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Morphological variability of tumour cells in T-cell-rich B-cell lymphoma

A histopathological study of 14 cases

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Abstract T-cell-rich B-cell lymphoma (TCRBCL) is an unusual lymphoma which is difficult to diagnose. A majority of reactive T-cells and numerous histiocytes mask the few large neoplastic B-cells. Fourteen cases of TCRBCL were studied in order to identify the main histological and cytological features useful for this diagnosis. Neoplastic cells are atypical and sometimes difficult to classify. Several types are seen; they are mostly centroblasts, which represent more than 50% of the tumour cells but are sometimes multilobated, immunoblasts- or Reed-Sternberg-like cells. Interestingly, at least two, and often three, types of tumour cell are present in all the cases. Epithelioid cells and histiocytes are always found and are often numerous. Hypervascularization and fibrosis are present in the majority of cases, but without annular bands. Necrosis is absent. All tumour cells express CD20 but EMA is expressed in less than half the cases. In two cases, the association of a diffuse large B-cell lymphoma in one site and a TCRBCL in another suggests that TCRBCL may be considered as a peculiar pattern of a diffuse large B-cell lymphoma with a strong stroma reaction. TCRBCL may not represent a clinicopathological entity.

Key words T-cell-rich B-cell lymphoma · B-cell lymphoma · Hodgkin's disease

Introduction

T-cell rich B-cell lymphoma (TCRBCL) was first described in 1984 by Jaffe and Mirchandani [11, 16] as a variety of B-cell neoplasm that mimics T-cell lymphoma morphologically and immunologically. The term TCRBCL was proposed by Ramsay et al. in 1988 [19]. This lymphoma is characterized by a majority of reactive T cells and histiocytes masking the tumour cells, which are atypical large B-cells. This pattern has been reported by various authors [1, 13, 15, 17], but the exact definition of TCRBCLs and its place in lymphoma classification remain unsettled. It is considered by the REAL classification [10] as a morphological variant of a diffuse large B-cell lymphoma. According to the published series, the proportion of reactive T-cells required for this diagnosis ranges from 50% to 90% [15, 17].

These lymphomas are a diagnostic pitfall. They are usually regarded as T-cell lymphomas, because of the predominance of T-cells, or Hodgkin's disease, because of the presence of atypical giant tumour cells, occasionally resembling Reed-Sternberg cells [6]. Its relationship to nodular paragranuloma (lymphohistiocytic Hodgkin's disease (L&H, HD), nodular) and that to its still debated diffuse variant are matters of discussion.

We report a study conducted in 14 cases with the goal of defining the main cytological and histological features that are required before this diagnosis is justified.

Materials and methods

Fourteen cases of TCRBCLs were identified during a 5-year period. Patients were evaluated for age, sex and clinical stage according to the Ann Arbor classification (Table 1).

We excluded from our study all the cases showing sheets of neoplastic B-cells even if they were associated with a T-cell-rich pattern, because they were easily classified as diffuse large B-cell lymphomas. Therefore, we studied 14 cases, in which large tumour cells were not obviously identified at low magnification, and where at higher magnification (×40) fewer than 20 dispersed tumour cells were seen to be present.

Lymph node biopsies were available in all cases, and extranodal tumour was seen in 4 cases. The morphological features were assessed on haematoxylin-eosin-safran, Giemsa, periodic-acid-Schiff (PAS) stains and Gordon-Sweet's silver impregnation performed on formalin or Bouin's liquid-fixed, paraffin-embedded tissue sections. The arrangement of tumour cells (alone or in small clusters) and their cytological type were analysed. The proportion of each subtype was evaluated with a semi-quantitative method: not obviously present, less than 25%, 25–50%, 50–75%, and more than 75% of the tumour cells.

