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TITLE:

Peripheral T-cell lymphomas with a follicular growth pattern are derived from follicular helper T cells (T_{FH}) and may show overlapping features with angioimmunoblastic T-cell lymphomas

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ABSTRACT

Rare cases of peripheral T-cell lymphomas with follicular growth pattern (PTCL-F) have been recently reported, and their association with t(5;9)(q33;q22) involving *ITK* and *SYK* has been suggested. However, the clinicopathologic aspects of PTCL-F are poorly described and the normal cell counterpart of this subgroup of lymphoma is still unknown.

Therefore, we analysed the pathological, phenotypic and cytogenetic features of a series of 30 patients (range 33 – 88 years) which showed histopathologic features of PTCL-F in at least one biopsy (n=30), either at initial presentation (n=26) or at relapse (n=4). Neoplastic cells were medium-sized clear cells that were CD4+ (24/27, 89%), CD10+ (21/29, 72%), BCL-6+ (14/19, 74%) and expressed PD-1 (27/27, 100%), CXCL13 (23/27, 85%), and ICOS (11/11, 100%), markers of follicular helper T cells (T_{FH}). Four out of 22 patients (18%) had t(5;9)(q33;q22) detected by fluorescent in situ hybridization. Patients with clinical data available had multiple lymphadenopathies (25/28, 89%), stage III-IV disease (17/26, 65%), B symptoms (7/27, 26%) and skin lesions (6/23, 26%). Three patients with sequential biopsies disclosed clinical and histopathological features of AITL at initial presentation.

Our results show that this rare form of PTCL-F (1) has an immunophenotype indicative of derivation from T_{FH} cells, (2) is associated with t(5;9) in a proportion of cases, and (3) shows some overlapping features with AITL, raising the question of a possible relationship.

Key words: peripheral T-cell lymphoma, angioimmunoblastic T-cell lymphoma, follicular helper T cell.

INTRODUCTION

Peripheral T-cell lymphomas (PTCL) comprise around 12% of all non-Hodgkin's lymphomas in Western countries. Among PTCL, those with a nodal presentation, include two well-defined clinicopathologic entities, angioimmunoblastic T-cell lymphomas (AITL) and ALK-positive anaplastic large cell lymphomas (ALCL) (36). The other cases are classified as either ALK-negative ALCL, or more frequently, peripheral T-cell lymphomas, unspecified (PTCL-u). Most cases of PTCL-u involve lymph nodes in a diffuse pattern and it was suggested that most PTCL-u were derived from CD4+ central memory T-cells (13) or activated peripheral T-cells (28). However, de Leval et al (8) recently reported three cases of PTCL-u with follicular involvement, and some cases with perifollicular growth pattern or involving the mantle zone have been described in other case reports (16, 32). Recently, Streubel et al (35) reported a novel t(5;9)(q33;q22) translocation in five out of 30 PTCL-u, including three with follicular growth pattern and one with perifollicular involvement. However, as for most PTCL, the normal cell counterpart of these PTCL with follicular involvement (PTCL-F) is unknown, and it is not established whether these PTCL-F represent a distinct subtype or a morphological variant showing overlap with other PTCL-u or AITL. In this respect, previous reports pointed out the common CD4+/CD10+/BCL-6+ phenotype of these PTCL-F, resembling that described in AITL, an entity which is now thought to be derived from a peculiar minor subset of T cells, designated follicular B helper T cells (T_{FH}) (7, 11, 14, 21, 42) and shows a morphological spectrum from cases demonstrating hyperplastic follicles to those with a diffuse pattern (1).

In this study, we aimed to investigate a series of PTCL-u with follicular growth pattern (PTCL-F) in order to describe their pathological and clinical aspects, to determine their normal cell counterpart, and to attempt to approach their possible relationship with AITL.

MATERIALS AND METHODS

Case selection

Forty specimens (lymph nodes = 36, skin = 2, nasopharynx = 2) from 30 patients were retrieved from the files of Departments of Pathology at Centre Hospitalo-Universitaire Henri Mondor in Créteil (n=22, 14 patients), at Centre Hospitalo-Universitaire de Nantes (n=12, 10 patients), and at Vienna General Hospital (n=6, 6 patients) during the period from 1994 to 2007. All patients had a diagnosis of PTCL-F either at diagnosis (n = 26) or at relapse (n=4). Parts of clinical, histologic, immunohistochemical, and genetic data of five patients have already been reported in two earlier publications (29, 35). Each biopsy was reviewed by at

least three of the four hematopathologists (P.G., A.M., Y.H., A.C.). Although none of the 30 samples classified as PTCL-F fulfilled the complete morphological and phenotypic criteria of the recognized patterns of AITL (1), special attention was given to the presence of the following features of AITL such as neoplastic clear cells, vascular hyperplasia, polymorphous cellular infiltrates including eosinophils and plasma cells, and scattered B immunoblasts. Cases had been previously stained with a panel of antibodies, directed against B (CD20) and T-cell markers (CD2, CD3, CD5, CD7, CD4, CD8) and a follicular growth pattern had been demonstrated by staining follicular dendritic cells (FDC) with CD23 and/or CNA.42 antibodies and mantle zone B-cells with IgD.

