Incidence and clinicopathological features of Follicular T-cell lymphoma in Finland; a population-based immunohistochemical study

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Highlights: The incidence of FTCL, The differential diagnosis between FTCL and AITL

Abstract

Follicular T-cell lymphoma (FTCL) is a rare subtype of mature T-cell lymphoma. It was recently recognized as a separate lymphoma entity in the 2017 revised 4th edition of the WHO classification. The main goals of the present study were to gain better knowledge of the incidence and histopathological and clinical features of FTCL in Finland. In this study, we reviewed all angioimmunoblastic T-cell (AITL) and peripheral T-cell lymphomas, not otherwise specified, from the patient records in three hospital districts in Finland over a 10-year period, to identify FTCL cases and estimate its incidence. Five patients rediagnosed with FTCL and thirty-four with AITL were analyzed. Hodgkin/Reed-Sternberg (HRS)-like cells were observed in 24 of the 34 AITL cases and 4 of the 5 FTCL cases. We found that the main features that differentiated FTCL from AITL were the presence of clear cells, rosetting of T-cells around HRS-like cells, follicular dendritic cell meshwork, and T-cell monoclonality. Our estimated incidence of FTCL is 0.20 per 100,000 people in Finland.

Keywords: PTCL; FTCL; AITL; rosetting; clonality; incidence

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1.1 Introduction

T-cell neoplasms are clonal tumors of immature and mature T cells at various stages of differentiation. T-cell lymphomas are an extremely complicated entity to classify because of the remarkable heterogeneity of these neoplasms. The WHO classification of hematopoietic and lymphoid tissue tumors is based on all available information explaining these disease entities. The origin of neoplastic cells is the most commonly used feature to identify lymphoid neoplasms [1].

Peripheral T-cell lymphomas (PTCLs) are a group of non-Hodgkin lymphomas (NHLs), representing a noticeably heterogeneous, usually aggressive group of diseases. The mortality rate of PTCLs is frequently high, and the prognosis is worse than that of B-cell lymphomas of the same stage. PTCLs can be roughly divided into three subgroups: nodal, extranodal, and leukemic. The most common PTCL, peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), accounts for approximately 30% of all T-cell lymphomas. PTCL-NOS is a heterogeneous group of nodal and extranodal PTCLs that cannot be categorized into any existing lymphoma subtypes defined by WHO.

Follicular T-cell lymphoma (FTCL) is a newly recognized PTCL. It was previously considered a follicular variant of PTCL-NOS [2] until the 2017 revised 4th edition of the WHO classification of Tumours of Haematopoietic and Lymphoid Tissues [3], where it was recognized as a separate lymphoma entity. In this classification, FTCL, angioimmunoblastic T-cell lymphoma (AITL), and other nodal PTCLs with the T-helper cell (Tfh)-phenotype were included under the same category: nodal T-cell lymphomas with a Tfh-phenotype. These diseases share a common cellular origin and are major differential diagnoses for each other. The plasticity of FTCL to AITL and vice versa has been observed in biopsies from different time points, and multiple overlapping features between these diseases have been described [3], [4], [5]. Currently, there is no consensus on whether these two diseases represent two biologically distinct entities or are two morphological variants of a single disease.

FTCL is a rare disease, and its identification is challenging. At the beginning of this study, the diagnosis of FTCL had not been used previously in Finnish hospitals, and the exact

incidence of FTCL in Finland was unknown. Furthermore, the clinical presentation and prognosis of FTCL are unknown, and the best treatment options are currently under investigation. The differential biology of FTCL and other Tfh-phenotype lymphomas remains unresolved. To study these issues, our panel of highly experienced hematopathologists conscientiously analyzed 39 cases, initially diagnosed as PTCL-NOS or AITL, to identify FTCL cases among them.

2.0 Materials and Methods

2.1 Patient material

We investigated 39 PTCL cases from Oulu, Tampere, and Kuopio University hospitals diagnosed between 2005 and 2014. Together, the Oulu, Tampere, and Kuopio hospital districts and their specific catchment areas cover a population of 2.5 million. The original diagnoses included AITL and PTCL-NOS with AITL-like features or its follicular variant. Patient data were collected from hospital records and included sex, age, presence of B-symptoms, plasma lactate dehydrogenase (LDH) level, WHO-performance status, Ann Arbor stage, International Prognostic Index (IPI) score, extranodal infiltration and follow-up data, relapse, progression, and mortality.

The study was approved by the Ethical Committee of the Northern Ostrobothnia Hospital District, Oulu University Hospital, and the Finnish National Supervisory Authority for Welfare and Health granted permission to use patient samples for research purposes. According to the national legislation of Finland, tissues gathered for diagnostic purposes can be used in scientific studies without informed consent from the patient; therefore, it was not obtained and is irrelevant for the current study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

2.2 Histology, immunohistochemistry, and clonality

A board of highly experienced hematopathologists (KMH, SK, MV, ME) reviewed the cases to confirm the diagnoses of FTCL according to the 2017 revised 4th edition of the WHO classification [6]. This was performed as a joint review using a multi-head microscope (Olympus BX53). In the classic WHO definition, FTCL is described by the presence of the follicular growth pattern, lacking the other typical features of AITL, such as the proliferation of high endothelial venules or extrafollicular follicular dendritic cells. Neoplastic T-cells express pan-T-cell antigens and have a Tfh phenotype.