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Table 1 Clinical features (*NA* not available)

Case	Age (years)	Sex	Clinical stage
1	71	M	IV
2	72	F	I
3	79	F	III
4	8	M	III
5	33	F	II
6	34	M	IV
7	44	M	III
8	64	F	III
9	35	M	IV
10	42	M	IV
11	24	F	II
12	61	F	IV
13	52	M	I
14	72	M	NA

Immunohistochemistry was performed on paraffin-embedded tissue using a three-stage indirect immunoperoxidase technique or an APAAP method. The antibodies used were anti-CD20 (L26, Dakopatts), DBB42 (Immunotech), anti-CD3 (Dakopatts), anti-CD45RO (UCHL1, Dakopatts), anti-CD15 (8OH5, Immunotech), anti-CD30 (HRS4, Immunotech), anti-EMA (E29, Immunotech), and anti-CD57 (NC1, Immunotech).

Monoclonality was tested by either immunohistochemistry or in situ hybridization in 8 and 3 cases, respectively, with either kappa and lambda light chain antibodies (Dakopatts) or oligonucleotides (Dakopatts).

Results

The clinical features of 14 patients are summarized in Table 1. There were 8 men and 6 women; the mean age was 49, ranging from 8 to 79 years of age. Superficial polyadenopathies were present in 12 cases, associated with mediastinal or abdominal involvement in 7 cases. Hepatosplenomegaly was observed in 5 cases. Among the 14 patients, 2 had stage I disease, 2 had stage II, 4, stage III, and 5, stage IV, while in 1 the stage was unknown.

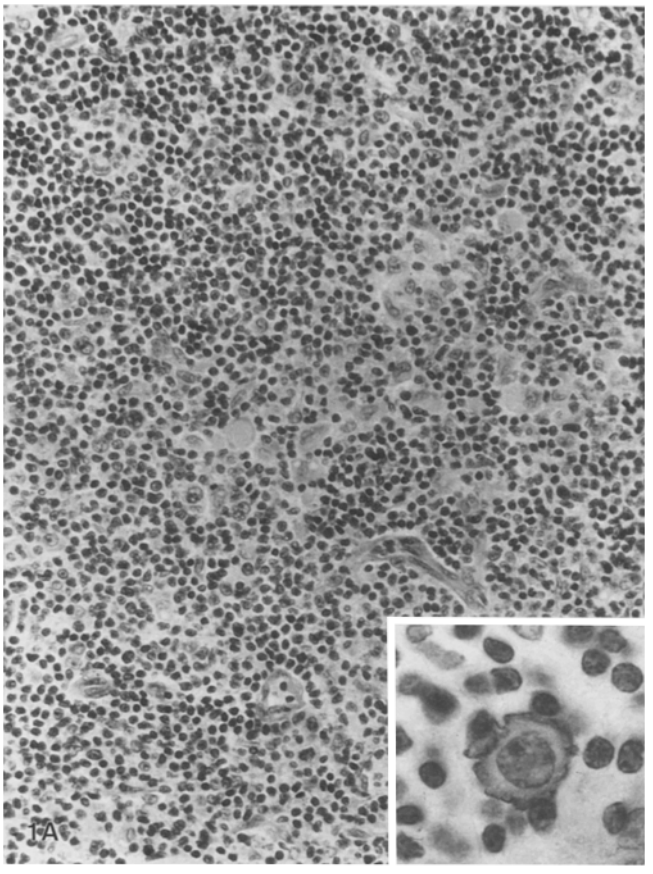
Histologically, all 14 cases were roughly homogeneous. There was complete obliteration of the nodal architecture in 12 cases (Fig. 1). In 2 cases in which this was not complete, tumour cells had an interfollicular spread, as in interfollicular Hodgkin's disease. Reticular sclerosis was seen in 10 cases and extensive sclerosis in 5, but without annular bands. Hypervascularization with high endothelial venule hyperplasia was observed in 12 cases. Areas of necrosis were absent. The reactive cells were predominant, mainly composed of T-lymphocytes, often small, sometimes medium-sized with a clear nucleus, a small nucleolus and pale cytoplasm. These cells were associated with histiocytes and epithelioid cells, grouped in small clusters, but sometimes numerous (Fig. 2). Eosinophils were obviously present in 4 cases. There was neither plasma cell nor mastocyte hyperplasia.

