Causes of double-negative T-cell lymphocytosis in children and adults

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ABSTRACT

Aims The causes and diagnosis of 'double-negative' (CD3+CD4−CD8−) T-cell lymphocytosis are not well studied. We aimed to define the causes of double-negative T-cell lymphocytosis in children and adults, and to identify simple clinical and laboratory features that would help to differentiate between the underlying conditions.

Methods We collected clinical and laboratory data on 10 children and 30 adults with significantly increased peripheral-blood double-negative T-cells (>10% of total lymphocytes). We identified conditions associated with double-negative T-lymphocytosis with flow cytometry, peripheral-blood morphology and T-cell receptor-gene rearrangement studies. Patients were assigned to diagnostic categories on the basis of these test results.

Results and conclusions The causes of double-negative T-cell lymphocytosis in children were autoimmune lymphoproliferative syndrome (ALPS) and reactive γ/δ T-lymphocytosis. T-cell large granular lymphocyte (T-LGL) leukaemia, reactive γ/δ T-lymphocytosis and hepatosplenic T-cell lymphoma (HSTL) were the the most common disorders underlying double-negative T-cell lymphocytosis in adults. Less common causes included hypereosinophilic syndrome, peripheral T-cell lymphoma, ALPS and monoclonal, double-negative T-lymphocytosis of uncertain significance. CD5/CD7/Vδ2 expression and absolute double-negative lymphocyte count (<1.8×10^9/L) were useful discriminators for distinguishing patients with reactive γ/δ T-lymphocytosis from those with γ/δ lymphoproliferative disorders. Differentiating between γ/δ T-LGL and HSTL can be difficult. Expression of CD57 and cellular morphology (pale cytoplasm with distinct granules) would support a diagnosis of γ/δ T-LGL.

INTRODUCTION

During T-cell development, the CD3+CD4−CD8− immunophenotype is typical of immature (thymic-derived) T-cells. A small subset of mature (post-thymic) T-cells with this paradoxical phenotype ('double-negative' T-cells) is normally found in peripheral-blood samples of healthy individuals (usually <2.5% of total lymphocytes). This minor component of the immune system is heterogeneous, consisting of cells that express gamma/delta T-cell receptors (TCRγδ) or alpha/beta T-cell receptors (TCRαβ).[1] Gamma/delta (γ/δ) T-cells are separated into Vδ1+ and Vδ2+ based on their V (variable) region.[2] The physiological and pathological roles of double-negative T-cells are not fully understood, but evidence suggests that they recognise highly conserved stress and heat-shock proteins expressed by parasites, bacteria (notably mycobacteria) and stressed mammalian cells. It is believed that double-negative T-cells recognising autologous stress-induced molecules may play a role in autoimmunity.[1–5]

It has been found that double-negative T-cells are increased in autoimmune disease, for example, systemic lupus erythematosus (SLE) and infections, though a great increase in this T-cell subpopulation representing >10% of peripheral-blood lymphocytes ('double-negative T-cell lymphocytosis') is very rare.[5 6] Double-negative T-cell lymphocytosis is a condition of multiple aetiology. The differential diagnosis of its causes poses a particular diagnostic challenge as a consequence of their rarity and potential unfamiliarity to many pathologists.

There have been no systematic studies of the causes of double-negative T-cell lymphocytosis in children and adults. Also, the morphological and immunophenotypical markers that could aid the differential diagnosis are not well studied. The aims of this study were to define the underlying causes of double-negative T-lymphocytosis in children and adults and to identify simple features that would help to differentiate between the underlying conditions.

METHODS

We did a retrospective study of double-negative T-cell lymphocytosis in four Greek hospitals from January 2010 to February 2019. Patients were eligible if they had CD3+CD4−CD8− T-cells measuring >10% of peripheral-blood lymphocytes by flow cytometry. Most were recruited from haematology clinics or inpatient units. All clinical samples and data were collected for routine patient care. The presence of lymphocytosis, abnormal or atypical lymphocytes in the blood film, neutropenia, autoimmune haemolytic anaemia (AIHA) and immune thrombocytopenia (ITP) and the investigation of possible immunodeficiency were the most common reason for flow-cytometric immunophenotyping. Cases of CD3 +CD4-CD8- T-lymphoblastic leukaemia/lymphoma were excluded from this study. Leukaemic lymphoblasts were identified by their light-scattering properties and staining with

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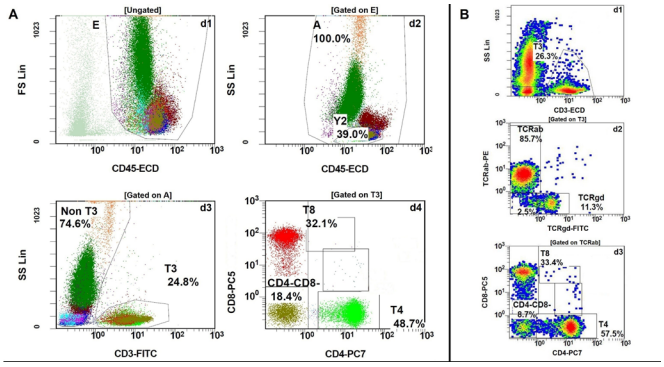