Immunohistochemical studies

Immunohistochemistry was performed on deparaffinized tissue sections using a three-step immunoperoxidase technique (for all antibodies except CXCL13). After antigen retrieval either with 1mM EDTA buffer at pH 8.0 (DakoCytomation, Glostrup, Denmark) in a water bath for 40 minutes (for most antibodies) or with 1X citrate buffer at pH 6 (Diapath, Martinengo, Italy) in a 750W microwave oven for 15 minutes (for CXCL13), sections were stained for CD2, CD4, CD5, CD7, CD10, CD23, CD57 (Novocastra, Newcastle, UK); CD3, CD8, CD20, CNA.42, IgD, BCL-6 (DakoCytomation, Glostrup, Denmark) and CXCL13 (53610, R&D Systems, Minneapolis, MN) antigens. PD-1 was produced by one of the authors (G.R.) (31). For CXCL13, a tyramide signal amplification system (CSAII kit, DakoCytomation) was applied as previously described (11). ICOS staining was performed by one of the authors (T.M) (24).

Epstein-Barr virus (EBV) studies

The detection of EBV infected cells was performed using either immunohistochemistry with a monoclonal antibody to LMP-1 (CS1-4, DakoCytomation) or in situ hybridization (ISH) with fluorescein-conjugated EBV (EBERs 1 and 2) oligonucleotides (DakoCytomation, Glostrup, Denmark) complementary to nuclear RNA portions of the *EBER* 1 and 2 genes. The ISH procedure was performed on deparaffinized tissue sections, as previously reported (19).

Clonality analysis

Twenty specimens from 17 patients were investigated for clonality analysis. DNA was extracted from tumor samples using High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). T-cell receptor (TCR) γ chain gene rearrangements were studied by

using a GC-clamp multiplex PCR-γ-DGGE procedure as previously described (37, 38) and the immunoglobulin (Ig) gene rearrangements by using consensus primers from European BIOMED-2 concerted action (41).

Fluorescent in situ hybridization (FISH) study

Fluorescent in situ hybridization study was performed on deparaffinized tissue sections according to the protocol described previously (35). The following bacterial artificial clones (BACs) were selected to identify *ITK-SYK* rearrangements: RP11-563G12 and RP11-1091N2 flanking *ITK* for *ITK* translocations, RP11-31B18 and RP11-47O12 flanking *SYK* for *SYK* translocations, and RP11-956N15 and CTB4E7 spanning *ITK* and *SYK* for *ITK–SYK* translocations. BAC probes were directly labelled with SpectrumOrange- and SpectrumGreen-dUTP using nick translation (Vysis). Twenty-four specimens from 23 patients were studied with all above-mentioned clones. For interphase FISH, 300 cells were analyzed. The cutoff value for the diagnosis of each set of probes was the mean percentage of cells with a false-positive signal constellation plus three standard deviation as assessed with the use of tissue specimens obtained from 10 reactive lymph nodes.

RESULTS

Clinical characteristics of PTCL-F

Sixteen patients were male and 14 were female. Median age was 67 years (range 33-88). Among the patients with clinical data available, the clinical presentation appears similar to what is usually observed in nodal forms of PTCL (data are given at the time of the first episode with PTCL-F histology): 25/28 patients (89%) had multiple lymphadenopathies, 6/27 (22%) splenomegaly, 7/27 (26%) presented with B symptoms (ie. fever, weight loss or night sweats) and 6/23 patients (26%) had skin rash or other skin lesions. Most patients (17/26, 65%) had disseminated, Ann Arbor stage III or IV disease. Eight out of 23 patients (35%) had an elevated LDH level at diagnosis. Interestingly, some patients presented with peculiar biological features characteristic for PTCL, and AITL in particular: hypergammaglobulinemia (5/23, 22%), eosinophilia (1/21, 5%) or a positive Coombs' test (6/12, 50%). Owing to the retrospective nature of this study including many referral cases, treatments given were heterogeneous and follow-up data was not available for all patients, making it difficult to draw any firm conclusion on therapeutic results in these patients. Among 23 patients with available data regarding response to treatment (which ranged from oral alkylating-agent monotherapy to high-dose chemoetherapy with stem cell support), 20 (87%) eventually

