomas have also been shown to react with CD5 and CD117 (77). Mucinous and enteric types of thymic adenocarcinoma have been described (78,79) and have been frequently associated to thymic cysts. The well-known occurrence of cystic tumors in the mediastinum (80) points to the need of an adequate sampling of every removed cystic lesion of the mediastinum (81,82). Magnetic resonance imaging (MRI) had been described as a powerful tool to distinguish benign cysts from mediastinal neoplasms (83,84). Nuclear protein of the testis (NUT) midline carcinoma [or lethal midline carcinoma, or TC with t(15;19) translocation], also included among TC, is a highly malignant undifferentiated type of carcinoma occurring at times in the thymus (85,86), but also in the lung (Figure 4A). This diagnosis requires a specific immunohistochemical (87) (Figure 4B) and fluorescence in situ hybridization (FISH)-approach in order to be diagnosed (88). In this paper, we present examples of a thymic well differentiated SQCC (Figure 5A) (89), an example of clear cell carcinoma (Figure

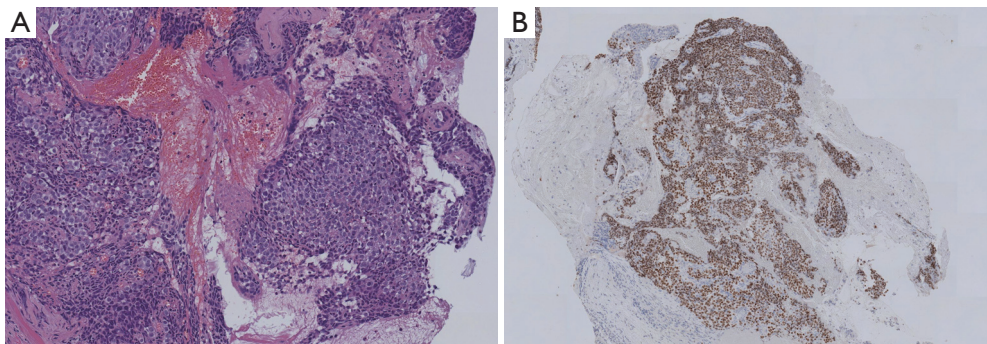


Figure 4 Nuclear protein of the testis (NUT) carcinoma: (A) H&E, 50x; (B) NUT stain, 50x. NUT carcinoma or nuclear midline carcinoma (NMC) is a variant of poorly differentiated squamous cell carcinoma (85-87). NUT midline carcinoma [or lethal midline carcinoma, or thymic carcinoma with t(15;19) translocation], are composed of a mixture of primitive, undifferentiated and squamous cells.

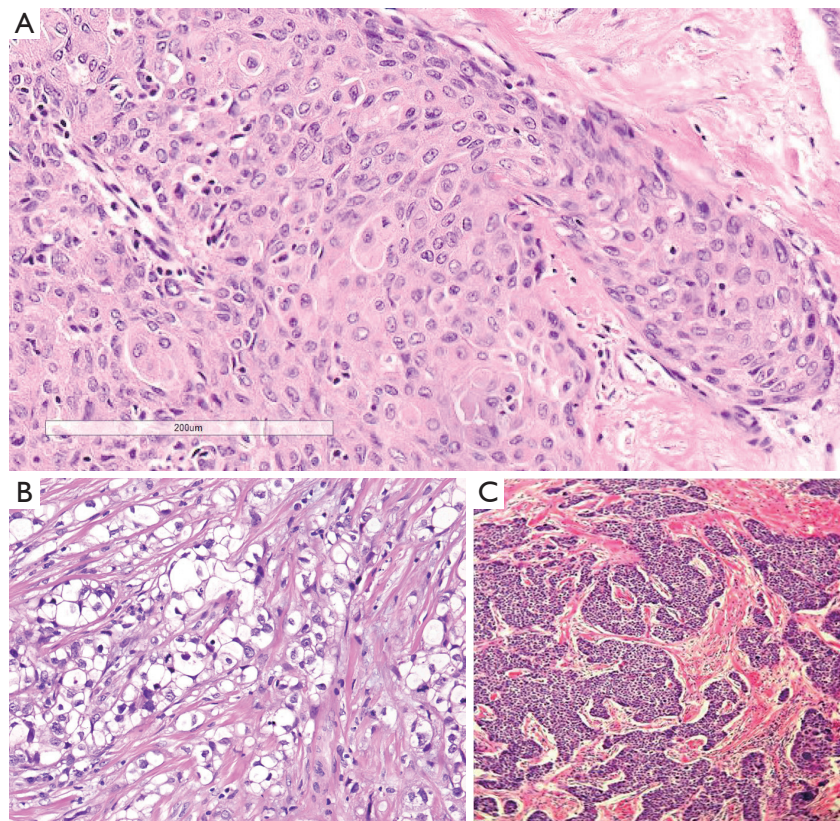


Figure 5 Examples of thymic carcinoma (TC): (A) well differentiated squamous cell carcinoma (SQCC), H&E, 200x; (B) clear cell carcinoma, 200x, courtesy of Prof. John Chan; (C) anaplastic carcinoma, 100x, courtesy of Prof. Libero Lauriola.

5B) (90), and of an “anaplastic” carcinoma (91) metastatic to the liver (Figure 5C).

Neuroendocrine thymic tumors (tNETs)

In the thymus, the very rare occurrence of primary neuroendocrine tumors (tNETs) is reported, and their

nomenclature and subtyping follow the general rules applied also for neuroendocrine lung tumors (42). A recent review reports an update of morphological features, immunohistochemical staining and reporting rules in combined cases (92). The well differentiated form of tNET,

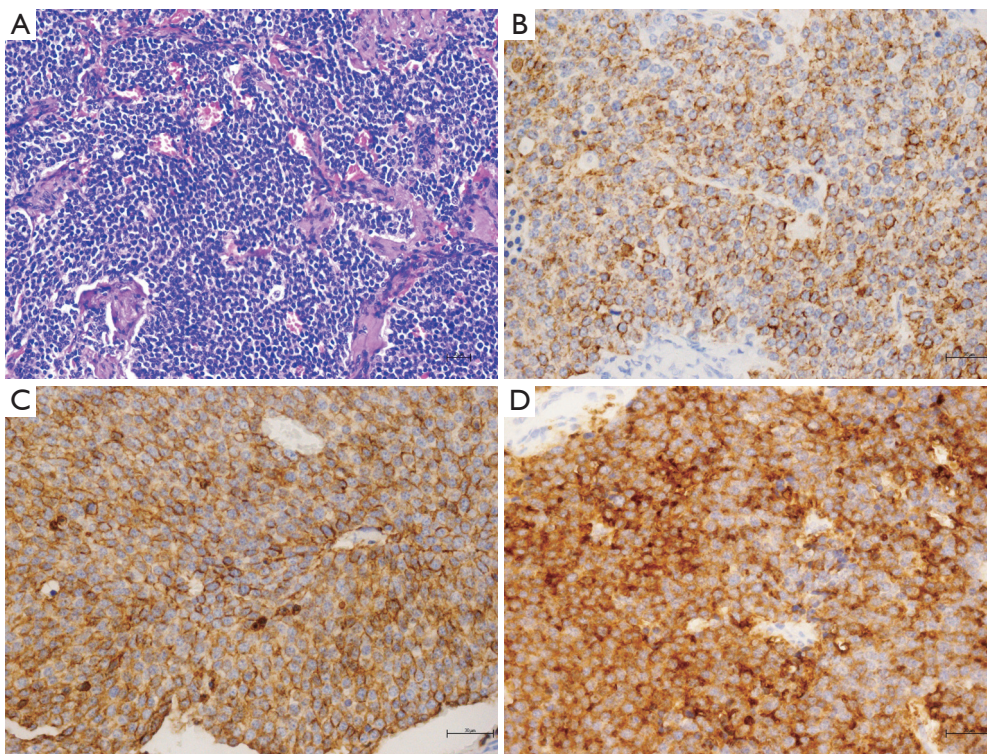


Figure 6 Thymic atypical carcinoid, a subtype of primary thymic neuroendocrine tumors (tNET): (A) H&E, 200 \times , sheets of monotonous EC with moderate amount of cytoplasm; (B) CK 19, 200 \times , cytoplasmic, predominantly dot-like positivity; (C) CD56, 200 \times , diffuse positivity; (D) synaptophysin, 200 \times , diffuse positivity. According to the WHO classification (42), in atypical carcinoid the number of mitoses should be 2–10/10 high power fields (hpf).

the carcinoid tumors of the thymus, also arises in the framework of MEN type 1. An association with smoking was reported (93) and tNET accounts for almost 20% of multiple endocrine neoplasia type 1 (MEN1)-associated mortality (94). Immunohistochemical markers of tNET include those currently utilized for lung neuroendocrine tumors, whereas genetic markers appear to be different (95). *Figure 6* shows the main morphological and immunohistochemical features of a case of atypical thymic carcinoid. Moreover, PAX8 may support the DD of tNET according to the contexts, but antibody clone and clinical information should also be considered (96,97).

Primary lymphoma in the anterior superior mediastinum

Lymphomas in the mediastinum account for 15% of mediastinal masses; they arise either in the thymus or from the mediastinal lymph nodes and only about 10% are primary (98). In biopsies performed on a lymphoid mass, it is difficult to find thymic remnants and other morphological

findings that could indicate a thymic origin due to the fact the neoplastic growth has a destructive action. However, when these remnants are seen they should be correctly recognized as such. We will focus here on the specific lymphomas arising or considered to derive from the thymus itself.

Due to the intrathymic presence of an immature T cell compartment as well as to the existence of peripherally derived B and T cells and of a specific intrathymic B-lymphopoiesis, it is not surprising that both lymphoma of immature precursor type (T and B) (99,100) as well as cases of peripheral T and B-cell lymphoma occur in the thymus. Hodgkin lymphoma of classic type (cHL) of thymic origin represents the most frequent mediastinal lymphoma (101). Among the lymphoma occurring in the mediastinum, T cell lymphomas of precursor type predominate in pediatric age, whereas, in the young adult and in adult age, the most frequent lymphomas are of B cell origin or are cHL of the thymus (102-104).