The immunohistochemistry (IHC) stainings used for initial diagnostics were CD3, CD21, CD30, and hematoxylin-eosin staining for some of the cases. We also stained biopsies for CD4, CD10, CD15, CD20, PAX5, PD1, and BCL6. In addition, EBER *in situ* hybridization

was performed according to the manufacturer's instructions and using Leica BOND-III stainer. For a more specific protocol, see Suppl. Appendix S1. Staining for CD15, CD20, CD30, PAX5, and EBER *in situ* hybridization was performed to define the immunophenotype of HRS-like cells. Staining followed diagnostic laboratory protocols. The growth pattern and presence of clear cells, epithelioid, or HRS-like cells were evaluated in all cases. The presence of rosettes forming neoplastic T-cells around HRS-like cells was also assessed. T follicular helper cell markers (PD1, Bcl6, CD10) and other markers expressed in the HRS-like cells (CD15, PAX5, EBER, CD30, CD20) were scored positive when at least 60% of the cell population was positive. For the presence of rosetting T cells around HRS-like cells, the presence of at least one complete rosette was assessed as positive. The stainings CD3, CD4, CD21/CD23 were performed and evaluated as positive and negative according to the standard clinical practice.

Samples were fixed in formalin and embedded in paraffin, and 3-µm sections from the paraffin blocks were cut and placed on SuperFrost Plus glass slides (Menzel-Gläser, Braunschweig, Germany). The slides were incubated at 37 °C overnight before deparaffinization in a clearing agent, Histo-Clear (National Diagnostics, Atlanta, GA, USA), and rehydrated in a descending ethanol series. Antigen retrieval was performed in a microwave oven for 15 min or with trypsin. Slides were allowed to cool at room temperature for 20 min and then incubated in a 3% H_2O_2 solution for 5 min to block endogenous peroxidase activity. The primary antibodies were incubated at room temperature. Staining was continued using the Dako REAL EnVision Detection System (Dako Denmark A/S, Glostrup, Denmark) (except CD21 antibody stain) according to the manufacturer's instructions. Diaminobenzidine was used to detect the immunoreaction, and nuclei were stained with Mayer's hematoxylin (Reagena, Toivola, Finland). Finally, the slides were dehydrated and mounted using Histomount (National Diagnostics). All washes between different steps were performed with phosphate-buffered saline containing 0.05% Tween-20. Primary antibodies, manufacturers, antigen retrievals, dilutions and incubation times are listed in the Suppl. Table S2.

T-cell receptor (TCR) gamma genes were analyzed to detect T-cell clonality (T-cell receptor gamma gene rearrangement assay 2.0; Invivoscribe Technologies, San Diego, CA, USA). The tests were performed according to the manufacturer's instructions. In addition, immunoglobulin heavy chain rearrangements were tested. Four of the five FTCL cases and

thirty-two of the thirty-four AITL cases were analyzed. The terms clonal, polyclonal and clonal with background are defined in the review article by Langerak et al [8] and in the Suppl. Appendix S3.

2.3 Statistical analysis

The chi-square test was used to detect statistically significant correlations between the clinicopathological variables. Overall survival (OS) was defined as the time from the day of diagnosis to the day of death or the date of the last follow-up. The Kaplan-Meier method was used to estimate the OS distribution. IBM SPSS Statistics for Macintosh, version 26.0. (SPSS Inc, Chicago, IL, USA) was used for all the analyses. Statistical significance was set at $P < 0.05$.

3.0 Results

3.1 Results of reclassification

According to the original classification, 3 cases presented with PTCL-NOS, 1 case with PTCL with AITL-like features, 1 case with PTCL with AITL- and NOS-like features, and 33 cases presented with AITL. One patient was suspected of having FTCL. During reclassification, we identified 5 FTCL and 34 AITL cases. The suspected FTCL case was confirmed to be FTCL. Two of the founding FTCL cases were initially diagnosed as PTCL-NOS and two as AITL. The results of the reclassification are presented in Figure 1.

3.2 Morphological and immunohistochemical differences between FTCL and AITL

The results are summarized in Table 1. The presence of clear cells and a follicular dendritic cell (FDC) meshwork was more characteristic of AITL than FTCL (P = 0.011 and 0.00000044, respectively). HRS-like cells were observed in both groups (P = 0.73). However, T-cell-rosetting around HRS-like cells was typical for FTCL but not for AITL (P = 0.000011). In AITL, HRS-like cells were more often positive for EBER, CD30, and CD20, than in FTCL (P = 0.055, 0.00025, and 0.0060, respectively). Representative hematoxylin-eosin-stained samples of one AITL and two FTCL cases are shown in Figures 2–4.

