CASE REPORT

Unusual immunophenotype of T-cell large granular lymphocytic leukemia: Report of two cases

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ABSTRACT

Large granular lymphocytes (LGL) leukemias are commonly of the T-cell or NK-cell type. T-cell LGL leukemia is typically a disorder of mature CD3, CD8 and T-cell receptor TCR (TCR — T cell receptor)-αβ positive cytotoxic T-cells. Rare variants include TCRγδ$^+$ variants and CD4$^+$ TCRαβ$^+$ cases. We report a case of each of these rare variants. An 83-year-old female presented with anemia and lymphocytosis with LGLs on peripheral smear. Six-color multiparametric flowcytometric analysis showed expression of CD3, heterogeneous CD7, dim CD2 and TCRγδ and lacked expression of CD5, TCRαβ, CD56, CD4 and CD8. A final diagnosis of TCRγδ$^+$ T-cell LGL leukemia was made. Differentiation between TCRγδ$^+$ T-cell LGL leukemia and other γδ$^+$ T-cell malignancies is of utmost importance due to the indolent nature of the former as compared to the highly aggressive behavior of the latter. An 85-year-old male diagnosed with liposarcoma was identified to have lymphocytosis during preoperative evaluation. Peripheral smear showed presence of LGLs. Flowcytometric immunophenotyping showed expression of TCRαβ, CD3, CD2, CD5, CD4, dim CD8, CD56 with aberrant loss of CD7 expression. Vβ repertoire analysis by flowcytometry showed 97% cells with Vβ14 clonality. A final diagnosis of TCRαβ$^+$ CD4$^+$ T-cell LGL leukemia was made. CD4$^+$ T-cell large granular lymphocytic leukemias have an indolent, less aggressive course when compared to their CD8$^+$ counterparts and are not necessarily associated with cytopenias. However, their association with secondary neoplasia (29% of the cases) warrants a high degree of suspicion in the diagnosis as also noted in the index case. Use of a wide panel of antibodies and newer modalities such as Vβ repertoire analysis helps in accurate subtyping of LGL leukemia.

KEY WORDS: Alpha beta large granular lymphocytes, CD4$^+$ large granular lymphocytes leukemia, gamma delta large granular lymphocytes, large granular lymphocytes, large granular lymphocytes leukemia

INTRODUCTION

Large granular lymphocytes (LGLs) normally constitute about 10-15% of peripheral blood mononuclear cells.[1,2] Majority are derived from NK cell (CD3$^-$ CD8$^+$) lineage, whereas cytotoxic T-cells (CD3$^+$ CD8$^+$) form a minor component.[2] The wide spectrum of LGL proliferations ranges from a polyclonal reactive lymphocytosis seen in association with a variety of causes including splenectomy, HIV infection, other viral infections, allogeneic stem cell transplantation, and solid organ transplantation to a typical indolent leukemic disease.

T-cell large granular lymphocytic leukemia (T-LGL) leukemia is a heterogeneous disorder, first described by McKenna *et al.* in 1977,[3] characterized by a persistent (>6 months) increase in the number of peripheral blood LGLs, usually between 2 and 20 × 10^9/L, without a clearly defined cause.[4] Although the underlying pathologic mechanisms of the disease are not well-understood, its frequent association with autoimmune disorders suggests, that sustained immune stimulation plays a role in the pathogenesis.

CD3$^+$ T-LGL leukemia represents approximately 85% of the LGL leukemia cases and the vast majority shows a TCR αβ$^+$/CD4$^-$/CD8$^+$ phenotype (95% of all T-LGL leukemias).[1,5] Rare variants include TCR γδ$^+$ and TCR αβ$^+$, CD4+ phenotypes. We report one case each of the above mentioned rare phenotypes.