The clinical presentation is usually an important part of

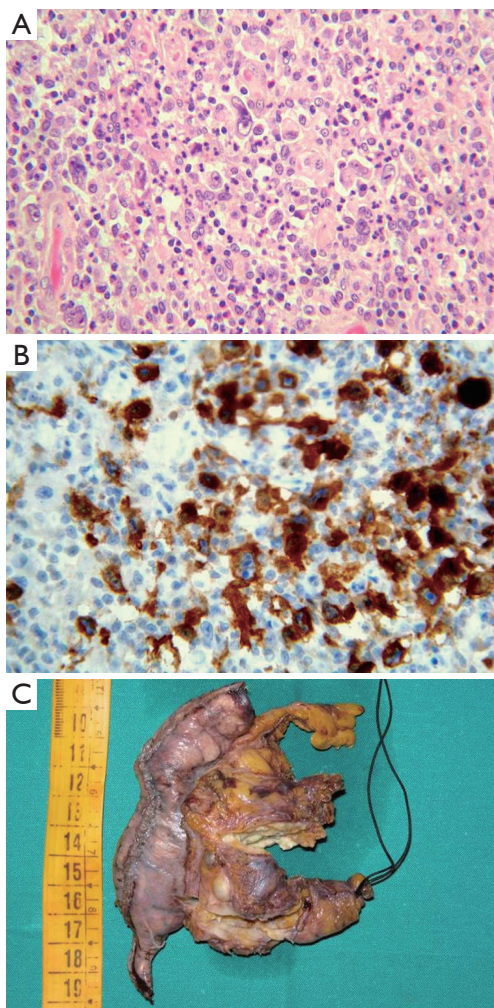


Figure 7 Classical Hodgkin lymphoma (cHL) of the thymus. (A) Polymorphic lymphoid cell population including scattered large atypical cells with the morphological features of Hodgkin's cells, H&E, 200 \times ; (B) CD30 staining of large atypical Hodgkin's cells 200 \times ; (C) macroscopy of a case of cHL in the thymus: mediastinal Hodgkin lymphoma is often cystic. In this case the mediastinal mass was adherent to lung parenchyma. Therefore extensive sampling of cystic mediastinal lesions is recommended.

the diagnostic process because T-lymphoblastic lymphomas (T-Lb lymphomas) arise, and are usually associated with T-cell lymphocytosis, in pediatric age or in a younger age group, by comparison with TET. cHL of the thymus is predominantly a tumor of the young age and primarily of females. B-cell lymphoma of primary mediastinal B cell type (PMBL), which primarily arises in the thymus, usually shows an acute or sub-acute presentation, with symptoms that include fast growing mass and respiratory

difficulties, the female sex and young or young-adult ages being predominantly affected (105,106). Moreover, among the tumors of mature B lymphocytes, the mucosa associated lymphoid tissue lymphoma (MALT lymphoma) of the thymus should be also considered (107), mostly when occurring in patients affected by autoimmune diseases (108,109). We present here in detail the prevailing lymphoma types in the Mediastinum, and we provide examples of neoplasias that derive from the accessory cells of the thymic lymphatic tissue.

cHL

The most frequent subtype of cHL in the mediastinum is the nodular sclerosis (NS) variant (110), constituting about 50–70% of primary mediastinal lymphoma (cHL-NS). It arises from a thymic B cell (111). The diagnosis relies on the demonstration of typical C30+ cells, which are often very rare, in a mixed fibroinflammatory background (112) (*Figure 7A,B*). Regarding the thymus, the cHL or infiltrates it or it is sharply demarcated from the thymus. Moreover, in cHL reactive EC proliferation and/or cystic changes (*Figure 7C*) can be elicited, simulating a thymoma. Therefore, again we suggest sampling every mediastinal cystic lesion extensively because foci of HL could be found in the wall (110). The lymphoma usually forms large firm masses with foci of necrosis and eosinophilic abscesses. cHL frequently constitutes a differential diagnostic problem with the primary mediastinal B cell lymphoma (PMBL), which is also associated to sclerosis. In fact, cHL-NS and PMBL shows the same (B) cell origin, similar morphology and may show the similar clinical presentation. Furthermore, some immunophenotypical and gene expression profile similarities exist (113). In *Table 2*, a list of the main immunophenotypical characteristics of neoplastic lymphoid cells in HL, in PMBL and in the entity denominated “Gray Zone Lymphoma” (GZL) (118) (B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma in the 2017 WHO classification) (49) is provided.

PMBL

This lymphoma was first described by Menestrina *et al.* (119) and named mediastinal B cell lymphoma with sclerosis. In fact, a distinctive fibrosis is associated, with large and medium-large pleomorphic cells, with clear cytoplasm in the central part and peripherally distributed lymphocytes in the sclerotic background (*Figure 8A,B*). This lymphoma usually forms bulky, solid masses >10 cm, with local symptoms of rapid growth, invasion and compression of vital structures. The tumor is limited to the thorax with no

Table 2 Main immunophenotypical characteristics of neoplastic lymphoid cells in HL, in PMBL and in the entity denominated “Gray Zone Lymphoma” (GZL)

Antibodies	cHL	PMBL	GZL
CD30	+ (85–96%, Memb.+ Golgi area)	>80%, weak and heterogeneous	Usually +
CD15	+ (75–85%)	Usually –	May be expressed
CD45	–	+	+
CD20	<20–40% +, variable intensity	+	+ usually
CD79a	Rarely+	+	+ usually
PAX5	+ weak in comparison with B cells	+	+ usually
OCT2	–/+ usually	+	+ usually
BOB1	–	+	+ usually
MUM1	+	+ (75% of cases)	+ usually
EBER (EBV)	Only 10–25% of cases	Mostly –	Mostly –
LMP-1 (EBV)	19% (114)	Mostly –	Mostly –
MAL	Negative	Positive	Positive in a subset of cases
CD23	Negative	+ (70% of cases)	NA
BCL6	Negative	+ (45–100%)	Variably +
Cyclin E	+ 79% (114)	Negative	NA
P63	NA	Positive	NA
Fascin	Positive	negative	NA

The reactivity patterns reported derive, modified, from the WHO classification of thymic epithelial tumors and of lympho hematopoietic tumors and from several review dealing with immunohistochemistry of mediastinal lymphoma (42,49,114-117) to which the reader is referred for further details.

involvement of lymph nodes or other lymphoid organs (only supraclavicular nodes are eventually reached). Neoplastic infiltrating CD20+ B cells (*Figure 8C*) have pleomorphic nuclei, ranging from regular, round nuclei to irregular, multilobulated forms. The cytoplasm is pale to clear. In certain cases, the neoplastic cells are large with prominent eosinophilic nucleoli, which resemble Reed-Sternberg cells or variants. CD30 is weakly or focally expressed (*Figure 8D*), but MAL, a protein involved in lymphocyte signal transduction, is expressed (120) by PMBL, and the inflammatory background is absent. The MAL antibody is directed against a membrane protein and targets a minor subpopulation of thymic medullary B cells (121). Specific genetic features are exhibited by the PMBL such as gains in loci of 9p24 and 2p15 as well as Xp11.4-21 and Xq24-26 (122,123).

GZL

GZL or “B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and

classical Hodgkin lymphoma” according to the 2017 WHO classification (49) is a lymphoma with morphological (*Figure 9A,B*) and immunohistochemical characteristics (*Figure 9C,D,E,F,G,H*) intermediate between HL and PMBL (124). The pathological tissue often contains “lacunar” cell-forming aggregates, in the subtype known as “syncytial variant”. In the case showed here neoplastic cells were coexpressing CD20, CD30, PAX5 and OCT2. GZL originates in the Mediastinum from a thymic medullary B cell (118). It arises in young patients (20 to 40 years) with a large anterior mediastinal mass eventually involving the supraclavicular lymph nodes. The genetical and epigenetic alterations reported show similarities and differences with those reported for PMBL and HL (125,126). The diagnostic criteria were recently refined in a retrospective large series from Sarkozy et al, in the framework of the lymphoma study association (LYSA) (115) and in the recently published WHO classification of haematological malignancies (49). A diagnostic scoring system was also proposed (116). We

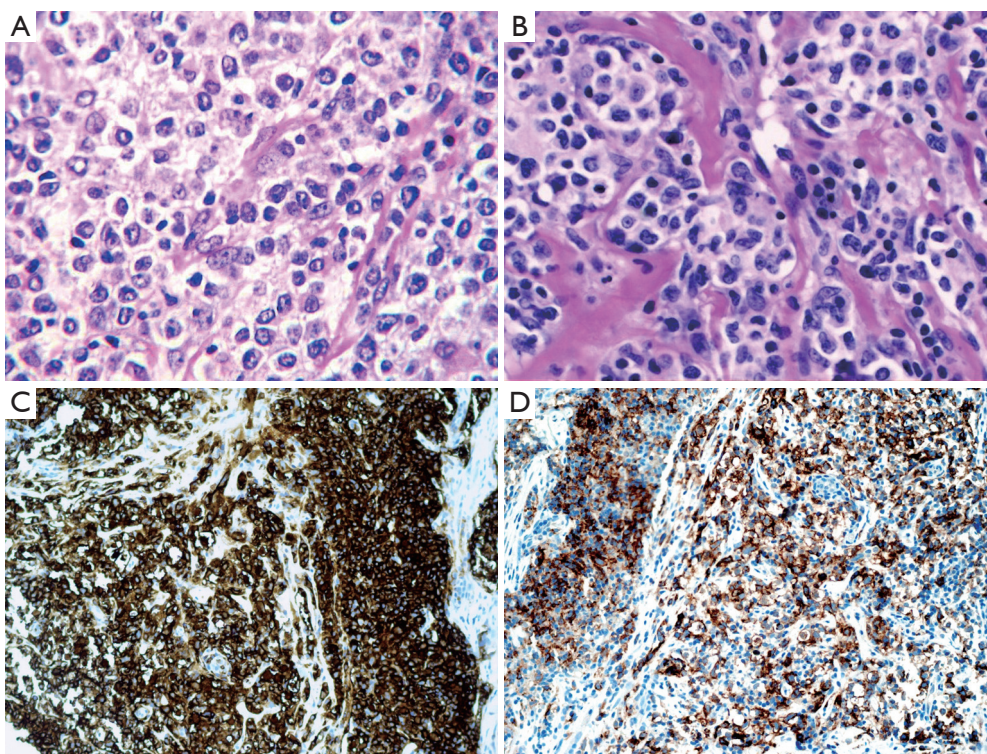


Figure 8 Primary mediastinal B cell lymphoma (PMBCL): (A) H&E, 400 \times ; (B) H&E, 400 \times ; (C) CD20, 200 \times ; (D) CD30, 200 \times . In (A & B), the sclerotic background is seen, as well as the irregular nucleus and the clear cytoplasm of the neoplastic cells B cells in PMBL in the sclerotic background (119). CD20 positivity is the hallmark of the disease, but CD30 is often coexpressed. (C & D) Courtesy of Prof. Fabio Menestrina.

previously listed in *Table 2* the main differential diagnostic characteristics of these lymphoma types (114,115,117). The adequate diagnosis is crucial due to clinical differences and treatment decisions (127,128).

MALT lymphoma of the thymus

Mature, peripheral B-cell lymphomas also occur in the thymus, the most common being the mucosa-associated B cell lymphoma of MALT type (MALT-LYM) (129). MALT-LYM of the thymus is often cystic. This rare lymphoma type usually develops in autoimmune diseases (130-133) and is characterized by scant residual thymic EC network, and by lymphoepithelial (LE) lesions involving HC (*Figure 10*). The biological substrate of autoimmune disease could stimulate the formation of acquired MALT (107,134).