3.3 T-cell receptor gene and immunoglobulin rearrangement analyses in FTCL and AITL

TCR gamma and IgH rearrangements were tested in 4/5 and 32/34 patients with FTCL and AITL, respectively. Clonal T-cell receptor gamma rearrangements were found in almost every AITL (91%) and all FTCL (100%) cases. In AITL, rearrangements were typically monoclonal (56%), but polyclonal (13%) and clonal (22%) cases with background were also observed. FTCL cases were either polyclonal (50%) or clonal with background (50%). All FTCL cases and 75% of AITL cases showed polyclonal IgH rearrangements. The remaining AITL cases were either clonal with background (16%) or monoclonal (9%). The results are shown in Table 2. There were no statistically significant differences between FTCL and AITL in TCR gene and IgH rearrangement analyses.

3.4 Clinical disease presentation in FTCL and AITL

The five FTCL patients studied included three men (60%) and two women (40%), and three of the five patients (60%) were 60 years old or older. The mean age at the time of diagnosis was 58 years. Two patients (40%) had B-symptoms, and three (60%) patients had Ann Arbor stage III or IV disease. There were no statistically significant differences in clinical disease presentation between the AITL and FTCL groups. Patient demographics are summarized in Table 3.

Three FTCL patients received at least six cycles of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone), resulting in two cases exhibiting complete responses (CR) and one case with a partial response (PR). In both CR cases, the disease relapsed. The relapse of patients who received six CHOP cycles was treated with gemcitabine and carboplatin, with no response. Another patient received CHOP × 8, and his recurrence was treated with intensive therapy, and CR was achieved. One patient had one cycle of epirubicin and one of CHOEP (cyclophosphamide, doxorubicin, vincristine, and etoposide), with no response. One patient was treated with four cycles of ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine), resulting in CR. Salvage radiotherapy was also administered. Two of the five patients were still alive at the latest follow-up.

Most of the 31 AITL patients studied were male (71%) and aged 60 years or older (79%). B-symptoms, elevated LDH levels, and advanced stage III–IV disease were also common (63, 80, and 90%, respectively). Extranodal spread with two or more sites was observed in only 24% of cases. The WHO performance score was two or higher in 60% of the cases.

3.5 Clinicopathological correlations in FTCL and AITL

Statistically significant associations were observed among the five FTCL cases. Ann Arbor stages III–IV was associated with CD10 expression ($P = 0.046$) and high WHO status with B-symptoms ($P = 0.025$). Rosetting around HRS-like cells negatively correlated with the number of extranodal metastases and the IPI score ($P = 0.046$ and 0.046, respectively). Monoclonal TCR rearrangement and CD30-expression in HRS-like cells were more common in patients with FTCL aged 60 years or older ($P = 0.046$ and 0.046, respectively).

In the AITL cases, B-symptoms were associated with advanced stage ($P = 0.019$) and CD10-expression ($P = 0.061$). An age of 60 years or more was associated with a diffuse growth pattern ($P = 0.043$). Stage correlated with BCL6-expression in lymphoma cells and Pax5-

expression in HRS-like cells (P = 0.001 and 0.019, respectively). LDH was elevated in 21 of 22 AITL cases with polyclonal IgH-rearrangement but not in any cases with monoclonal rearrangement (P = 0.0002). LDH was not elevated in cases with rosette formation (P = 0.042). A high WHO-score was associated with extranodal infiltration (P = 0.018). The IPI score did not divide the cases in terms of survival. None of the clinical variables showed a significant correlation with progression-free survival time (PFS) (data not shown).

3.6 Prognosis of FTCL and AITL

The median follow-up time for patients with FTCL was 30 months (range, 5–105 months) and 17 months for those with AITL (range, 1–150 months). The 2-year overall survival rate was 60% for FTCL and 55% for AITL. The 5-year overall survival rate was 40% for both FTCL and AITL.

3.7 Incidence of FTCL in Finland

During the 10-year period from 2005 to 2014, we identified 5 FTCL cases in Oulu, Kuopio, and Tampere University Hospital districts and their specific catchment areas. This means that the estimated incidence of FTCL in Finland is 0.20 per 100,000 people per year.

4.0 Discussion

This was a population-based, sample-survey study investigating the incidence, histopathological, and clinical features of FTCL in Finland. Our study indicates that the incidence of FTCL in Finland is 0.20 per 100,000 people per year. In our study, most of the patients were men aged 60 years or older. We found that the absence of clear cells and an FDC meshwork, as well as a high incidence of T-cell rosetting around HRS-like cells, were the main features differentiating FTCL and AITL. Although several relevant clinical factors were retrospectively collected, we were unable to find significant differences between the clinical presentations of AITL and FTCL.

Our estimated incidence of FTCL in Finland is 0.20 per 100,000 people per year, making it extremely rare. It has been suggested that FTCL likely accounts for <1% of all T-cell neoplasms [1]. In Finland, the incidence of FTCL has not been previously evaluated. Furthermore, the incidence may be underestimated because we did not include Hodgkin lymphoma cases (HLs) in the present study. Since FTCL is a new lymphoma entity, we speculate that some PTCL-NOS or even HL cases could be reclassified as FTCL. However, because of the favorable prognosis of HL in comparison to FTCL, we strongly believe that the actual incidence does not differ much from our estimate.