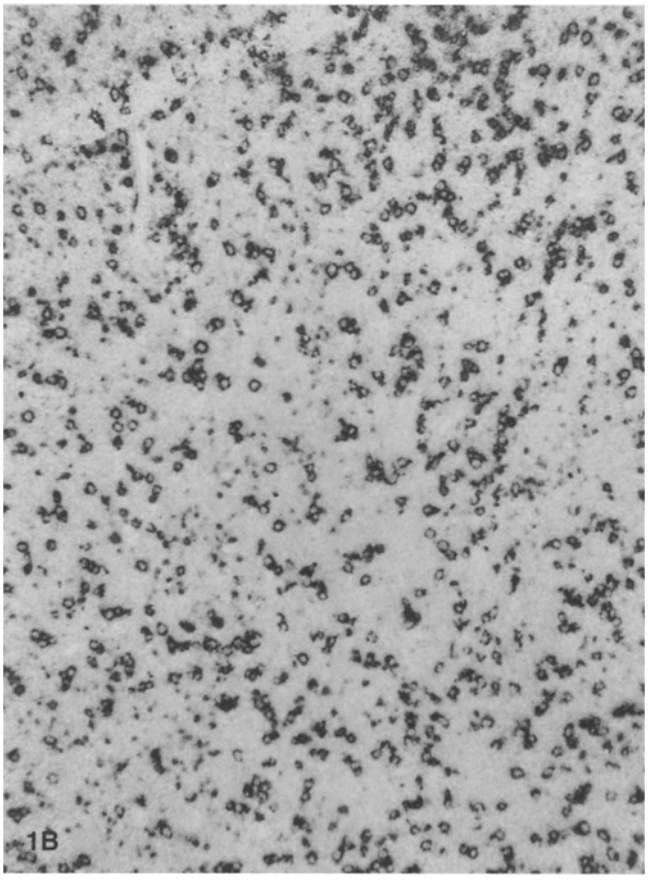
Tumour cells were few and were difficult to detect at low magnification, because of the wide-ranging reactive lymphocytic and histiocytic infiltrate. They were isolated or grouped in small clusters (Fig. 1B). Each cluster comprised a group of three to ten tumour cells. Tumour cells varied in size and form and were sometimes difficult to classify (Fig. 3). Four main subtypes of tumour cells were characterized. Typical centroblasts were often present (Fig. 4). In addition, some other cells with nuclei resembling centroblasts and with a wider cytoplasm that was basophilic to a varying degree with Giemsa stain were seen. Multilobated centroblasts (Fig. 5), sometimes looking like multinucleated centroblasts (Fig. 6), constituted a second subtype. Immunoblasts (Fig. 7) characterized by a prominent nucleolus and a large basophilic cytoplasm, sometimes with plasmacytic differentiation, represented a third subtype. Reed-Sternberg-like cells are the fourth main subtype. Some cells were unilobated, with an irregular and sometimes folded nucleus associated with a large solitary eosinophilic nucleolus and a wide cytoplasm. Other cells were bilobated, like a typical Reed-Sternberg cell, but with a smaller nucleolus (Fig. 8).

In all cases, different subtypes of tumour cells were present in variable proportions (Table 2). In each case, at least three subtypes of tumour cells were identified. In 8 cases more than 50% of tumour cells were centroblasts, and these cells associated with multilobated centroblasts, immunoblasts or Reed-Sternberg-like cells. Two cases (4, 7) were particularly rich in multilobated centroblasts, but all the other subtypes were present in low percentages. In 1 case (14) the tumour was mainly composed of immunoblasts. Reed-Sternberg-like cells were the predominant population in 3 cases (6, 8, 12), but these cells did not express CD15 and CD30 and almost all of them expressed CD20. Moreover, they were associated with typical centroblasts and immunoblasts.

Two patients who had TCRBCL in a lymph node, simultaneously had diffuse large B-cell lymphoma at another site. The first case (12) was that of a 61-year-old woman who had hepatosplenomegaly and pancytopenia. Bone marrow and liver biopsies showed a destructive infiltrate mainly composed of small T lymphocytes and histiocytes, with very few large cells suggesting a lymphoma localization, making it difficult to differentiate between Hodgkin's disease and non-Hodgkin lymphoma. A laparotomy was performed and histological analysis revealed a typical TCRBCL in a splenic hilar lymph node and a centroblastic, polymorphic B-cell lymphoma in the spleen. The second patient (7) was a 44-year-old man who had a mesenteric tumour associated with superficial polyadenopathies. Histopathological analysis revealed a TCRBCL in the inguinal lymph node, while the mesenteric tumour was a centroblastic B-cell lymphoma.

