

Peripheral T-cell lymphoma or Hodgkin's disease in a HIV seropositive patient: a histopathological study

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Summary. Malignant lymphomas occurring in patients with AIDS are usually derived from the B-cell lineage while T-cell malignant lymphomas are very rare in these patients.

We report a HIV seropositive 29-year-old homosexual man in whom cervical lymph node biopsy showed an atypical lymphoproliferative process. On morphological and paraffin section immunohistochemical grounds the possibility of Hodgkin's disease (HD) mixed cellularity was initially suggested, but frozen section immunohistochemical studies revealed that the cellular infiltrate exhibited an aberrant pan T immunophenotype and consequently the diagnosis of peripheral T-malignant lymphomas (T-ML) was made. However, genotypic studies would be required to definitely confirm this diagnosis, in such cases.

In our case, varying numbers of small and medium-sized cells were positive for both Leu 3/CD4 and Leu 2/CD8 whereas some large cells reacted only with Leu 3/CD4 antibody. Some medium-sized, large and giant cells showed cytoplasmic positivity for Leu M1/CD15. Furthermore, the positivity of many large and giant cells with the activation markers BerH2/CD30, Ki-1/CD30, Tac/CD25 and HLA-DR suggested an activation state for these cells. Our findings emphasize the usefulness of frozen section immunohistochemical methods in order to investigate the spectrum of lymphoid malignancies occurring in HIV seropositive patients, and confirm results of previous studies which stressed the diagnostic difficulties that may appear in distinguishing HD from peripheral T-ML.

Key words: HIV-infection, Hodgkin's disease, Peripheral T-malignant lymphoma, Frozen section immunohistochemistry, Monoclonal antibodies

Introduction

Human immunodeficiency virus (HIV) infection is associated with increased incidence of non-Hodgkin malignant lymphomas (NHL) (Ioachim et al., 1985; Di Carlo et al., 1986; Ioachim and Cooper, 1986; Raphael et al., 1986) and the development of Hodgkin's disease (HD) with unusually aggressive clinical features (Unger and Strauchen, 1986). The Centers of Disease Control (CDC) now include in their definition of Acquired Immunodeficiency Syndrome (AIDS), all NHL occurring in patients with positive serologic or virologic test for HIV, even in the absence of opportunistic infections or Kaposi's sarcoma (Centers for Disease Control, 1985). NHL associated with AIDS are usually B-cell lymphomas of high grade malignancy and have a predilection for extranodal sites (Levine et al., 1984; Ioachim et al., 1985; Kalter et al., 1985; Ioachim and Cooper, 1986; Raphael et al., 1986). Only a few reports documented the occurrence of T-cell lymphomas (T-ML) in patients affected by AIDS (Kobayashi et al., 1984; Nasr et al., 1988; Sternlieb et al., 1988) or positivity for antibody to HIV (Howard and McVerry, 1987; Present et al., 1988).

In the report, we describe the morphological and immunohistochemical features of an unusual atypical lymphoproliferative process occurring in a HIV seropositive patient. Although the possibility of Hodgkin's disease (HD) mixed cellularity was initially considered, frozen section immunohistochemistry suggested that this process was a peripheral T-ML.

Materials and methods

In november 1987, a 29-year-old homosexal man was admitted to Lariboisiere Hospital with a 2-month history of intermittent fever, weight loss and night sweats. Physical examination revealed bilateral cervical and left inguinal lymphadenopathy. Biopsy of a left cervical lymph node initially disclosed Hodgkin's disease

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mixed cellularity, but frozen section immunohistochemistry was considered to be more consistent with peripheral T-ML. No hepatosplenomegaly, ascites or cutaneous abnormalities were present and chest X-ray was normal. Abdominal CAT-scan showed abdominal lymphadenopathies. Laboratory studies yielded the following values: Hemoglobin 8.6 gr/dl; leucocyte count $4.4 \times 10^9/l$ (with 10% lymphocytes); erythrocyte count $3.17 \times 10^{12}/l$ and platelets $166 \times 10^9/l$. The T4/T8 ratio was 0.3. The patient's serum was HbS+, anti HbS-, anti Hbc+, Hbe+, anti Hbe-, IgM anti HbC-, and anti Delta-. Serum electrophoresis demonstrated increased alpha-1, alpha-2, beta and gamma globulins ($a_1 = 4.5$ g/l; $a_2 = 11.8$ g/l; $b = 12.4$ g/l; $g = 24.9$ g/l). HIV (HTLV-III) antibodies were found in the patient's serum by ELISA test and by immunoblot (western blot) assays. Bone marrow biopsy and aspirate were free of malignant lymphoma and ORL examination was normal. Chemotherapy was begun with decrease in lymphadenopathies and the patient was alive six months after admission.

