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RESEARCH ARTICLE

Outcome of T- Large Granular Lymphocyte Leukemia from a Tertiary Care Centre in North India

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Abstract

Introduction: T-cell large granular lymphocytic leukemia (T-LGL) is a rare disorder with a frequency of less than 5% of the lymphoproliferative disorders (LPD) . T-LGL is characterized by persistent increase in LGLs (2 to 20×10^9 /L) on peripheral blood in absence of a reactive cause. **Material and methods:** In this retrospective study for a period of 66 months (January 2019 to June 2024), all the samples received in the flow cytometry lab with a suspicion of LPD were screened. A stain-lyse-wash protocol was used and samples were stained with Two to three tubes of 8-10 color combinations. The clinical and laboratory features of the patients diagnosed as T-LGL were retrieved from computerized Hospital Information System and were further analyzed. **Results:** A total of 341 samples were analysed during this period which were diagnosed as B cell neoplasm 87%, T cell neoplasm 8%, NK-cell neoplasm 1% and reactive lymphoid proliferation 4%. The T LGL comprised of 10 (2.9%) cases. Mean age of presentation was 57.3 years, with a male:female ratio of 1.25:1.Approximately 60% patients had BM involvement, 50% had autoimmune disorder and 40% had splenomegaly. Patients were treated with corticosteroids, weekly methotrexate and cyclosporine, if required. 7/10(70%) patients are on follow up, are stable and in remission. Two patients died while one was lost to follow up. **Conclusion:** The frequency of T LGL noted in our study was 2.9% of the lymphoproliferative disorders. T LGLs had an indolent course and responds well to treatment.

Keywords: T- cell large granular lymphocytic leukemia- Diagnosis- Treatment

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Introduction

Large granular lymphocytes (LGL) comprise of cytotoxic T-cells and NK cells, and morphologically have round to reniform nucleus, clumped chromatin, moderate cytoplasm with large azurophilic granules that contain proteins involved in cell lysis (perforin and granzyme B) [1, 2]. These cells play a role in destroying virus infected cells and tumor cells [2]. In normal adults they constitute ~10-15% of mononuclear cells in peripheral blood [1]. LGL leukemia is characterized by clonal proliferation of these lymphocytes,which depending upon the type of cell, can be T-LGL leukemia or NK cell leukemia.

Earlier, the diagnosis was made by an observation of increase in T-LGL level (2-20×10^9 /L) in the peripheral blood for >6 months in absence of any reactive cause [2-5]. Currently, the diagnosis can be made with evidence of clonality (determined by TCR rearrangement via PCR or Vβ reterpoire by flow-cytometry)even on lower cell count (≥0.5-2 x10^9 /L) [1, 2, 4, 6].

It is a rare disorder, with an incidence of <5% of Chronic Lympho-Proliferative Disorders (CLPD) [3, 5, 6, 7, 8]. T-LGL leukemiais seen in 6th decade of life with equal incidences in both male and females [2, 5, 8, 9]. Patients usually present with anemia and neutropenia, apart from an increase in number of LGL [1, 3, 5]. Around one third patients remain asymptomatic and are diagnosed incidentally on routine blood tests [1, 3, 6]. Recurrent respiratory tract infection and association with autoimmune disorders like immune thrombocytopenia (ITP), autoimmune hemolytic anemia AIHA), pure red cell aplasia (PRCA), rheumatoid arthritis (RA) is seen

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[1, 3, 8]. Infiltration of LGL can occur in bone marrow, spleen and liver leading to cytopenia, splenomegaly and/or hepatomegaly [1, 5]. Typical 'B-symptoms' and lymphadenopathy is usually not seen [2, 7]. NK-LGL leukemia on the other hand is known to be an aggressive disease, presenting with organomegaly, B-symptoms, increased LGL count and massive bone marrow infiltration. It is more common in Asian population and associated with Epstein-Barr virus infection. It is refractory to treatment, hence prognosis is poor [8, 9].