Immunohistochemical studies showed a small number of large CD20-positive cells among a majority of small T-cells (CD3 or CD45RO positive). All the tumour cells expressed CD20 (Fig. 1A, inset). The B-cell phenotype of tumour cells was also demonstrated with DBB42 in 4





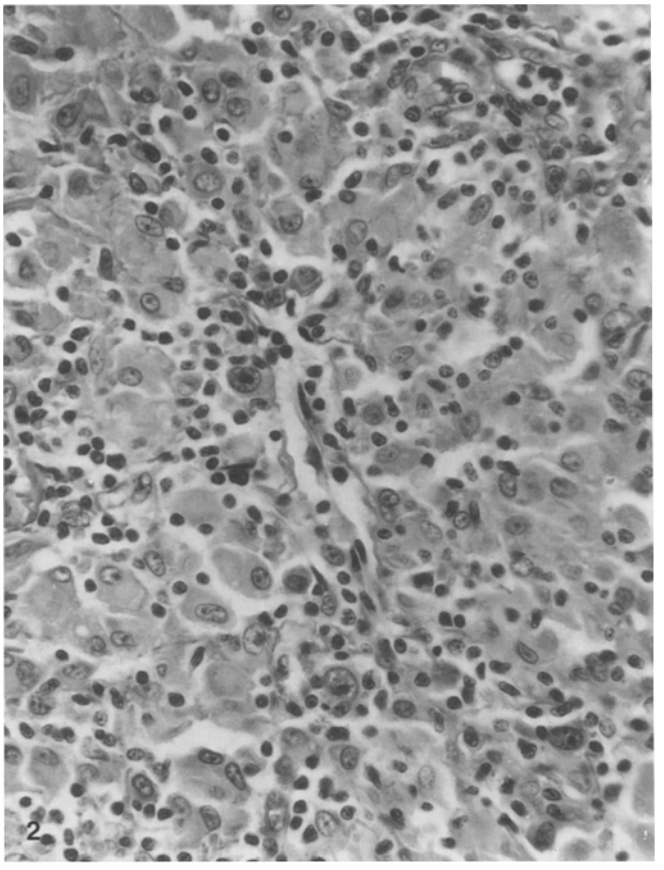


Fig. 1 **A** Obliteration of the nodal architecture by numerous small T cells. H&E, G×240. *Inset* Membranous staining with anti-CD20 of a large cell. H&E, G×945. **B** CD20-positive large cells are isolated or grouped in small clusters. NBT/BCIP, ×125

Fig. 2 Sheets of epithelioid cells are present in some areas of the lymph node. H&E, ×240

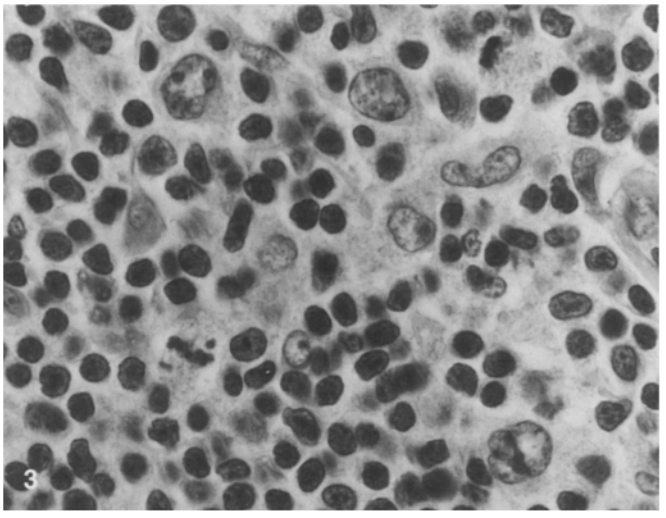
out of 6 cases. Neoplastic cells did not express CD15 in the 9 cases tested and expressed CD30 in only 2 out of 9 cases. Out of 11 cases tested for EMA expression, 4 cases were positive. In 12 cases, anti-CD57 antibody stained scattered small lymphocytes without rosette formation around tumour cells.