The cervical lymph node was fixed in Bouin's fluid and in 10% buffered formalin. Sections of 3 μ m were made from paraffin-embedded specimens and stained with hematoxylin-eosin-saffran (HES), periodic acid-Schiff (PAS), and methods of May-Grunwald-Giemsa (MGG), Gordon-Sweet, Brown and Brenn, Ziehl and Perls.

For electron microscopy tissue samples were immediately fixed in 3% cacodylate-buffered glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in graded acetone and embedded in epoxy-araldite. Ultrathin sections were stained with uranyl acetate and lead citrate.

Fresh tissue samples were snap frozen at -170° C in isopentane with liquid nitrogen and cryostat sections of 6 μ m were performed. Frozen sections were tested with a panel of monoclonal antibodies which included anti-Leu 14/CD22, Leu 1/CD5, Leu 2/CD8, Leu 3/CD4, Leu 4/CD3, Leu 5/CD2, Leu 6/CD1, Leu 7/NKcell, Leu 9/CD7, HLA-DR, Tac/CD25, Leu M1/CD15 (Becton-Dickinson, Mountain View, CA), Ki-1/CD30 (Dakopatts, Glostrup, Denmark). Paraffin-embedded specimens were tested with three polyclonal (anti-lysozyme, alpha-1-antitrypsin and S-100 protein, Dakopatts) and some monoclonal antibodies [anti-epithelial membrane antigen (EMA), anti-leucocyte common antigen (LCA), Ber H2/CD30 and UCHL1, (Dakopatts), anti-cytokeratin (Immunotech, Marseille, France) and MB2, MT1 (Eurodiagnostics BV, Holland)].

For monoclonal and polyclonal antibodies the avidin-biotin-peroxydase complex method (Hsu et al., 1981) and the threestep procedure (Ancelin et al., 1984) were used. 3-3 diamino-benzidine tetrachloride was finally used in each method. Appropriate dilutions and positive and negative controls were performed.

Results

Histological evaluation of the cervical lymph node revealed complete obliteration of the normal architecture by a diffuse lymphoproliferative process composed of a

mixed population of small, medium-sized, large and giant cells. Many cells contained irregular nuclei but in some areas a considerable number of small lymphoid cells with round or ovoid nuclei were also observed. Coarse chromatin was observed in medium-sized cells and one or multiple prominent nucleoli were frequently seen in large and giant cells (Figs. 1, 2). The cytoplasm was moderately abundant, pale and PAS-negative. Some multinucleated giant cells with faintly basophilic cytoplasm and prominent eosinophilic nucleoli closely resembled Reed-Sternberg cells (R.S.).

Tumor cells were intermingled with reactive components such as plasma cells, eosinophilic leucocytes and histiocytes. Ultrastructural study confirmed the light microscopic findings and revealed a mixed population of small, medium-sized, large and giant cells with nuclear outlines similar to those described in light microscopy. Heterochromatin was abundant, frequently speckled with marginal condensation. One or multiple large nucleoli were observed in giant and large cells (Fig. 3). The cytoplasm was moderately abundant and contained free ribosomes, mitochondria, and rough and

Table 1. Immunohistochemical findings

MARKER	CELL TYPE			
	SMALL	MEDIUM-SIZED	LARGE	GIANT
EMA*	-	-	-	-
Cytokeratin	-	-	-	-
LCA*	+++	+++	+	-
Leu 14/CD22	+	-	-	-
MB ₂	+	-	-	-
MT ₁	+	+	-	-
UHcL ₁	+	+	-	-
Leu6/CD1	-	-	-	-
Leu1/CD5	+	+	-	-
Leu4/CD3	+++	+++	++	-
Leu5/CD2	-	-	-	-
Leu9/CD7	+	+	-	-
Leu2/CD8	+	+	-	-
Leu3/CD4	+	++	+	-
Leu7/NK cells	-	-	-	-
BerH2/CD30	-	-	++	++
Leu M1/CD15	-	+	+	+
Ki-1/CD30	-	-	++	++
Tac/CD25	-	-	++	++
HLA-DR	-	-	++	+
Lysozyme	-	-	-	-
AAT*	-	-	-	-
S-100 protein	-	-	-	-