Neoplasms of accessory cells of the lymphatic tissue

This group of rare neoplasias includes those deriving from Langerhans cells (sarcoma and Langerhans cell histiocytosis), histiocytic sarcoma, Follicular dendritic cell sarcoma,

and interdigitating reticulum cell (IDC) sarcoma (42). These tumors as a group should be differentiated from the other sarcoma/mesenchymal tumors, which occur in the mediastinum, reviewed and discussed by den Bakker *et al.* in the same issue (51). Moreover, it should be pointed out that Langerhans cell histiocytosis often occurs in children, leading to widespread involvement, whereas in adults, thymic involvement is often marginal, and limited to small aggregates of LC in otherwise thymic hyperplasia or in a thymic cyst (135). Accessory cell sarcomas have been rarely reported to develop in GCT of the mediastinum (42). The follicular dendritic cell sarcomas (FDCS) often arise in the framework of Castleman disease (CD). CD is a heterogeneous group of non-neoplastic lymphoproliferative disorders of the lymphatic tissue, occasionally giving rise to neoplasms of the constituting cells (both lymphoid and “accessory”) (136-138) (*Figure 11*). In the mediastinum, the most frequent type of CD is the Hyaline-vascular type (HV), but a plasmacellular type (PC) is also described and

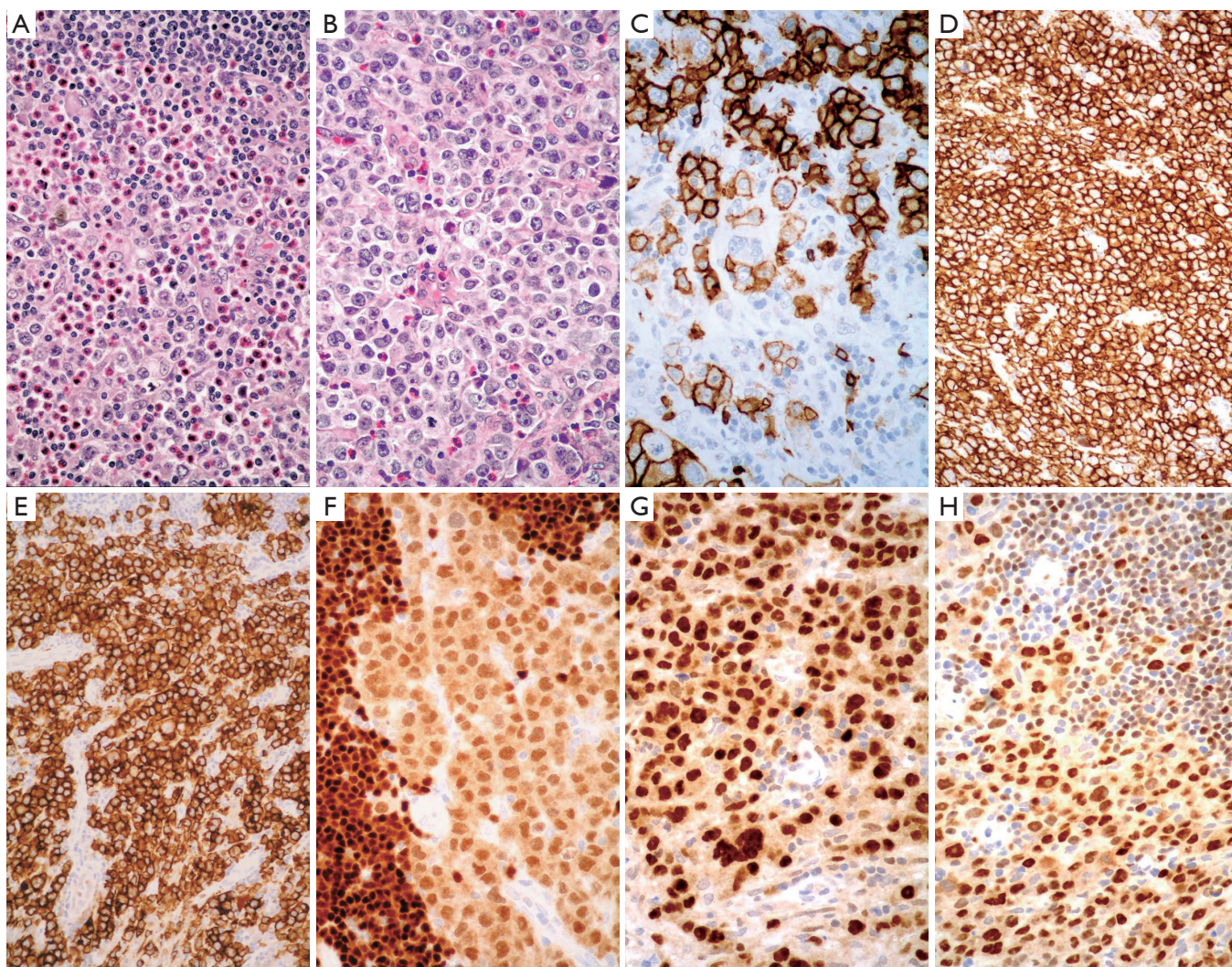


Figure 9 Gray zone lymphoma (GZL) is a lymphoma with morphological and immunohistochemical characteristics intermediate between HL and PMBL (49,114-117). (A) H&E, 200x; (B) H&E, 200x; (C) CD20, 200x; (D) CD20, 200x, different field; (E) CD30, 200x; (F) PAX5, 200x; (G) MUM1, 200x; (H) OCT2 200x. In the GZL case showed here neoplastic cells coexpress CD20, CD30, PAX5 MUM1 and OCT2.

usually associated to multicentric disease (139-141). Most of the CD of multicentric types are associated with polyclonal hypergammablobulinemia, elevated VES rate, increase in LDH or IL-6 or with thrombocytopenia; moreover multicentric CD is often associated with HHV-8 and HIV infections (142,143).

Some of the neoplasias described may simulate sclerosing (or fibrosing) mediastinitis (SM), which will be discussed later.

Other tumors of the thymus, such as GCT and soft tissue tumors have been presented by other authors in this issue (41,51) and will not be discussed here.

Metastasis in thymus/mediastinum

The incidence of thymic metastases is difficult to establish due to the possibility of secondary thymic involvement by diseases which first involve the mediastinal lymph nodes. This statement was first made by Castleman (144). Middleton (145) first performed a detailed autoptic study of the thymus in patients that had deceased due to multiple pathologies. He described the patterns of involvement of the thymus in several cases. According to Suster and Moran, the metastases constitute the prevailing neoplastic pathology in the mediastinum (146). Usually the metastases involve the middle mediastinum or hilar lymph nodes. Teratoma

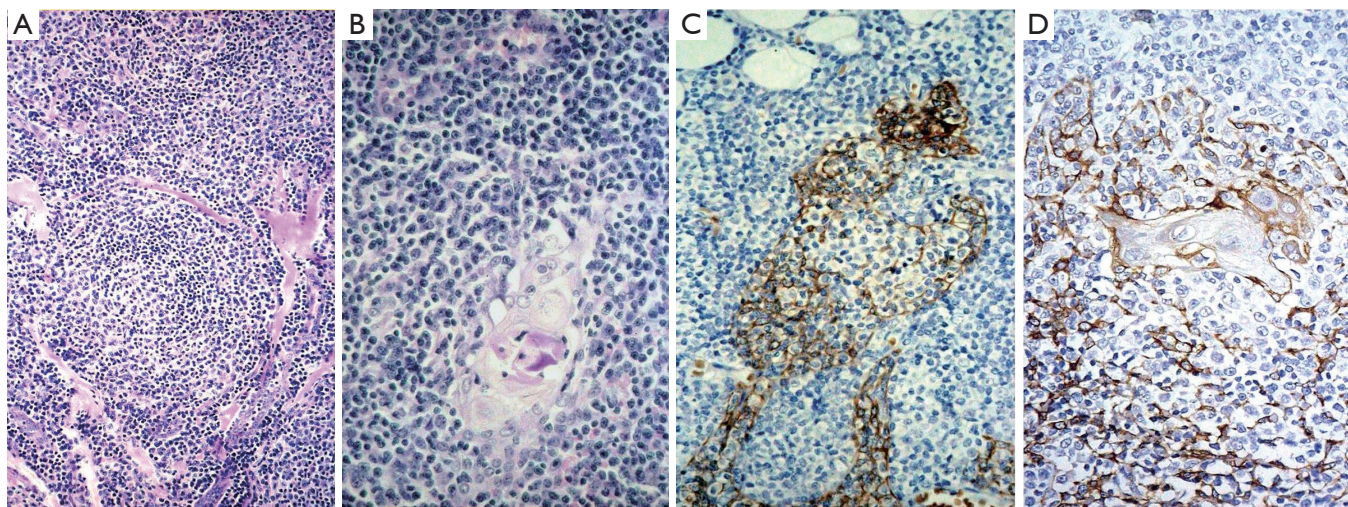


Figure 10 Mucosa associated lymphoid tissue (MALT) lymphoma of the thymus. (A) H&E, 100 \times , a residual follicle is seen among the small monocytoid B cells; (B) H&E, 200 \times , Hassall's corpuscle surrounded by neoplastic B cells; (C) pancytocheratin stain, 200 \times : residual thymic epithelial networks in the lymphoid background; (D) lymphoepithelial (LE) lesions in a Hassall's corpuscle, pancytocheratin stain, 200 \times .

metastasis from the testis also occurs in the mediastinal lymph nodes (*Figure 12A*). Metastatic lung carcinomas involve the mediastinal lymph nodes more frequently than other tumors. Melanoma metastasis is also frequently reported in the thymus (*Figure 12B,C,D*). However, also tumors from the breast could involve the thymus. We personally had the occasion to study a breast cancer metastasizing in a thymoma, likely through the lymphatic drainage (not shown here). Moreover, thyroid carcinoma metastases are reported as being more frequently localized to the upper mediastinum (147,148). Immunohistochemistry plays a relevant role in the identification or confirmation of the primary site (42,46,72).

Section 2: practical approach to the DD of lesions of the anterior mediastinum

A variety of different diagnostic/surgical approaches are adopted by clinicians when facing a mediastinal mass of unknown origin. The clinical examination should include an immunological check-up including anti-acetylcholine receptors and anti-nuclear antibodies, together with a complete examination of blood cells and serum protein electrophoresis. The imaging, the clinical setting of TET and of the main different neoplasms occurring in the mediastinum have been reported extensively by Carter *et al.* (3,106) and informative studies have also been published by den Bakker *et al.* (38), by Marchevsky *et al.* (105) and

in recently published books/book chapters (50,52), so we will not report on them here in detail. The work-up of tumors which appear to be resectable and have the radiologic appearance of TET, such as a distinct lobulated architecture, does not include a diagnostic pre-treatment biopsy (149).

In well fixed and stained cases, the diagnosis of TET, as also confirmed in the WHO classification (42) can usually be carried out on H&E stains of well-sampled surgical cases. The sampling of tumors must include at least one paraffin block every centimeter size (150). The use of immunohistochemistry in the routine setting is first of all needed in the evaluation of surgical biopsies and of core biopsies (105). These specimens must be evaluated in an expert center because an experienced pathologist in this particular type of tumor/anatomical area is recommended. The hematolymphoid diagnostic experience has also been recommended because, as previously described, TET and lymphomas or other haematological diseases often require DD and/or the awareness of rare diseases is needed. A panel of immunohistochemical stains is suggested as mediastinal masses require interpretation of patterns, recognition of normal remnants of background structures and awareness of the variety of neoplastic/pseudoneoplastic patterns (38,70,151). The previously reported *Table 1* reports a list of antibodies which are useful in the DD of TET from other primary malignancies and from metastases (45,46,48,69,72,73,152-156).

