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ORIGINAL ARTICLE

Thymoma with loss of keratin expression (and giant cells): a potential diagnostic pitfall

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Abstract Due to its profound therapeutic consequences, the distinction between thymoma and T-lymphoblastic lymphoma in needle biopsies is one of the most challenging in mediastinal pathology. One essential diagnostic criterion favouring thymoma is the demonstration of increased numbers of keratin-positive epithelial cells by immunohistochemistry. Loss of keratin expression in neoplastic epithelial cells could lead to detrimental misdiagnoses. We here describe a series of 14 thymic epithelial tumours (11 type B2 and B3 thymomas, 3 thymic carcinomas) with loss of expression of one or more keratins. Cases were analysed for expression of various keratins and desmosomal proteins by immunohistochemistry and immunofluorescence and compared with 45 unselected type B thymomas and 24 thymic carcinomas arranged in a multitissue histological array. All 14 cases showed highly reduced expression of at least one keratin, three cases were completely negative for all keratins studied. Of the 14 cases, 13 showed strong nuclear expression of p63. Expression of desmosomal proteins was preserved, suggesting intact cell contact structures. Loss of expression of broad-spectrum-keratins and K19 was observed in 3 and 5 % of unselected thymomas and in 30 and 60 % of thymic carcinomas. A proportion of keratin-depleted thymomas contained giant cells, reminiscent of thymic nurse cells. Loss of keratin expression in type B2 and B3 thymomas is an important diagnostic pitfall in the differential diagnosis with T-lymphoblastic lymphoma and can be expected in 5 % of cases. A panel of epithelial markers including p63 is warranted to ensure correct diagnosis of keratin-negative mediastinal tumours.

Keywords Thymoma · Keratin · T-lymphoblastic leukaemia · Lymphoma · Mediastinum · Thymic nurse cell · Thymic carcinoma

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Introduction

Thymomas are rare epithelial tumours of the thymus. According to the current WHO classification of tumours of the thymus [7], thymomas are separated from thymic carcinomas based on H&E morphology, presence of generally abundant immature T cells and immunohistochemistry (e.g. expression of CD117 in thymic carcinomas). The only other malignant neoplasm in the human body containing immature T cells is T-lymphoblastic lymphoma (T-LBL). T-LBL typically involves the thymus and often mediastinal lymph nodes [2]. Both thymoma and T-LBL can present as a bulky mass in the mediastinum and the differential diagnosis can be challenging in small biopsies [3], especially when dealing with the lymphocyte-rich thymoma subtypes B1 and B2 [9, 10]. The epidemiology in both diseases is different: T-LBL is usually a disease of adolescence and young adulthood and often rapidly progressive, while thymomas usually occur in the elderly and are usually slowly growing. However, aggressive type B thymomas occur also in young adults [16] and these epidemiologic considerations cannot replace histology in an individual case. Since the therapeutic management of thymomas and T-LBL is fundamentally different (surgical resection in

thymomas vs. chemotherapy in T-LBL) [1, 17], a correct pretherapeutic diagnosis is of utmost clinical relevance. One basic diagnostic clue is the analysis of the meshwork of keratin-positive cells that constitutes the epithelial component of the normal thymus. Demonstration of an intact or often crowded meshwork of keratin positive thymic epithelial cells is one of the hallmarks of thymoma [5], while the epithelial meshwork is destroyed in T-LBL, resulting in highly reduced or negative keratin staining in T-LBL. We here report on a series of 14 thymomas and thymic carcinomas with subtotal loss of one or several keratins as a potential pitfall in the differential diagnosis with T-lymphoblastic lymphoma. Analysis in a larger unselected thymoma series showed that loss of keratin expression as revealed by the most widely used antibodies is rare.

Material and methods

The study was performed with approval of the Ethics committee of the Medical Faculty Mannheim (2013-802R-M). Tumour classification was performed on tissue sections stained with H&E and Giemsa, according to the World Health Organisation (WHO) Classification of Tumours of the Lung, Pleura, Thymus and Heart [8].

