

A Rare Variant of Aggressive T-Cell Large Granular Lymphocyte Leukemia Associated With Hepatic Fibrosis and Trisomy 8: A Case Report and Literature Review

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Abstract

Aggressive T-cell large granular lymphocytic leukemia (T-LGL) is a rare entity with a poor prognosis, the main clinical findings being splenomegaly, lymphadenopathy, thrombocytopenia and a CD3⁺/CD56⁺ phenotype. A 21-year-old lady presented with hepatosplenomegaly, lymphadenopathy, “B” symptoms and thrombocytopenia. She subsequently developed jaundice, ascites and deranged liver function. LGL was detected in the peripheral blood and marrow. They had an aberrant CD3⁺/CD4⁻/CD8dim/CD56⁺ phenotype. Karyotyping showed trisomy 8 which has not previously been reported in aggressive T-LGL. A liver biopsy showed hepatic fibrosis with a sinusoidal LGL infiltrate. Her ascitic fluid also showed involvement by T-LGL. She had a poor response to cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) chemotherapy and opted for palliation. We performed targeted sequencing to detect mutations in the *STAT3*, *STAT5b* and *PTPRT* genes which were absent, suggesting the presence of an alternative genetic driver in our patient. We report an unusual case of aggressive T-LGL with an aberrant immunophenotype, trisomy 8 and hepatic fibrosis with absence of mutations in the STAT pathway. Our case describes novel features of aggressive T-LGL and the need for further studies to elucidate the genetic basis of this rare disease.

Keywords: Large granular lymphocyte; Leukemia; Hepatic perisinusoidal fibrosis; CD56; Trisomy 8

Introduction

T-cell large granular lymphocytic leukemia (T-LGL) is a rare

lymphoproliferative disorder characterized by persistent, monoclonal large granular lymphocytosis with a characteristic immunophenotype [1]. The association of T-LGL with autoimmune disorders suggests that sustained immune stimulation and dysregulated FAS ligand-mediated apoptosis may be important in the pathogenesis [2]. Symptoms are usually due to anemia or neutropenia and autoimmune manifestations such as rheumatoid arthritis are common [2]. Splenomegaly is the most common finding on physical examination [2]. The majority of patients have an indolent clinical course, although rare cases of aggressive disease have been described [3]. The diagnosis is established by the presence of circulating large granular lymphocytes (LGL) above $0.4 \times 10^9/L$ with an activated T cell immunophenotype (CD3⁺/CD8⁺/CD56⁻/CD57⁺ and/or CD16⁺) [3]. Clonality can be demonstrated by T-cell receptor (TCR) gene rearrangement studies while bone marrow (BM) studies may be required in patients with low circulating LGL numbers in the right clinical context [3]. Therapeutic options for indolent disease include immunosuppressive therapy with responses reported using methotrexate, cyclosporine and cyclophosphamide [3].

Although hepatic involvement in T-LGL has been reported, association with liver fibrosis has not to our knowledge been previously described [4]. We report a case of aggressive T-LGL associated with hepatic fibrosis and trisomy 8 on karyotyping with an atypical immunophenotype.

Case Report

A previously well 21-year-old Vietnamese lady presented in May 2012 with bruising, menorrhagia and abdominal distension of 5 months duration. She also reported fevers of 39 °C associated with weight loss of 9 kg and night sweats. Physical examination revealed hepatosplenomegaly with ascites. Initial investigations in Vietnam revealed a hemoglobin of 9 g/dL, a white cell count of $4.0 \times 10^9/L$ and a platelet count of $34 \times 10^9/L$. Serology tests for hepatitis B, C, HIV, cytomegalovirus and Epstein-Barr virus were negative. Anti-nuclear antibody and anti-double stranded DNA were negative, lactate dehydrogenase was normal and a direct Coomb's test was negative. Bone marrow aspiration (BMA) was not conclusive.