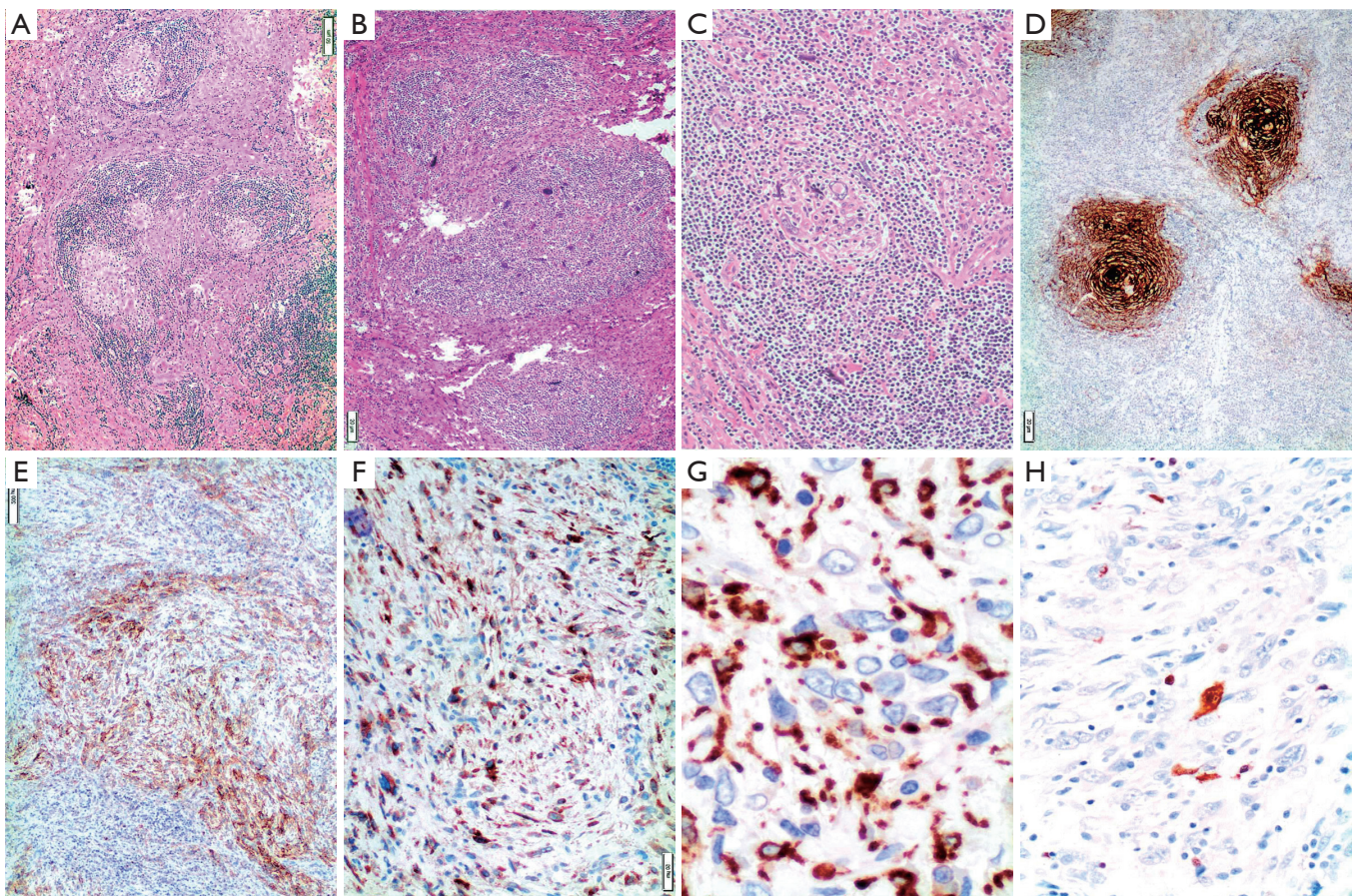


Figure 11 Follicular dendritic cell sarcoma (FDSC) arising in Castleman disease (CD). CD is a heterogeneous group of non-neoplastic lymphoproliferative disorders (136-138) of the lymphatic tissue, occasionally giving rise to neoplasms of the constituting cells, both lymphoid and “accessory”. (A) H&E, 100 \times ; (B) H&E, 100 \times ; (C) H&E, 100 \times ; (D) CD21, 100 \times ; (E) EMA, 100 \times ; (F) Clusterin, 200 \times ; (G) CD163, 400 \times ; (H) S100, 200 \times . (A-C) Residual follicles of CD surrounded by a polymorphic neoplastic population. (D-G) Neoplastic cells are positive for CD21, EMA, Clusterin, CD163, and negative for S100 (H).

A contribution of cytology in the DD of thymic tumor remains controversial (157). Certainly, fine needle aspiration (FNA) may reveal ECs in an immature T-cell component, thus providing elements for a diagnosis of thymoma. However, the thymoma diagnosis and subtyping should be performed on histological specimens, due to the complexity and variety of cellular patterns. In fact FNA and also core biopsy provide scant material (158). When immunohistochemistry can be performed, an antibody panel should be considered given that the use of single markers could be misleading (38,105).

In this overview, our aim is to present first examples of (I) lymphoid-cell-rich lesions of T-cell type; (II) lymphoid-cell-rich lesions of B-cell type; (III) the spectrum of CD34+ neoplasias in mediastinum (or rare haematological

localizations and/or vascular tumors); (IV) lymphocyte poor, fibrosing lesions with different pathogenesis and biological meaning; (V) solid or vascular tumors with overlapping/aberrant immunophenotypical features. It should be taken into account that the diagnosis of lymphoma in Europe is largely based on histological and immunohistological findings, whereas, in the United States, the cytofluorimetric approach is generally used for lesions suspected to be lymphoma. Therefore, we show our routine experience in cases which could represent a difficult DD.

The spectrum of lymphoid-cell-rich lesions of T-cell type

Most lymphocyte-rich lesions in the anterior mediastinum contain T-cells, with immature phenotypes predominating.

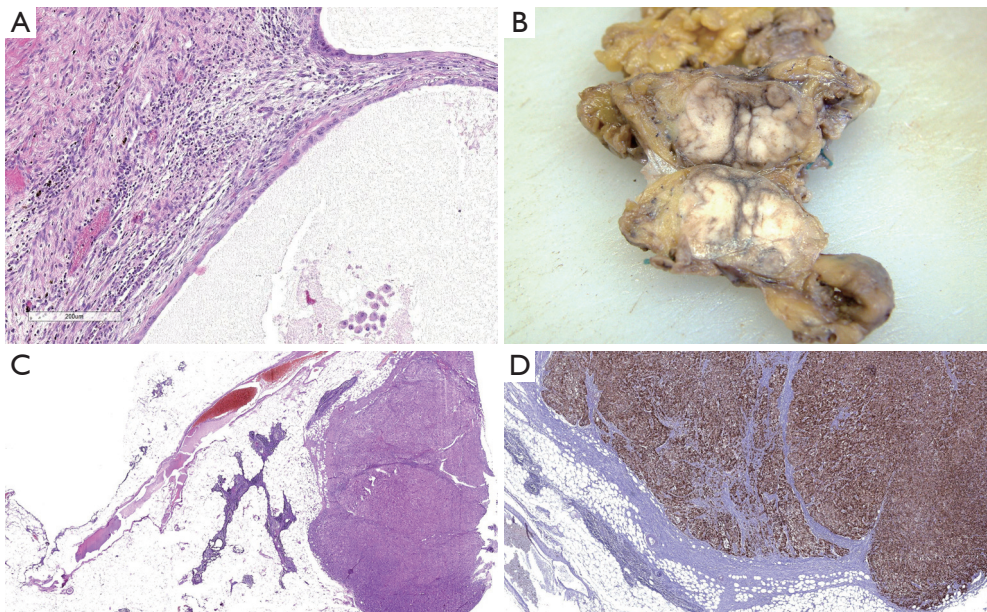


Figure 12 Metastases. (A) Testicular teratoma metastasis in a mediastinal lymph node, H&E, 100x; (B) thymic metastasis of melanoma, surgical specimen; (C) H&E, 50x, the melanoma metastasis shown in (B) is located in the thymic tissue, residual thymus is clearly seen also outside; (D) melanoma metastasis in the thymus, HMB45 stain, 100x.

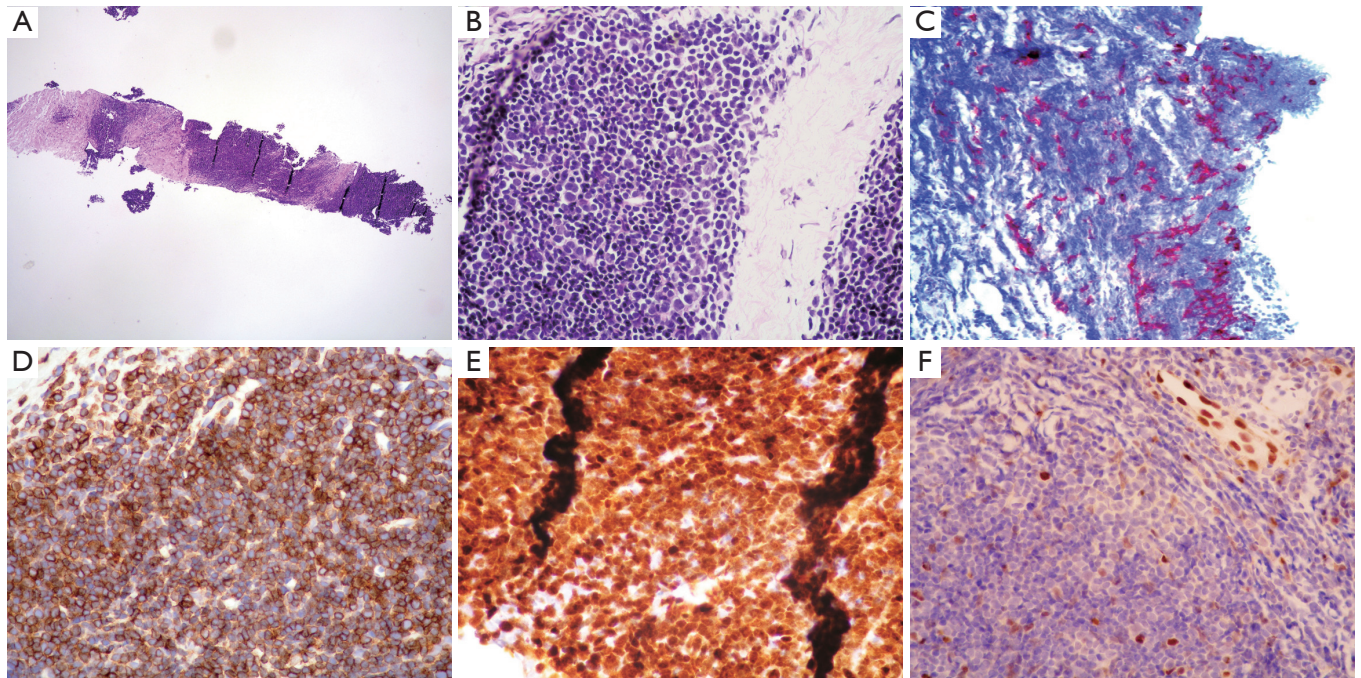


Figure 13 The morphologic spectrum of T cell rich lesions: B1 thymoma. (A) FNBA of a T lymphoid-rich mass: epithelial cells at low magnification are not easily seen, 40x; (B) higher magnification highlight an epithelial cell component, 200x; (C) CK AE1/3 stain, 100x; (D) CD3, 100x; (E) Tdt stains the background of immature T-cells, 100x; (F) LMO2 however, doesn't react with these immature T cells, confirming their benign nature, 100x.