Immunophenotyping is usually done in the peripheral blood which shows the neoplastic clone to be derived from T-cell lineage (in ~85%) and from Natural Killer cell lineage (in rest ~15%) [1].T-LGL Leukemia cellsmay express (i) CD3+, CD8+, CD57+ and TCRαβ+; (ii) CD3+, CD8-, CD4+, CD57+, TCRαβ+ and (iii) CD3+, CD4-, CD8-, TCRγδ+ (rare, ~10%) [1, 4, 7, 9]; while NK-LGL Leukemia cells express CD2, cCD3, CD16, CD56 and CD57 [1, 6]. T-LGL leukemia is characterized by downregulation of CD5 and/or CD7 [2, 7], while CD27 and CD28 is negative [9]. CD56 expression is related to aggressive disease and recurrent infections, hence require different treatment than usual phenotype [2, 3, 6, 8]. Flowcytometric analysis of TRBC-1 (constant region 1 of T-cell receptor β-chain) expression has been recently proved to be a simple, specific, rapid and accurate method to determine Tαβ-cell clonality [10].

Treatment is required when there is cytopenia (ANC <500/cumm), recurrent infection, progressive splenomegaly, pure red cell aplasia/transfusion dependent anemia, autoimmune disease and B symptoms [3, 6, 7, 9]. Immunosuppresants are the mainstay of treatment; consisting of low dose methotrexate as first line, cyclosporine A and corticosteroids as second line treatment [5, 7]. Prednisone is used as adjunct to these drugs and provides rapid stabilization of blood counts6. G-CSF (for neutropenia) and EPO (for PRCA) were not found effective on long term treatment [9]. Newertreatment options include Purine analogs andmonoclonal antibodies like Alemtuzumab (anti CD52) [4, 7]. Role of targeted therapy against membrane receptors JAK-STAT (Tofacitinib) and NkFB is under trial [4, 8].

The disease is known to be indolent and responds well to treatment [1]. Spontaneous regression of disease is also reported [5, 9]. Median survival is more than 10 years [3]. Death if occurs (<10%), is usually due to neutropenia associated severe infection and sepsis rather than disease progression [4, 5, 6, 8, 9]. Thus, treatment is indicated to correct cytopenia and reduce proliferation of clonality rather than to eliminate T-LGL clone [4].

The present study was conducted to study the clinical features, immuno-phenotype, laboratory parameters and the course of disease in an Indian scenario over a time periodof 5.5 years.

Materials and Methods

This was a Retrospective, observational, single center study over a period of 66 months (January 2019 to June 2024). All the patients with suspicion of CLPD for which peripheral blood/ bone marrow aspirate samples were received in flow-cytometry lab, Hematology departmentwere included.Of the total CLPDs, we retrospectively retrieved cases diagnosed as T-LGL leukemia on flow-cytometry. The clinical and laboratory features of these patients were retrieved from computerized Hospital Information System.

Immunophenotypic study was performed on peripheral blood or bone marrow sample using flow cytometry. Samples were stained as per standard stain-lyse-wash protocol with 2-3 tubes of 8-10 color combinations. The antibodies in the panel included CD45, CD19, CD20, CD10, Kappa, Lambda, CD56, CD2, CD3, CD4, CD5, CD7, CD8 and CD57. CD26, CD28, TCR alpha beta, TCR Gamma Delta and TRBC1 was added in all cases. Data acquisition and analysis was performed on BD FACS Lyric cytometer using BD FACSuite software.

Clinical details and follow up was obtained from electronic medical records.

Results

A total of 341samples with a suspicion of CLPD were received for flow-cytometry analysis in this duration (January 2019 to June 2024). The final diagnosis rendered after evaluation was mature B-cell neoplasm in 87% cases, T-cell neoplasm in 8%, NK cell neoplasm (1%) and reactive lymphoid proliferation in 4% cases. T-LGL leukemia comprised of 10 (2.9%) cases out of total CLPDs. Mean age of presentation was 57.3 ± 11.4 year with a range of 38-74 year. No sex predilection was noted (M:F= 1.25:1).