As her blood counts remained stable, she was managed expectantly until June 2012 when she underwent a splenecto-

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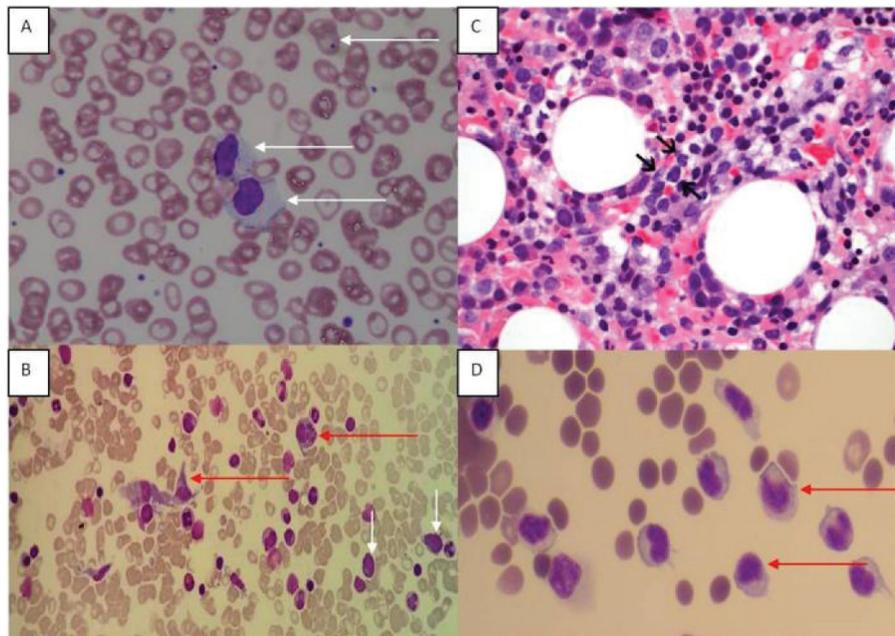


Figure 1. (A) Peripheral blood film showing large granular lymphoid cells. Howell Jolly bodies are seen in some red cells in keeping with a post-splenectomy picture. (B) Bone marrow aspirate (MGG stain) showing hemophagocytic activity and atypical lymphoid cells. Red arrows point to histiocytes, and white arrows indicate atypical lymphoid cells. (C) Bone marrow trephine showed a sinusoidal infiltrate of small to medium sized lymphocytes (arrows) with irregular nuclei and indistinct nucleoli (H&E, original magnification, $\times 600$). (D) Ascitic fluid showing atypical lymphoid cells.

my in view of persistent splenomegaly and worsening thrombocytopenia. Histopathology of the spleen revealed “lymphoid proliferation” but no evidence of malignancy. Her fever and thrombocytopenia improved transiently after the splenectomy but recurred in August 2012 at which point she was treated with pulsed methylprednisolone for 1 week with a transient improvement in the cytopenias. In October 2012, she was

found to have worsening jaundice with conjugated hyperbilirubinemia. A fibroscan score of F4 was recorded and she underwent a liver biopsy which suggested cholestatic liver injury with cirrhosis.

She was transferred to our center in January 2013 for further evaluation of her thrombocytopenia. At the time her main symptoms were those of worsening jaundice, ascites, fever

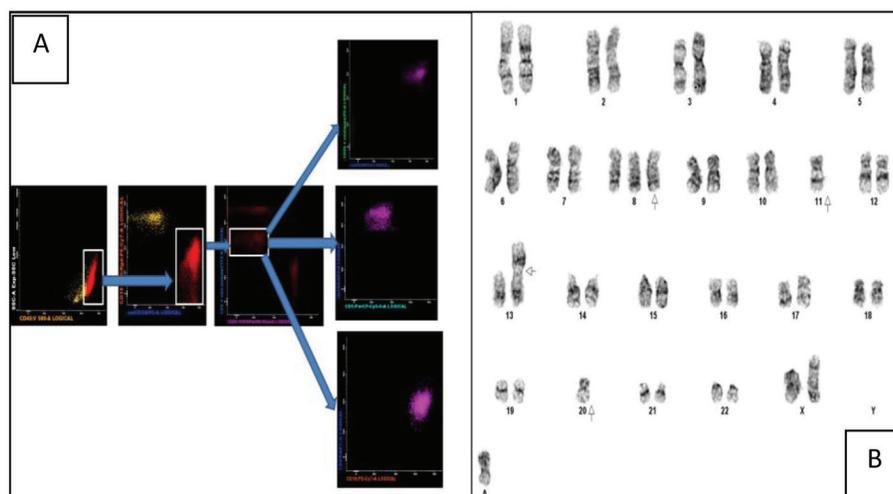


Figure 2. (A) Multiparametric flow cytometric analysis of the bone marrow specimen. The abnormal events are $CD45^+$ with a low side scatter. They are T cells as evidenced by $CD3$ positivity and lack of $CD19$ expression (the yellow events in the $CD19/CD3$ scatter plot are normal B cells). Among the $CD8^+$ T cells there is an abnormal $CD8dim$ sub-population which constitutes the neoplastic clone. These events are $CD5^-/CD56^+/CD16^+$ and $CD94^-$. (B) Karyogram showing trisomy 8, loss of chromosomes 11 and 20, a non-clonal abnormality of chromosome 13 and a marker chromosome.