Tumour histology was type B2 thymoma in eight cases, type B3 thymoma in three cases, one thymic squamous cell carcinoma, one lymphoepithelioma-like carcinoma and one basaloid carcinoma. Four cases were Masaoka stage II, six cases in stage III and four cases in stage IV. Two of the cases had received preoperative chemotherapy. All 11 thymoma cases presented here were referral cases sent for consultation and were resection specimens. As controls, we used 30 type B2 thymomas (14 males, 16 females; age range 29–75 years), 15 type B3 thymomas (7 males, 8 females; age range 33–81 years) and 24 thymic carcinomas (17 squamous cell carcinomas (2×G1, 5×G2, 10×G3), one basaloid carcinoma, one clear cell carcinoma, one lympho-epithelioma-like carcinoma, two sarcomatoid carcinomas and two large cell neuroendocrine carcinomas) arranged in multitissue arrays [15]. Each tumour in the array was represented by five carefully selected punches designed to best account for tumour heterogeneity.

Tumour paraffin sections were immunostained with monoclonal antibodies (all purchased from Dako, Hamburg, Germany) against a large spectrum of keratins: clone AE1/3 (pH9, 1:1,000), K19 (clone RCK108, pH9, 1:50), K5/6 (clone D5/16 B4, pH9, 1:50), K8/18 (clone Cam5.2, pH6, 1:100), p63 (clone 4A4, pH9, 1:200), E-cadherin (clone NCH38, pH9, 1:100) and ß-catenin (pH6, 1:200) Ki67 (clone MIB-1, pH6, 1:800). A polyclonal anti-FOXN1 antibody was purchased from Abcam, Cambridge, UK (pH9, 1:50). Nomenclature of keratins followed the consensus nomenclature for mammalian keratins [12]. Keratin staining was considered negative in a given case if no staining of tumour cells was detected throughout; if an otherwise negative case showed focal staining (up to 15 % of total area of a given slide) or single cell staining, this was noted separately (see Table 1). Weak staining was defined as faint diffuse staining seen best at

Table 1 Summary of immunohistochemical findings in keratin-depleted thymomas and thymic carcinomas

Case	Histology	CT	GC	AE1/3	K19	K5/6	K8/18	P63	CTNB	ECad
1	B2	No	No	++	—/F	+	+++	+++	+	+++
2	B2	Yes	No	—	—	—	—	+++	+	+
3	B2	No	*Yes*	++	—/F	—	—/F	+++	++	—/F
4	B2	No	No	+	+	+	—	+++	+++	+++
5	B2	No	No	—	+	—	n.a.	+++	n.a.	n.a.
6	B2	No	No	—	—	—/F	—	++	n.a.	n.a.
7	B2	No	No	—	—	—	+	++	++	+++
8	B2	No	No	—	—	—	—	++	+	+
9	B3	No	No	+++	—	+++	++	+++	+++	+++
10	B3	No	*Yes*	—/F	++	++	—	+++	+++	+++
11	B3	No	*Yes*	—/F	—/F	—	—	—/F	—/F	—/F
12	TSCC	Yes	No	++	++	—	—	+++	+++	+++
13	LELC	No	No	—	—	—	—	+++	+++	+++
14	BTC	No	No	—	n.a.	+	n.a.	+	n.a.	n.a.
Antigen deficiency				9/14	9/13	9/14	9/12	1/14	1/11	2/11

TSCC thymic squamous cell carcinoma, *LELC* lymphoepithelioma-like carcinoma of the thymus, *BTC* basaloid thymic carcinoma, *CT* chemotherapy prior to resection, *GC* intratumorous giant cells, *K* keratin, *CTNB* β-catenin, *ECad* E-cadherin, *F* focal or single cell staining, *n.a.* not available

— negative, + weak, ++ moderate, +++ strong

higher (×20 objective) magnification. Moderate staining was defined as unequivocal staining seen at low (×10 objective) magnification throughout. Strong staining was defined as diffuse positive staining in the saturation range of the detection system. The presented cases were collected based on our routine practice to analyse for the epithelial component by K19 and AE1/3 staining and were included if at least one of the two showed either only unusually weak (case #4) or negative staining or absence of at least one of the two markers (all other thymoma cases). The three thymic carcinomas presented here were included based on absent or weak staining of K5/6.