Clinical features: Most common presenting symptom was fatigue and shortness of breath (70%), followed by splenomegaly (40%), hepatomegaly (30%), fever (20%), joint pain (20%) and lymphadenopathy (10%). One patient was completely asymptomatic and was diagnosed with lymphocytosis on routine blood examination. The clinical features are better depicted in bar chart (Figure 1). Associated autoimmune disorder was noted in some patients which included Pure Red Cell Aplasia most commonly (30%), followed by Rheumatoid arthritis (10%) and Inflammatory polyarthritis (10%).

Lab findings: Lymphocytosis was the universal finding in peripheral blood with presence of large granular

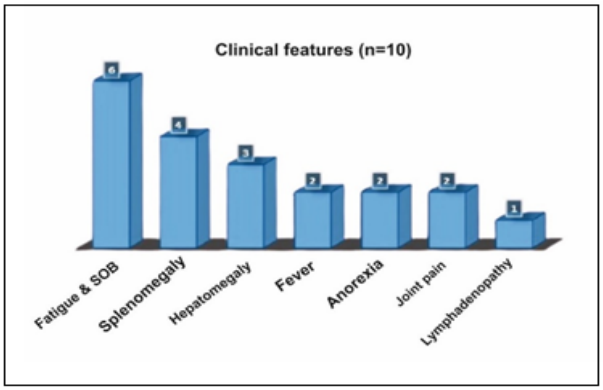

Figure 1. Clinical Features in Bar Diagram

Table 1. Immunophneotypic Features on Flow Cytometry of Individual Cases. (only T cell markers are shown)

Case No	CD3	CD2	CD4	CD8	CD5	CD7	CD16	CD56	CD57	TCR-αβ
1	+	+	-	+	ND	Dim	-	-	ND	+
2	+	+	-	+	ND	Dim	-	-	ND	ND
3	+	+	-	+	Dim	+	-	-	Dim	+
4	+	+	-	+	Dim	-	-	-	ND	ND
5	+	+	-	+	Dim	Dim	-	-	ND	ND
6	+	+	-	+	ND	+	-	-	+	ND
7	+	+	-	+	Dim	-	-	-	+	ND
8	+	+	-	+	Dim	Dim	-	-	+	ND
9	+	+	+ in one population	+ in other population	Dim	Dim	ND	+	+	+
10	+	+	-	+	Dim	Dim	-	-	+	+

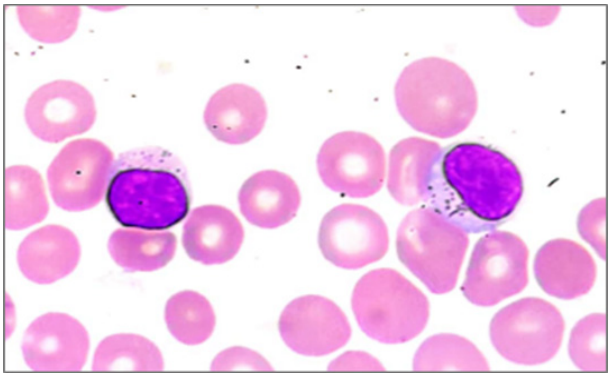

Figure 2. Peripheral Smear Shows Large Granular Lymphocytes withabundant Cytoplasm and Azurophilic Granules. (Leishman stain 40x)

lymphocytes (Figure 2). Anemia was present in 6 out of 10 patients (60%) with very severe anemia (Hb <6 g/dl) in 3 patients. Transfusion dependent anemia was seen in 4 (40%) patients. Leucocytosis was seen in 5 out of 10 patients (50%). One patient presented with thrombocytopenia, whereas one patient had thrombocytosis. Bone marrow examination was performed in 6 out of 10 patients and all showed lymphomatous infiltration (60%). Interstitial lymphocytic infiltration was most commonly noted. Immunophenotyping was performed by flow-cytometry and results of individual cases are shown in Table 1. One of the case of asymptomatic T LGL Leukemia showed two different clones, one expressing CD4 while other expressing CD8. Representative scatter plots are shown in Figure 3.