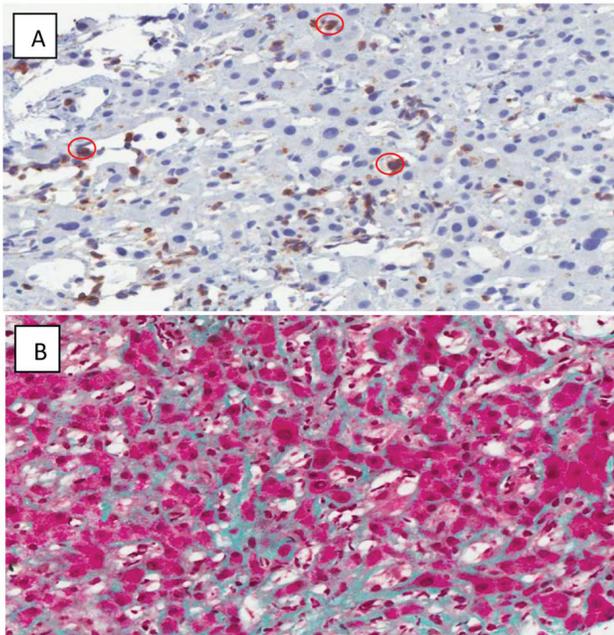


Figure 3. (A) CD 3 Immunoperoxidase highlights the T-cells circled in red. ($\times 400$) Some T-cells are somewhat larger than others. (B) Sinusoidal fibrosis. (Masson's trichrome stain, $\times 200$) The hepatocytes are red, and the collagen is green. Collagen is normally restricted to portal tracts and the cuff of central veins, but here is seen running along sinusoids between plates of hepatocytes.

and night sweats. She also described menorrhagia and gingival bleeding. Physical examination revealed marked cachexia with ascites, hepatomegaly and scleral icterus. She also had inguinal lymphadenopathy.

Her investigations were repeated, a full blood count showed hemoglobin 11.8 g/dL, white cell count $14.5 \times 10^9/L$, neutrophils $5.3 \times 10^9/L$, lymphocytes $5.0 \times 10^9/L$ and platelets $57 \times 10^9/L$. Liver function tests showed serum albumin of 34 g/L (38 - 48 g/L), elevated bilirubin at 40 $\mu\text{mol/L}$ (5 - 30 $\mu\text{mol/L}$) and predominantly conjugated (32 $\mu\text{mol/L}$). Aspartate transaminase and alanine transaminase were within normal limits. She was coagulopathic with a prothrombin time of 16.8 s (12.0 - 14.5 s) and an activated partial thromboplastin time of 42 s (27.0 - 35.6 s).

The peripheral blood film revealed target cells and a large granular lymphocytosis (Fig. 1A). Her BMA showed LGL comprising 9% of nucleated cells (Fig. 1B). The BM trephine showed a sinusoidal infiltrate of small to medium sized lymphocytes with irregular nuclei (Fig. 1C). Flow cytometry of the BM showed an abnormal population of large T cells with the following phenotype: $\text{CD}3^+/\text{CD}4^+/\text{CD}5^+/\text{CD}8^{\text{dim}}/\text{CD}56^+/\text{cyGranzymeB}^+/\text{cyPerforin}^+/\text{CD}16^+$. The aberrant $\text{CD}8^{\text{dim}}/\text{CD}5^-/\text{cyPerforin}^-$ phenotype supported the suspicion of a neoplastic T-LGL population (Fig. 2A). A 20-metaphase cytogenetic analysis of the BM revealed a karyotype of $46,XX,+8,-11,-20,+mar(cp3)/46,XX(17)$, indicating three metaphases with trisomy 8, loss of chromosomes 11 and 20, along with a marker chromosome (Fig. 2B). TCR gene rearrangement studies from the BM sample confirmed the presence of a clonal

T-cell population. In view of the presence of trisomy 8 on karyotyping, we performed immunohistochemical staining for C-Myc on the patient's BM trephine, and the malignant cells did not stain positive for C-Myc.