Our study is in line with previous studies [4], [6], [9], which demonstrated a male predominance (60%) and most patients being aged 60 years or older (60%). Surprisingly, in a study by Miyoshi et al. (2012) [7], 58.8% of patients were women. Even if most of the patients are elderly, FTCL can also occur in young patients. In 2015, Delas et al. described the first case of pediatric FTCL [10]. The patient was an 11-year-old boy with advanced-stage disease. He received four cycles of CHOEP (cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisolone) and two cycles of DHAP (cisplatin, cytarabine, and dexamethasone). Partial remission was achieved, but the disease relapsed after 13 months.

Most patients with FTCL have advanced disease, and current data suggest that approximately 50% of patients die within 2 years of diagnosis [6], [4]. In our study, the 2-year overall survival rate was 60% for FTCL and 55% for AITL, and the 5-year overall survival rate was

40% in both patient groups. Naturally, no robust conclusions can be drawn from these five cases.

Histopathologically, FTCL is characterized by a follicular growth pattern of neoplastic T-cells with a Tfh phenotype. According to the WHO definition, FTCL lacks other typical AITL features, such as the extrafollicular proliferation of FDCs and proliferation of high endothelial venules. The neoplastic T-cells are positive for multiple Tfh markers, such as PD1, CXCL13, BCl6, CD10, and ICOS. Clear cells were also observed. Interfollicular B-immunoblasts and HRS-like large cells can be present in a subset of cases. B-immunoblasts express CD20 and often show Epstein-Barr virus (EBV) reactivity. HRS-like cells are frequently surrounded by neoplastic T-cells, forming rosette-like structures [6], [9]; they may be positive for CD30, CD15, and PAX5 (weakly) [6]. Both EBV-positive and -negative HRS-like cells have been previously reported [11].

In the present study, the features that best differentiated FTCL from AITL were the presence of clear cells, rosette-forming of T-cells around HRS-like cells, FDC meshwork, and T-cell monoclonality. Clear cells were seen in most AITL cases, but only in one FTCL case. Rosetting around HRS-like cells was a common finding in FTCL (75%) but was very rare in AITL (3%). Fifty-seven percent of AITL cases presented with monoclonal T-cell gamma receptors, while FTCL cases were either polyclonal or clonal with background.

Our findings support the idea that FTCL and AITL may have biologically distinct features. Whether these differences fulfill the criteria for two distinct entities is subject to further study. Autonomous growth is an integral feature of malignant diseases. However, this is not an absolute truth, and several malignant tumors still need a cytokine or growth factor-mediated survival and growth support from their surroundings. In the field of lymphomas, this seems to be the case in the early phase of gastric marginal zone lymphomas and HLs. The presence of an extensive FDC meshwork is a typical feature of AITL [12], and our study supports this. This FDC meshwork produces CXCL13 [13], a ligand for CXCR5. CXCR5 is expressed by malignant cells [14], thus controlling the homing of malignant T-cells and giving them a survival signal. In contrast, we found that among FTCLs, only 20% of the cases presented with an FDC meshwork. Instead, most of the FTCL cases showed T-cell rosetting around large HRS-like cells. We speculate that in FTCL, this rosetting may represent the reliance of the tumor cells on the stimulus received from HRS-like cells. This

phenomenon might be integral to the pathogenesis of FTCL. This is further supported by the fact that rosetting is associated with limited-stage disease and the lack of extranodal dissemination. However, this hypothesis requires further verification. Another finding supporting the differential biology of FTCL and AITL is the lack of monoclonal gamma T-cell receptors.

The highly esteemed panel of hematopathologists and an extensive IHC repertoire used in the study to add value to current knowledge. However, our work clearly has some limitations, the most important of which is the small number of FTCL patients. The small number of FTCL patients leaves room for occasional findings where even a single case may change the statistics; thus, the results need to be validated using a larger patient population. Further studies on this subject are needed.

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Abbreviations:

ABVD = Doxorubicin, bleomycin, vinblastine, dacarbazine
AITL = Angioimmunoblastic T-cell lymphoma
CHOEP = Cyclophosphamide, doxorubicin, vincristine, and etoposide
CHOP = Cyclophosphamide, doxorubicin, vincristine, and prednisolone
CR = Complete response
EBV = Epstein-Barr virus
FDC = Follicular dendritic cell
FTCL = Follicular T-cell lymphoma
HRS = Hodgkin/Reed-Sternberg
IHC = Immunohistochemistry
IgH = Immunoglobulin heavy chain
IPI = International Prognostic Index
LDH = Lactate dehydrogenase
NHL = Non-Hodgkin lymphoma
OS = Overall survival
PFS = Progression free survival time
PR = Partial response
PTCL = Peripheral T-cell lymphoma
PTCL-NOS = Peripheral T-cell lymphoma not otherwise specified
TCR = T-cell receptor
Tfh = T-helper cell
WHO = World Health Organization