Treatment: Immunosuppressants were the mainstay of treatment. Weekly Methotraxate, Cyclosporine and Wysolone were most commonly used.

Follow up: Follow-up was available in 7 out of 10 patients, while 1 patient was lost to follow-up. Duration of follow-up period was median 30 months and ranged from 24 to 60 months.7 out of 9 patients responded well to treatment and are currently under observation. Two patients died due to severe upper respiratory tract infection. One asymptomatic patient is still under observation with regular blood counts. Overall response rate and overall survival was seen in 77.8% of patients.

Discussion

T-LGL is uncommon T cell neoplasm with varied presentation. The pathogenesis of T-LGL leukemia remains unclear, but several mechanisms are proposed. Chronic antigenic stimulation may drive cytotoxic T-cell clonal expansion, aided by escape from Fas-mediated apoptosis and activation of survival pathways [9]. Platelet-derived growth factor and interleukin-15 play critical roles in promoting LGL survival through apoptosis dysregulation. Some patients exhibit antibodies against proteins homologous to HTLV1/2, suggesting retroviral involvement. Mutations in STAT3, a key JAK-STAT pathway protein, promote leukemic T-cell

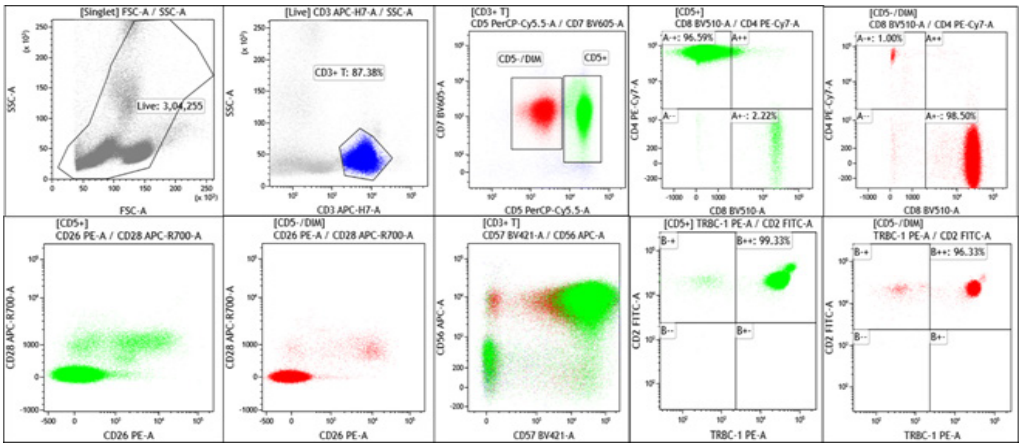

Figure 3. Flowcytometry Plots Demonstrating a Case of Asymptomatic T-LGL with Two Clones

Table 2. Comparison of the Present Study with other Similar Studies

S. No.	Studies	No of cases	Study duration (years)	Median age (years)	M:F	M/C Associated autoimmune condition	M/C presentation	Abs lymphocyte count	Neutro-penia	Anemia	Thrombo-cytopenia	BM involved	Treatment given
1	Our study (India)	10	5.5	57.3	1:25:1	PRCA	Fatigue and breathlessness, f/b mild splenomegaly	1 – 21x 10^9	20%	80%	10%	60%	Methotrexate & Cyclosporine
2	Aribi A.1 (USA)	42	8	58.5	1:01	Rheumatoid arthritis	Asymptomatic 74%	0.6 – 24 x 10^9/L	47.60%	40.50%	14.30%	100%	Cyclosporine
3	Rashid A.3 (Pakistan)	4	15	61	3:01	None	Asymptomatic 75%	7.8 – 20 x 10^9/L	-	-	-	100%	Methotrexate
4	Gupta N5 (India)	1	--	61	F	Rhematoid arthritis	Fatigue, breathlessness, and mild splenomegaly	8.1 x 10^9/L	Present	Present	No	Yes	Methotrexate
5	Sylvia MT2 (JIPMER, India)	1	--	38	F	--	Abdominal pain and fever	81 x10^9/L	Present	Present	Present	Yes	Prednisolone

proliferation and anti-apoptosis. Rare STAT5b mutations (~2%) are linked to aggressive CD4+ T-LGL leukemia [2, 6, 7]. These factors collectively highlight a complex interplay of immune dysregulation and genetic alterations.

