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CASE REPORT

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Common acute lymphoblastic leukaemia-lymphoma expressing cytokeratin: a case report

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Abstract This report presents a case of common acute lymphoblastic leukaemia-lymphoma expressing low molecular weight cytokeratin but no leukocyte common antigen (CD45) in a 57-year-old man. The unusual morphology and clinical course together with the aberrant immunohistochemical results suggested a diagnosis of undifferentiated carcinoma. A detailed immunohistochemistry study on frozen and paraffin sections and molecular analysis prevented a diagnostic mistake.

Key words Cytokeratin
Acute lymphoblastic leukaemia; lymphoma
Immunohistochemistry

Introduction

The role of immunohistochemical analysis in the diagnosis and classification of malignant lymphomas is widely recognized [13, 24, 31, 34]. Distinguishing between malignant lymphoma and undifferentiated carcinoma is the most frequent diagnostic dilemma [10]. On practical grounds, the combined use of antibodies against the leukocyte common antigen (LCA/CD45) and cytokeratins (CKs) is sufficient to reach the correct diagnosis in the large majority of cases [1, 9, 25, 36]. However, the lack or aberrant expression of such antigens may cause diagnostic pitfalls [35]. In lymphoid neoplasia, the lack of staining for CD45 is a relatively frequent phenomenon [7, 8, 16], whereas the aberrant expression of CKs has been occasionally recognized in cases of lymphoplasmacytic-, immunoblastic-, or anaplastic large cell-lymphomas and plasmocytomas [4, 5, 6, 12, 23, 30, 37].

To our knowledge, cases of acute lymphoblastic leukaemia/lymphoma (ALL) expressing CKs have never been reported.

In this paper, we present a case of CD10+ lymphoblastic leukaemia-lymphoma characterized by positive staining for CKs and absence of CD45 antigen.

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Case report

In August 1987 a 57-year-old man was admitted to hospital with chronic sinusitis and hyperpyrexia. Peripheral blood count was normal (white cell count 4.3×10^9/l; haemoglobin 13.7 g/dl; platelets 190×10^9/l) but rare lymphoid-looking blasts were observed. A bone marrow aspirate showed diffuse infiltration by medium-sized cells with the morphological aspect of lymphoblasts with minimal cytoplasm, a large nucleus, fine chromatin and distinct nucleoli. Myeloperoxidase and nonspecific esterase stains were negative; periodic acid-Schiff stain showed coarse granularity. Immunocytochemical analysis demonstrated the phenotype of common ALL (TdT+, CD10+, CD19+, HL-DR+, Slg–, CD3–, CD7–). On the basis of this diagnosis the patient was treated with a chemotherapeutic regimen for ALL based on the use of prednisone, vincristine and daunorubicine. After the achievement of complete remission the patient received radio- and chemo-prophylaxis to the central nervous system, several courses of reinduction treatment and constitutional maintenance therapy. A year later he was admitted to a neurological department for hemiparetic syndrome of a vascular nature and subsequently to an infectious disease department, having developed a nonA-nonB viral hepatitis. In May 1990, ischaemic heart disease resulted in readmission to the hospital. On that occasion a swelling was noticed in the left thigh and an area of osteolysis 8 cm in length was detected in the proximal third of the femur at X-ray examination. At surgical inspection, infiltration of the muscles of the thigh was evident and a biopsy of the mass was performed. On microscopy the neoplasm was characterized by solid areas which sometimes formed cell "ribbons". Immunohistochemical analysis on formalin-fixed material showed that neoplastic cells were negative for LCA/CD45 and positive for CKs. A metastatic carcinoma in the bone was suspected and an extensive search for a primary site, including a total body CT scan and gastric endoscopy, was per-

Table 1 Results of immunohistochemical analysis on paraffin embedded and cryostat (in parentheses) sections (*CK* cytokeratin, *LCA* leukocyte common antigen, *EMA* epithelial membrane antigen, ± positive, – negative, +/– variable)

Reagent (CD)	Source	First specimen	Second specimen
CK Cam 5.2	Becton-Dickinson	+	+ (+)
CK AE1/AE3	Ortho	–	–
CK Cytokeratin-A	Ortho	–	–
LCA (CD45)	Dakopatts	–	–
TdT	Seralab		(+)
CD10	Becton-Dickinson		(+)
CD19	Becton-Dickinson		(+)
HLA-DR	Biotest-Clonab		(+)
L26 (CD20)	Dakopatts	–	–
MT1 (CD43)	Biotest-Clonab	+	+ (+)
UCHL1 (CD45RO)	Dakopatts	–	–
MT2 (CD45R)	Biotest-Clonab	–	–
LN2 (CD74)	Biotest-Clonab	+(dot)	+
HLA-DR	Biotest-Clonab	+/–	+
IgM	Ortho	–	–
IgG	Ortho	–	–
IgA	Ortho	–	–
Kappa	Ortho	–	– (–)
Lambda	Ortho	–	– (–)
EMA	Dakopatts	–	–
BerEP4	Dakopatts	–	–
S100	Dakopatts	–	–
Neurofilaments	Dakopatts	–	–
Desmin	Dakopatts	–	–
Vimentin	Boehringer	+/–	+/–
Actin (muscle specific)	Dako	–	–

