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CASE REPORT

Primary mediastinal anaplastic alk-1-positive large-cell lymphoma of T/NK-cell type expressing CD20

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Abstract We describe an unusual case of ALK-1-positive primary mediastinal lymphoma with the morphology of an anaplastic large-cell lymphoma (ALCL) of T/NK cell type but expressing CD20. This tumour had T/NK morphology and immunophenotype, as demonstrated by its expression of CD30, EMA, ALK-1, CD7 and TiA-1 and the lack of expression of B-cell markers other than CD20. The significance of such a co-expression of a B cell-associated antigen in a case of ALCL of T/NK cell type is discussed.

Keywords Anaplastic ALK-1 positive large-cell lymphoma T/NK · Aberrant CD20 expression · Mediastinum lymphoma

Introduction

CD20 is a membrane protein involved in regulating B-cell activation. It is considered to be a specific marker for cells of the B lineage [3]. We report, here, a case of anaplastic large-cell lymphoma (ALCL) of the T/NK type expressing CD20 and discuss, based on a short review of the literature, the significance of this expression.

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Clinical history

In 2002, an 11-year-old boy was referred to the Pathology Department of Hôtel-Dieu Hospital (Paris, France) from Setif Hospital (Setif, Algeria). This Algerian boy presented with a 1-month history of cough, chest pain, and weight loss, with no sign of immunodepression. Thoracic computed tomography (CT)-scan revealed a 14×7 cm mass located in the postero-inferior mediastinum, invading the subcutaneous and paravertebral spaces. This mass compressed the left bronchus, causing segmental atelectasia. There were no signs of bone lysis or mediastinal adenopathy. A biopsy was performed on this mass.

Materials and methods

Tissues were fixed in 10% formalin, embedded in paraffin and cut into 5 μm sections, which were stained with haematein–eosin, Giemsa stain and impregnated with silver by the Gordon–Sweet method. Immunohistochemical studies were performed on paraffin sections, using the three-step immunoperoxidase method with microwave pretreatment (Table 1).

Results

Histology

We received one paraffin block with two fragments, each measuring 1 cm and showing the same histological pattern. Both presented a diffuse infiltrate, masking the normal tissue. This infiltrate consisted of medium to large cells with an abundant pale cytoplasm (Fig. 1a). The nuclei

Table 1 Summary of immunohistochemistry materials and results

Antigen	Name	Source	Result
CD30	Ber-H2	Dako, Glostrup, Denmark	+ Membranous and Golgi punctate pattern
EMA	E29	Dako	+ Membranous and cytoplasmic
ALK-1	ALK-1	Dako	+ Cytoplasmic and nuclear
CD20	L26	Dako	+ Membranous
CD79a	JCB117	Dako	−
Kappa	Polyclonal	Dako	−
Lambda	Polyclonal	Dako	−
CD7	272	Tebu, Le Perray en Yvelines, France	+ 30–40% of tumour cells
TiA-1	TiA-1	Immunotech, Luminy, France	+ cytoplasmic granular
Granzyme B	GRANZ-B	Tebu	+ 10% of tumour cells, cytoplasmic granular
CD2	AB75	Tebu	−
CD3	polyclonal	Dako	−
CD5	4C7	Tebu	−
CD8	114B	Dako	−
CD56	1B6	Tebu	−
CD57	NK1	Dako	−
Bcl2	124	Dako	−
Cytokeratins	KL1	Immunotech	−
LMP-1	CS1/4	Dako	−

contained finely dispersed chromatin and one or two prominent nucleoli. The nuclei varied considerably in shape: round, ovoid, bilobulated and horseshoe-shaped or multilobulated (Fig. 1b). Some large multinucleated cells were also present. Mitotic figures were frequent. A few small lymphocytes and histiocytes were scattered between the tumour cells. Small necrotic areas were also dispersed throughout the infiltrate. In some places, the tumour had been destroyed by large areas of ischaemic necrosis.

Immunohistochemistry

The tumour cells strongly expressed CD30 (Fig. 2a) and EMA. Punctate ALK-1 staining was observed around the nucleus (Golgi apparatus) in most cells, but well-defined nuclear staining was observed in some cells, suggesting nucleophosmin-ALK-1 rearrangement [1] (Fig. 2b). Approximately 30 to 40% of the tumour cells expressed CD7 (Fig. 2c). All expressed TiA-1. Only a few (10%) showed strong granzyme B expression. The demonstration of perforin was not interpretable, probably due to the fixative which has been used (Bouin's solution) and also to the very low number of tumour cells present on the remaining section. However, all the other T/NK markers studied were negative (CD2, CD3, CD5, CD4, CD8). Surprisingly, the membranes of all the tumour cells were strongly stained for CD20 (Fig. 2d), whereas CD79a staining was negative. Staining for the kappa and lambda immunoglobulin light chains was also negative. The expression of Pax5 gene