CASE REPORTS

Case 1

An 83-year-old female was referred to our hospital with transfusion dependent anemia and lymphocytosis. She had complaints of frequency of micturition and dysuria since 8 months. On examination there was no organomegaly or lymphadenopathy. The complete blood count (CBC) showed anemia (Hb 7.7 g/dl), thrombocytosis

(platelets 611 × 10^3/uL) and absolute lymphocytosis (total leucocyte count-9.81 × 10^3/uL with 89% lymphocytes). Peripheral smear examination showed presence of many LGLs [Figure 1]. The serum lactate dehydrogenase (LDH) was above normal limits (793 IU/L).

Flow cytometric analysis of the peripheral blood was carried out using stain — lyse — wash method. A six color immnunotyping panel for chronic lymphoproliferative disorders was used and samples were run on a BD FACS Canto II instrument. All the antibodies were sourced from BD Biosciences India

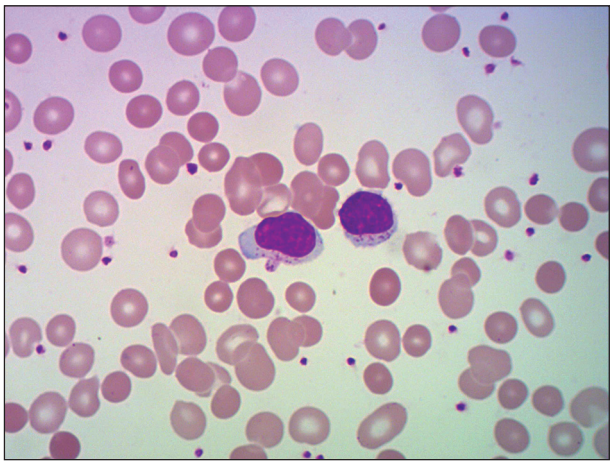

Figure 1: Large granular lymphocytes on PS

The panel used is shown in Table 1:

CD45 versus side scatter analysis showed a single cluster in the lymphocyte region [Figure 2a normal lymphocytes are displayed in blue color and the abnormal lymphocytes are displayed in red]. Majority of the lymphocytes showed CD3 and heterogenous CD7 positivity [Figure 2b] along with TCR γδ and dim CD2 expression [Figure 2c and e]. These cells were CD4 and CD8 dual negative [Figure 2d]. There was lack of expression of TCR αβ, CD5 and CD56 [Figure 2c, e and f]. Few normal B-cells were seen in the background. Based on the clinical details, morphological findings and above immunophenotpye a diagnosis of T-cell LGL leukemia of the TCR γδ subtype was made.

Table 1: Panel of antibodies used for analysis

Fluorochromes	*FITC*	*PE*	*PerCP*	*PE Cy7*	*APC*	*APC H7*
Tube 1	Kappa	Lambda	5	19	23	20
Tube 2	8	7	3	4	56	45
Tube 3	FMC7	200	38	19	10	45
Tube 4	γδ	αβ	3	5	2	45