A CT scan of the abdomen showed an enlarged caudate lobe of the liver and extensive ascites with inguinal lymphadenopathy. A liver biopsy was repeated and showed diffuse perisinusoidal fibrosis, extramedullary hematopoiesis and $\text{CD}3^+$ lymphocytic infiltrate (Fig. 3). These were morphologically larger than normal lymphocytes and were negative for CD4 and CD8 on immunohistochemistry, strongly suggesting that these were T-LGL infiltrating the sinusoids.

She was treated with six courses of cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) chemotherapy (she was not fit enough to receive a more aggressive treatment protocol) and had a transient response with an improvement of her cytopenias. Within 6 months of completing her treatment, she developed a recurrence of cytopenias, T-LGL lymphocytosis and worsening ascites and lymphadenopathy. Cytology of the ascitic fluid showed involvement by T-LGL (Fig. 1D); the flow cytometric immunophenotype of the lymphoid cells detected in the ascitic fluid was identical to that seen in the BM previously.

In view of the availability of a clinical trial of a *STAT3* inhibitor in our center, we performed targeted sequencing of the patient's diagnostic BM sample to identify *STAT3* mutations which would have made her a candidate for the clinical trial. Unfortunately the *STAT3* mutation was negative which made her ineligible for the study. She subsequently returned to Vietnam for palliative treatment.

Discussion

Most patients with T-LGL have an indolent clinical course; although aggressive T-LGL has been reported, these cases are rare [5]. Gentile et al reported three patients with aggressive LGL leukemia - the main clinical features present in all three cases were splenomegaly, lymphadenopathy and B symptoms [6]. Recurrent infections and rheumatoid arthritis which are characteristic of indolent LGL were notably absent in these patients [6]. Neutropenia which is commonly reported in indolent T-LGL was not reported in any of the patients while all three had thrombocytopenia which is unusual in indolent T-LGL [6]. Peripheral blood immunophenotyping revealed a $\text{CD}3^+/\text{CD}4^+/\text{CD}8^-/\text{CD}16^+/\text{CD}57^-/\text{CD}56^+$ expression in this series [6]. Based on the differences in clinical features and consistent $\text{CD}3^+/\text{CD}56^+$ phenotype, the authors proposed $\text{CD}3^+/\text{CD}56^+$ aggressive T-LGL as a distinctive clinical entity [6]. Matutes and colleagues described a case of T-LGL which transformed into a large T-cell lymphoma 11 years from the original diagnosis [7]. The main clinical features at transformation were those of splenomegaly, fever and lymphadenopathy with a $\text{CD}3^+/\text{CD}4^-/\text{CD}8^+/\text{CD}56^+$ T-cell phenotype on immunohistochemistry of the lymph node biopsy [7]. Our patient had clinical features similar to the cases described by Gentile et al as well as a $\text{CD}3^+/\text{CD}56^+$ phenotype. Given the $\text{CD}4^-/\text{CD}8^{\text{dim}}$ phenotype, hepatic infiltration and aggressive disease course, hepatosplenic

T-cell lymphoma (HTL) was considered a differential diagnosis. The T cells were however negative for TCR- γ/δ which together with the positive expression of granzyme B (rare in HTL) makes T-LGL the more likely diagnosis [1].

Cytogenetic abnormalities associated with T-LGL have not been clearly defined, mostly due to the rarity of the disease and the classification of some cases as T-chronic lymphocytic leukemia [8]. Loughran et al described two cases of indolent T-LGL with trisomy 14 and trisomy 8, respectively [9]. Trisomy 8 was also described in a case of CD3- NK- LGL leukemia with aggressive transformation [10]. Trisomy 8 has been shown to predict an adverse outcome in acute myeloid leukemia and has been implicated in blast phase transformation of chronic myeloid leukemia [11, 12]. Amplification of the C-Myc oncogene located on chromosome 8q has been postulated as a mechanism of the adverse outcome associated with this cytogenetic abnormality [12]. Ours is the second report of trisomy 8 in T-LGL and the first in the rare, aggressive subset of this disease. It is possible that trisomy 8 may play a role in the transformation of T-LGL from an indolent to an aggressive disease and may have prognostic significance.