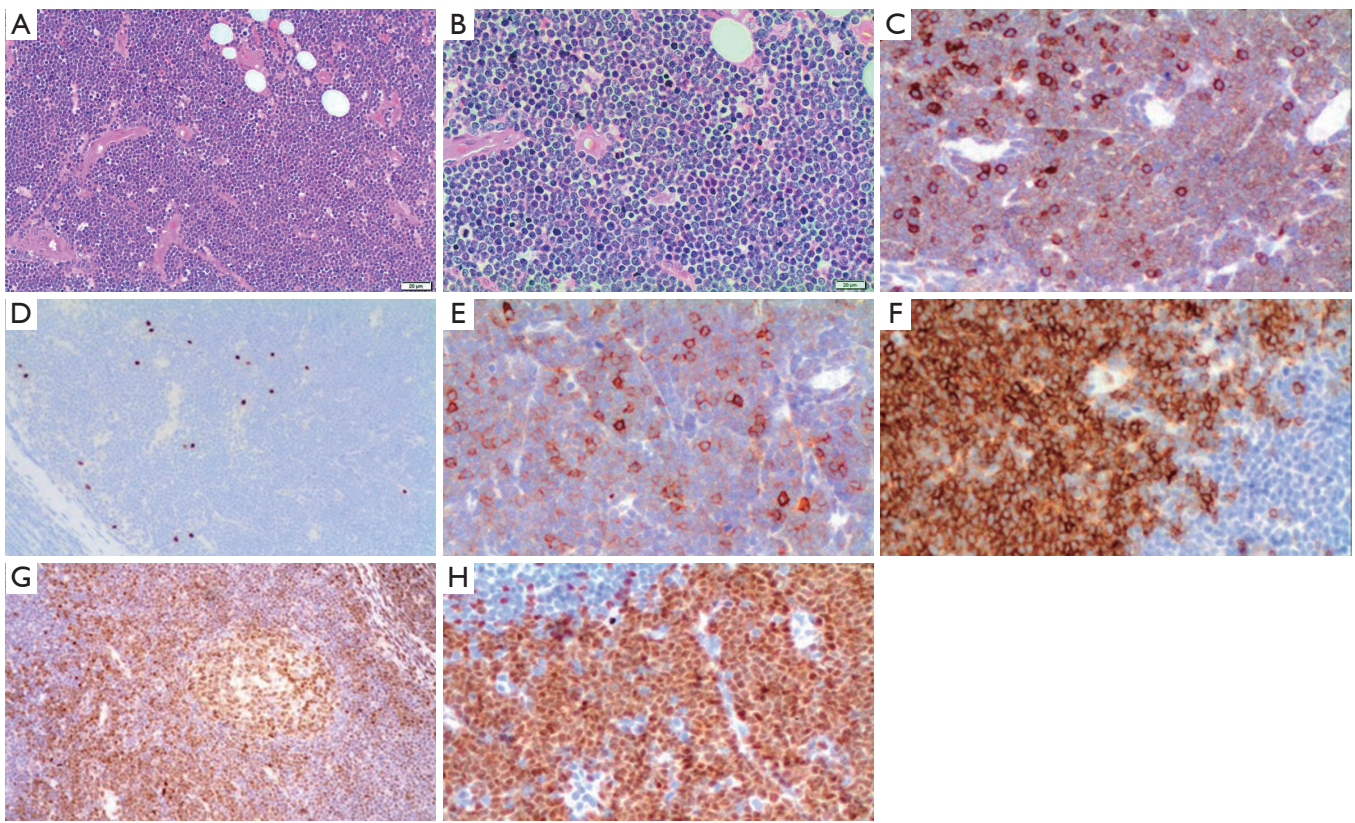


Figure 14 The morphologic spectrum of T cell rich lesions: T-cell lymphoma, lymphoblastic (T-Lb). (A) A 22-year-old male, H&E, 100 \times ; (B) H&E, 200 \times ; (C) CD3, 200 \times ; (D) Pax5, 100 \times ; (E) CD1a, 200 \times ; (F) CD10, 200 \times ; (G) LMO2, 200 \times ; (H) Tdt, 200 \times . In the case shown, the monotonous, atypical neoplastic cells (A,B) are positive for CD3, CD1a, CD10, LMO2, Tdt (C,E,F,G,H), and negative for PAX5 (D).

Both B1 thymoma and T-lymphoblastic (T-Lb) lymphoma may be similar in appearance at first glance, and both tumors in addition are frequently necrotic. Demographical data and clinical presentation are usually of support in the diagnosis, so underlying—once again—the need for continuous clinico-pathological correlation. *Figure 13A* shows a core biopsy of a B1 thymoma. In the area shown, the high lymphocytic content almost obscures the epithelial network. However, at higher magnification (*Figure 13B*) and by the cytokeratin staining (*Figure 13C*), the EC network is highlighted, at the periphery of the lymphoid areas. The figure shows that CD3⁺ lymphocytes (*Figure 13D*) in B1 thymoma are also Tdt positive (*Figure 13E*) but LMO2 negative (*Figure 13F*). Otherwise, the routine immunophenotypical features of T-lymphocytes overlap in the 2 neoplasias, B1 thymoma and T-Lb lymphoma, as shown in *Figure 14*. Please note, in addition, that in T-Lb lymphoma exceptionally EC framework remnants could still be present—such as thymic or cystic epithelial

structures. Therefore, the evaluation of the structure formed is equally important as the EC network detection, and the under-evaluation of these different characters may constitute a pitfall for the diagnosis. Furthermore, the morphological features of lymphocytes shown in the two cases are very different, as in T-Lb lymphoma the T-lymphoblasts are highly atypical (*Figure 14A,B*). The neoplastic T lymphoblasts were also positive for CD3 (week), CD1a, CD10, Tdt (*Figure 14C,E,F,H*) and negative for PAX5 (*Figure 14D*). Phenotypic refinement is of further support: a recently available antibody, LMO2, proved to be useful in the characterization of lymphoblast-containing proliferations in the mediastinum (159). In fact, this antibody (Ab) does not stain normal lymphoblasts in the thymus but only neoplastic lymphoblasts (160) (compare *Figure 13F* of B1 Thymoma with *Figure 14G* of T-Lb lymphoma). Similar findings were reported with NOTCH1 intracellular domain immunohistochemistry, positive with T-lymphoblastic leukemia cells and negative in lymphocyte-

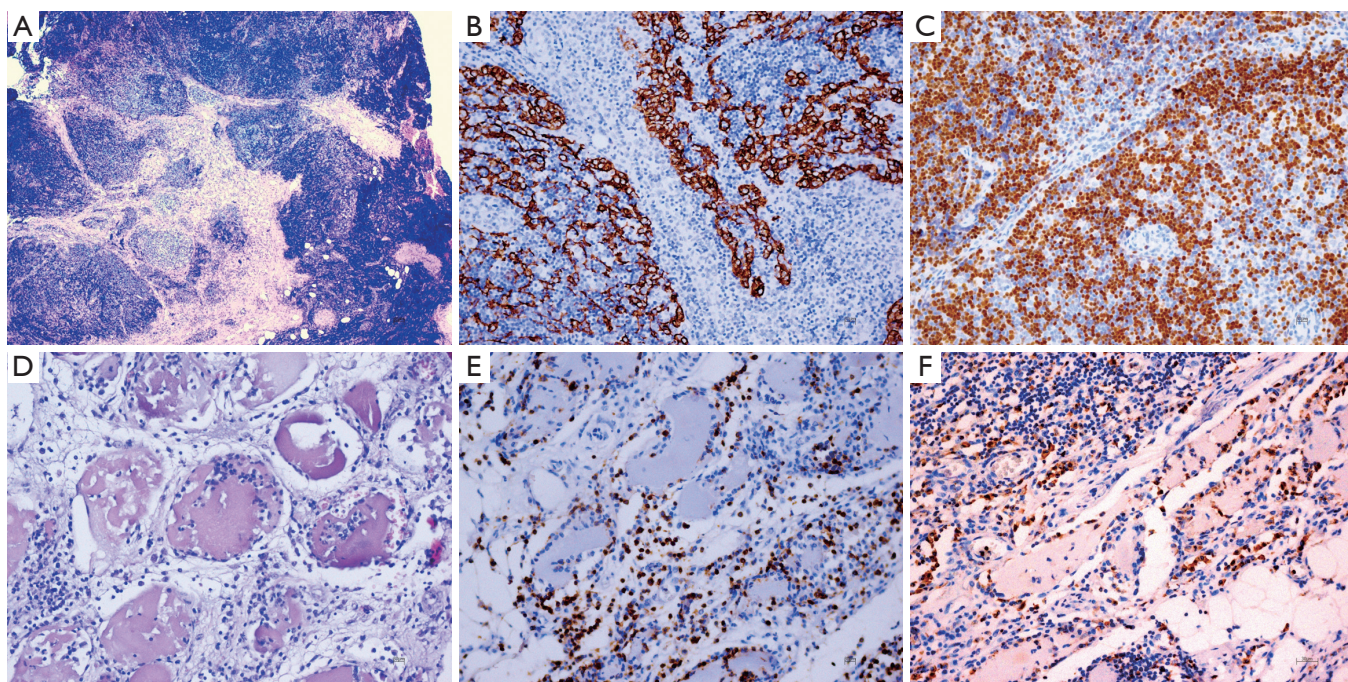


Figure 15 The morphologic spectrum of T-cell rich lesions. (A) B2 thymoma, H&E, 40 \times . This case was associated with a prominent polyclonal T-cell lymphocytosis, requiring differential diagnosis with a T (lymphoblastic) lymphoma. (B) 200 \times : the CK MNF116 stain highlights the prominent and disorganized epithelial cell network. (C) 200 \times : Tdt stain of T immature cells in the tumor. (D) Skeletal muscle, autophagic tissue: this case was associated to a myositis and the patient died in spite of therapy. (E) CD3, 200 \times stain of skeletal muscle. (F) CD68, 200 \times stain of skeletal muscle.

rich thymoma (161). In this case, the clinical/demographical setting is also very different, as T-Lb lymphoma is predominantly if not always a tumor of childhood or young age and is associated to or develops a lymphocytosis.

There are further difficulties in evaluating immature T-cell containing mediastinal lesions. In fact, it should be mentioned that thymoma could be associated with T-cell lymphocytosis, and that cases of thymoma/TC and T-lymphoblastic lymphoma occurring in the same patient have been recorded (162). In similar cases, T-clonal rearrangement evaluation should be associated with the routine investigations. In *Figure 15A,B,C*, the main morphological and immunohistochemical features of a B2 type thymoma associated with T-cell lymphocytosis are shown (163). A polyclonal T cell receptor rearrangement was found both in the tumor and in the peripheral blood (PB) (not shown). Polymyositis and myocarditis were also associated (*Figure 15D,E,F*) (164). The interpretation of these cases requires a careful multidisciplinary approach due to the fact that the clinical picture could also be very severe and complex.