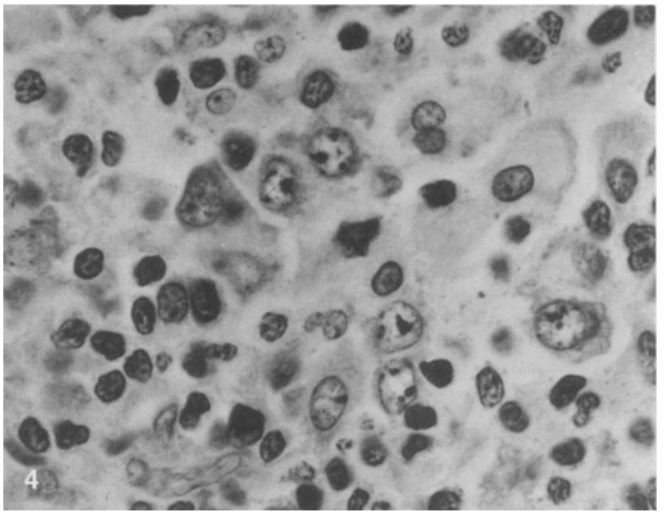
Light chain restriction was demonstrated in 3 out of 9 cases, either by immunohistochemistry (2 cases) or by in situ hybridization (1 case).

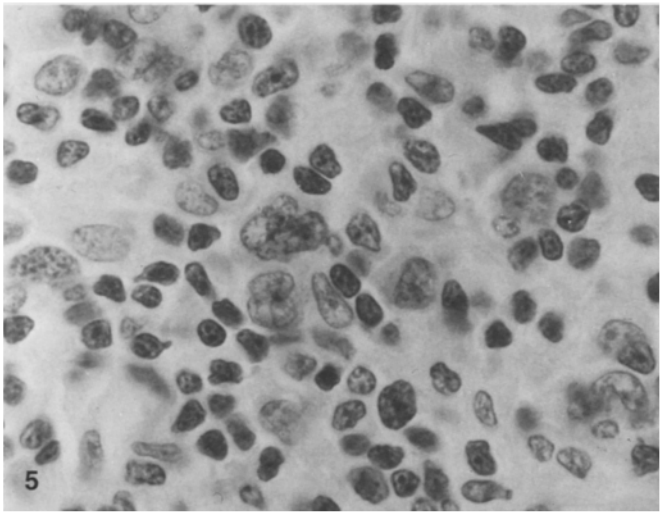
Discussion

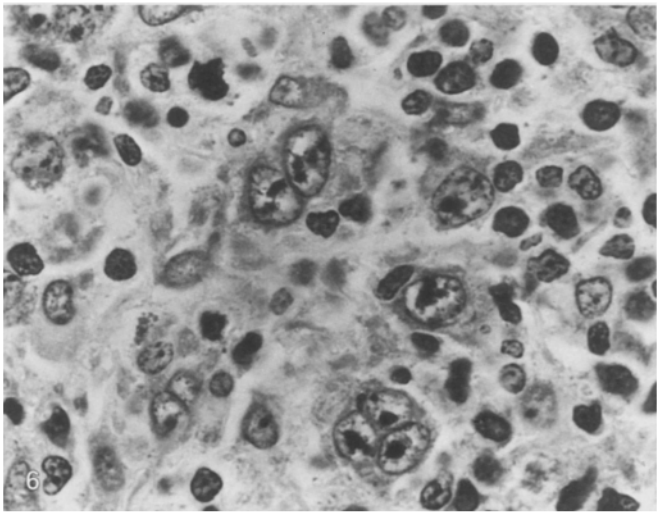
In each of our 14 cases of T-cell-rich B-cell lymphoma there was a small number of atypical large B-cells scattered among a majority of reactive T-lymphocytes and associated with a variable number of histiocytes and epithelioid cells.