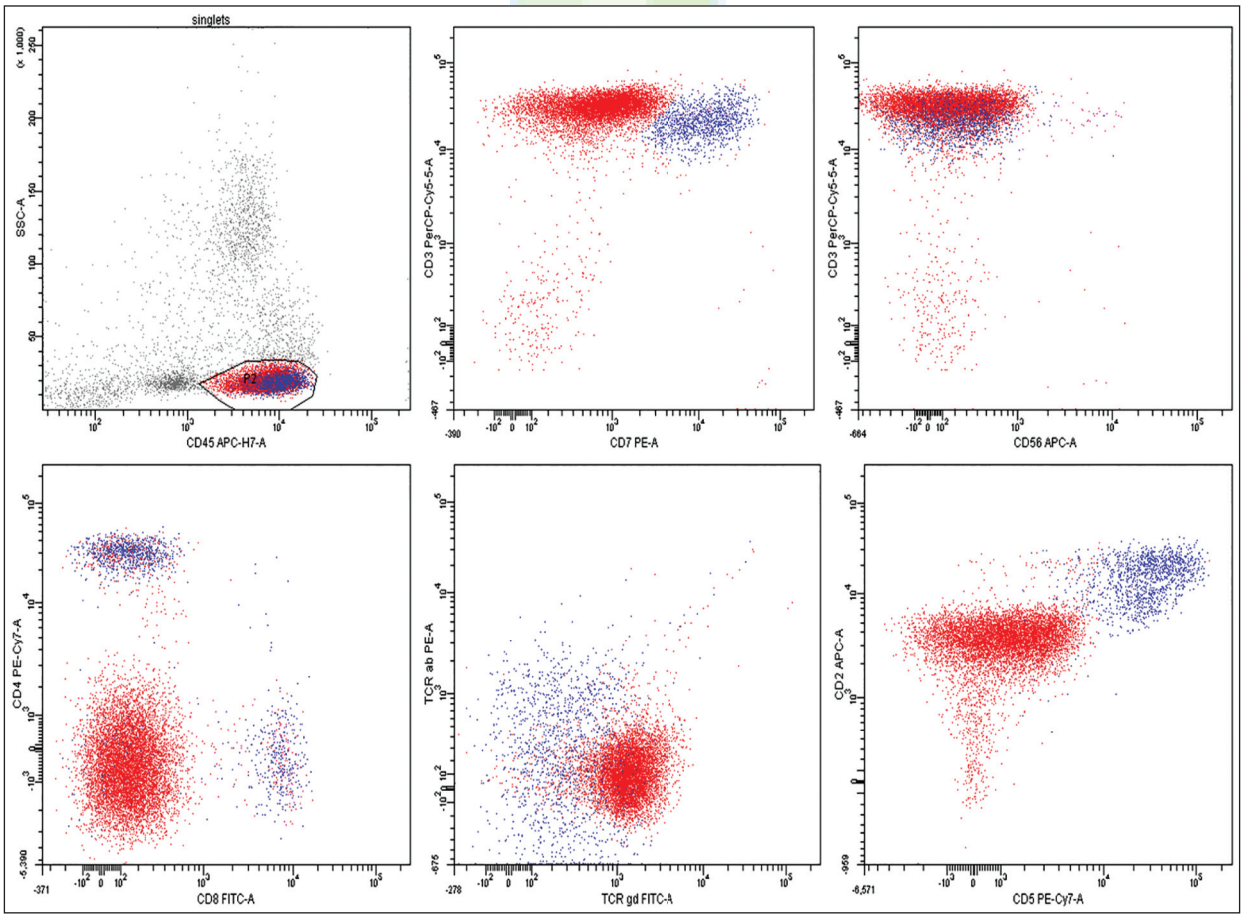

Figure 2: (Blue — normal lymphocytes, Red — Abnormal population). The abnormal lymphocytes express a brighter CD3, heterogeneous CD7, dim CD2 and TCR γδ as compared to the normal lymphocytes. Also they lack CD4, CD8, CD5 and CD56 expression

Case 2

A 85-year-old had complaints of abdominal fullness for 3 months. Examination showed presence of an umbilical hernia and an ill-defined lump in the left lumbar region. The computed tomography scan revealed that the lump extended from the left hypochondriac to the left lumbar region, measuring 19 cm × 17 cm × 12 cm. The diagnosis of well-differentiated liposarcoma with focal dedifferentiation was made on histopathological examination of biopsy specimen. He was mainly being evaluated for the surgical removal of the tumor and was referred to the hematologist as preoperative investigations showed evidence of lymphocytosis. A peripheral blood sample was send to our hospital for the evaluation of lymphocytosis. The CBC reports showed Hb-14.3 g/dl, total leukocytic count-9.50 × 10^3/uL, and platelets-98 × 10^3/uL. Peripheral smear showed 65% lymphocytes with presence of LGLs.