Hepatic fibrosis has not previously been described in T-LGL. Loughran et al described four patients with aggressive T-LGL, two of whom underwent a liver biopsy in view of abnormal liver function tests [13]. A neoplastic lymphoid infiltrate was detected in the hepatic sinusoids, with hepatocyte apoptosis demonstrated by TUNEL positivity [13]. It was postulated that the hepatocyte apoptosis was mediated by dysregulated FAS-FAS ligand signaling, as this was supported by the finding of increased serum FAS ligand levels and FAS being expressed on the hepatocytes [13]. They also demonstrated hepatocyte and pneumocyte apoptosis in FAS ligand transgenic mice [13]. The reason for hepatic fibrosis in our patient was not clear. It is possible that the fibrosis was a response to hepatocyte injury resulting from T-LGL infiltration. As dysregulated expression of growth factors such as transforming growth factor β has been shown to be important in the pathogenesis of hepatic fibrosis, it may be postulated that T-LGL infiltration leads to aberrant expression of such mediators [14].

Mutations in the *STAT3* (signal transducer and activator of transcription 3) gene have been demonstrated in LGL [15]. Whole exome sequencing (WES) of 77 patients with LGL identified *STAT3* mutations in 31 patients (40%), Y640F in 22 (17%), D661Y in 12 (9%) and N647I in 12 (9%). The presence of *STAT3* mutations was associated with neutropenia and rheumatoid arthritis in this cohort. In another study looking at T and NK LGL patients, it was shown that 48 of 170 (28%) of patients had a *STAT3* mutation, with similar incidence among T and NK subtypes of LGL [16]. Importantly it was demonstrated that even patients without *STAT3* mutations had increased expression of *STAT3* target genes [15]. The gene expression profile of LGL patients with and without *STAT3* mutations had also been shown to be similar [17]. This led to the hypothesis that other genes involved in *STAT3* signaling may be dysregulated in patients with LGL without *STAT3* mutations. Protein tyrosine phosphatase receptor 3 (PTPR3) is a tumor suppressor gene whose normal function is to reverse tyrosine phosphorylation on *STAT3* leading to a deactivation of *STAT3* signaling. WES of a patient with LGL without *STAT3* mutations identified a mis-

sense mutation of PTPR3 (V995M). It was hypothesized that this PTPR3 mutation may be responsible for abnormal *STAT3* signaling in LGL patients who lack *STAT3* mutations [17].

Rajala and colleagues recently demonstrated that *STAT5b* mutations may be a driving genetic lesion in LGL without *STAT3* mutations [18]. They described three T-LGL patients with *STAT5b* mutations, two of them had the Y665F mutation while the other one had the N642H mutation. The patients with the Y665F mutation had an indolent clinical course with no treatment required but the N642H patient had a very aggressive disease with extensive organ infiltration and a poor response to chemotherapy. Interestingly the N642H patient had a CD3⁺, CD56⁺ phenotype similar to our patient [18].

Given the clinical and immunophenotypic similarity of our patient with the *STAT5b* N642H mutated patient described above, we performed targeted capillary sequencing to detect somatic missense mutations in the *PRPRT* and *STAT5b* genes. Primer sequences were adapted from previous reports [17, 18], but PCR conditions for targeted capillary sequencing of *STAT5b* exons 14, 15, 16, 17, 18 and *PTPRT* exon 23, were optimized in-house. We did not identify any mutations at these loci (and she had no *STAT3* mutations) suggesting that an alternative genetic driver may be present in our patient.

Although *STAT3* inhibitors are in clinical trials, studies investigating *STAT5b* inhibition are awaited. It is important to further clarify the underlying genetic abnormalities in *STAT3* mutation negative LGL, especially those with the rare aggressive variant like ours who had a suboptimal response to conventional chemotherapy [19].

Conclusion

In conclusion, our patient has a rare aggressive variant of T-LGL with the unusual features of trisomy 8 on cytogenetics and hepatic fibrosis. This case adds to the limited data on aggressive T-LGL and supports the proposal that CD3⁺/CD56⁺ aggressive T-LGL is a distinct clinical entity. Further research is required to better define the clinicopathological and genetic features of aggressive T-LGL and elucidate the effects of tissue infiltration by this rare leukemia. There is an urgent need for international clinical trials to determine the optimal therapy for this aggressive disease. For young patients, early consideration of stem cell transplant is important in view of the poor outcome with currently available therapy.

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Conflict of Interest

None.

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None.

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