formed without finding any clinical evidence of neoplastic disease in other sites. Bone marrow aspirates did not show any abnormal cells. A second biopsy was performed, preserving fresh material to better evaluate the immunohistochemical features of the tumour with a larger panel of antibodies. Immunohistochemical and molecular biology studies performed on this specimen definitively demonstrated the lymphoblastic nature of the neoplastic cells (see pathological findings). On the basis of these data and of the apparent lack of bone marrow and peripheral blood involvement, a diagnosis of CD10+ lymphoblastic lymphoma (recurrence of ALL) was given. Later, the disease showed an accelerated course and the patient died of severe shock. At autopsy, multiple deposits of the neoplasm were observed in both adrenals, in the liver, the spleen and the base of the skull. The bone marrow was not involved.

Tissue fragments from the first and the second biopsies obtained from the thigh were fixed in 10% formalin and embedded in paraffin. Sections 5 μm thick were stained with haematoxylin and eosin and Giemsa and prepared for immunohistochemical analysis. Unstained bone marrow and/or peripheral blood smears of 20 cases of ALL were also obtained to compare the reactivity of CKs. Immunohistochemical analysis was performed using the standard avidin/biotin immunoperoxidase (Dako) or the alkaline phosphatase/antialkaline phosphatase (Dako) techniques, following the methods suggested by the manufacturer.

Snap-frozen fragments of the second biopsy were cut in a cryostat and 5 μm thick sections were stuck onto glass slides covered with 0.5% polylisine (Sigma) as adhesive. The antibodies used for the immunohistochemical analysis on cryostat and paraffin sections are listed in Table 1.

The configuration of heavy and k light chain immunoglobulin gene loci was analysed by Southern blot hybridization of the DNA purified from the residual frozen tissue used for immunohistochemistry as previously described in detail [29].

Pathological findings

At microscopic observation, the first and second biopsies were very similar, showing a wide infiltration of neoplastic cells forming irregular aggregates within connective and fatty tissues. The pattern of cell infiltration was mostly diffuse but in some areas the cells formed ribbon-like structures or nests simulating a carcinomatous infiltration. The cells were of medium to large size, with relatively abundant cytoplasm, while the nuclei were usually round and contained one or more nucleoli (Fig. 1).

A large panel of monoclonal antibodies reacting with different CKs was used, as shown in Tables 1 and 2. Positive staining was observed with Cam 5.2, Lu.5, CK2 and K8.13 antibodies, all including CK 18 from Moll's classification [19], but was negative with the others (see Table 2). The pattern of staining was generally diffuse and strong in the cytoplasm of neoplastic cells, occasionally focal (Fig. 2). The majority of cells were also reactive with MT1 (CD43), vimentin and major histocompatibility complex class II antibodies on paraffin sections.

Immunohistochemical analysis performed on frozen sections showed the typical phenotype of common ALL (TdT+, HLA-DR+, CD10+, CD19+; Fig. 3). Cytological smears of bone marrow aspirates obtained at first hospitalization were still available and showed a strong dot positivity for Cam5.2 (Fig. 4). By contrast, all 20 cases of ALL used as controls were negative for all tested CKs.

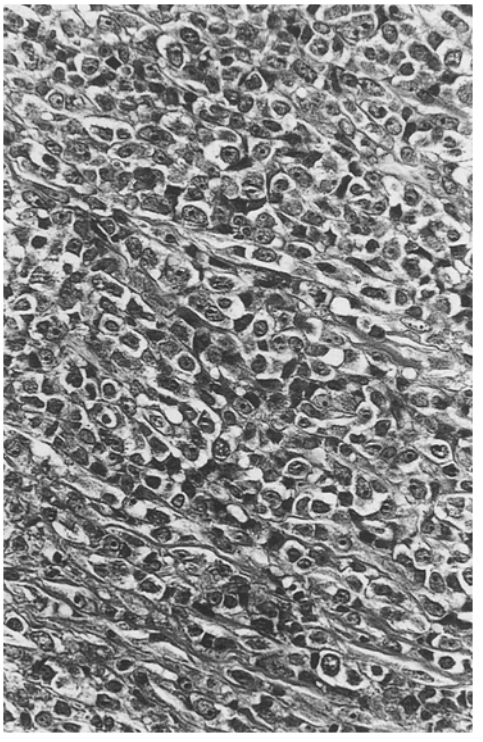

Fig. 1 Infiltration of monotonous cell population aggregated in vaguely ribbon-like structures (H&E, Original magnification ×250)