Flow cytometric analysis of the peripheral blood was carried out using stain-lyse-wash method and a similar six colour immnunotyping panel was used and samples were run on a BD FACS Canto II instrument. Flow cytometric analyses showed a cluster of lymphocytes on CD45 versus side scatter gating [Figure 3a]. Majority of the lymphocytes expressed CD3, CD2 and CD5 along with TCR αβ [Figure 3b and f]. The cells showed predominantly CD4 expression with a very small percentage of CD4 positive lymphocytes showing dim CD8 positivity [Figure 3e]. There was aberrant loss of CD7 expression [Figure 3b]. TCR γδ was negative [Figure 3d]. CD56 showed heterogenous expression [Figure 3b]. Additional antibodies were evaluated where CD16, CD25 and CD11c were not expressed and HLA DR (Human Leukocyte Antigen — DR) showed dim positivity.

A small proportion of normal CD7 expressing T-cells with normal CD4 and CD8 expression were seen in the background along with normal CD7 and CD56 positive NK cells.

For the confirmation of clonality, a Vβ repertoire analysis by flow cytometry was done. The $CD4^+$ TCR αβ T-cells showed 97% cells with Vβ 14 clonality [Figure 4]. The normal T-cells in the background showed a polyclonal pattern.

DISCUSSION

Large granular lymphocytes leukemia is predominantly a disease of the elderly; however cases have been reported at all ages. Clinically patients usually present with fever, recurrent infections, weight loss and splenomegaly. Studies have revealed that high levels of Fas produced by the leukemic clone block