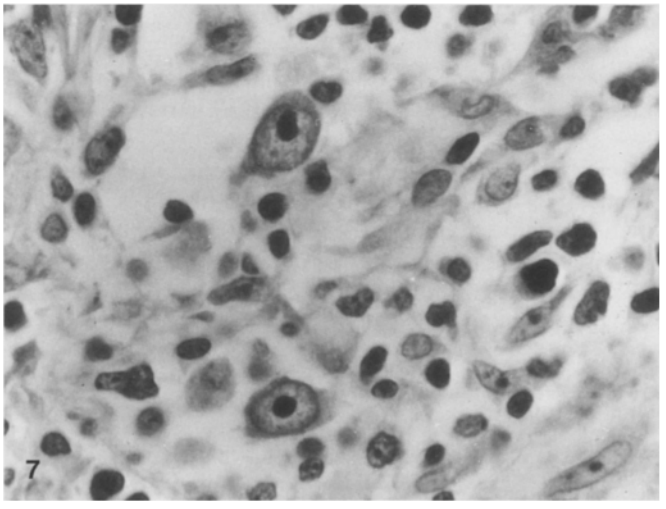
At diagnosis, most of the patients cases presented with an advanced clinical stage, III or IV (9/14), fitting in perfectly with the series already published [1, 13, 15, 19]. TCRBCL has been described mainly in lymph











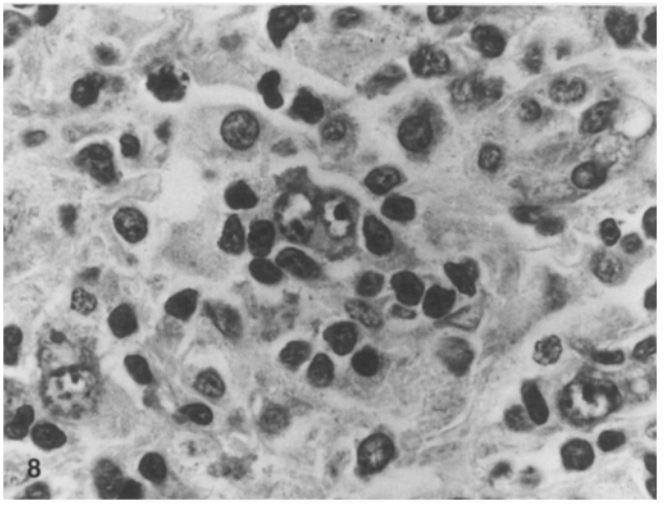


Fig. 3 Tumour cells are sometimes difficult to classify. H&E, ×945

Fig. 4 Centroblasts. H&E, ×945

Fig. 5 Multilobated centroblasts. H&E, ×945

Fig. 6 Multinucleated centroblasts. H&E, ×945

Fig. 7 Immunoblasts. H&E, ×945

Fig. 8 Reed-Sternberg-like cells. H&E, ×945

nodes and spleen [2], but also in several extranodal localizations [21] such as liver [12], common bile duct [4] and lung [5].

All our 14 cases shared common histological features. In all of these cases, tumour cells, although polymorphic and sometimes difficult to classify, were often associated with immunoblasts, centroblasts and Reed-Sternberg-like

Table 2 Cytological features of tumour cells (percentage of each cytological subtype among all tumour cells: *0* cells not obviously present, *A* less than 25% of the tumour cells, *B* between 25 and 50%, *C* between 50% and 75%, *D* more than 75%)

Case no. Tumour cells	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Centroblast (Cb)	C	C	C	A	D	A	B	B	C	D	C	B	D	A
Multilobated Cb	0	0	0	C	0	0	B	0	0	0	0	0	0	0
Immunoblast	0	A	A	A	A	A	A	A	0	A	A	A	A	C
Reed-Sternberg like cells	B	0	A	A	0	C	A	C	B	A	B	C	A	A

cells. Centroblasts were often predominant. Multilobated centroblasts were rarely seen, making up more than 50% of tumour cells in only two cases. Reed-Sternberg-like and immunoblast-like cells were often present in small numbers, but were rarely seen as the predominant population. The tumour cells express CD20, EMA in 36% of the cases, rarely CD30, but not CD15. The monotypic expression of the immunoglobulin light chain proteins or mRNAs on paraffin sections supporting the clonality of the few large B-cells was demonstrated in only 25% of our cases.