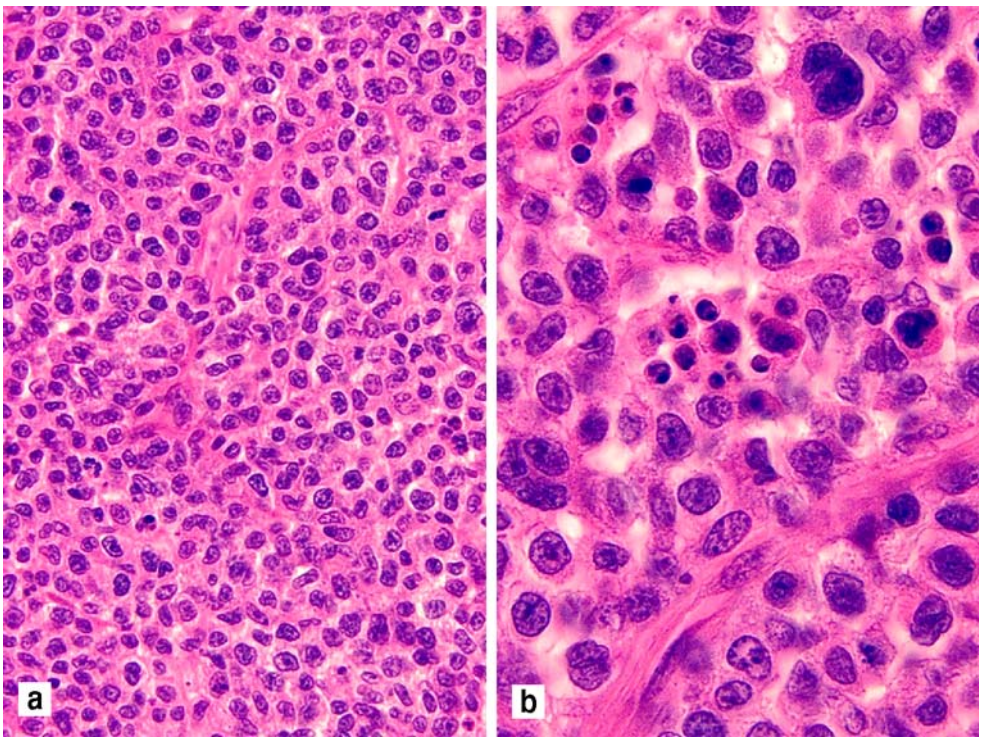

Fig. 1 Haematein and eosin. **a** G×40. Diffuse infiltration by large cells with irregular nuclei. **b** G×100. Note the presence of cells with multilobulated nuclei and with a horseshoe-shaped nucleus

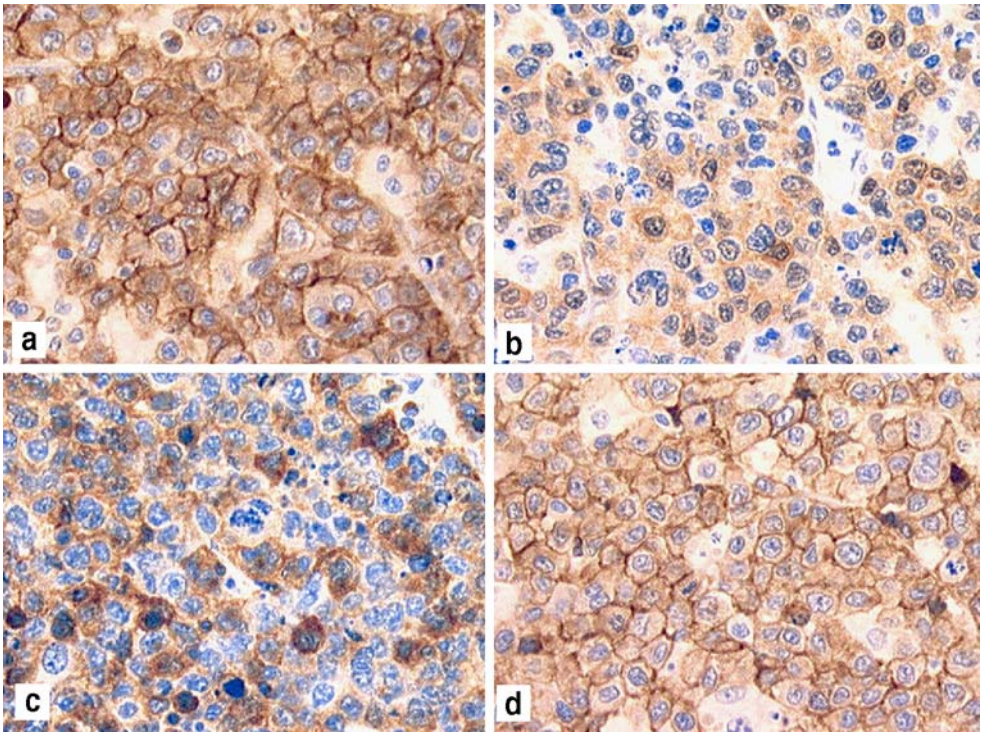

Fig. 2 Immunoperoxidase: G× 100. The tumour cells express CD30 with a cytoplasmic and a Golgi staining (**a**), ALK-1 with a cytoplasmic, membranous and sometimes nuclear staining (**b**), CD7 with a cytoplasmic staining (**c**) and CD20 on the membrane (**d**)