showed at least a partial response to treatment, the others being refractory. At the time of last follow-up, 11 patients died of their disease or disease related-complications after a median follow-up of 24 months, 13 were alive including seven disease-free (follow-up ranging from 12 to 87 months, median 35 months) and six patients were lost to follow-up. Four patients reached a persistent complete remission following multiagent, anthracyclin-based chemotherapy, lasting for as long as 5 years.

Among the seven patients with sequential biopsies, three (#8, 15, 30) had clinical and biological features of AITL at initial presentation - that is multiple lymphadenopathies, splenomegaly, B symptoms, hypergammaglobulinemia, eosinophilia or a positive Coombs' test.

Histopathological features of PTCL-F

Among the 40 biopsies from these 30 patients, 30 disclosed features of PTCL with follicular growth pattern (Table 1). Among those 30 samples with a diagnosis of PTCL-F (Figure 1 A-F), neoplastic cells in eight samples were found to be confined within sharply demarcated lymphoid follicles with attenuated mantle zones or marginal zones, responsible of a pattern mimicking follicular lymphoma (FL-like). However, unlike centrocytes, neoplastic cells displayed round or slightly irregular, medium-sized nuclei, with usually abundant cytoplasm. The 22 remaining samples predominantly showed large, regular or irregular nodules reminiscent of progressive transformation of germinal centers (PTGC-like). These nodules were composed of mature IgD+ B lymphocytes admixed with small aggregates of neoplastic cells displaying medium-sized nuclei with abundant clear cytoplasm. In five of these samples (#9, 11, 21, 23, 28), a mixed FL-like and PTGC-like pattern was present. Three samples (#15-2nd, 18, 26) classified in this category showed a more extensive background of small IgD+ B lymphocytes interpreted as confluent B-cell nodules in view of the presence of some remnants of FDC meshwork, with scattered aggregates of atypical clear T cells. At careful examination, in nine samples (#9, 10, 12-1st, 14, 15-2nd, 16, 20, 24, 25), some limited foci of paracortical hyperplasia containing a few plasma cells, eosinophils, and/or hyperplastic high endothelial venules were observed.

Pathological features (histological evolution) in patients with consecutive biopsies (Table 2)

Among the seven patients (#4, 5, 8, 12, 13, 15, 30) with consecutive biopsies, it is noteworthy that three patients at first presentation disclosed typical histological and clinical features of AITL (#8, 15, 30), but at relapse showed PTCL-F (Figure 1 G-H). Another patient (#4)

presenting with skin rash, disclosed polymorphous skin infiltrate consistent with cutaneous lesions in AITL at first diagnosis, but the axillary lymph node biopsy three years later revealed a PTCL-F with a FL-like pattern. Among the three remaining patients, one patient (#13) with PTCL-F in the first biopsy at diagnosis relapsed as skin lesions with diffuse, haphazard infiltrates of lymphoma cells suggestive of AITL, and the other two patients (#5, 12) with PTCL-F in the first lymph node biopsies then relapsed with a T-cell lymphoma showing features of PTCL-F associated with areas reminiscent of AITL in the second lymph node biopsies four and 14 years later, respectively.

Immunohistochemical findings of PTCL-F

Neoplastic cells were T cells with a CD2+ (22/22, 100%), CD3+ (29/29, 100%), CD5+ (21/21, 100%) phenotype with a common loss of CD7 (5/23, 22%) and frequent expression of CD10 (21/29, 72%) and of T_{FH} markers [PD-1+ (27/27, 100%), CXCL13+ (23/27, 85%), BCL-6+ (14/19, 74%), CD57+ (4/19, 21%), ICOS (11/11, 100%)]. A CD4+ helper T-cell immunophenotype was demonstrated in 24 out of 27 studied cases (89%). All three CD4- cases were also negative for CD8. The 11 cases investigated for expression of TIA1 and/or Granzyme B cytotoxic proteins were negative. Two of the 9 cases investigated for CD25 antigen disclosed heterogeneous staining on neoplastic cells. Presence of scattered CD20+ B-immunoblasts was scored in 13/23 cases (56%) in the vicinity of the atypical T cells and a few EBV-positive cells in 13/19 cases (68%). In addition, a population of small IgD+ B lymphocytes surrounding the neoplastic cells was evidenced in 14 cases. With respect to the three newly described T_{FH} markers CXCL13 (11, 14), PD-1 (31), and ICOS (20), CXCL13 was found to be positive in 23 out of the 27 interpretable specimens with a cytoplasmic and/or dot-like staining whereas PD-1 disclosed membrane postivity on neoplastic cells of all 27 interpretable specimens and ICOS in the all 11 tested cases. The growth pattern was often highlighted by the aid of CXCL13, PD-1 and/or ICOS staining. Between these three T_{FH} markers, PD-1 and ICOS usually stained more neoplastic cells, often with a sheet-like pattern, than did CXCL13.