Figure 1 Immunophenotype of peripheral-blood T-lymphocytes in a child who presented with enlarged cervical lymph nodes due to ALPS. The haemoglobin level was 100 g/L, white cell count 5.17×10^9/L (lymphocytes 44.1% and neutrophils 43.4%), and platelets 243×10^9/L. (A) Gating strategy for T-cell subsets. Initial gating with CD45 versus forward scatter (diagram d1) was used to identify white blood cells (region E). CD45 versus SS (diagram d2) on the white blood cells was then used to identify the cellular elements based on which region they appear (A denotes the white blood cell region and Y2 denotes the lymphocyte region). A CD3 versus SS display (diagram d3) was used to identify the T-cell gate (T3 region). The T-cell gate was analysed for CD4 and CD8 expressions (diagram d4). An increased CD3+CD4−CD8− ('double-negative') T-cell subpopulation (18.4%) was observed. (B) The identification of increased double-negative T-cells led to further characterisation of these cells. For this purpose, the T-cell gate was stained for TCRαβ and TCRγδ (diagram d2) and, then, a CD4 versus CD8 display was gated on TCRαβ+ T-cells (diagram d3). It showed 8.7% double-negative T-cells positive for TCRαβ. These CD3+TCRαβ+CD4−CD8− double-negative T-cells constituted 7.5% of T-lymphocytes, consistent with ALPS. An increased proportion of reactive CD3+TCRγδ+CD4−CD8− double-negative T-cells was also noted (11.3% in diagram d2). ALPS, autoimmune lymphoproliferative syndrome; ECD, phycoerythrin-Texas Red conjugate (energy coupled dye); FITC, fluorescein isothiocyanate; FS, forward light scatter; SS, side light scatter; PC5, phycoerythrin-cyanine5 conjugate; PC7, phycoerythrin-cyanine7 conjugate; PE, phycoerythrin; TCRαβ, alpha/beta T-cell receptor; TCRγδ, gamma/delta T-cell receptor.

monoclonal antibodies against CD99, CD1a, and primitive-cell markers (CD34, TdT) in conjunction with other laboratory analyses (eg, morphology and karyotyping).

A total of 10 children (6 boys and four girls) aged 3–16 years (median, 11) and 30 adults (16 men and 14 women) ranging in age from 22 to 84 years (median, 59.5) with double-negative T-cell lymphocytosis were identified. Tests that were done for all patients included differential white-cell counts and assessment of lymphocyte morphology on Giemsa-stained peripheral-blood films. The features considered were: cell size, nuclear chromatin, nuclear shape, nucleoli, amount and basophilia of cytoplasm, and azurophilic (red) granules. Human T-cell lymphotropic virus type 1 (HTLV-1) and HIV antibodies were tested in each of these patients.

Initial screening for peripheral-blood T-lymphocytes was done by standard flow-cytometric techniques with a panel of antibodies against the T-lineage-associated markers CD2, CD3, CD4, CD5, CD7 and CD8. The identification of an increased proportion of double-negative T-cells led to further characterisation of these cells with monoclonal antibodies against the TCRαβ and the TCRγδ. Monoclonal antibodies against Vδ1, Vδ2 and 24 Vβ-chains of the TCR were also used, as were monoclonal antibodies against natural killer (NK) cell-associated markers (CD16, CD56 and CD57). The aberrant expression of CD2, CD5 and CD7 was evaluated by comparing the mean expression value on the double-negative T-cells with the remaining normal T-cells. If clinically indicated, further markers (CD25, CD30, TIA1, CD26, CD27 and TCL1) were used. In a substantial number of cases, bone marrow, lymph node and skin biopsy specimens were available and in some cases of particular diagnostic difficulty, liver biopsy. Cell staining was analysed by a five-colour flow cytometer with at least 100 000 events counted for each sample. Conventional consensus-primer TCR-γ or TCR-β PCR was used to identify monoclonal populations of T-cells.[7] Individuals who were diagnosed as having reactive double-negative T-lymphocytosis had a polyclonal population of lymphocytes.

RESULTS

Seven of the 10 children fulfilled the criteria for diagnosis of autoimmune lymphoproliferative syndrome (ALPS).[8] The median age at presentation was 12 years (6–16). Cervical lymphadenopathy and splenomegaly were the most common symptoms; four of these children had evidence of AIHA. The median white cell count was 6.4×10^9/L (4.5–8.3) with 43.6% lymphocytes (20.7–66.3). Children with ALPS had double-negative TCRαβ+CD2+CD5+CD7+CD16−CD56− T-cells that were polyclonal (figure 1). The abnormal T-cells constituted 10%–20% of circulating lymphocytes (median, 11.7%).

Three children had reactive double-negative γ/δ T-lymphocytosis with a proportion of double-negative T-cells ranging from 23.6% to 45.5% (median, 28.2%), which was higher than ALPS (p=0.016). Disorders underlying reactive γ/δ T-lymphocytosis were primary immunodeficiency, ileal–lymphoid–nodular hyperplasia and recurrent aphthous stomatitis.