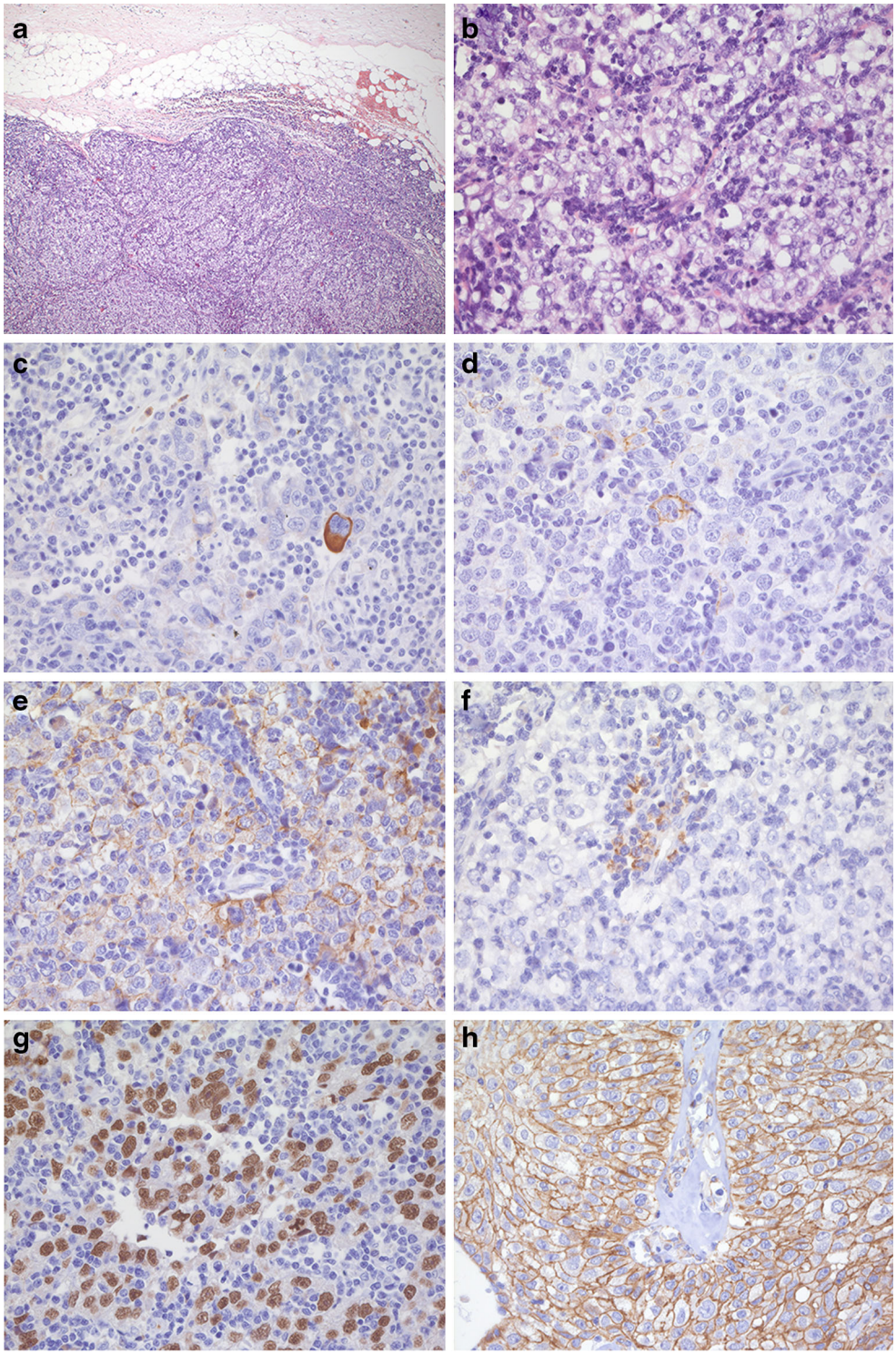

Fig. 1 Loss of keratin expression in a type B2 thymoma (case no. 7). H&E staining showing invasion into the mediastinal fat (**a**) and typical cell morphology with clustering of atypical epithelial cells (**b**). Immunohistochemical stainings showing subtotal loss of pan keratin (**c**), keratin 19 (**d**), keratin 8/18 (**e**) and keratin 5/6 (**f**), but strong nuclear expression of p63 (**g**) and membranous staining of E-cadherin (**h**) (immunoperoxidase on paraffin)

Immunofluorescence

Immunofluorescence double staining for the cell-cell contact protein plakophilin-1 (PKP1) (Clone PP1-5C2, Progen Biotechnik, Heidelberg, Germany) and K19 (guinea pig serum K19, Progen Biotechnik, Heidelberg, Germany) was performed as described [14].