Appendices

Supplementary data

Table 1 Morphological and immunohistochemical differences between FTCL and AITL

	AITL, n (%)	**FTCL, n (%)**	**p-value**
Clear cells	26/34 (76 %)	1/5 (20 %)	0.011
Epithelioid cells	30/34 (88 %)	3/5 (60 %)	0.102
HRS-like cells	24/33 (73 %)	4/5 (80 %)	0.731
Rosetting around HRS-like cells	1/24 (4 %)	3/4 (75 %)	0.000178
CD3 positive neoplastic cells	34/34 (100 %)	5/5 (100 %)	-
CD4 positive neoplastic cells	33/34 (97 %)	4/5 (80 %)	0.106
CD10 positive neoplastic cells	23/34 (68 %)	3/4 (75 %)	0.765
PD-1 positive neoplastic cells	21/34 (62 %)	4/5 (80 %)	0.427
BCL6 positive neoplastic cells	28/34 (82 %)	4/5 (80 %)	0.898
CD15 positive HRS-like cells	2/24 (8 %)	0/4 (0 %)	0.549
PAX5 positive HRS-like cells	17/24 (71 %)	2/3 (67 %)	0.882
EBER positive HRS-like cells	17/23 (74 %)	1/4 (25 %)	0.055
CD30 positive HRS-like cells	25/25 (100 %)	2/4 (50 %)	0.000248
CD20 positive HRS-like cells	23/24 (96 %)	2/4 (50 %)	0.0060
CD21 or CD23 positive meshworks or large meshwork (vs nodular)	28/28 (100 %)	1/5 (20 %)	0.000000444

Table 2 Results of T-cell receptor gamma gene and immunoglobulin rearrangement tests

	AITL, n (%)	**FTCL, n (%)**	**p-value**
T-cell receptor gamma rearrangement			
None	3/32 (9.4%)	0/4 (0%)	0.080
Monoclonal	18/32 (56%)	0/4 (0%)	
Polyclonal	4/32 (13%)	2/4 (50%)	
Clonal with background	7/32 (22%)	2/4 (50%)	
Immunoglobulin heavy chain clonality			
Monoclonal	3/32 (9%)	0/4 (0%)	0.526
Polyclonal	24/32 (75%)	4/4 (100%)	
Clonal with background	5/32 (16%)	0/0 (0%)	

Table 3 Patient demographics after reclassification

	AITL, n (%)	**FTCL, n (%)**	**p-value**
Number of cases	31	5	
Male	22/31 (71 %)	3/5 (60 %)	0.621
Age 60 years or older	23/29 (79 %)	3/5 (60 %)	0.347
Patients with B-symptoms	19/30 (63 %)	2/5 (40 %)	0.324
Stage III-IV	26/29 (90 %)	4/5 (80 %)	0.536
Extranodal spread 2 or higher	7/29 (24 %)	2/5 (40 %)	0.458
Elevated LDH*	24/30 (80 %)	4/5 (80 %)	1
WHO performance score 2 or higher	18/30 (60 %)	2/5 (40 %)	0.403
IPI high 4–5	15/28 (54 %)	2/5 (40 %)	0.576

*LDH indicates lactate dehydrogenase

Table 4 Clinicopathological correlations in FTCL and AITL

Clinicopathological correlations in FTCL

	CD10-positive neoplastic cells	**CD10-negative neoplastic cells**	***P*-value**			
Ann Arbor 1–2	0/4 (0%)	1/4 (25%)	0.046			
Ann Arbor 3–4	3/4 (75%)	0/4 (0%)				
	Patients with B-symptoms	**Patients with no B-symptoms**				
WHO performance score 0–1	0/5 (0%)	3/5 (60%)	0.025			
WHO performance score >1	2/5 (40%)	0/5 (0%)				
	Extranodal spread 0–1	**Extranodal spread >1**		**IPI-score 1–3**	**IPI-score 4–5**	***P*-value**
Rosetting around HRS-like cells	3/4 (75%)	0/4 (0%)	0.046	3/4 (75%)	0/4 (0%)	0.046
No rosetting around HRS-like cells	0/4 (0%)	1/4 (25%)		0/4 (0%)	1/4 (25%)	

Clinicopathological correlations in AITL

	Patients with B-symptoms	**Patients with no B-symptoms**	***P*-value**			
Ann Arbor 1–2	0/29 (0%)	3/29 (10%)	0.019			
Ann Arbor 3–4	18/29 (62%)	8/29 (28%)				
CD10-positive neoplastic cells	15/30 (50%)	5/30 (17%)	0.061			
CD10-negative neoplastic cells	4/30 (13%)	6/30 (20%)				
	Age ≥ 60 years or older	**Age under < 60 years**	***P*-value**			
Diffuse growth	21/28 (75%)	4/28 (14%)	0.043			
Nodular growth	1/28 (4%)	2/28 (7%)				