Genotypic findings

T-cell receptor γ rearrangement analysis was performed in 20 specimens from 17 patients. Among the 16 patients with interpretable results, 14 patients were found to be clonal, including one case disclosing, in addition to a predominant clone, oligoclonal bands (#21). Clonality could be investigated in consecutive biopsies of two patients (#8, 15) showing

identical bands in the consecutive biopsies. Five out of the six cases (#9, 10, 13-1st, 16, 21) investigated for immunoglobulin gene rearrangement were polyclonal, the remaining (#12-2nd) showing an oligoclonal pattern, without any evidence of composite B-cell lymphoma under microscopic examination.

Fluorescent in situ Hybridization findings

Fluorescent in situ hybridization for t(5;9)(q33;q22) was performed in 24 biopsies from 23 patients. One (#3) failed due to lack of signal. Among the 22 patients with adequate signals, four patients (18%) were found to have t(5;9)(q33;q22) (Figure 1 I), including two patients previously reported (35). One additional patient (#29) was investigated by conventional cytogenetics and it was negative for t(5;9). Among the four patients positive for t(5;9), one (#20) presented with symptoms - intense pruritus, general weakness, multiple lymphadenopathy, haemolytic anemia, and splenomegaly - known to be associated with AITL. Another patient (#5) was investigated by FISH in two consecutive biopsies: this patient initially presented as a disseminated disease with multiple lymphadenopathies showing PTCL-F (FL-like) and recurred 4 years later as a stage II disease with some areas reminiscent of AITL in addition to features of PTCL-F. Translocation t(5;9) was demonstrated on both episodes.

DISCUSSION

We here report a series of 30 patients with peripheral T-cell lymphomas showing a follicular growth pattern, the largest so far reported. We describe their morphological spectrum and show their immunophenotype which is highly suggestive of derivation from T_{FH} cells. Based on several observations including that of some patients with consecutive biopsies, we suggest their possible relationship with AITL.

Peripheral T-cell lymphomas with a nodular growth pattern have been recently recognized and regarded as part of the morphological spectrum of PTCL-u. To the best of our knowledge, around 30 cases have been reported (3, 8, 15, 16, 18, 23, 29, 32, 34, 39, 40) that have variable morphological appearance. In fact, several of these cases have been referred as PTCL with either “follicular” or “perifollicular” involvement, comprising cases in which neoplastic cells seem to localize within the germinal center, and other cases where neoplastic cells are distributed within the marginal zone with a so-called perifollicular distribution or involve expanded mantle zones of the follicles. Interestingly, most cases have been reported to derive from helper CD4+ T cells (8, 16, 32), with frequent expression of BCL-6 and CD10

(8, 15, 16, 18, 34) and many cases have been reported to be associated with FDC network (8, 16, 18, 34, 40). It is yet unknown whether these variants represent the morphological spectrum of the same disease. In the present series, we emphasized that PTCL with follicular growth pattern include cases resembling those previously described by de Leval et al (8) and by Ikonomou et al (16) with a predominant involvement of the germinal center and patchy involvement of the expanded mantle zones of the B-cell follicles, respectively.