Results

Immunohistochemical findings in keratin-depleted thymomas and thymic carcinomas

The immunohistochemical findings are summarized in Table 1 and illustrated in Figs. 1 and 2.

In brief, all cases showed loss of expression (either complete or in ≥85 % of the section) of at least one of the four keratin markers used here. Three cases showed loss of two keratins, two cases showed loss of three and four cases showed loss of all four keratin markers. Of note, expression of p63 was readily detectable in 13 out of 14 cases and is therefore a safe marker to demonstrate the epithelial background in thymomas and squamous cell thymic carcinomas in the absence of keratin expression. Another interesting finding was moderate to strong membranous expression of E-cadherin and ß-catenin in 9/11 cases. In a comparison with 45 unselected type B2 and B3 thymomas and 24 thymic carcinomas (mostly squamous cell type) arranged in a multitissue array, loss of expression of broad-spectrum keratin AE1/3 and K19 (a widely expressed thymic keratin [14]) was seen in only 3 and 5 % of thymoma cases, but was common (30 and 61 %) in thymic carcinomas. In contrast to keratin-depleted tumours, strong expression of ß-catenin and E-cadherin was seen in only about 30 % of unselected thymomas and 4 and 21 % of thymic carcinomas (Table 2). Interestingly, on

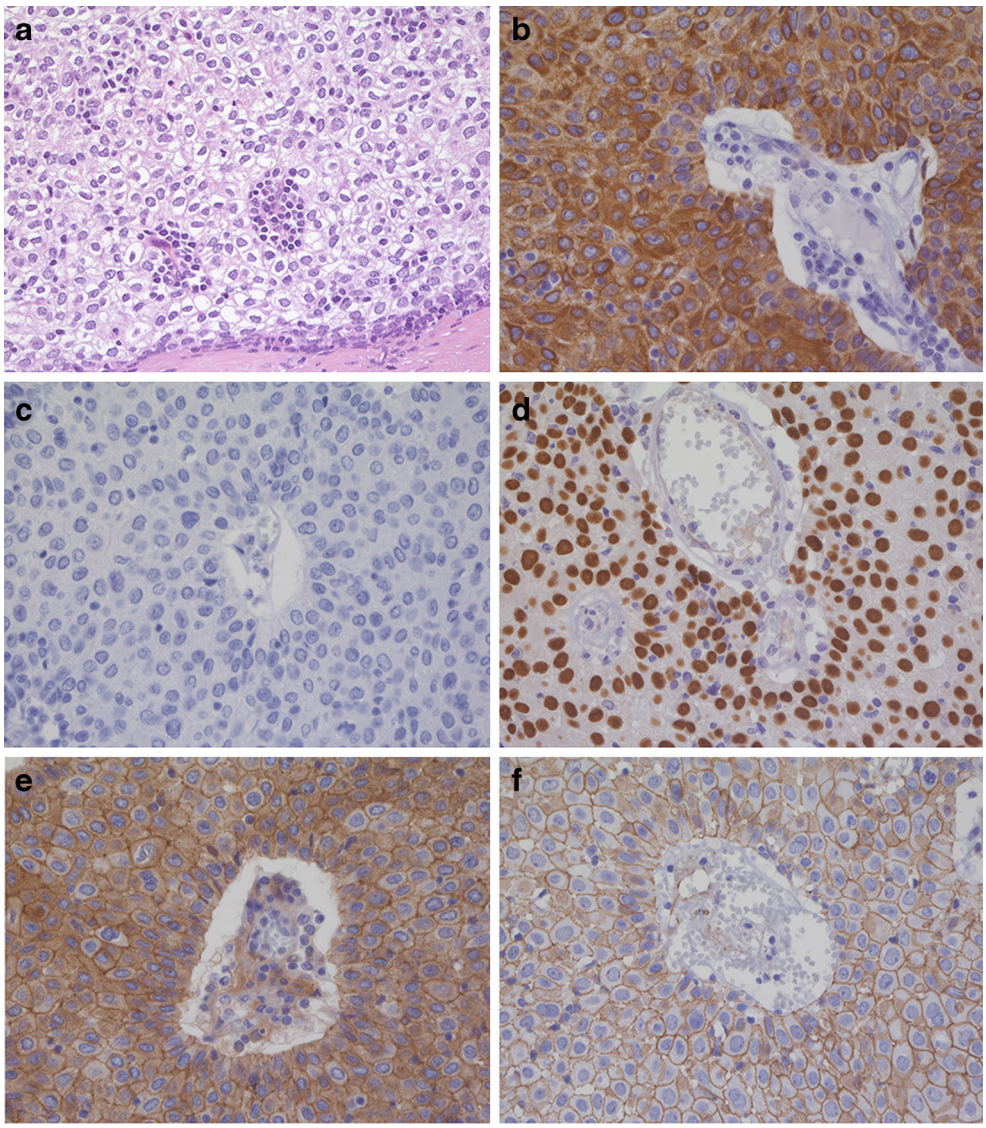

Fig. 2 Selective loss of keratin 19 but not other keratins in a type B3 thymoma (case no. 10). This case of an otherwise typical type B3 thymoma on H&E morphology (**a**) showed strong expression of AE1/3 (**b**), but complete loss of keratin 19 (**c**). Expression of p63 (**d**), E-cadherin (**e**) and b-catenin were preserved