* EMA : epithelial membrane antigen

LCA : leucocyte common antigen

AAT : alpha-1-anti-trypsin

+++ : 50% or more positive cells

++ : 25-50% positive cells

+ : 10-25% positive cells

+/- : 5-10% positive cells

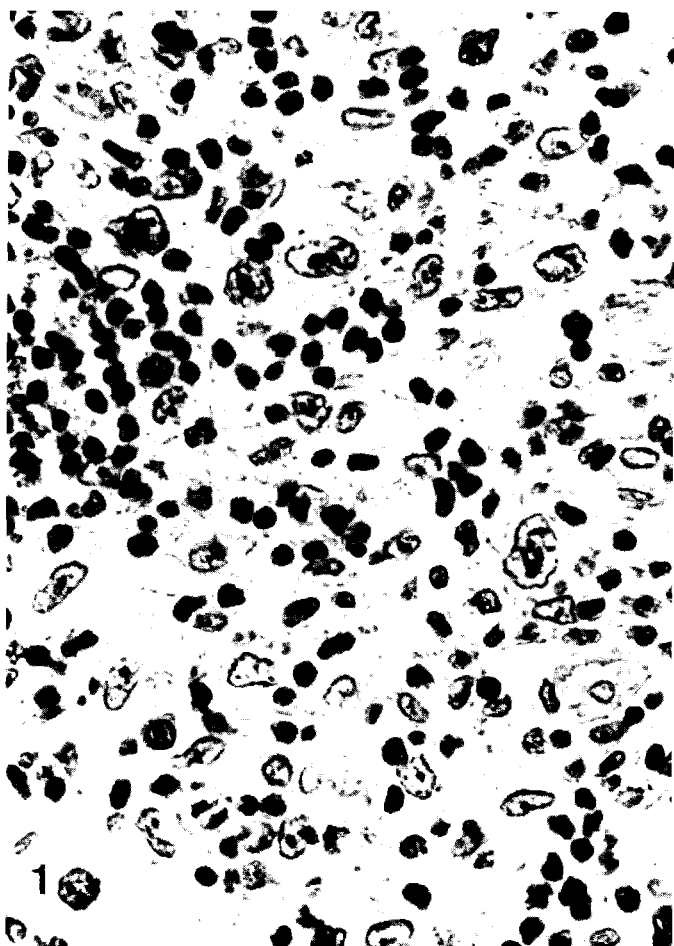


Fig. 1. Hodgkin-like histological aspect of the peripheral T-ML. Hematoxylin-eosin saffran. $\times 300$

smooth endoplasmic reticulum. A Golgi apparatus was occasionally seen.

Immunohistochemical studies (Table 1) disclosed that many small, medium-sized and large cells were positive for anti-LCA/CD45, while no reactivity was observed with anti-EMA and anti-cytokeratin. Scattered small cells reacted with anti-Leu 14/CD22. Varying numbers of small and medium-sized cells demonstrated a surface staining for anti-Leu 1/CD5, Leu 4/CD3, Leu 9/CD7, whereas some large cells were reactive only for anti-Leu 4/CD3 (Fig. 4). No cells reacted with anti-Leu 5/CD2 antibody. Small, medium-sized and some large cells reacted for anti-leu 3/CD4 and some small and medium-sized cells were positive for anti-leu 2/DC8 antibody. No cells reacted with Leu 6/CD1, Leu 7/NK cells, anti-lysozyme, anti-alpha-1-antitrypsin and anti-S 100 protein. Some medium-sized, large and giant cells showed focal paranuclear or diffuse cytoplasmic staining for anti-Leu M1/CD15. Varying numbers of large and giant cells reacted with BerH2/CD30, Ki-1/CD30, Tac/CD25 and anti-HLADR antibodies. Scattered small and medium-sized cells were positive for UCHL1 and MT1 antibodies and some small cells reacted with MB2.