Only 1 of the 30 adults had double-negative T-cell lymphocytosis associated with ALPS. She was evaluated at 37 years of age and was the mother of two children with ALPS. The presenting features and final diagnoses of the 30 adults are shown in table 1.

Eleven (36.6%) patients had T-cell large granular lymphocyte leukaemia (T-LGL), making it the the most common cause of double-negative T-lymphocytosis in adults. Diagnosis of T-LGL was based on the presence of increased peripheral-blood lymphocytes with distinct azurophilic granules and/or expression of at least one NK cell marker with detectable T-cell receptor clones. Seven of the 11 cases were positive for TCRγδ (γ/δ T-LGL); two cases were positive for TCRαβ (α/β T-LGL); and two cases had two distinct populations of aberrant T-cells (patients 3 and 4). The lymphocytes had a low nuclear:cytoplasmic ratio and pale cytoplasm with several fine (rarely coarse) azurophilic granules; occasional cells with the same morphology were agranular. Double-negative T-LGL presented in an insidious fashion except for patient 8, who had aggressive disease. Membrane markers in patient 8 showed double-negative, TCRγδ+CD2+CD5+C-D7weak+CD16-CD56+CD57+CD25−TCL1− T-cells and cellular morphology large granular lymphocytes and 'transformed' immunoblast-like cells with basophilic cytoplasm and nucleoli (figure 2).

Five patients (16.6%) had peripheral-blood involvement by hepatosplenic T-cell lymphoma (HSTL), four of whom had typical γ/δ HSTL and one had atypical HSTL with absence of surface expression of TCR (TCRαβ−/TCRγδ−). The HSTL of α/β type was not observed in our study. As expected, patients with HSTL had aggressive disease; common presenting symptoms included weight loss, fever, abdominal pain, and tiredness or breathlessness related to anaemia. Notably, two patients had

Table 1 Presenting features and final diagnoses in the 30 adults with double-negative T-cell lymphocytosis

Patient number	Sex/age (year)	Splenomegaly	Lymphadenopathy	Cutaneous presentation	Associated disease	Haematological findings				Tissue biopsy	Diagnosis
						Anae mia*	* Thrombocytopenia	* Neutropenia	Lympho cyte count		
1	M/67	No	No	No	Rheumatoid arthritis	No	No	No	High–normal		α/β T-LGL
2	F/50	No	No	No	Multiple sclerosis	No	No	Mild	Lymphopenia		α/β T-LGL
3†	M/75	No	No	No	No	No	Mild	Mild	Lymphocytosis		Biclonal: α/β T-LGL and T8-LGL
4†	F/66	Yes	No	No	SEL and marginal-zone B cell lymphoma	Mild	No	Severe	Lymphocytosis	Bone marrow	Biclonal: α/β T-LGL and γ/δ T-LGL
5	M/69	Yes	No	No	No	Severe	No	Mild	Lymphocytosis	Bone marrow	γ/δ T-LGL
6	F/57	Yes	No			No	No	Severe	Normal		γ/δ T-LGL
7	M/84	No	No	No	No	No	No	Mild	Lymphocytosis	Bone marrow	γ/δ T-LGL
8‡	F/59	Yes	Yes	Purpuric lesions	Rheumatoid arthritis, rheumatoid vasculitis and AIHA	Moderate	Severe	Severe	Lymphocytosis	Bone marrow and lymph node	Aggressive γ/δ T-LGL
9	M/80	Yes	No	No		Mild	No	Moderate	Lymphocytosis		γ/δ T-LGL
10	M/52	No	No	No	No	No	No	Moderate	Lymphopenia		γ/δ T-LGL
11	M/23	Yes	No	No	ITP	No	Severe	No	Lymphopenia	Bone marrow, liver	γ/δ T-LGL
12	M/49	Yes	No	Yes	No	Severe	Moderate	Severe	Lymphopenia	Bone marrow	HSTL
13§	M/67	Yes	No	No	No	Mild	Mild	No	Lymphocytosis	Bone marrow	HSTL
14§	M/82	Yes	No	No	ND	No	No	No	Lymphocytosis	Bone marrow	HSTL
15¶	F/47	Yes	No	No	ND	No	No	No	Lymphocytosis	Bone marrow, spleen and liver	Atypical HSTL
16	M/70	Yes	No	No	ND	Moderate	Severe	Moderate	Low–normal	Bone marrow	HSTL
17**	F/52	No	Yes	Yes	No	No	No	No	Lymphocytosis	Bone marrow, skin and lymph node	L-HES
18	F/34	No	No	No	No	No	No	No	Lymphocytosis	Bone marrow and skin	L-HES
19	F/82	No	No	Yes	CIDP and AIHA	Severe	No	Mild	Lymphopenia	Bone marrow and skin	α/β peripheral T-cell NHL
20	F/61	Yes	Yes	No		Mild	Mild	No	Normal	Bone marrow, lymph node and CSF	α/β peripheral T-cell NHL
21	M/70	No	Yes	Yes		No	Severe	No	High–normal	Bone marrow, skin and CSF	Relapsed-refractory cutaneous γ/δ T-cell NHL
22††	M/50	Yes	Yes	Pruritus	Hodgkin's lymphoma	No	No	No	Lymphocytosis	Bone marrow and lymph node	Reactive γ/δ lymphocytosis
23††	22/M	No	No	No	Chronic idiopathic neutropenia	No	No	Moderate	Normal		Reactive γ/δ lymphocytosis
24††	F/70	No	No	No	No	No	No	No	Lymphocytosis		Reactive γ/δ lymphocytosis
25††	F/42	ND	No	No	β-thalassaemia	ND	No	No	Lymphocytosis		Reactive γ/δ lymphocytosis