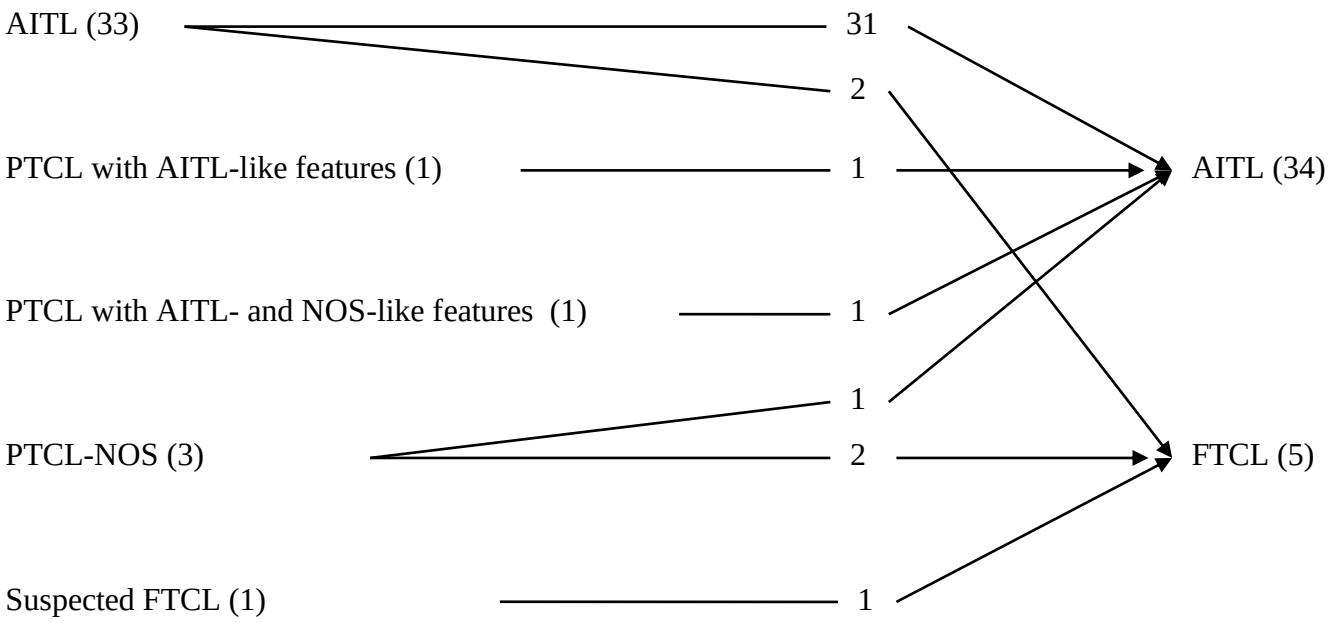

Figure 1 Results of reclassification

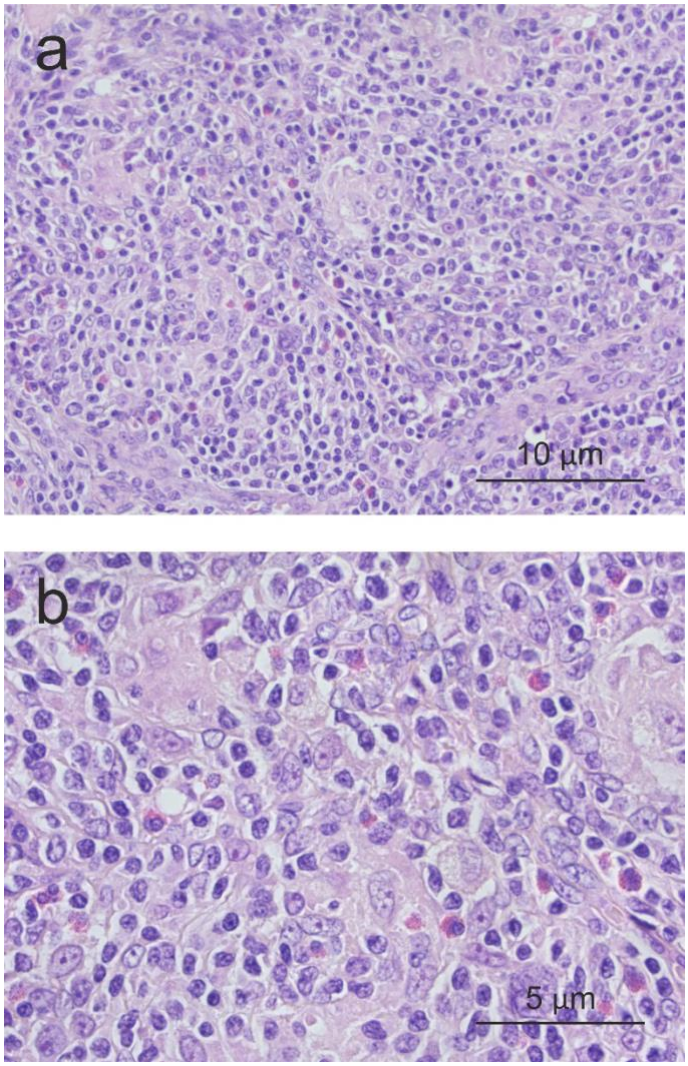

Figure 2 A case of reclassified AITL with a growth pattern 3 showing neoplastic lymphocytic cells with a mixture of clear cells, epithelioid cells, and HRS-like cells with a background meshwork of dendritic cells and variable inflammatory cell population (a). Neoplastic cells were positive for CD3, CD4 and CD10 but negative for Bcl-6 and PD-1. HRS-like cells were expressing CD20 and CD30 but were negative for CD15 antigen, EBER was positive. No rosetting was seen in this case (b). The case was monoclonal for TCR-gamma and polyclonal for IgH gene. Hematoxylin and eosin staining.