By definition, all cases disclosed a peculiar distribution of neoplastic cells within the germinal center and/or mantle zone of the B-cell follicles, therefore suggesting a possible derivation from T_{FH} cells. Thus, we investigated by immunohistochemistry the expression of several T_{FH} markers (CD10, CD57, BCL-6, CXCL13, PD-1, ICOS). In addition to the frequent expression of CD10, CD57, BCL-6 in agreement with previous reports, we demonstrate here expression of CXCL13, PD-1 and/or ICOS - three characteristic T_{FH} markers recently shown to be applicable in diagnostic practice - in all 27 evaluable cases of PTCL-F (3, 9, 11, 25, 26, 30, 31). This profile strongly supports that the T_{FH} cell can be regarded as the normal cell counterpart of these lymphomas. CXCL13 is a chemokine that promotes follicular homing by B and T cells as well as the migration of a subset of dendritic cells into B-cell follicles in a lymphotoxin-dependent fashion (46). Originally designated as B-cell-attracting chemokine 1 (BCA1), it interacts with its receptor, CXCR5, to help follicular homing process. In view of these functions, its secretion by neoplastic T_{FH} cells could explain the capacity to induce follicular structures with frequent expansion of IgD small lymphocytes together with that of FDC in PTCL-F. Programmed death-1 (PD-1) and inducible costimulator (ICOS) are members of the CD28 family of receptors that also includes CD28, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and B- and T-lymphocyte attenuator (BTLA) (9). These receptors play a role in the cellular immune response. These molecules play an important role in T-cell dependent antibody responses and are expressed at very high levels on T_{FH} cells within the light zone of germinal centers (5, 33). Recently, several reports described that CXCL13, PD-1 and ICOS are overexpressed in T_{FH} cells as well as in AITL not only at the transcriptional level (6, 7) but also at the protein level as documented by immunohistochemistry (11, 14, 24, 31)

It is questionable whether there might be some overlap between those PTCL-F and some variants of AITL. AITL is a distinct entity characterized by systemic manifestations including B symptoms, multiple lymphadenopathies, skin rash, effusions, arthritis, typically associated with polyclonal hypergammaglobulinemia and autoimmune manifestations (25). However, the constellation of clinical and biological symptoms seen in the classic form of AITL is not

present in every patient (25) and the diagnosis of AITL – at least in its classic form – can rely on a combination of morphologic, immunohistochemical and/or molecular criteria (2). AITL has peculiar pathological features with a diffuse polymorphous infiltrate including variable proportions of medium-sized neoplastic cells associated with prominent arborizing blood vessels, proliferation of FDC and the presence of EBV-positive large B-cell blasts (10, 17). Detection of markers such as CD10 or T_{FH} molecules may help (1, 9, 11, 14, 31). In a recent study, we have shown its molecular link with T_{FH} (7), a finding further confirmed by Piccaluga et al (27), therefore strongly supporting its derivation from the minor T_{FH} cell population. By contrast, PTCL-u is regarded as a diagnosis of exclusion (36). Therefore, this latter category is recognized as heterogeneous and may include cases with overlapping features with other T-cell lymphoma entities (43), as recently suggested at the molecular level between PTCL-u and AITL (7, 27, 28). In most of the previous studies, PTCL-F has been included in the category of PTCL-u. Contrary to AITL, they do not show a diffuse effacement of the architecture, lack polymorphous infiltrate in most cases and had not been so far reported to have the clinical and biological features typical of AITL. From these previous reports, most patients appear to present with multiple lymphadenopathies, disseminated stage III-IV disease as well as frequent B symptoms, but the presence of skin lesions and biological manifestations such as hypergammaglobulinemia and autoimmune haemolytic anemia were not recorded. However, in addition to the common expression of CD10 and BCL-6 in a proportion of cases, it is noteworthy that proliferation of high endothelial venules was noted in several reports (8, 16, 32), and marked plasmacytosis, presence of large B-immunoblasts and/or EBV-positive scattered cells have been reported (32, 34). In our study, we emphasize these previous pathological overlapping findings with AITL: the neoplastic cells in PTCL-F have medium-sized nuclei and abundant clear cytoplasm, are frequently admixed with occasional large immunoblasts and also scattered EBV-positive blasts in a proportion of cases. In addition, despite the fact that complete clinical and biological data could not be recorded for all patients, some clinical and biological characteristics of AITL such as skin manifestations, hypergammaglobulinemia and positive Coombs' test were recorded in a percentage of patients in the present series. Finally, we show the common T_{FH} immunophenotype in both diseases and describe some patients that may switch from AITL to PTCL-F over time.

Altogether, these morphologic and phenotypic similarities and our observation of three patients in this series disclosing typical clinical and pathological features of AITL at initial presentation and PTCL-F in other sequential biopsies, support a relationship between PTCL-F