Continued

Table 1 Continued

Patient number	Sex/age (year)	Splenomegaly	Lymphadenopathy	Cutaneous presentation	Associated disease	Haematological findings			Lympho cyte count	Tissue biopsy	Diagnosis
						Anae mia*	* Thrombocytopenia	* Neutropenia			
26††	M/74	No	No	No	Pneumonia	Mild	No	Increased	Lymphocytosis		Reactive γ/δ lymphocytosis
27††	F/45	No	No	No	CVID	No	No	Mild	High–normal		Reactive γ/δ lymphocytosis
28††	F/60	No	No	No	No	No	No	No	Lymphocytosis		Reactive γ/δ lymphocytosis
29‡‡	M/41	No	No	Yes		No	No	No	Lymphocytosis	Bone marrow	Monoclonal TCRαβ+ lymphoproliferation of uncertain significance
30††	F/37	No	Yes	No	No	No	No	No	Lymphocytosis		ALPS

*According to Common Terminology Criteria for Adverse Events V.5.0 (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf). Normal haemoglobin 120-147 g/L, platelet number 140–400×10^9/L, neutrophils 2.0–7.5×10^9/L and lymphocytes 1.5–3.5×10^9/L.
†Patient 3 had two subpopulations of abnormal double-negative T-cells. One had alpha/beta T-cell receptor, and the other expressed gamma/delta T-cell receptor. Patient 4 also had two abnormal T-cell subpopulations: one was consistent with double-negative α/β T-LGL and the other with classical CD3+CD4-CD8+ T-LGL (T8-LGL). Patient 3 had a history of systemic lupus erythematosus and had been diagnosed with marginal-zone B cell lymphoma 4 years earlier.
‡Patient eight had rheumatoid arthritis treated with azathioprine. She presented with fever, pronounced hepatosplenomegaly, abdominal lymphadenopathy, increased lactate dehydrogenase (LDH) concentration, leucocytosis (14.5×10^9/L), increased lymphocyte count (12.1×10^9/L), anaemia (haemoglobin 100 g/L), neutropenia (0.87×10^9/L) and thrombocytopenia (15×10^9/L).
§Patients 13 and 14 presented with an acute leukaemia-like picture with markedly elevated white cell count (63.5×10^9/L and 150×10^9/L, respectively). In patient 13, leucoerythroblastosis was noted on peripheral-blood film on admission.
¶Patient 15 presented with febrile splenomegaly and pancytopenia (haemoglobin 95 g/L, white blood cells 2.2×10^9/L, platelets 60×10^9/L). She underwent splenectomy with improvement of cytopenia (spleen histology was non-diagnostic); 9 months postsplenectomy, progressive leucocytosis (68.1×10^9/L), haemoglobin, 141 g/L, platelets 205×10^9/L and hepatic dysfunction developed.
**Transition to overt T-cell lymphoma occurred in patient 17, manifested by the rapid increase of leucocytes (69.1×10^9/L), lymphocytes (41.8×10^9/L) and eosinophils (9.3×10^9/L) in peripheral blood, complex cytogenetics (+7, del7q, −9, −10), skin infiltration and lymph node enlargement.
††Individuals who were diagnosed as having reactive double-negative T-lymphocytosis or double-negative T-lymphocytosis associated with ALPS had no evidence of a clonal population of lymphocytes by PCR.
‡‡Patient 29 has been studied for 4 years. There is no notable medical history and he has remained stable without any lymphadenopathy, splenomegaly, eosinophilia or other symptom to date.
. AIHA, autoimmune haemolytic anaemia; ALPS, autoimmune lymphoproliferative syndrome; CIDP, chronic inflammatory demyelinating polyneuropathy; CSF, cerebrospinal fluid; CVID, common variable immunodeficiency; F, female; HSTL, hepatosplenic T-cell lymphoma; ITP, autoimmune thrombocytopenia; L-HES, lymphocytic variant of the hypereosinophilic syndrome; M, male; ND, not determined; NHL, non-Hodgkin's lymphoma; SEL, systemic lupus erythematosus; T-LGL, T-cell large granular lymphocyte leukaemia.