product, a good marker for a B cell origin, could not be evaluated due to the absence of material. Moreover, the Bouin fixation strongly decreases the chance to detect such nuclear antigen by immunohistochemistry.

Molecular biology

Polymerase chain reaction (PCR) was carried out on formalin-fixed and paraffin-embedded tissue, to check for B and T rearrangement. Unfortunately, this approach was unsuccessful due to DNA degradation as a result of fixation.

Discussion

We report a case of mediastinal large-cell lymphoma presenting the morphology of a T/NK anaplastic large-cell lymphoma defined according to the WHO classification [7, 9]. The immunophenotype of this tumour was also typical, with the neoplastic cells expressing CD30, EMA and ALK-1. The T/NK origin of the tumour is demonstrated by the expression of CD7, TiA1 and granzyme B, with no staining observed for any other T-cell marker. However, this T/NK ALCL also strongly expressed one B cell-associated antigen, CD20. We were unable to demonstrate the T-cell origin of the tumour by molecular biological analysis of paraffin-embedded tissue because the fixation process had resulted in nucleic acid degradation. Unfortunately, the absence of results for perforin and Pax5 gene product did not help us to confirm a T cell origin or to demonstrate a B cell origin of the tumour cells.

The mediastinal site of this tumour does not rule out a diagnosis of ALCL of T/NK origin, as several similar cases have been reported in adults and children. In the GELA (*Groupe d'Etude des Lymphomes de l'Adulte*) series [12], mediastinal involvement was found in 24% of ALCL of T/NK-cell type.

Differential diagnoses for our case include ALCL of B-cell phenotype, a rare condition [5]. ALCL of B-cell phenotype have a similar anaplastic morphology and express CD20. However, they also express other B-cell markers, such as CD79a, and no T-cell markers. They may be positive for CD30 in some cases, and they lack EMA. They also differ from ALCL of T/NK origin in having no ALK-1 protein or typical translocation. They are now regarded as a cytological variant of diffuse large-cell lymphomas of B-cell type (DLBCL) [5].

DLBCL expressing ALK-1 is the second main differential diagnosis, but can easily be ruled out on the basis of morphology and phenotype. Histologically, this type of lymphoma shows a mixture of large immunoblasts and plasmablasts, but lacks the hallmark cells of ALCL. These DLBCL do not express CD30, CD20 or T cell-associated antigens, but do, as in our case, express EMA and ALK-1. However, ALK-1 is generally exclusively cytoplasmic, due to clathrin-ALK-1 rearrangement [4] and is only rarely cytoplasmic and nuclear, as in our case.

Finally, our case should also be distinguished from peripheral T/NK-cell lymphoma, which may aberrantly

express some B cell-associated antigens, particularly CD20 and even CD79a [13], but without the morphology and immunophenotype of ALCL.

We must also consider the likelihood of CD20 expression by malignant T cells. First, it is unlikely that a molecule other than CD20 reacted with the L26 monoclonal antibody, as shown in previous studies of T-cell neoplasias expressing CD20 [10, 14]. Indeed, in such cases, the malignant cells were shown to be CD20-positive with two different monoclonal antibodies directed against different epitopes of the CD20 molecule.

Second, some cases of peripheral T/NK cell lymphoma may result from the neoplastic proliferation of a normal subset of peripheral T cells expressing CD20 [10]. Such cells, accounting for 12% of normal T cells are known as CD20dimT lymphocytes and display weaker membrane CD20 expression than B lymphocytes on flow cytometry [6]. They are present in the bone marrow [2] and have been associated with clonal T-cell proliferation in flow cytometry studies, suggesting clonal transformation of the CD20dim subset into peripheral T/NK cells [8, 11].

As a last possibility, the malignant T/NK cells may display aberrant CD20 expression as an aberrant characteristic frequently observed in malignancy. Based on the available evidence, we diagnosed this tumour as a cytotoxic T/NK ALCL expressing CD20.

This case also demonstrates that the use of a single marker, such as CD20, as a specific marker of the B-cell lineage may, in some cases, lead to the misclassification of cell lineage, particularly in the absence of CD3 expression.

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