and AITL. Therefore, in agreement with a recent case report (3), it might be tempting to speculate that, in at least some cases, PTCL-F could represent a peculiar stage of AITL in which neoplastic cells remain located within the B-cell follicles as highlighted by PD-1, CXCL13, and ICOS stainings. This hypothesis would imply several differences especially in terms of cytokine secretion capacities which would explain why the *bona fide* clinical, biological and histological features of AITL are lacking in most PTCL-F (such as marked polymorphous infiltrates and florid hyperplasia of high-endothelial venules). This would also imply that the criteria used to define AITL should be reviewed, including cases without the constellation of clinical symptoms and pathological features of AITL observed in its classic form. This hypothesis may be also supported by the observation that a proportion of T_{FH}-derived PTCL-u shows at least some pathological and/or clinical features of AITL (7, 11, 30). Interestingly, EBV was observed in 68% of the PTCL-F tested cases with a pattern reminiscent of B-immunoblasts, a finding closer to that reported in most AITL than in PTCL-u (2, 25, 44). Whether the presence of EBV should be regarded as an initiating event in PTCL-F, as it has been proposed for AITL (10), is questionable. Therefore, our findings may suggest that at least some PTCL-F cases may reflect the broad morphologic spectrum of AITL and/or the lack of precise criteria for distinguishing borderline cases between PTCL-U, PTCL-F, and AITL. However, it cannot be formally excluded that AITL and PTCL-F represent distinct subtypes that, despite a common derivation from T_{FH} cells, differ according to some oncogenic alterations.

Translocations of *ITK* on chromosome 5q33 and *SYK* on 9q22 which result in an *ITK-SYK* fusion transcript were observed in four patients of the present series, but have not been reported in 32 AITL cases investigated by FISH (12, 35). ITK belongs to a group of five related kinases comprising the Tec family and plays a role in T cell development, differentiation, and T cell receptor signalling (45). SYK is a non-receptor protein tyrosine kinase that functions as a key regulator of multiple signal transduction pathways (4). In comparison with *ITK*, the translocation of *SYK* has already been shown in myelodysplastic syndromes in addition to t(5;9) (22). The biological significance of this translocation and its specificity remain unclear, and studies aiming to investigate the genetic abnormalities of PTCL-F compared to AITL are needed. Interestingly, overexpression of SYK has been recently recorded in the vast majority of PTCL-u and AITL, independent of *ITK-SYK* translocation, suggesting alternative alterations resulting in the involvement of SYK pathway (12).

Whatever their relationship with other PTCL entities is, the peculiar histologic appearance of PTCL-F generates a list of differential diagnoses. PTCL with follicular growth pattern should be distinguished from reactive conditions and some B-cell lymphomas. At low power magnification, nodular growth pattern could be mistaken as a follicular lymphoma. Follicles in both diseases have decreased numbers of tingible body macrophages, attenuated mantle zone, and neoplastic cells could be both BCL-6 and CD10 positive. Lack of cytologic characteristics of centrocytes, abundant clear cytoplasm, and expression of T-cell markers in addition to CXCL13, PD-1 and/or ICOS argue for a diagnosis of PTCL. Other cases with large irregular nodules should be distinguished from a reactive process, specially PTGC, or nodular lymphocyte predominant Hodgkin lymphoma and other small B-cell lymphomas such as mantle cell lymphomas and marginal zone lymphomas. Immunohistochemistry showing aggregates of atypical medium-sized T cells – sometimes with an abnormal T-cell phenotype - within the follicles and salient morphology of background B cell infiltrates could help in making a correct diagnosis. T_{FH} markers are useful to highlight the presence of aggregates of atypical T cells in hyperplastic follicles.

In conclusion, our study shows that, in agreement with the distribution of the neoplastic cells within zones of the B-cell follicle, the subgroup of PTCL with follicular growth pattern derives from T_{FH} cells. It also indicates that at least some of them may present with the clinical and pathological features of AITL. These findings may support a possible link between PTCL-F and AITL. In routine practice, immunohistochemistry with T_{FH} markers is mandatory to distinguish these lymphomas from reactive lymphoid hyperplasia and some B-cell lymphomas. The biological significance of the association with t(5;9) warrants further study.

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FIGURE LEGEND

Two types of peripheral T-cell lymphoma with follicular growth pattern. A, Hematoxylin and eosin (H&E)-stained sections showing a follicular lymphoma-like PTCL-F (#1) characterized by numerous nodules in a back-to-back fashion mimicking a follicular lymphoma, composed of medium-sized neoplastic cells with clear cytoplasm and irregular nuclei (inset). The neoplastic cells are surrounded by (B) CD20+ B cells and positive for (C) CD5 and CXCL13 (inset, #8). D, H&E sections showing a progressive transformation of germinal center-like PTCL-F (#29) characterized by small aggregates of neoplastic cells dispersed in large irregular nodules mimicking progressive transformation of germinal center. The inset shows that the neoplastic cells have irregular nuclei and clear cytoplasm. The neoplastic cells are surrounded by (E) IgD+ small, mature B cells and positive for (F) CD3 and CXCL13 (inset, #15). G and H, H&E sections showing features of (G) angioimmunoblastic T-cell lymphoma in the first episode then relapsed as (H) PTCL-F in patient #15. I, FISH for the detection of the t(5;9)/*ITK*-*SYK*. Separate green and red signals for *ITK* (left panel) and *SYK* (right panel) demonstrate rearrangements of the genes in a t(5;9)/*ITK*-*SYK* positive patient (#5-1st). (Original magnification A and D x2.5; B, C, E, and F, x10; G, and H, x25; A inset, C inset, D inset, and F inset, x40).