Figure 2 Double-negative γ/δ T-LGL . (A) Peripheral-blood findings in a typical case of γ/δ T-LGL (patient 7). The cells have a low nuclear:cytoplasmic ratio and show several distinct azurophilic granules in the abundant pale blue cytoplasm. (B) However, in patient 8, who had a particularly aggressive form of the disease, the morphological features were different, with large granular lymphocytes and immunoblast-like cells with more basophilic cytoplasm and nucleoli (arrows). (C) In patient 7, the abnormal CD3+CD4−CD8− ('double-negative') T-cells constituted 44.5% of the circulating T-lymphocytes (C3 region in diagram d1) and expressed large amounts of surface CD57, as shown in diagram d2. The CD3+ T-cells in diagram d1 were gated from the total lymphocyte population (data not shown). (D) PCR showed clonal rearrangements of the γ-chain of the T-cell receptor (patient 7). γ/δ T-LGL, γ/δ T-cell large granular lymphocyte leukaemia. SS, side light scatter.

an acute leukaemia-like picture at diagnosis with total white-blood count exceeding 60×10^9/L (patients 13 and 14). By contrast, total white blood count was <20×10^9/L in patients with T-LGL.

The immunophenotypical profile of HSTL was very similar to that of γ/δ T-LGL. Furthermore, the median percentage of double-negative T-cells did not differ significantly between T-LGL and HSTL (p=0.36). Loss of expression or decreased expression of CD5 was found in all HSTL versus 77.8% T-LGL (p=0.36). Loss of expression or decreased expression of CD7 was found in 57% of γ/δ T-LGL and 60% of HSTL. In most instances, HSTL expressed Vδ1 (75%), but γ/δ T-LGL was not consistently Vδ1+ or Vδ2+. Sixty per cent of HSTL had expression of CD56 and/or CD16 vs 44.4% of T-LGL (p=0.74). CD57 was positive in 71.4% of T-LGL and 20% of HSTL. We saw that the morphology of circulating HSTL cells varied greatly from medium-sized lymphoid cells with abundant pale cytoplasm and condensed chromatin without nucleoli resembling virally activated lymphoid cells to medium-sized and large-sized cells with high nuclear:cytoplasmic ratio, nuclear irregularity (or even convoluted nuclei) and immature nuclear chromatin

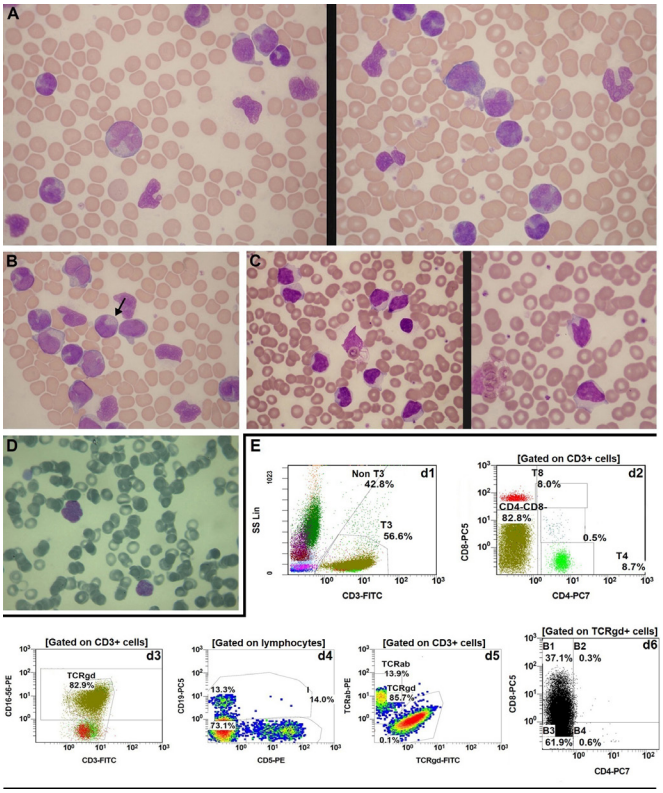

Figure 3 Leukaemic manifestations of hepatosplenic lymphoma. (A) The morphological features of the circulating cells are medium-sized and large-sized cells with a high nuclear:cytoplasmic ratio, basophilic cytoplasm, immature nuclear chromatin and irregular nuclear outline. Some blastic cells are also seen (patient 14). (B) Occasional cells with the same morphology contain faint, 'dust-like' granules (arrow). (C) There are also instances in which HSTL is characterised by medium-sized lymphocytes with abundant cytoplasm without granules and clumped nuclear chromatin with indistinct nucleoli resembling virally (immunologically) activated lymphoid cells (patient 15). (D) Small or large cerebriform cells may also be seen in HSTL (patient 12). (E) Membrane markers revealed CD3+CD4−CD8− ('double-negative') TCRγδ+TCRαβ−CD5−CD16+CD56+ T-cells (patient 14). In addition to CD8− cells, the tumour-cell population contained cells with weak staining of CD8, as shown in diagram d2. Analysis of the TCRγδ+ cell gate for CD8 expression (diagram d6) showed 61.9% CD4−CD8− (B3 region) and 37.1% CD4−CD8weak (B1 region) cells. This finding is not uncommon in γ/δ HSTL, in which a minority of tumour cells may be weakly or focally positive for CD8.[13] HSTL, hepatosplenic T-cell lymphoma. SS, side light scatter; FITC, fluorescein isothiocyanate; PC5, phycoerythrin-cyanine5 conjugate; PC7, phycoerythrin-cyanine7 conjugate; PE, phycoerythrin

resembling blasts. Fine azurophilic granules were not seen except in one patient in whom few cells had faint, 'dust-like' granules (figure 3).