Table 2 Expression of keratins and associated proteins in unselected type B2 and B3 thymomas and thymic carcinomas

Antibody	Positive (*n*)	Total (*n*)	%positive
B2 and B3 thymomas			
AE1/3	34	35	97
K19	42	45	93
K5/6	35	45	78
K8/18	19	30	63
p63	32	39	82
CTNB	9	30	30
Ecad	8	29	28
Thymic carcinomas			
AE1/3	16	23	70
K19	9	23	39
K5/6	3	23	13
K8/18	7	22	31
p63	7	23	30
CTNB	1	24	4
Ecad	5	24	21

K keratin, *CTNB* β-catenin, *ECad* E-cadherin

immunofluorescence, desmosomal structures appeared well preserved even in keratin-depleted tumour areas, indicating a selective process specifically affecting intermediate filaments, while leaving cell adhesion contacts intact (Fig. 3).

A subset of keratin-depleted thymomas contains epithelial tumour giant cells

H&E morphology of keratin-depleted thymomas and thymic carcinomas was generally unremarkable and not different from conventional thymomas. However, five of the 14 cases showed foci of coagulative necrosis and three cases (cases #1, 10, 11) harboured highly unusual tumour giant cells. The giant cells measured up to 120 μm with nuclear sizes between 20–60 μm. A few of these giant cells showed strong cytoplasmatic expression of keratins as well as nuclear FOXN1 and p63 expression (Fig. 4), proving their epithelial nature. Some giant cells showed engulfment of lymphocytes, a phenomenon also known as emperipolesis (Fig. 4b, d).

Discussion

The differential diagnosis between thymoma and T-lymphoblastic lymphoma (T-LBL) is one of the most critical issues when dealing with needle biopsies of mediastinal masses with potentially devastating consequences when