Peripheral T-cell lymphomas have also been reported in the thymus/mediastinal lymph nodes (165), so any possibility should be considered when faced with a T-cell-rich lesion in the mediastinum.

The spectrum of lymphoid-cell-rich lesions of B-cell type

B-lymphoblastic lymphomas (B-Lb), although rare, may affect the mediastinal area/structures, as shown in *Figure 16*. This tumor occurred as a pericardial mass in a 56-year-old woman (*Figure 16A*) and the immunohistochemistry was fundamental in order to characterize the specific phenotype of the B Lymphoblasts, positive for CD34, CD10, PAX5 and CD79A (*Figure 16B,C,D,E*) but negative for CD20 (*Figure 16F*). The coexpression with CD34 of CD10, CD79a, PAX5 and CD38 (not shown) together made it possible to establish the diagnosis of B-Lb lymphoma even in the absence of CD20 positivity.

B-Lb lymphomas in the Thymus usually occur in the setting of a systemic disease, with BM involvement, but it is peculiar to note that a primary mediastinal mass may also

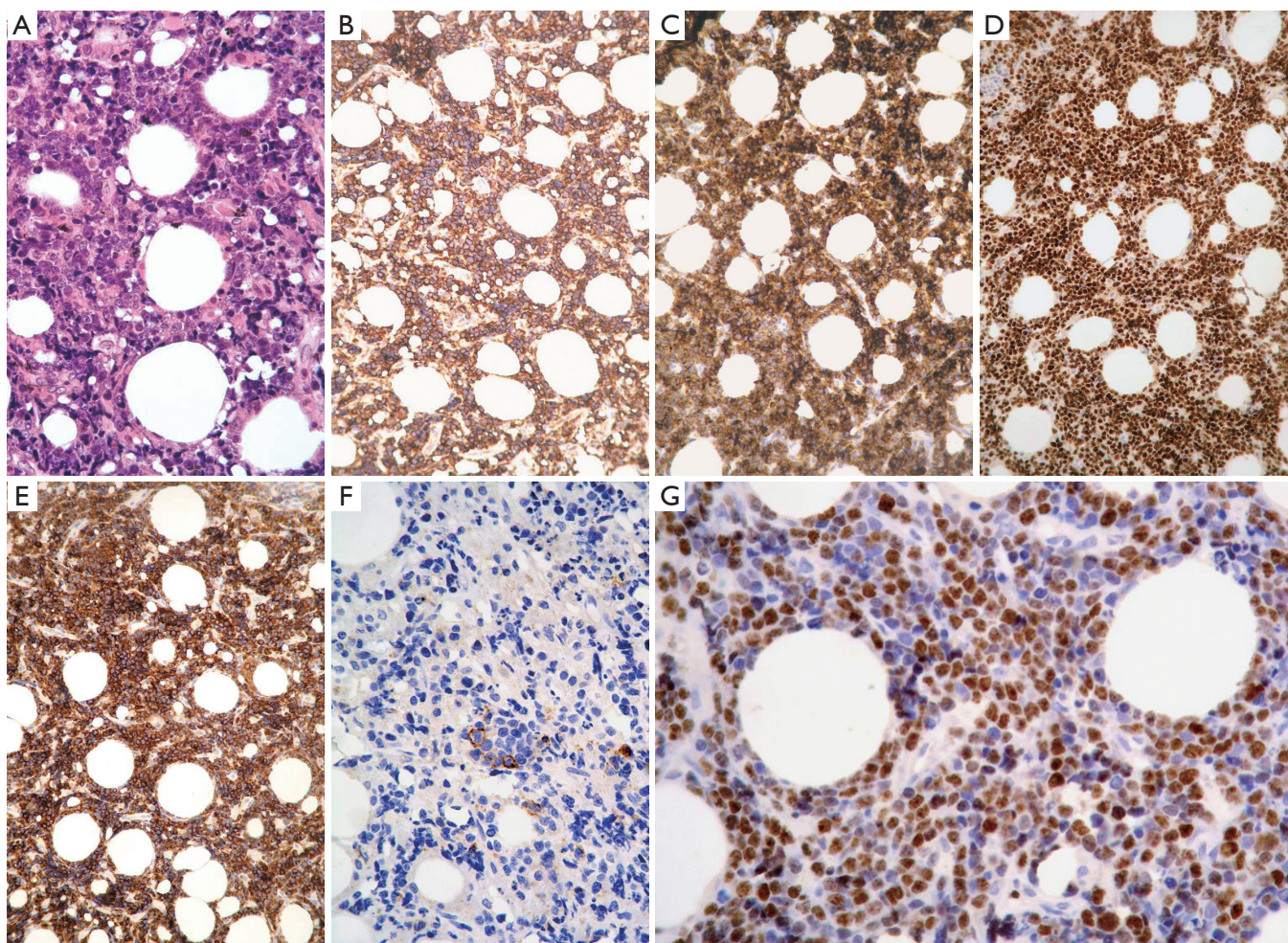


Figure 16 The morphologic spectrum of B-cell rich lesions: lymphoblastic B (B-Lb) lymphoma, pericardium. (A) H&E, 200 \times ; (B) CD34, 200 \times ; (C) CD10, 200 \times ; (D) PAX5, 200 \times ; (E) CD79a, 200 \times ; (F) CD20, 200 \times ; (G) KI67, 200 \times . In the case shown, although CD20 (F) is negative, other B cell markers (D-E) are positive in the immature (A), CD34+, CD10+ (B,C) B lymphoblastic population. A very high proliferative activity is shown by the KI67 stain (G).

occur (166,167).

Among low-grade B-cell lymphoma, precursor lesions of MALT lymphoma have been described by Moran *et al.* (168,169). We also previously reported the removal of a thymic mass showing follicular lymphatic hyperplasia with a monoclonal B lymphocyte background (170). Among precursor lesions, the description of the rare occurrence of monoclonal B cell populations/low grade-B cell lymphoma occurring in micronodular thymoma (MNT) was previously carried out (Figure 17). A pathogenetic link between MALT lymphoma of the thymus and a MNT-type proliferation was proposed (171). This topic is still a matter of debate (65) and the description of other cases in the future will provide

new insights.

The spectrum of CD34+ neoplasias in mediastinum (or rare haematological localizations and/or vascular tumors)

CD34 gp10-120, mucosialin, is a MY10 sialomucin and it is a common marker of vascular tumors as well as of hemopoietic progenitor cells. This antibody can be used in the context of wide panels to characterize tumors of hemopoietic lineage progenitors. Among CD34+ neoplasia in the mediastinum, in fact, we would like now to mention the occurrence of CD34 positivity in blastic haematological neoplasias, such as the tumor-forming myeloid sarcoma

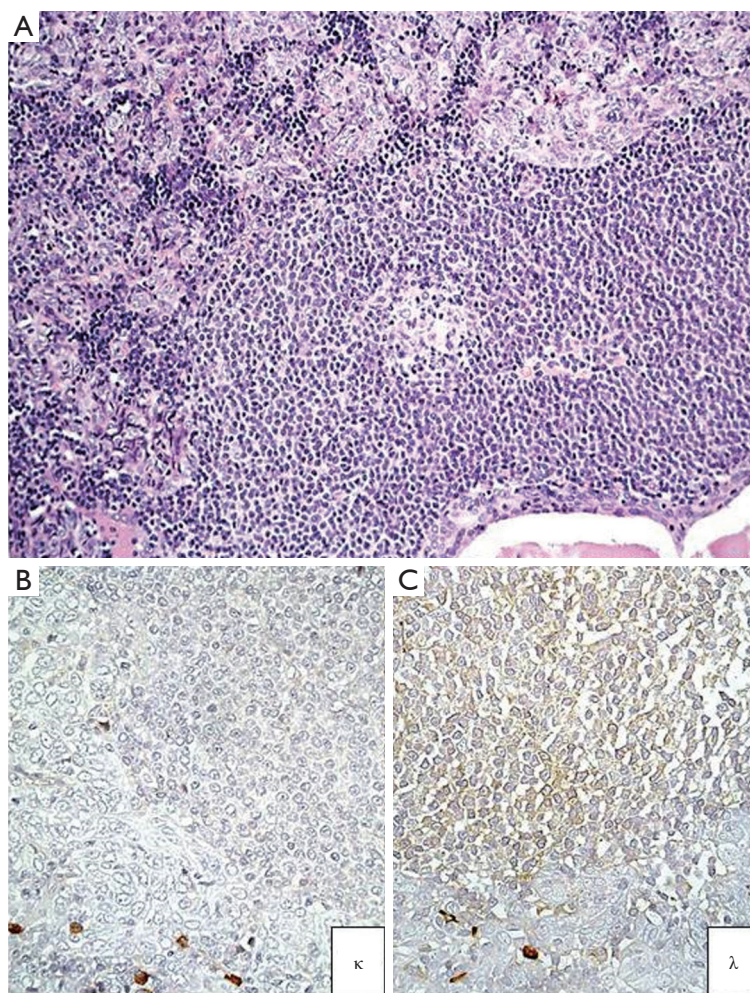


Figure 17 The morphologic spectrum of B-cell rich lesions. (A) MALT lymphoma arising in micronodular thymoma (MNT): lymphoid infiltrate as in MALT lymphoma between nodules of epithelial cells in MNT, 200 \times ; (B) surface κ (400 \times) versus (C) lambda (400 \times) (++) positive lymphocytes of the MALT lymphoma in the infiltrate (courtesy of Prof. P. Stroebel and Prof. A. Marx) (171).

(MS) (172) (also named granulocytic sarcoma) arising in the mediastinum *de novo* (173,174) or in the setting of myeloproliferative diseases as a relapse (175) or as blastic crisis (176). In that field, we show here the case of a man affected by a chronic myeloid leukemia (CML) who developed a mediastinal mass and only subsequently was faced with a blastic crisis. *Figure 18* shows the reasons for difficulty in understanding, in a biopsy, the morphological features of cells infiltrating a sclerotic mediastinal stroma (*Figure 18A*), even with the positivity for CD45 (*Figure 18B*) and CD34 (*Figure 18C*) of these cells. Subsequently, the complete history of the patient was referred and the FISH hybridization procedure confirmed the BCR/ABL fusion positivity (*Figure 18D*) in these blasts arising in

CML. It should be mentioned here that extramedullary hematopoiesis (EMH), which usually occurs in a posterior mediastinum localization, has been exceptionally reported in an anterior mediastinal localization (177,178). It should be also remembered here that haematological malignancies may arise also in the framework of GCT of the mediastinum (179,180).