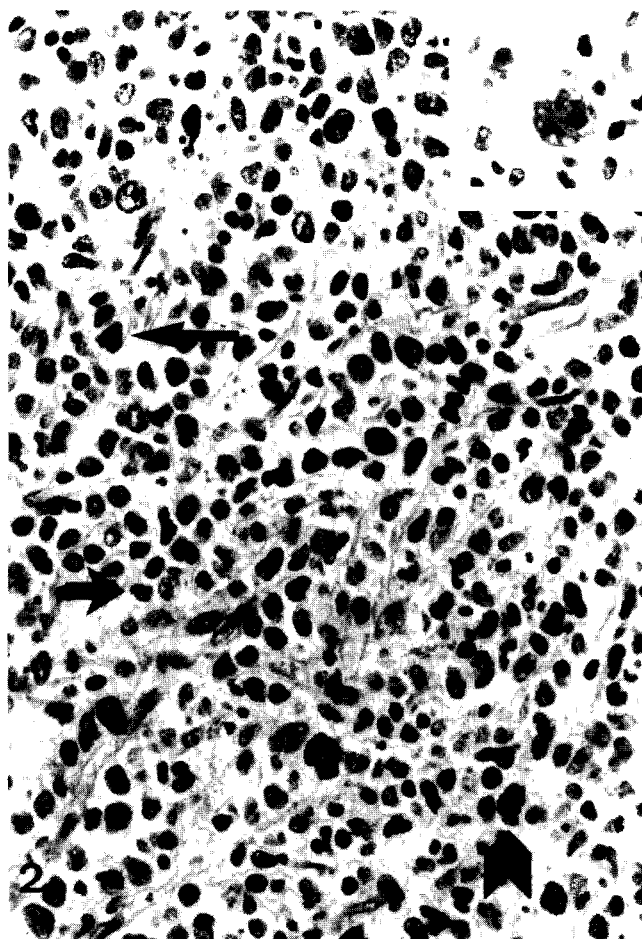


Fig. 2. Histological features of the peripheral T-ML composed of small (short arrow), medium sized (long arrow) and large cells (arrow head). Inset: Reed-Sternberg like cell. Hematoxylin-eosin saffran. $\times 300$

Discussion

The histological and immunohistochemical features displayed by the lympho-proliferative process in our case may suggest the diagnoses of HD and peripheral T-ML.

The possibility of HD mixed cellularity was initially considered because of the presence of multinucleated giant cells closely resembling R.S. cells, associated with an accompanying polymorphous cellular background (small lymphoid cells, plasma cells, eosinophils and histiocytes). Paraffin section immunohistochemistry provided further evidence in support of HD since many giant cells displayed usual phenotypic features of R.S. cells in HD (LCA/CD45-, Leu M1/CD15+, BerH2/CD30+) (Falini et al., 1987), and large as well as giant cells remained negative with the T-cell markers MT1 and UCHL1.

However, the variations in the size of the lymphoid cells which were associated with the giant cells was greater than that usually seen in typical cases of HD. These findings which were confirmed by electron microscopic study, prompted frozen section immunohistochemical investigations which revealed that the small

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Fig. 3. Large tumor cell with irregularly shaped nucleus and large nucleolus. $\times 6,600$

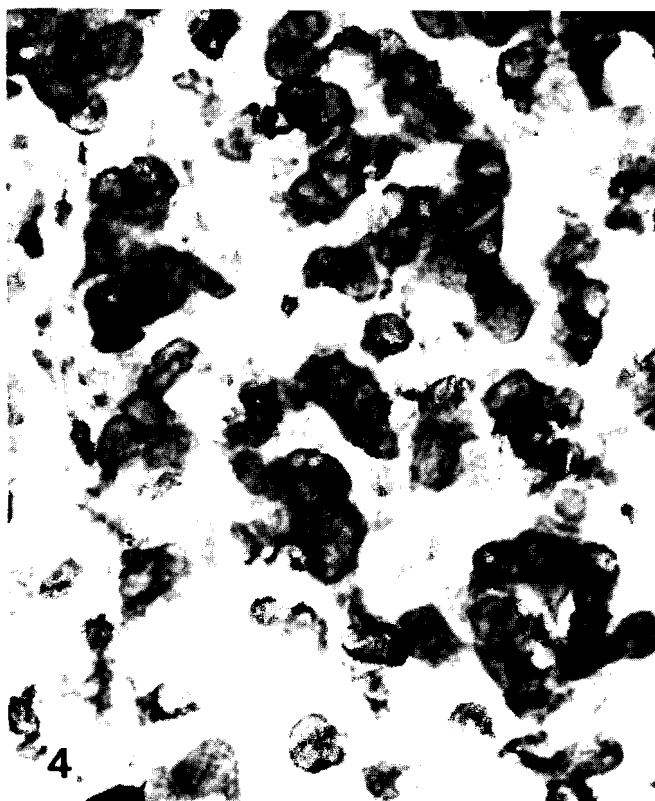


Fig. 4. Lymphoma cells show membrane positivity for Leu 4/CD3 antibody. Immunoperoxidase staining. $\times 300$