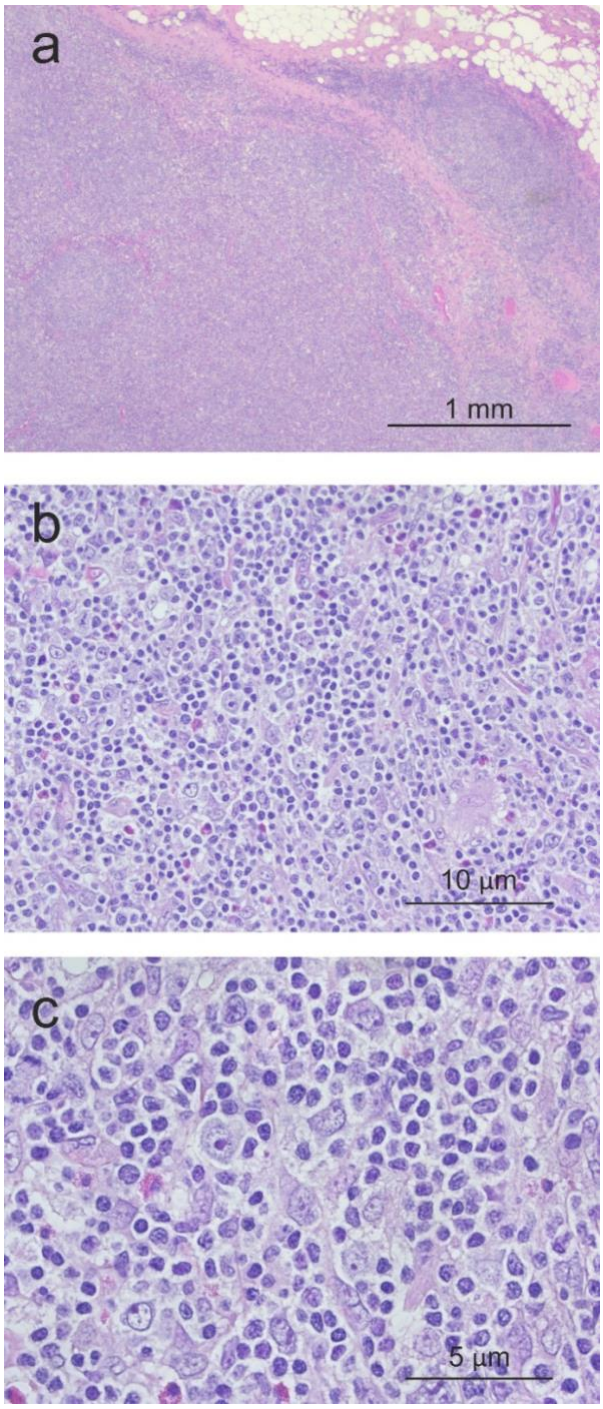

Figure 3 A case of reclassified FTCL from a previous PTCL NOS diagnosis. It has a nodular growth pattern (a) consisting of neoplastic lymphocytic cells with pale and clear cytoplasm, epithelioid cells and HRS-like cells (b-c) with a background of dendritic cells in nodules. No rosetting was seen in this case. Neoplastic cells were positive for CD3 and CD10 but negative for CD4, Bcl-6 and PD-1. HRS-like cells were positive for CD20 and negative for CD30, CD15 and EBER. The case was polyclonal for TCR-gamma and polyclonal for IgH gene. Hematoxylin and eosin staining.