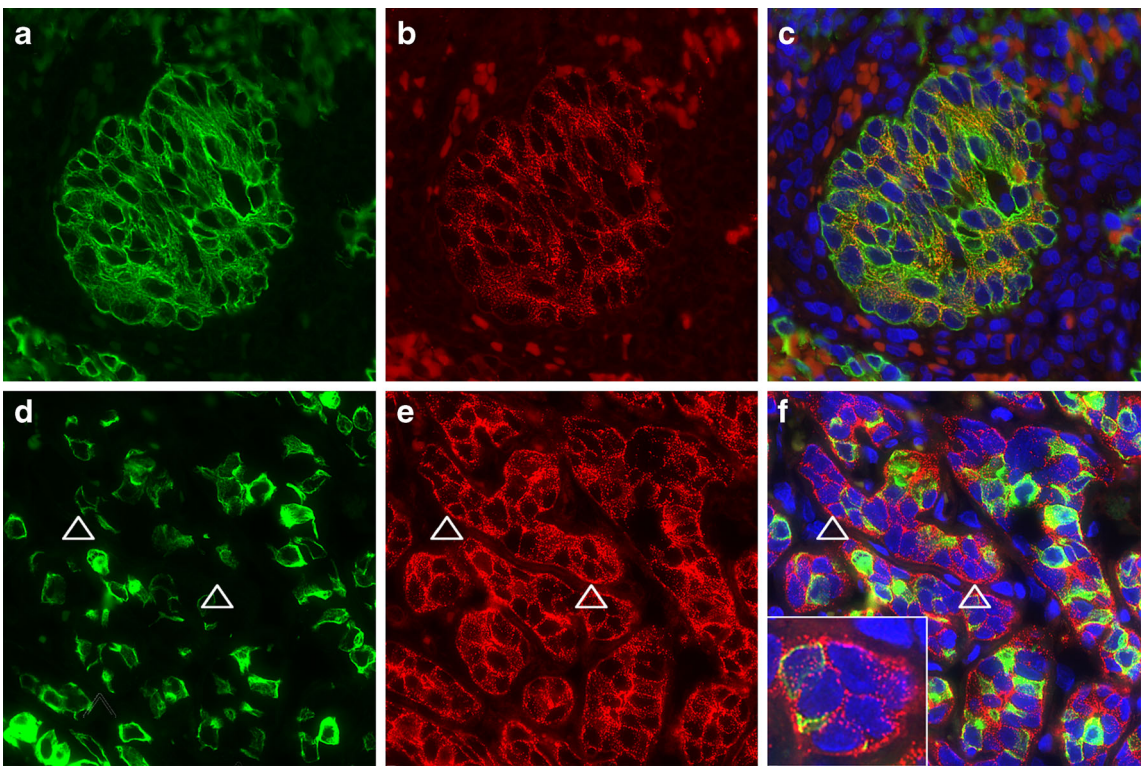

Fig. 3 Preservation of desmosomal structures in the absence of keratin expression. Comparison of a conventional type B3 thymoma with normal keratin expression (**a–c**) and a thymoma with focal loss of keratin expression (**d–f**). **a**, **d** (*green*): K19-alexa 488, **b**, **e**) (*red*): plakophilin-1-Cy3, **c**, **f**: overlay. **d–f** Preserved desmosomal structures (delicate granular *red lines*) even in areas without stainable keratins (*arrowheads*) and *inset* in panel **f**