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Tables

Table 1. Summary of predominant histology, immunophenotype, clonality, and t(5;9) in patients with first diagnosis of PTCL-F.

Patient no.	Age/sex	Biopsy site	CD4	CD10	BCL6	CXCL13	PD-1	ICOS	EBV*	Clonality	t(5;9)
1	60/F	LN	+	+	NI	+	+	NI	+		-
2	74/F	LN	+	+	+		+		+		-
3	78/M	LN	+	-	NI	+	+	+	+		NI
4	86/M	LN	+	-	+	+	+				-
5	79/F	LN	+	+	-	+	+	+	+	+	+
6	51/M	LN	+	+	+	+	+	+		+	+
7	77/M	LN	+	-	-	-	+	+			
8	62/M	LN		+	+	+	+			+	
9	69/F	Nasopharynx				-			+	+	
10	45/M	LN	+	+		-	+		-	+	-
11	88/F	LN	+	+	+	NI			-	NI	
12	45/M	LN	+	+	+				+	+	-
13	81/M	LN	+	-		+	+	+	+	-	-
14	77/F	LN	+	+	+	+	+	+			-
15	64/F	LN	+	+		+	+		+	+	-
16	81/M	Nasopharynx	+	+		+	+	+	+	+	-
17	71/F	LN	+	+	+	+	+		+		
18	54/F	LN	+	+	+	+	+		+		-
19	70/M	LN	+	-	-	-	+		-		-
20	80/F	LN	+	+	+	+	+		+		+
21	65/F	LN	+	+	+	+	+			+	-
22	72/M	LN	+	+	+	+	+	+	-	+	-
23	45/F	LN	-	-	-	+	+	+		+	-
24	74/F	LN	-	+		+	+		-		-
25	33/M	LN	-	-	+	+	+	NI		-	-
26	72/M	LN	+	-		+	+		NI		-
27	55/M	LN	+	+	-	+	+	+		+	+
28	71/F	LN	+	+		+	+	+		+	-
29	50/M	LN	+	+	+	+	+		+		-†

Patient no.	Age/sex	Biopsy site	CD4	CD10	BCL6	CXCL13	PD-1	ICOS	EBV*	Clonality	t(5;9)
30	45/M	LN		+		+	+		-	+	

*: defined as the presence of scattered EBV-infected cells positive for LMP-1 and/or EBER in situ hybridization, reminiscent of CD20+ B immunoblasts, †: by conventional cytogenetic study, LN: lymph node, NI: non-interpretable.