The same marker, CD34, can be used in the context of wide panels either in the study of dubious epithelial rich thymoma of A type in order to exclude mesenchymal tumors such as synovial sarcoma (SS) (181,182), or solitary fibrous tumor (SFT) (183) or in the context of vascular tumors. Mesenchymal tumors and their DD have already been widely discussed by den Bakker *et al.* in a paper of

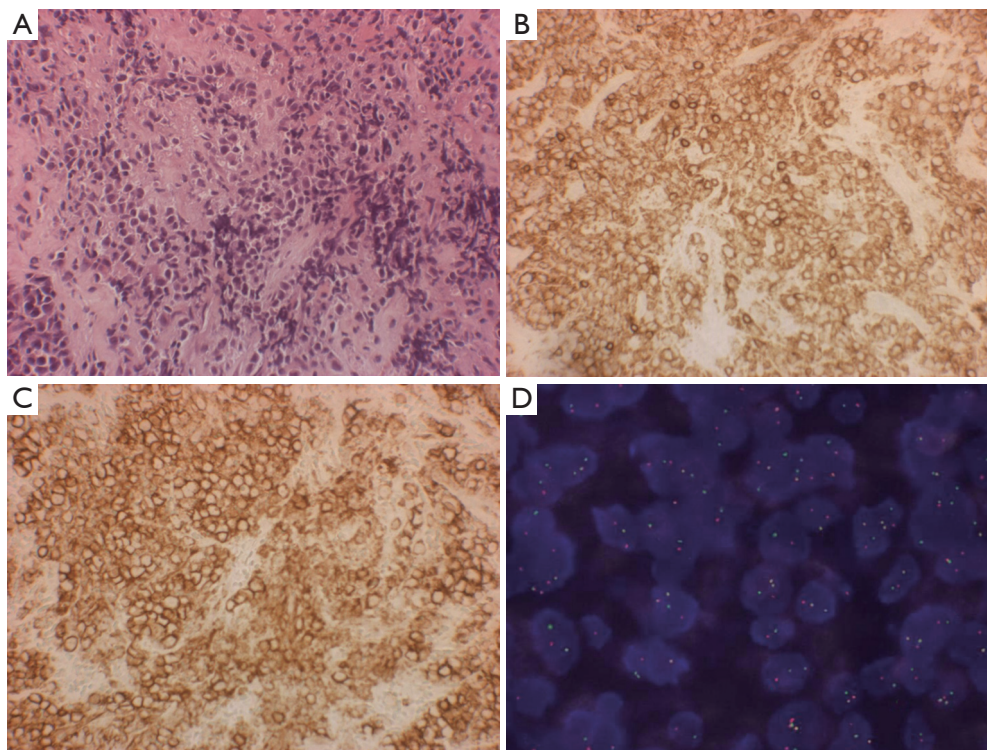


Figure 18 The morphological spectrum of CD34+ lesions: mediastinal localization of blastic crisis in chronic myeloid leukemia (CML) (176). (A) H&E, 200 \times ; (B) CD45, 200 \times ; (C) CD34, 200 \times ; (D) fluorescence *in situ* hybridization analysis, 1,000 \times . FISH BCR-ABL results in the mediastinal biopsy using LSI bcr-abl dual colour Extra Signal (Vysis, Abbott): the LSI BCR probe labeled with spectrum green and LSI ABL probe labeled with spectrum orange. The presence of the yellow BCR/ABL fusion signal confirms the presence of t(9;22) translocation between BCR gene located on chromosome 22 and ABL located on chromosome 9 (courtesy of Dr. R. Merola).

this issue (51). CD34 staining is of support in the diagnosis of a rare Cystic lymphangioma of the mediastinum (184) (*Figure 19*), a rare tumor occurring in a 66-year-old man (185). Moreover, the CD34 positivity was the only positive vascular marker (186) in the evaluation of a large mediastinal mass expanding also outside the thorax in the supraclavicular soft tissue (*Figure 20A,B*). In this case, the high grade cytology and the positivity for the endothelial marker CD34 together with the negativity for several other markers—among them CD31, another fundamental vascular tumor marker—made it possible to identify an epithelioid angiosarcoma in a 26 year- old young male.

The spectrum of fibrosing lesions in the mediastinum

The spectrum of fibrosing tumors/pseudotumors in the mediastinum includes on the one side sclerosing thymoma, described in the WHO classification manual as an epithelial tumor with overwhelming fibrosclerotic stroma (42) but it also includes the cases of HL of the

mediastinum. In particular, the NS subtype of cHL is associated with diffuse sclerosis (110) (*Figure 7*); the same sclerotic background is characteristic of the PMBL, previously denominated Mediastinal large cell lymphoma of B type with sclerosis (119) (*Figure 8*). In *Figure 21*, a further case of cHL is shown, with a peculiar increase in IDC, which could simulate an IDC sarcoma. Moreover, the sclerosing mediastinitis (SM), a peculiar disease with immune/autoimmune pathogenesis which rarely affects the mediastinum is known to produce abnormal fibrous tissue in the mediastinum.

SM

IgG4-related disease (IgG4-RD) is an idiopathic fibroinflammatory disorder associated with hypergammaglobulinemia and increased serum levels of IgG, particularly IgG4. The clinical spectrum of the disease includes sclerosing (autoimmune) pancreatitis (AIP), sclerosing cholangitis, retroperitoneal fibrosis (IRF), sclerosing sialadenitis (Küttner tumor), orbital pseudotumor,

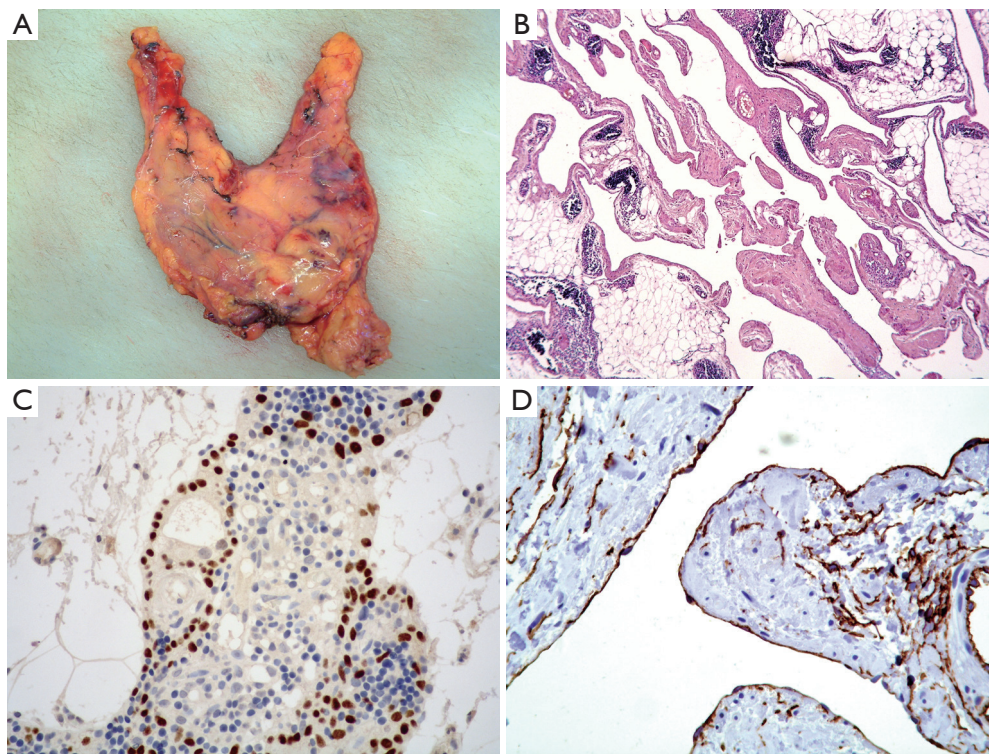


Figure 19 The morphologic spectrum of CD34+ lesions: thymic lymphangioma. (A) Macroscopical picture of the tumor, recapitulating the thymus shape; (B) H&E stain of the tumor, 50x; (C) p63 stain of thymic epithelial cells (EC), 200x; (D) CD34 stain of lymphatic endothelium, 100x.

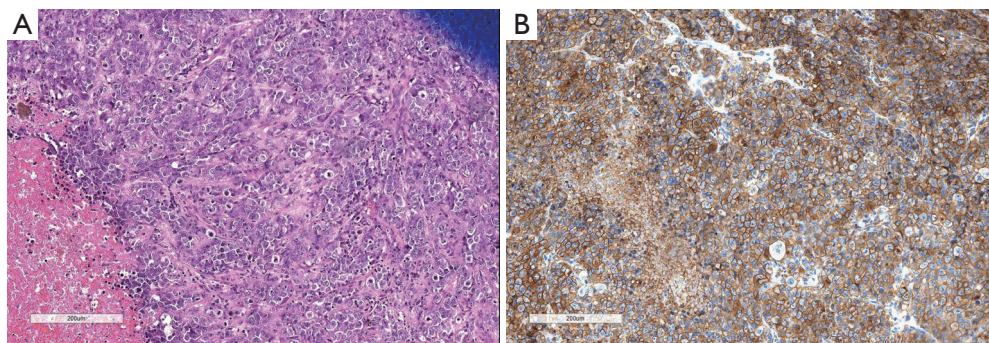


Figure 20 The morphologic spectrum of CD34+ lesions: epithelioid angiosarcoma with a mediastinal localization in a 28-year-old man. Epithelioid angiosarcoma is a highly aggressive tumor of endothelial cell origin. Here pleomorphic epithelioid cells, with abundant eosinophilic cytoplasm, vesicular nuclei, and prominent nucleoli are seen. (A) H&E, 200x: highly atypical cell population, necrosis; (B) CD34 positivity of neoplastic cells, 100x. CD34 positivity ranges in different series of epithelioid angiosarcoma. Factor VIII, CD31, Fli-1, vimentin are usually also positive. Pancytokeratins may be also positive.

lymphadenopathy, nephritis, thyroiditis and interstitial pneumonia. All these disease forms lead to inflammatory pseudotumors, which develop in different organ/systems, including mediastinum, with a marked IgG4+ plasma cell

infiltrate (187,188). *Figure 22* shows a case of IgG4 disease developing in a salivary gland (Küttner tumor) in an old woman. Cellular and storiform fibrosis, lymphoplasmacytic infiltration, increased numbers of IgG4-positive plasma

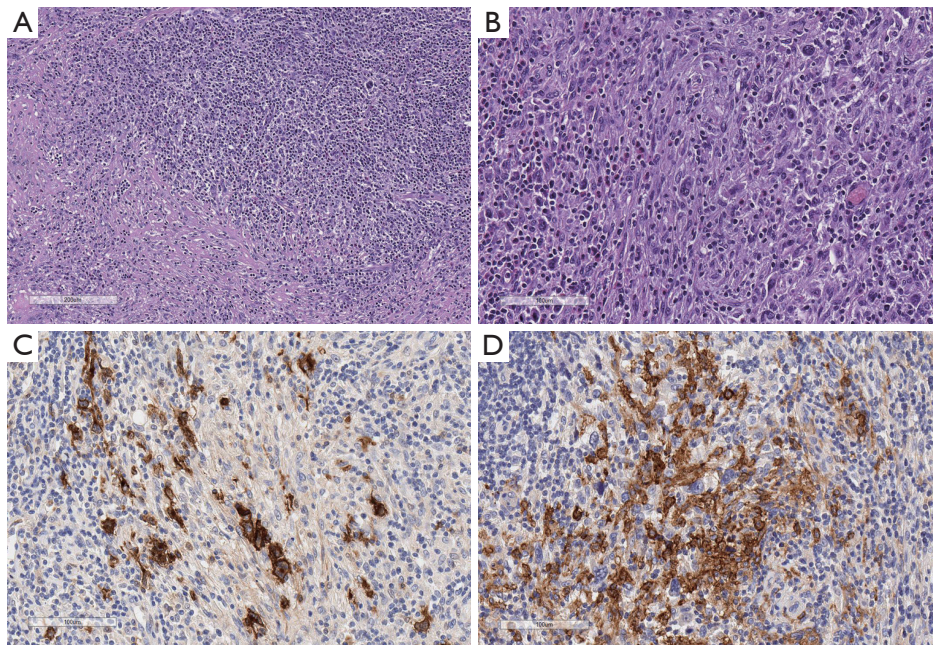


Figure 21 The morphologic spectrum of fibrosing lesions: Hodgkin lymphoma, nodular sclerosis (cHL, NS): the case shown is particularly rich in interdigitating reticulum cells (IDC). (A) H&E, 100 \times ; (B) H&E; 200 \times : (A and B) show the polymorphic lymphoid infiltrate; (C) CD30, 200 \times , stains the large and atypical Hodgkin's cells; (D) CD1a, 200 \times : this stain highlights the abundance of IDC.