Patients 17 and 18 had eosinophilia (>5×10^9/L), a pruritic rash and monoclonal, double-negative, TCRαβ+CD2+CD5+CD7- CD16-CD56-CD57−CD25−CD27+TCL1−CD30- T-cells, consistent with the 'lymphocytic variant' of the hypereosinophilic syndrome (L-HES). Figure 4 shows morphological and immunophenotypical features of double-negative L-HES.

Patients 19 and 20 had peripheral-blood involvement by double-negative, TCRαβ+CD2+CD5-CD7+CD16−CD56−CD25− peripheral T-cell lymphoma (T-cells from patient 19 also expressed CD30), and patient 21 had relapsed-refractory, double-negative, CD2+CD5-CD7+CD16+CD56+CD30− cutaneous γ/δ T-cell lymphoma.

Seven patients (23.3%) had reactive double-negative γ/δ T-lymphocytosis; four had an underlying condition (common variable immunodeficiency, Hodgkin's lymphoma, pneumonia and chronic idiopathic neutropenia); and three had no underlying condition. In each of these patients, the double-negative T-cells were TCRγδ+CD2+CD5+CD7+Vδ2+. Features that suggested a diagnosis of reactive γ/δ T-lymphocytosis rather than of γ/δ lymphoproliferative disorder (T-LGL/HSTL) were absolute double-negative lymphocyte count <1.8×10^9/L (receiver operating characteristic curve(ROC)=0.79) and CD5/CD7/Vδ2 expression.

Finally, we noted a case of long-standing, monoclonal, double-negative T-lymphocytosis with a benign clinical course and an unusual phenotype, the T-cells being TCRαβ+CD2+CD5+CD7−CD16−CD56−CD57−CD25−TCL1−CD30− and exhibiting increased levels of CD26 (a T-cell activation marker). The lymphocytes were small with slight nuclear irregularities without visible nucleoli or azurophilic granules. This patient, who did not clearly fit into one of the diagnostic groups, was categorised as having 'monoclonal, double-negative TCRαβ+lymphoproliferation of uncertain significance' (patient 29).

Patients were categorised into five groups according to their primary diagnosis. Table 2 shows the laboratory results for each diagnostic group. HTLV-I and HIV antibodies were negative in all patients.

DISCUSSION

ALPS has been well recognised as the disorder most commonly associated with double-negative T-cells in children, and this finding was confirmed by our study. We also showed that reactive γ/δ T-lymphocytosis is an important cause, whereas none of the children had monoclonal T-cells.

The most common causes of double-negative T-lymphocytosis in adults were T-LGL, reactive γ/δ T-lymphocytosis and HSTL, which comprised 36.6%, 23.3% and 16.6% of cases, respectively. Diagnosis of reactive γ/δ T-lymphocytosis is usually straightforward with PCR to identify clonal T-cell receptor-gene rearrangements. It should be noted, however, that PCR for T-cell receptor genes is not widely available in all countries. Our finding that a double-negative T-cell count <1.8×10^9/L and CD5/CD7/Vδ2 expression can help to distinguish between reactive lymphocytosis and T-LGL may be a simple diagnostic tool for use in the differential diagnosis of double-negative T-lymphocytosis.

We found that double-negative T-LGL is often associated with cytopenias (usually neutropenia) and splenomegaly. As in classical T-LGL, 45.4% of our patients presented with concomitant autoimmune disorder (rheumatoid arthritis, SLE, AIHA, ITP and multiple sclerosis). The other series of patients with γ/δ T-LGL reported in the literature show similarities with our own, from both immunophenotypical and clinical points of view.[9–12] The study by Chen and coworkers,[12] however, indicated that double-negative γ/δ T-LGL is mostly associated with lymphopenia, by contrast with our results and those of other studies in which almost half of the patients had absolute lymphocytosis. This is likely to be attributable to the recruitment of patients since lymphocytosis was the the most common reason for immunophenotyping in our study.

Distinguishing between double-negative T-LGL and HSTL is important because they require different management strategies.