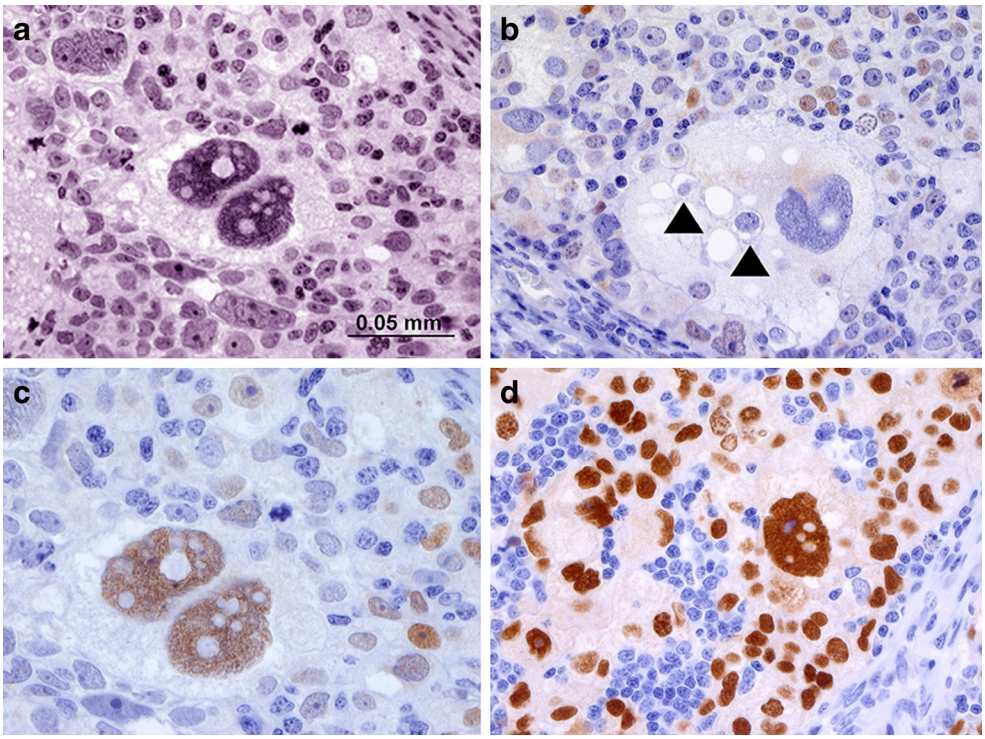

Fig. 4 Giant epithelial cells in a keratin-depleted B3 thymoma. **a** H&E morphology showing binuclear giant cell with foamy cytoplasm. **b**, **c** FOXN1 stainings, showing a FOXN1-negative giant cell with inclusion of lymphocytes (emperipolesis) (**b**, *arrowheads*) and another FOXN1-positive giant cell of the same case (**c**). Several giant cells with strong expression of p63 (**d**)