and medium-sized cells reacted with CD3, CD5, CD7, CD4 and CD8 antibodies whereas they did not stain with CD2 antibody. The emergence of an aberrant pan T immunophenotype in our case indicated that small and medium-sized cells represented more a neoplastic population than a reactive T-cell hyperplasia, since it has been previously reported that the loss of one or more pan T antigen may support the diagnosis of a T-ML (Weiss et al., 1985; Borowitz et al., 1986; Sheibani et al., 1986). Furthermore, in a view of this aberrant pan T immunophenotype, the variation in size of T-lymphoid cells which constituted the cellular background of the giant cells could be interpreted, in accordance with a previous report (Krajewski et al., 1988), as morphological evidence in favour of T-ML. Taking all into consideration, these data could be regarded as features consistent with the diagnosis of peripheral T-ML, pleomorphic medium and large cell type (Stansfeld et al., 1988). However, genotypic studies would be required in order to definitely confirm this diagnosis in such cases. With respect to the diagnosis of T-ML, the lack of reactivity of the large and giant cells with most pan T markers, which is a finding previously described in T-ML, and particularly of large cell morphology (Weiss et al., 1985; Ramsay et al., 1987), might be due to the loss of T-cell antigens in the blastic forms of the malignant proliferation.

In addition, the staining of some medium-sized, large and giant cells with Leu M1/CD15 agrees with results of

other studies which describe Leu M1/CD15-positive large and/or R-S-like cells in peripheral T-ML (Wieczorek et al., 1985; Sheibani et al., 1986).

Once the diagnosis of peripheral T-ML has been made, the occurrence of this non-Hodgkin lymphoma in our HIV seropositive patient should be considered as diagnostic of AIDS (Center for Disease Control, 1985).

Malignant lymphoma occurring in patients with AIDS are usually of B-cell phenotype (Levine et al., 1984; Ioachim and Cooper, 1986) while to our knowledge, there have only been a few case reports on the association of T-ML and AIDS (Kobayashi et al., 1984; Nasr et al., 1988; Sterlieb et al., 1988) or HIV infection (Howard and Mac Verry, 1987; Presant et al., 1988). A case of HTLV-1 positive T-cell leukemia/lymphoma (Leu 5+, Leu 1+, Leu 3a+ Leu 2a-) was reported in a Japanese patient (Kobayashi et al., 1984). More recently, a large cell immunoblastic pulmonary T-ML (CD5+, CD2+, CD4+, CD3-, CD1-, CD8-, HLADR+) (Nasr et al., 1988) and a diffuse mixed small and large cell pulmonary peripheral T-ML (CD7+, CD2+) (Sternlieb et al., 1988) were reported in patients with AIDS. The other two reports described a nodal lymphoblastic T-ML (T11+, Leu1+, Leu3+, TdT+, B1-, Common ALL-) in a patient with HIV infection (Presant et al., 1988) and a nodal large cell T-ML (UCHL1+) in a HIV seropositive and HTLV-1 negative haemophilic patient (Howard and Mc Verry, 1987).

In the current study, it was difficult to determine whether the lymphoma exhibited helper/inducer or cytotoxic/suppressor phenotype since varying numbers of small and medium-sized cells were positive for both Leu 3/CD4 and Leu2/CD8 antibodies whereas only some large cells, were reactive with Leu 3/CD4, and giant cells remained negative for both markers. These findings may be correlated to those of previous reports where some cases of peripheral T-ML showed both helper and cytotoxic/suppressor phenotype markers (Weiss et al., 1985; Borowitz et al., 1986). Assessment of activation associated markers in our case revealed expression of CD30, CD25 and HLA-DR on large and giant cells, reflecting an activation state of these cells. These findings may be in keeping with those of previous studies (Stein et al., 1985) which reported that large malignant cells in peripheral T-ML were more likely to express activation markers. Our study confirms previous reports which emphasized the diagnostic difficulties that may be encountered in differentiating HD from T-ML (Sheibani et al., 1986; Duggan et al., 1988; Krajewski et al., 1988) and suggests that precise identification and complete characterization of the lymphoid malignancies require not only detailed frozen section immunohistochemical investigations but also genotypic studies.

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