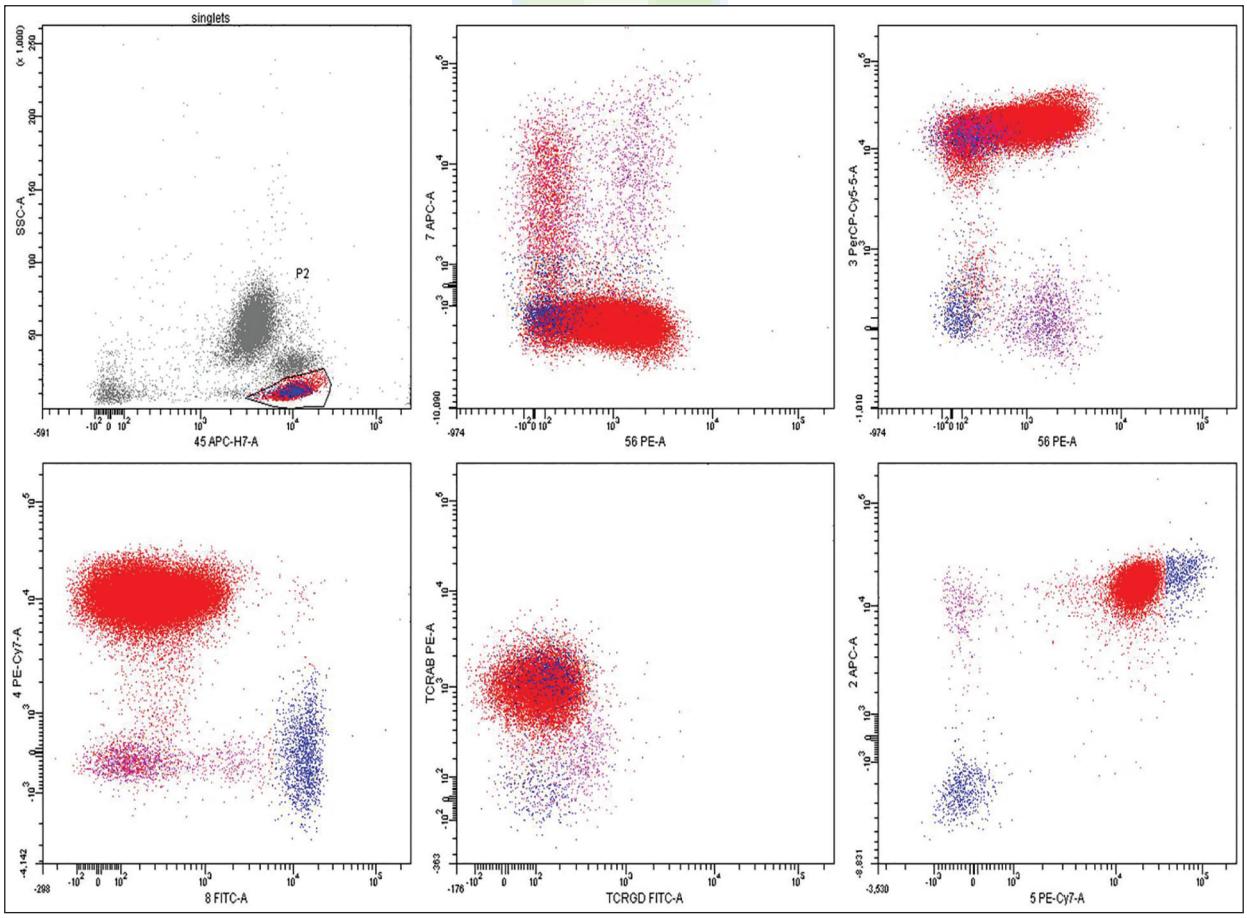

Figure 3: (Blue — normal lymhocytes, Pink — NK cells, Red– LGLs). The LGL leukemia cells express CD3, CD2, CD5, CD4, dim CD8, TCR αβ and heterogeneous CD56. Aberrant loss of CD7 is seen

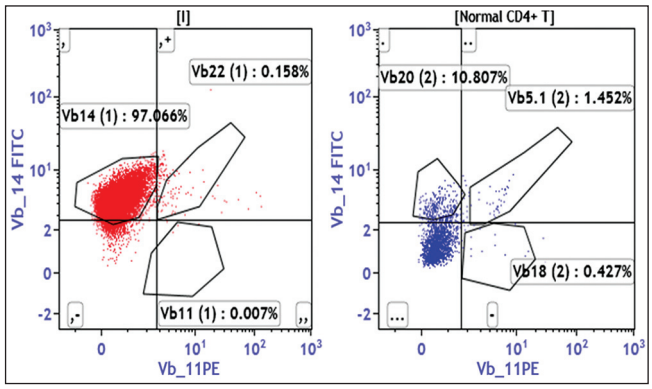

Figure 4: Vβ analyses showing clonality to Vβ14

the normal Fas mediated apoptosis of activated T-cells, thus contributing to the pathogenesis of this disorder.[2]

Most peripheral T lymphocytes express the αβ $CD3^+$ T-cell antigen receptor while only a few $CD3^+$ T-cells (1-6%) express an alternative γδ TCR. The γδ variant of T-LGL leukemia accounts for <5% of T-LGL leukemia cases.[1,5] Very few cases (around 30 cases only) of CD4/CD8 dual negative γδ T-LGL leukemia have been reported in literature.[5]

γδ T-LGL leukemias are frequently associated with neutropenia, anemia, thrombocytopenia and lymphocytosis similar to the more common αβ variants. The frequency of occurrence of anemia, neutropenia and lymphocytosis as found by Bourgault-Rouxel *et al*. in a study of 20 cases of γδ T-LGL leukemia was 10%, 70% and 60% respectively.[1] Although absent in the index case splenomegaly was reported in 35% of the cases. The frequency of anemia and splenomegaly was higher in the study by Chen *et al*. but that of neutropenia was similar. Chen *et al*., however reported that certain cases may show lack of peripheral blood lymphocytosis or increased LGLs despite bone marrow or splenic involvement.[5] Serological abnormalities occurring in these cases include antinuclear antibodies, rheumatoid factor, elevated LDH and β2-microglobulin levels. Associated autoimmune conditions include rheumatoid arthritis, autoimmune hemolytic anemia and pure red cell aplasia. The index case (case 1) of T-LGL leukemia of the TCR γδ subtype was not associated with any autoimmune condition, but did show presence of anemia, neutropenia (Absolute Neutrophil count 1.078×10^3/uL), lymphocytosis with LGLs and raised LDH levels.