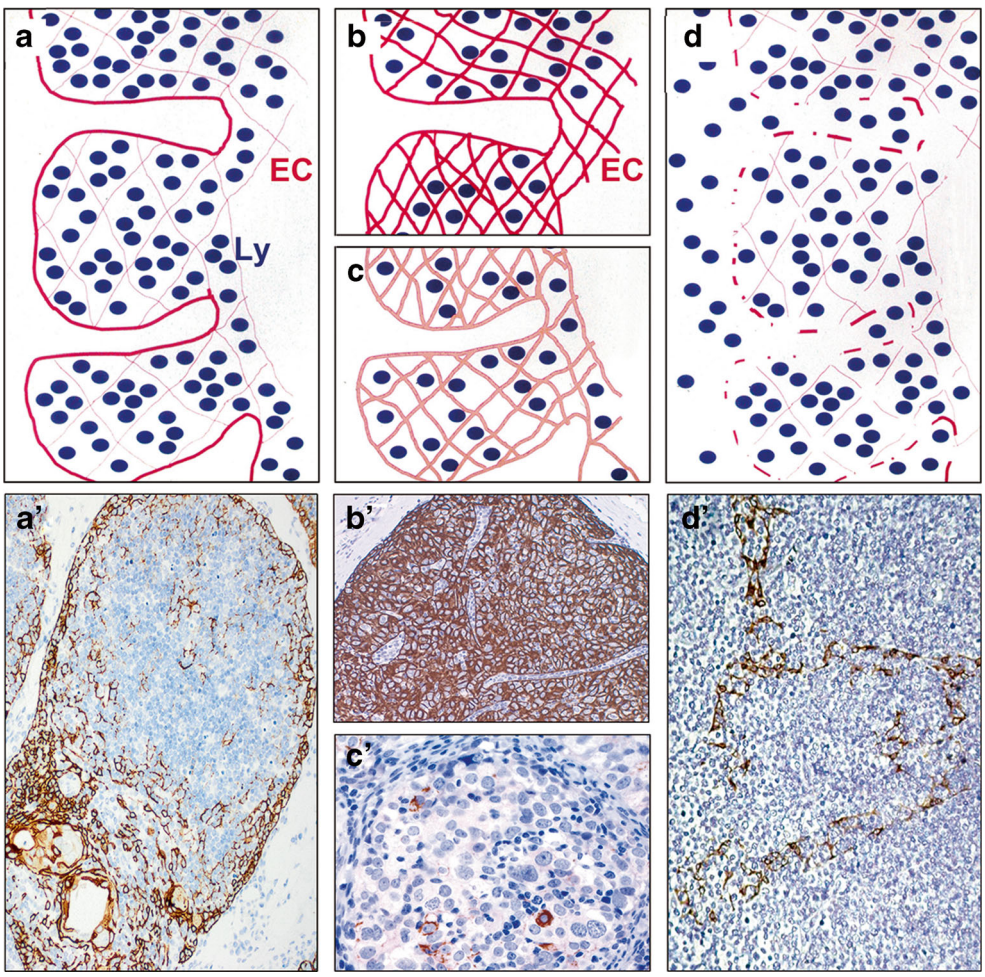

Fig. 5 Schematic representation (**a**–**d**) and immunohistochemical K19 staining (**a′**–**d′**) of epithelial cell meshwork in normal thymus, "conventional" type B3 thymoma, thymoma with keratin depletion and T-lymphoblastic lymphoma. **a**, **a′** Normal thymus showing a delicate epithelial cell (*EC*) meshwork in the background of numerous immature lymphocytes (*Ly*). Note pronounced staining of subcapsular cortical epithelium. **b**, **b′** Type B3 thymoma with increased numbers of large epithelial cells with strong expression of K19. **c**, **c′** B3 thymoma with increased numbers of large epithelial cells, but lost expression of K19. **d**, **d′** T-lymphoblastic lymphoma with colonization and destruction of epithelial cell meshwork

missed. The immune phenotype of T-LBL blasts can be very similar to that of precursor T cells in thymoma [6]. Assessment of proliferation is not helpful or even misleading, since most of the nonneoplastic immature thymocytes in the normal thymus and in thymomas are in cycle, resulting in a Ki67 index close to 100 %. Molecular clonality assays in T-LBL nearly always show clonal rearrangements, but may not be lineage-specific.

Thus, immunohistochemical assessment of the epithelial structures (preserved or even increased in thymomas, colonized and destroyed in T-LBL) is an important diagnostic clue (Fig. 5). We describe here loss of expression of several keratins as an important diagnostic pitfall in a series of prototypic cases. All cases described here belonged to the type B group of tumours that are the most challenging in the discrimination from T-LBL. Importantly, virtually all of the cases with keratin-depletion were strongly positive for p63 and most cases showed upregulation of E-cadherin and β-catenin. Antibodies against these additional epithelial antigens should therefore be included in doubtful cases. P63 has been shown to be essential for the proliferative potential of stem cells in stratified epithelia (including the thymus) [11, 13] and appears to play also a key role in the biology of thymic epithelial cells. Loss of keratin expression is an infrequent phenomenon: less than 5 % of type B thymomas in an unselected control series showed reduction of the most widely used keratins.

The reason why keratin expression is downregulated is not clear. The fact that this phenomenon was also observed in thymic carcinoma might indicate loss of epithelial differentiation and has been previously mentioned in a type B2 thymoma [9]. Interestingly, however, expression of cell-cell contact proteins (notably plakophilin-1) appeared preserved or even upregulated (E-cadherin, beta-catenin), which argues against, e.g. epithelial to mesenchymal transition. Five of 14 tumours (36 %) with keratin loss showed areas of coagulative necrosis, an uncommon finding in unselected thymomas, which might also suggest keratin depletion to be a degenerative phenomenon, although it is not apparent why loss of keratin expression affected only certain keratins in some cases (Fig. 2).

A highly interesting observation was the occurrence of an unusual population of keratin + FOXN1+p63 + giant cells with engulfment (emperipolesis) of lymphocytes in a subset of keratin-depleted thymomas. With these features, the giant cells resemble thymic nurse cells, a poorly understood cell population in the normal thymus with a purported role in positive and negative selection of thymocytes and in the maintenance of thymic epithelia [4]. In fact, these cells were shown in a figure of the chapter on type B2 thymomas in the WHO textbook, but were interpreted as anaplasia. Whether this is true or whether these cells could represent thymic nurse cells, remains to be shown.

In summary, loss of keratin expression in type B thymomas (and thymic carcinomas) is a rare, but potentially misleading phenomenon that must be considered especially when dealing with small preoperative biopsies of mediastinal masses.

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Conflict of interest Authors declare no conflict of interest.

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