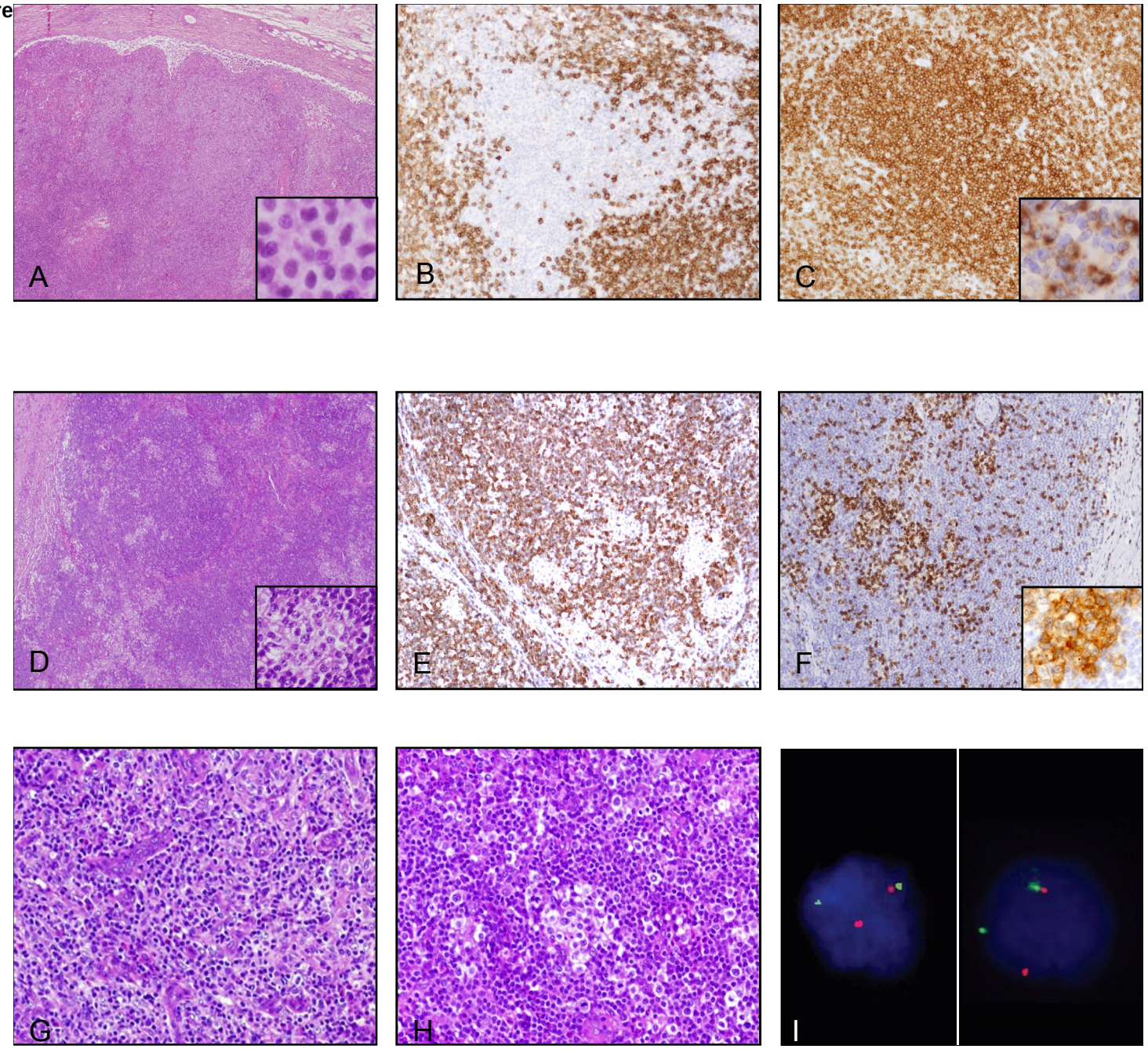
Tables

Table 2. Summary of clinical, histological, immunophenotypic and cytogenetic features in patients with consecutive biopsies.

Patient no.	Years to recur	Pattern	Site of biopsy	B symptoms	Skin rash	Multiple lymphadenopathies	Stage	Hyper-gammaglobulinemia	Coombs' test	CD10	BCL6	EBV*	CXCL13	ICOS	PD-1	t(5;9)
4	0	AITL	Skin													
		PTCL-	LN													
4	3	F					IV			-	+		+		+	-
		PTCL-	LN													
5	0	F		-		+	IV	-		+	-	+	+	+	+	+
		PTCL-	LN													
		F /														
5	4	AITL		-		-	II	-		-		+	+		+	+
8	0	AITL	LN	+	+	+	III	+	+	+	+		+		+	
		PTCL-	LN													
8	8	F		-	-	+		-	+	+	+		+		+	
			LN													
8	9	PTCL-u		-	-	-		-		+			+		+	
		PTCL-	LN													
12	0	F		+	-	+				+	+	+				
		PTCL-	LN													
		F /														
12	14	AITL								+		-			+	-
		PTCL-	LN													
13	0	F		-	+	+	II	-		-		+	+	+	+	-
13	1	AITL	Skin													
15	0	AITL	LN	-	+	+	IV	+		+		+				
		PTCL-	LN													
15	2	F		-		+	II	-	+	+		+	+		+	-
30	0	AITL	LN	+	-	+	III	-		+	+	-	+		+	
		PTCL-	LN													
		F /														
30	2	AITL		+	-	+	IV	+	+	+		-				
		PTCL-	LN													
30	8	F		+	+	+	IV	-		+		-	+		+	
30	13	AITL	LN	+	+	+	IV	-		+			+		+	

*: defined as the presence of scattered EBV-infected cells positive for LMP-1 and/or EBER ISH, reminiscent of CD20+ B immunoblasts, AITL: angioimmunoblastic T-cell lymphoma, LN: lymph node, PTCL-F / AITL: T-cell lymphoma showing features of PTCL-F associated with areas reminiscent of AITL, PTCL-u: peripheral T-cell lymphoma unspecified.

Figure



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