The immunophenotype of γδ T-LGL leukemias as published in literature typically shows CD3+, $CD2^+$, $CD4^-$, variable CD8, variable CD5 (60%),variable CD7 (100%), $CD16^+$ (86%), variable CD56 (38%) expression.[6] In comparison in the index case (case 1) CD5, CD56 expression was not seen and it was CD4/CD8 dual negative. This is similar to results of Chen *et al*. who showed in their study of 7 γδ T-LGL leukemia cases that majority of their CD4/CD8 dual negative cases (71%) lacked CD5 and the NK associated CD56 antigen expression.

35-40% of γδ T-LGL leukemia cases are $CD4^-$, $CD8^-$ (index case) as compared to 3% of αβ cases. The remaining cases are $CD4^-$, $CD8^+$, with CD8 expression often being dim.[1,7,8]

γδ T-cell neoplasms such as hepatosplenic γδ T-cell lymphoma (most common) and other γδT-cell lymphomas are known to have aggressive clinical course and require aggressive chemotherapy. In contrast, most case reports show that γδ T-LGL leukemia have a more indolent course and good prognosis.[9] Thus, it is of utmost importance to recognize this entity and distinguish it from the more aggressive malignancies. Expression of CD57 and CD5 in γδ T-LGL leukemia by flow cytometry and morphological presence of LGL's on peripheral smear can be helpful in differentiating it from hepatosplenic T-cell lymphoma.[5,9]

As shown above, case 2 was diagnosed as a TCR αβ/$CD4^+$/$CD8^-$ LGL, which is an uncommon phenotype with very few case reports in literature. There are certain differences between TCR αβ/$CD4^+$/$CD8^-$ and $CD4^-$/$CD8^+$ T-LGL clonal proliferations. Chiefly, $CD4^+$ TCR $\alpha\beta^+$ cases are not associated with cytopenias and splenomegaly. Even though, there is no association with autoimmune disorders, there have been reports of $CD4^+$ T-cell LGL leukemia being associated with secondary malignancies.[10,11]

Literature review shows that TCR αβ/$CD4^+$ T-LGL typically express variable levels of CD8 ($CD8^-$/dim^+) as seen in the index case, a bright and homogeneous CD2 expression, homogeneous CD5, constantly heterogeneous and either dim or nearly absent reactivity for CD7 and always express NK associated antigens CD56 or CD57.[10] Above findings are very similar to the immunophenpotype of case 2 reported above.

All patients with monoclonal expansions of TCR αβ/$CD4^+$/$CD56^+$/$CD8^{-/+}$dim T-LGL usually have a major TCR-Vβ expansion. Vβ test results by flow cytometry are considered clonal if >50% of T-cells express 1 Vβ subtype and suggestive of clonality if 40-49% are of 1 Vβ subtype or if >70% of tested cells fail to react with any of the Vβ subtypes tested. No specific Vβ subtype is associated with T-LGL leukemia.[6] Index case showed clonality to Vβ 14 in 97% of the cells. The normal lymphocytes showed a polyclonal pattern.

$CD4^+$ T-LGL leukemias have an indolent, less aggressive course as compared to their $CD8^+$ counterparts. However, their association with secondary neoplasia (29% of the cases) warrants a high degree of suspicion in the diagnosis as also noted in the index case.[10] It has been hypothesized that their proliferation is an effort of the immune system to control tumor growth. Monoclonal expansions of $CD4^+$ T-LGL have only been sporadically reported in the literature and hence inadequate data are available regarding course and overall outcome of the disease.

Diagnosis of T-cell LGL leukemia requires a multidisciplinary approach using clinical findings, serological and basic hematological investigations along with use of flow cytometric immunophenotyping. Using a wide panel of antibodies can

ensure that there is accurate lineage subtyping and identification of aberrant antigen expression. Vβ repertoire analyses using flow cytometry can conclusively distinguish between polyclonal and monoclonal expansions of LGLs. An accurate diagnosis means that γδ T-LGL leukemia can be differentiated from more aggressive γδ malignancies as well as the more indolent (αβ T-LGL) variants. The monoclonal expansions of $CD4^+$ T-cell LGLs may precede the diagnosis of secondary malignancies that are commonly associated with this group. A close follow-up of these cases is thus essential to detect the development of related symptoms.

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