Table 2 Specificity of different anti-CK monoclonal antibodies according to Moll's classification and staining results

Reagents/clones	CK subtype	Source	Results
Cam 5.2	8, 18, 19	Becton-Dickinson	+
Lu-5	1–19	Boehringer	+
CK2	18	Boehringer	+
K8.13	1, 5, 6, 7, 8, 10, 11, 18	Bio-Yeda	+
CK-A/35βH11	8	Ortho	–
CK-B/34βE12	1, 5, 10, 14	Ortho	–
CK-C/34βB4	1	Ortho	–
AE3	1–8	Ortho	–
AE1	10, 14, 15, 16, 19	Ortho	–
CK7	7	Amersham	–
K92	11	Dakopatts	–

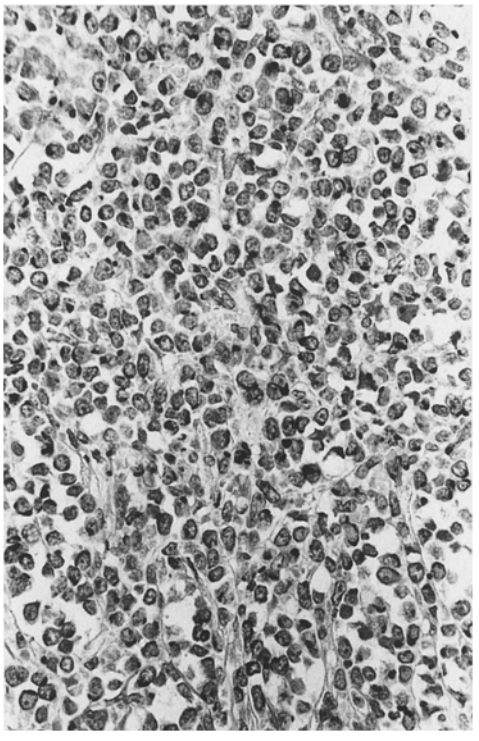

Fig. 2 The neoplastic cells show cytoplasmatic positive reaction to anticytokeratin antibody (Cam 5.2), avidin biotin immunoperoxidase complex (ABC)

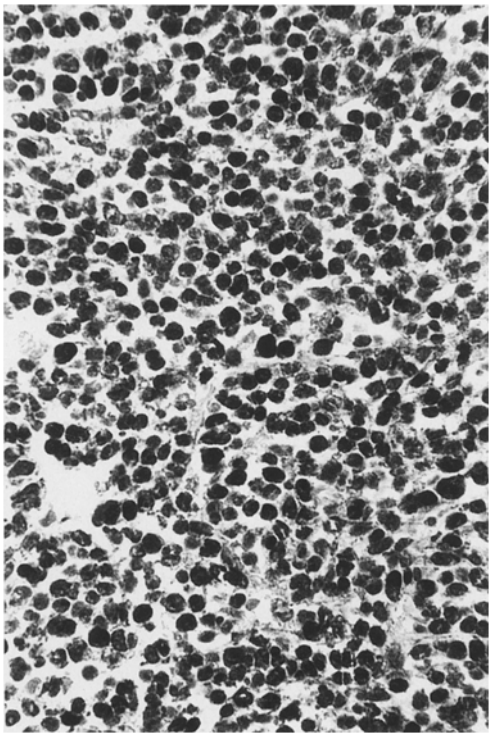

Fig. 3 On frozen sections the neoplastic cells show the typical phenotype of common acute lymphoblastic leukaemia lymphoma as defined by nuclear positivity for TdT, ABC

DNA samples showed rearranged band at Southern blot analysis for both immunoglobulin (Fig. 5) and k light chain genes, thus confirming the lymphoid nature of the neoplasm.

Discussion

This case of ALL was atypical in its clinical course, morphological pattern and immunohistochemistry. Clinically, after a good response to the therapy, the disease spread with large tumour masses, without bone marrow or peripheral blood involvement in a manner more in keeping with a solid tumour than a leukaemic disorder.