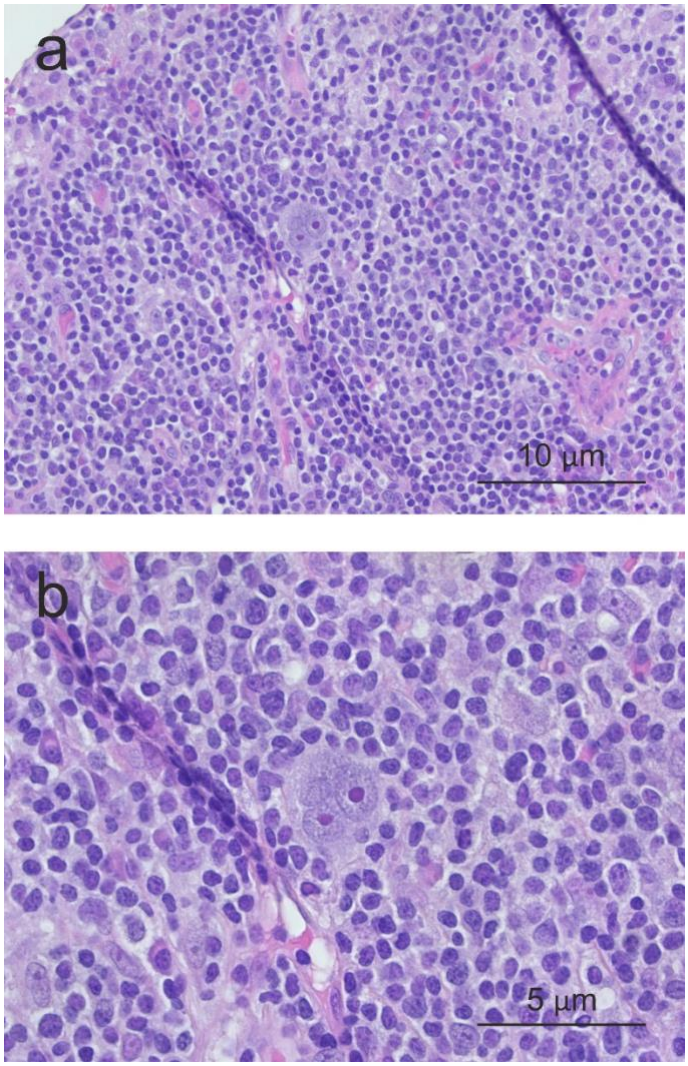

Figure 4 A case of reclassified FTCL from a previous AITL diagnosis. It has a nodular growth pattern with a background meshwork of dendritic cells. Neoplastic lymphocytic cells have a pale cytoplasm, but clear cells or epithelioid cells were not seen (a). Proliferation was positive for CD3, CD4, Bcl-6 and PD-1 and negative for CD10. HRS-like cells were negative for CD20, CD30, CD15 and EBER and they were seen with surrounding neoplastic cells forming rosettes (b). The case was polyclonal for TCR-gamma and polyclonal for IgH gene. Hematoxylin and eosin staining.

Suppl. Appendix S1 The protocol of EBER *in situ* hybridization

EBER *in situ* hybridization was prepared using the fully automated BOND-III stainer system (Leica Biosystems, Melbourne, Australia), Bond Dewax (AR9222), Bond EBER probe (PB0589) in combination with Bond Enzyme Pretreatment Kit, Enzyme 1 (AR9551), the Anti-Fluorescein Antibody (AR0222) and Bond Polymer Refine Detection (DS9800). The recommended staining protocol for EBER Probe 'ISH Protocol A' was operated. Appropriate tissue (EBER positive lymph node sample) and reagent controls were used.

Suppl. Table S2 Antibodies and dilutions applied for immunohistochemistry

	Primary antibody	Manufacturer	Antigen retrieval	Ab dilution	Ab incubation time	Detection system
CD3	NCL-CD3-PS1	Leica Biosystems Newcastle Ltd. (Newcastle Upon Tyne, UK)	Tris-EDTA pH 9	1:50	30 min	Dako REAL™ EnVision™ Detection System, Dako Denmark A/S (Glostrup, Denmark)
CD4	NCL-L-CD4-368	Leica Biosystems Newcastle Ltd. (Newcastle Upon Tyne, UK)	Tris-EDTA pH 9	1:40	30 min	Dako REAL™ EnVision™ Detection System, Dako Denmark A/S (Glostrup, Denmark)
CD10	NCLCD10-270	Leica Biosystems Newcastle Ltd. (Newcastle Upon Tyne, UK)	Tris-EDTA pH 9	1:100	30 min	Dako REAL™ EnVision™ Detection System, Dako Denmark A/S (Glostrup, Denmark)
CD15	M3631	Dako North America, Inc. (Carpinteria, CA, USA)	Tris-EDTA pH 9	1:150	30 min	Dako REAL™ EnVision™ Detection System, Dako Denmark A/S (Glostrup, Denmark)
CD20	M0755	Dako Denmark A/S (Glostrup, Denmark)	Tris-EDTA pH 9	1:1000	30 min	Dako REAL™ EnVision™ Detection System, Dako Denmark A/S (Glostrup, Denmark)
CD21	NCL-L-CD21-2G9	Leica Biosystems Newcastle Ltd. (Newcastle Upon Tyne, UK)	Trypsin	1:50	60 min	UltraVision ONE Detection System , Thermo Fisher Scientific/ LabVision Co
CD30	M0751	Dako Denmark A/S (Glostrup, Denmark)	Tris-EDTA pH 9	1:300	30 min	Dako REAL™ EnVision™ Detection System, Dako Denmark A/S (Glostrup, Denmark)
PAX5	CMC31231022	Cell Marque (Roclin, CA, USA)	Tris-EDTA pH 9	1:50	60 min	Dako REAL™ EnVision™ Detection System, Dako Denmark A/S (Glostrup, Denmark)
Bcl6	M7211	Dako Denmark A/S (Glostrup, Denmark)	Tris-EDTA pH 9	1:50	30 min	Dako REAL™ EnVision™ Detection System, Dako Denmark A/S (Glostrup, Denmark)
PD1	ab52587	Abcam plc (Cambridge, UK)	Tris-EDTA pH 9	1:100	30 min	Dako REAL™ EnVision™ Detection System, Dako Denmark A/S (Glostrup, Denmark)

Suppl. Appendix S3 The definition of terms clonal, polyclonal and clonal with background

The TCR/IgH profiles can be technically determined using the terms clonal, polyclonal, clonal with background, etc., with fluorescence intensity and fragment size. The term “clonal” represents a profile with one or two reproducible clonal peaks or bands, “polyclonal” means a profile where the Gaussian distribution is seen with/without minor reproducible peaks/bands. “Clonal with background” is a more detailed technical description of clonal profiles, and it refers to the impact of non-clonal, reactive cells, resulting in a polyclonal Gaussian profile with clonal findings.

Langerak AW, Groenen PJTA, Brüggemann M, et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. Leukemia 2012;26:2159. https://doi.org/10.1038/LEU.2012.246.