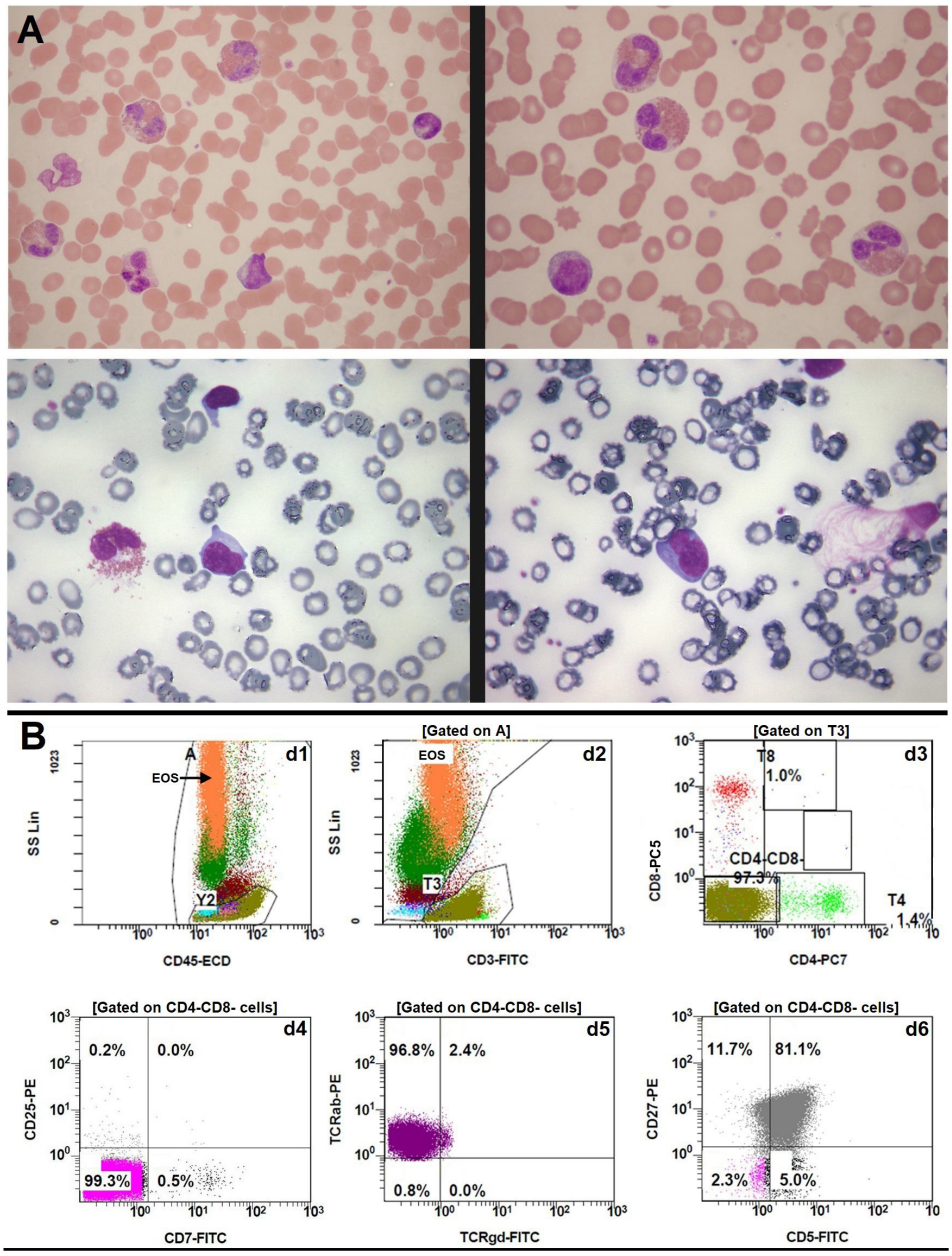

Figure 4 Double-negative L-HES. (A) Both patients had a considerable degree of eosinophilia, some degranulated eosinophils and atypical lymphoid cells with a lower nuclear:cytoplasmic ratio than normal and variable cytoplasmic basophilia resembling immunologically activated lymphocytes (top panel, patient 18; bottom panel, patient 17). (B) The diagrams d1–d6 show the gating strategy for identification of aberrant T-cells with a CD3+CD4–CD8–TCRαβ+TCRγδ–CD5+CD7- CD25–CD27+ immunophenotype in patient 17. CD45 versus SS (diagram d1) on the white blood cells (region A) was used to identify the cellular elements based on which region they appear (EOS denotes the eosinophil region and Y2 denotes the lymphocyte region). CD3 versus SS (diagram d3) was used to gate CD3+ T-cells (T3 region). The T-cell gate was then analysed for CD4 and CD8 expressions (diagram d4). The gating in diagrams d4, d5 and d6 was on the CD4–CD8– double-negative T-cells. EOS, eosinophil; L-HES, lymphocytic variant of hypereosinophilic syndrome. SS, side light scatter; FITC, fluorescein isothiocyanate; PC5, phycoerythrin-cyanine5 conjugate; PC7, phycoerythrin-cyanine7 conjugate; PE, phycoerythrin

Double-negative T-LGL is indolent in its natural course, while HSTL is notorious for its aggressive course and poor outcome.[9–13] Yet, the course of illness in patient 8 shows that γ/δ T-LGL may also have an aggressive clinical behaviour similar to HSTL ('aggressive γ/δ T-LGL'). This information is important in evaluating patients with double-negative T-lymphocytosis who have more bulky disease of the liver and spleen, severe constitutional symptoms, severe cytopenias and rapid clinical course. Similar cell morphology was noticed by Matutes and colleagues in a 40-year-old woman with 'transformed' T-LGL.[14]