The peculiar morphology and the aberrant immunohistochemical features of neoplastic cells at first favoured the diagnosis of a metastatic process. In fact, the solid ribbons and nests of neoplastic elements were strongly suggestive of an undifferentiated carcinoma. This diagnostic suspicion was apparently confirmed by the immunohistochemical demonstration of CKs and by negative staining for CD45. A second neoplasm complicating haematological disease is a phenomenon which has been reported, although not adequately evaluated [15, 38]. These tumours may be related to genetic mutations or to immunodeficiency either induced by the therapy or inherent in the disease itself. However, in the first biopsy immunophenotypic analysis on paraffin sections was not completely consistent with the diagnosis of carcinoma since the neoplastic cells exhibited a strong CD43/MT1 positivity. This marker, to our knowledge, has never been described in non haematopoietic neo-

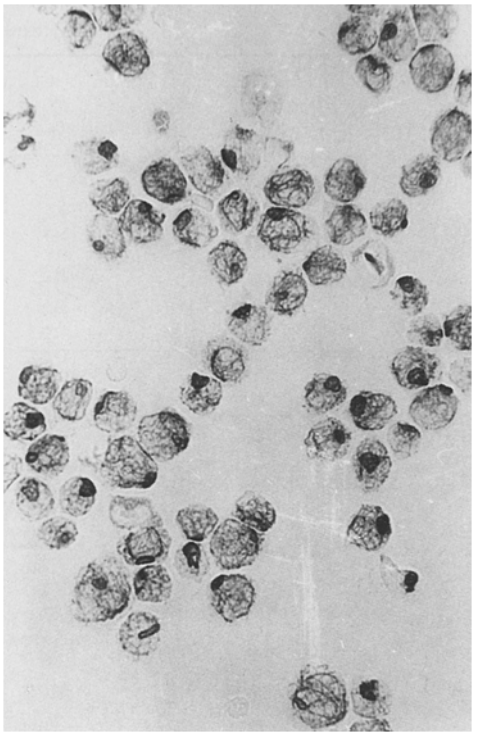

Fig. 4 Bone marrow smear at first diagnosis: leukaemic neoplastic cells show a strong dot-like cytoplasmatic positivity for Cam 5.2, ABC

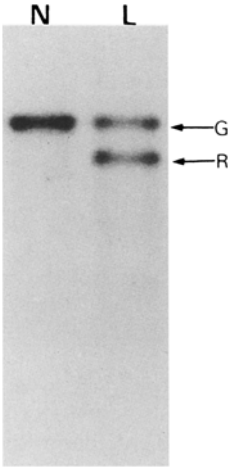

Fig. 5 DNA from the tumour (*L*) and peripheral blood of the same patient (*N*) was digested with *Eco*RI and hybridized with JH probe. The tumour sample shows a monoallelic rearrangement (*R*). *G* is the germline band

plasms [26]. On the basis of this apparent discrepancy we performed a more extended immunohistochemical analysis on frozen sections. On this material we were able to reach a final diagnosis of common ALL which was also confirmed by gene rearrangement analysis.

Experience in the application of antibodies has now clarified that different epithelial markers can be expressed in tissues other than epithelial in several normal and neoplastic conditions mainly related to the soft tissues [2, 3, 11, 17, 20, 21, 27]. Rare cases of lymphomas are also reported [4, 5, 6, 12, 23, 30, 37]. In these, the true nature of the disease can only be clarified by an extended panel of antibodies recognizing different lymphoid and non-lymphoid markers.

The cases of CK+ lymphoma reported by Sewell et al. [30], by Wotherspoon et al. [37], by De Mascharel et al. [5], by Doglioni et al. [6] and by Petruch et al. [23] were all characterized by plasmacytic or plasmablastic differentiation so that immuhistochemical studies were able to detect cytoplasmic light chain restricted immunoglobulins, proving the B monoclonal lymphoid nature of the disease. More complex is the case of CK+ large cell anaplastic CD30+ (Ki1) lymphomas reported by Del Sol et al. [4] and Gustmann et al. [12]. This problem can be only solved by a detailed immunohistochemical analysis and gene rearrangement studies. It is important to note that if CD30 and CKs are simultaneously expressed by the same cells, embryonal carcinoma has to be considered in differential diagnosis [22].

The significance of CK expression in non-epithelial neoplasms is still debated [18, 32]. Some criticism can be expressed about the positivity of CKs in haematopoietic neoplasia but Gustmann et al. [12] have confirmed true expression of CKs 8 and 18 in a single case of lymphoma by western blotting analysis.

It is interesting to note that in our case the expression of CKs analysed with antibodies detecting different CKs from Moll's classification was apparently restricted to the 18 CK. This is one of the CK subtypes more frequently detected in non-epithelial cells in non-neoplastic and neoplastic conditions [12, 14, 28, 33].

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