The common histological features shared by our cases are puzzling compared with the heterogeneity of the TCRBCL in other groups [8,13]. This may be due either to a selection bias or to the difficulty of differentiating multilobated centroblasts from L and H (lymphohistiocytic) cells of Hodgkin's disease, such as popcorn cells. Baddoura et al. [1] did not see any L&H cells, whereas Krishnan et al. [13] reported the presence of these cells in TCRBCL. However, none of our cases had a nodular pattern. Histiocyte-rich B-cell lymphoma has been described [7] as an entity, and in our cases, histiocytes were always present and sometimes numerous. They were variable in amount in different areas of the same case and although the clinical presentation of these cases (majority of stage III and IV) and the distribution and phenotype of large B-cells is similar to Delabie's series [7], we did not observe nodular pattern, as has been described in this entity.

The diagnosis of TCRBCL is difficult and should be considered after exclusion of T-cell lymphoma, Hodgkin's disease and non-Hodgkin large B-cell lymphoma. The main T-cell lymphoma that can be regarded as a diagnostic pitfall is the lympho-epithelioid lymphoma. This lymphoma has some features in common with TCRBCL, such as the presence of clusters of epithelioid cells associated with immunoblasts and some atypical cells looking like Reed-Sternberg cells, among a majority of small T-cells. In contrast with lympho-epithelioid T-cell lymphoma, immunohistochemistry shows a B phenotype of large cells in TCRBCL.

Several types of Hodgkin's disease must be considered in the differential diagnosis. To eliminate the cellular phase of nodular sclerosing Hodgkin disease (type 2), it is important to demonstrate the absence of either typical Hodgkin disease lacunar cells or a nodular pattern. The CD20 positivity of almost all the tumour cells without expression of CD15 and only rare expression of CD30 should also be found. A mixed cellularity Hodgkin disease (type 3) is sometimes difficult to exclude on morphological criteria alone, particularly in the lymphocyte-rich or epithelioid-rich subtype. It is characterized by typical Reed-Sternberg cells, which express CD15 and CD30. Nodular paragranuloma (L&H predominant HD, nodular) is characterized by the peculiar morphology of the tumour cells with an irregular, large and clear nucleus looking like popcorn in homogeneous nodules, corresponding to progressively transformed follicles. In our cases, we did not observe a nodular pattern, easily excluding this disease in the differential diagnosis. The diffuse counterpart of nodular paragranuloma (Hodgkin, type 1 diffuse) may or may not exist [9, 20]. It seems that this term was used in the past for several different entities, which may have included TCRBCL. However, the clinical presentation of diffuse lymphocyte-predominant Hodgkin disease seems to be completely different from TCRBCL; there is predominance of clinical stages I and II in most cases [3, 14, 22]. Therefore, it does not seem that TCRBCL represent the main group of what was called Hodgkin type 1 diffuse.

The definition of TCRBCL is variable according to previous studies [15,17]. In our series, we excluded cases showing sheets of neoplastic cells, which raises the problem of the borderline between TCRBCL and diffuse large B-cell lymphomas. In order to clarify this problem, we distinguish diffuse large B-cell lymphoma, which includes a T-cell-rich pattern and the typical TCRBCL as described by Ramsay et al. [19]. However, the concept of TCRBCL remains unclear and may represent only a morphological variant of a diffuse large B-cell lymphoma with a strong stroma reaction. This hypothesis is supported by the fact that TCRBCL can be associated with large B-cell lymphoma [15, 18], as in 2 of our cases.

The individuality of TCRBCL in lymphoma classification is a vexed question. It corresponds to a peculiar histological pattern but it is difficult to assert that this pattern is an entity in terms of clinical presentation and response to therapy. Prospective multicentre studies are necessary to evaluate whether or not this histological pattern is a real clinicopathological entity.

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