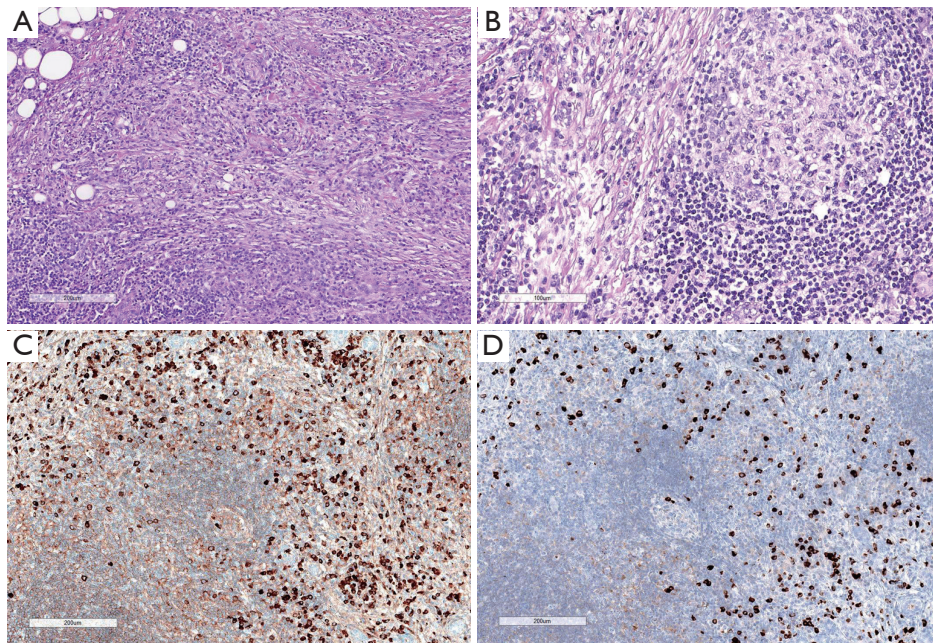


Figure 22 The morphologic spectrum of fibrosing lesions: IgG4-related disease (IgG4-RD), an idiopathic fibroinflammatory disorder associated with hypergammaglobulinemia and increased serum levels of IgG4 produces pseudotumors, which develop in different organ/systems, including mediastinum: example of IgG4, plasma cell rich-fibrosing lesion: (A) H&E, 100 \times , fibroinflammatory infiltrate, rich in plasma cells (pc); (B) H&E, 200 \times ; many pc are seen among the lymphoid cells, surrounding the reactive lymphoid follicle; (C) IgG, 100 \times : the plasma cells produce IgG; (D) IgG4: most plasma cells are IgG4 positive, 100 \times .

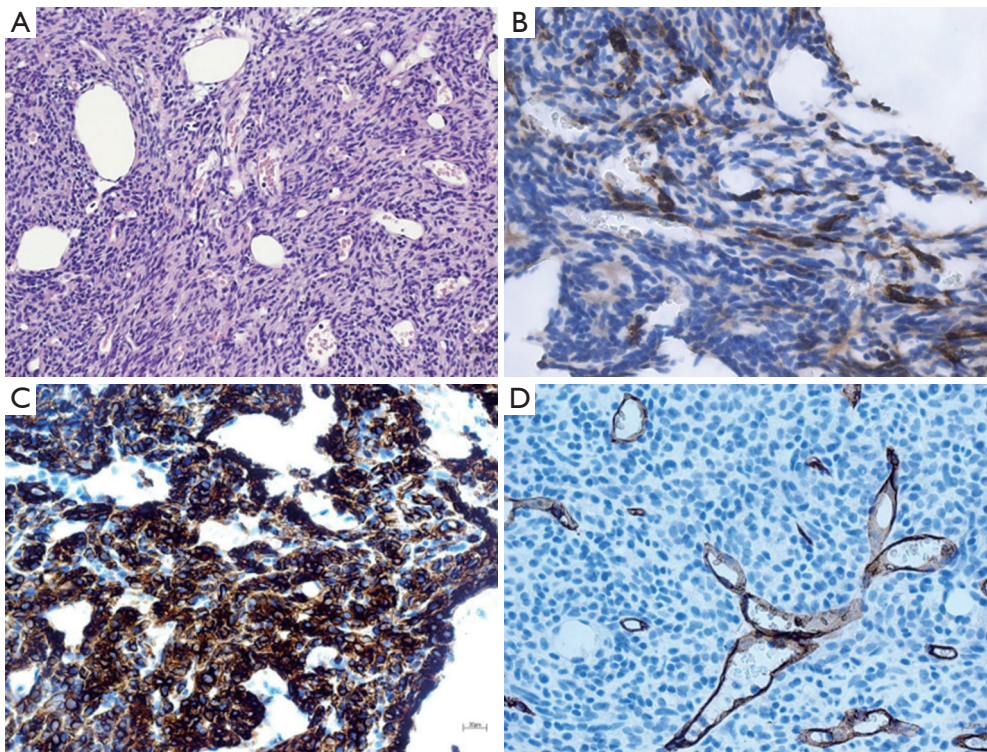


Figure 23 The spectrum of solid spindle or epithelial/epithelioid tumors: type A thymoma, fine needle biopsy aspiration of a mediastinal mass reaching the sternum and involving the lung. (A) Spindle cell tumor, H&E, 200 \times : the tumor was composed of bland appearing spindle cells, consistent with a thymic origin; (B) CD20 aberrant coexpression by EC, a feature described in type A and type AB thymoma, 200 \times ; (C) CK19 is expressed by most EC cells, 200 \times ; (D) CD34 negativity of the spindle EC in the type A thymoma, 200 \times .

cells and obliterative phlebitis are the hallmark histological features in IgG4-RD. In the mediastinum, lymph node enlargement is associated with lung disease and it is very frequent in patients with AIP (189). SM is an aggressive syndrome characterized by the formation of invasive fibrous tissue which substitutes the adipose tissue with dense sclerosis and eventually compromises vital structures in the mediastinum. SM can be idiopathic or secondary to infections and malignancies. In cases of fibrosing lesions of the mediastinum, occult malignancies should be excluded by extensive examination of the fibrous tissue. In fact, in some cases, SM was masquerading a malignancy, especially lymphoma (165,190), follicular dendritic sarcoma (191) and mesothelioma (192). Two forms of SM have been described, a focal granulomatous form and a diffuse usually non-granulomatous form (193). Histoplasmosis, tuberculosis, aspergillosis, blastomycosis, cryptococcosis and also sarcoidosis can be responsible for granulomatous SM. Instead, non-granulomatous SM is considered an idiopathic reaction to different autoimmune syndromes, to radiation

therapy, or to treatment with methysergide or in the setting of Behçet disease (194,195). All the previously mentioned diseases contribute to the wide clinical spectrum of IgG4-related disease (IgG4-RD) (195).

The spectrum of solid spindle or epithelial/epithelioid tumors in the mediastinum

The epithelial/epithelioid tumor spectrum in the anterior-superior mediastinum first includes the EC-rich types of TET. Type A thymoma is characterized by spindled or oval cells, with a fascicular/storiform or solid pattern, or which form glandular/adenoid structures, or pseudorosettes, or which have a pericytomatous pattern. Type A thymoma shows a very scant T-lymphocytic content of the mature type, as described by Marx *et al.* (44). Cytokeratins, p63/p40, with CD20 co-expression, in our opinion, are the best marker association in order to better identify Type A thymoma in doubtful cases (Figure 23). This TET subtype can often require a DD with other spindle cell tumors of the mediastinum (196), such as the already-mentioned SFT (183)

and SS (43,181). Primary TET arisen in the pleura are negative for Calretinin, a feature assisting in the DD with malignant pleural mesothelioma (MPM) (151,153). SS, especially monophasic fibrous SS arising in the mediastinum, can be diagnosed by a panel of markers (197), including the transcriptional corepressor TLE1 (198,199). The diagnosis can be confirmed by the SYT-SSX fusion gene via (72) fluorescence *in situ* hybridization (200) as reported by den Bakker *et al.* (51). Atypical type A thymoma, an entity that has yet to be recognized by the WHO, shows in addition signs of atypia and necrosis (201,202). Moreover, type B3 TET needs to be distinguished from B2 TET as well from thymic SQCC. These last topics are largely described in the paper published by Marx *et al.* in 2014 (44), in the WHO 2015 classification (42), in a recent book (52) and in the same focused issue (43).

The complex chapter of the DD of primary thymic SQCC *vs.* lung SQCC metastases is yet to be completed because a very specific immunohistochemical panel is difficult to establish. However, the WHO (42) and several papers have discussed the topic and proposed wide immunohistochemical panels in support of a meaningful clinical and pathological diagnosis (48,72).

Conclusions

The stimuli leading to EC proliferation in the thymus vary and not only neoplastic processes are responsible. Our awareness of the biology of EC in the thymus is very scant, regarding both their immunological interplay and reactivity to different types of local inflammation.

The complexity of structure, embryological derivation and functional activities render the thymus and its derived tumors very complex both for their diagnosis and functional interplay with the entire immune system and...beyond. No similar example of involuted organ in humans is responsible for so many interactions and effects in its pathological state. Assessment of the clinical setting and careful attention to all cell types included in a biopsy/tumor specimen should be applied in order to reach an adequate diagnosis.

Acknowledgments

The authors thank Dr. Enzo Gallo for the editorial assistance and Dr. Michael G. Kenyon for his review of the English language.

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Mediastinum* for the series “Diagnostic Problems in Anterior Mediastinum Lesions”. The article has undergone external peer review.

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/med.2018.12.01>). The series “Diagnostic Problems in Anterior Mediastinum Lesions” was commissioned by the editorial office without any funding or sponsorship. MM served as the unpaid Guest Editor of this series and serves an unpaid editorial board member of *Mediastinum* from May 2017 to Apr 2019. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/med.2018.12.01

Cite this article as: Marino M, Ascani S. An overview on the differential diagnostics of tumors of the anterior-superior mediastinum: the pathologist's perspective. *Mediastinum* 2019;3:6.