Peripheral-blood involvement by HSTL is uncommon at presentation but may occur late in the clinical course.[13] However, a leukaemic clinical presentation may also occur.[15 16] Because T-LGL and HSTL may express the same markers, the results of immunophenotyping need careful interpretation. CD57 expression and cellular morphology were identified in our study as diagnostic factors that could help distinguish double-negative T-LGL from HSTL: if double-negative T-cells are CD57+ and ≥30% of peripheral-blood lymphocytes have pale cytoplasm with distinct azurophilic granules, the diagnosis is likely to be

Table 2 Laboratory findings for each diagnostic group*

Diagnosis	Number	Male:female ratio	Age (years), median (range)	Total white cell count (×10^9/L), median (range)	Absolute lymphocyte count (×10^9/L), median (range)	Lymphocytes (%), median (range)	Double-negative T-cells (%), median (range)	Double-negative T-cell count (×10^9/L), median (range)
T-LGL	11	7:4	67 (23–84)	8.9 (3.1–14.5)	6.1 (0.9–12.1)	68.5 (19.6–88)	38.5 (10.1–88)	1.57 (90–10.9)
HSTL	5	4:1	67 (47–82)	61.8 (2.6–150)	40.4 (1.2–120)	63.7 (43.3–84.7)	75 (36–82.8)	29.7 (432–84)
L-HES	2	0:2	43 (34–52)	23 (10–36)	4.8 (3.8–5.9)	27.2 (16.4–38)	72 (54–90)	3.68 (2.05–5.31)
Peripheral T-cell NHL	3	1:2	70 (61–82)	4.9 (3.3–9.7)	2.06 (1.2–3.5)	36.3 (36.1–42.2)	58.9 (19.74–73.6)	0.70 (0.69–1.52)
Reactive	7	3:4	50 (22–74)	10.2 (3.6–31.8)	4.3 (2.2–18.1)	56.8 (40.7–61)	15.2 (10.1–46.9)	0.5 (0.3–1.75)

*Patients 29 and 30 were not included as they represented single cases.
HSTL, hepatosplenicT-cell lymphoma; L-HES, lymphocytic variant of hypereosinophilic syndrome; NHL, non-Hodgkin's lymphoma; T-LGL, T-cell large granular lymphocyte leukaemia.

T-LGL. We should emphasise, however, that accurate classification of these tumours often depends on bone marrow biopsy and cytogenetic analysis.[17] As observed in patients 11 and 15, hepatic biopsy may be required for diagnosis.

Less common disorders underlying double-negative T-lymphocytosis in our study were L-HES and peripheral-blood involvement by T-cell non-Hodgkin lymphoma (T-NHL). The haematological syndrome of (1) eosinophilia, (2) double-negative T-cell lymphocytosis, (3) detectable T-cell-receptor clones and (4) clinical manifestations almost exclusively restricted to the skin was characteristic of L-HES. Various T-cell immunophenotypes have been reported in L-HES.[18] Our study shows that L-HES should be included in the differential diagnosis of double-negative T-lymphocytosis. The diagnosis of double-negative L-HES is firm only when adult T-cell leukaemia/lymphoma has been excluded, since HTLV-1 may cause eosinophilia and double-negative T-cells.[19 20] None of our patients was infected with HTLV-1, which reflects the fact that HTLV-1 infection is extremely rare in Greece. Patients identified as having L-HES should be closely monitored for overt T-cell lymphoma. Finally, we identified three cases of peripheral-blood involvement by T-cell lymphoma. We consider these cases to be exceptional as T-cell lymphomas are not usually associated with a significant population of tumour lymphocytes in the peripheral blood.

Take home messages

- In the assessment of patients with double-negative T-cell lymphocytosis, T-lymphoblastic leukaemia/lymphoma and human T-cell lymphotropic virus 1 (HTLV-1) infection should be ruled out. Immunophenotyping of double-negative T-cells should include antibodies against alpha/beta T-cell receptor (TCRαβ) and gamma/delta T-cell receptor (TCRγδ).
- Autoimmune lymphoproliferative syndrome (ALPS) was the commonest cause of double-negative T-cell lymphocytosis in children followed by reactive γ/δ T-lymphocytosis.
- T-cell large granular lymphocyte leukaemia (T-LGL), reactive gamma/delta (γ/δ) T-lymphocytosis and hepatosplenic T-cell lymphoma (HSTL) were the most common disorders underlying double-negative T-cell lymphocytosis in adults followed by lymphocytic variant of the hypereosinophilic syndrome, peripheral T-cell lymphoma, ALPS and monoclonal, double-negative TCRαβ+ lymphoproliferation of 'uncertain significance'.
- Accurate differentiation between reactive γ/δ T-lymphocytosis and γ/δ lymphoproliferative disorders depends on PCR. Simple features that indicate reactive T-lymphocytosis are double-negative lymphocyte count <1.8×10^9/L and expression of CD5/CD7/Vδ2.
- HSTL may present with an acute leukaemia-like picture with markedly elevated white blood count. The total white cell count was <20×10^9/L in patients with T-LGL.
- γ/δ T-LGL can be at times difficult to distinguish from HSTL. A multifaceted approach, with morphological, immunophenotypical and histological analyses, is essential to make correct diagnoses.

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