The incidence of T-LGL leukemia was low (~3%) in our study, similar to that reported in western countries [1, 3, 4]. Sex ratio and Median age at presentation was similar to that reported in literature [1, 3]. In other studies, majority of the patients were asymptomatic at the time of presentation with persistent leukocytosis on routine blood investigations [1, 3] while in our study only one patient was asymptomatic. Persistent lymphocytosis was the universal finding as seen in other studies. Thrombocytopenia was not seen and its rare association is proven in literature [5, 7]. Recurrent infection and symptomatic anemia are the most common indications of treatment [1]. Lymphadenopathy was rare finding as known from literature [5]. Following Table 2 describes the comparison of T-LGL leukemia in various studies.

Flow-cytometric immunophenotyping results showed that majority of cases showed positive expression of CD3, CD2, CD8, CD57 and TCRαβ; CD5 and/or CD7 was downregulated, as found in other previous studies [5]. Rare cases with presence of uncommon variants which express CD4 ± CD8 (as one case in our study which showed two clonal populations) are known to occur in literature [5].

Gujral S. et al from India had studied immunophenotyping of mature T & NK cell neoplasm presenting as leukemia. They studied 380 consecutive samples of mature lymphoid neoplasm presenting as leukemia over a four year period (2003-2007) and found majority as B-NHL and only 9/380 as mature T & NK cell neoplasm. Of these nine, only four (1%) were diagnosed as T-LGL leukemia. Immunophenotyping of T-LGL cases revealed majority CD8+, CD3+, CD5+, TCRαβ+, CD4-, CD56- and CD7- [11].

Schreiber J et al had described two patients of T-LGL leukemia one was asymptomatic middle aged male with absolute lymphocytosiswithout anemiaor thrombocytopenia and no history of autoimmune disorder, lymphadenopathy or hepatosplenomegaly. Flow-cytometry showed ~45% clonal (γδ) T-cells which showed positive expression of CD2, CD3, CD5, CD7, CD16, CD56 but negative for CD4 and CD8. Bone marrow biopsy showed interstitial and sinusoidal infiltration of atypical lymphocytes. As the patient was asymptomatic he was kept under observation. The other patient was elderly male who presented with anemia and thrombocytopenia. Peripheral smear showed slightly increased number of circulating LGL. Serological examination showed antibodies against glycoprotein IIb/IIIa and Ib/IX, confirming ITP. Flow-cytometry showed abnormal population of γδ T-cells (~42%) which showed positive expression of CD3, CD8 (dim), CD57 (dim), CD56 (dim) and negative for CD16. Bone marrow revealed PRCA, dys-megakaryopoiesis and discrete along with intra-sinusoidal population of CD3 and CD8 positive T-cells. Parvovirus serology was negative. This patient was started on Cyclophosphamide and his symptoms improved after treatment [7].

Rabade N. et al from India also reported two cases of the rare variants of T-LGL leukemia, TCRγδ+ and CD4+ TCRαβ+. One was an 83 old female with anemia and lymphocytosis. Peripheral blood showed LGLs. Flowcytometric analysis revealed positive expression of

CD3, CD7 (heterogenous), CD2 (dim) and TCRγδ+; while negative for CD5, CD56, CD4, CD8 and TCRαβ. He also emphasized that TCRγδ+ T-LGL leukemia needs to be differentiated from other γδ+ T-cell malignancies due to aggressive nature of latter. The other patient was 85 year old male already diagnosed with liposarcoma,with lymphocytosis and LGLs on peripheral smear as incidental finding. Flowcytometric immunophenotyping showed positive expression of CD3, CD2, CD4, CD5, CD56 and TCRαβ; CD8 (dim) and loss of CD7 expression. CD4+ T-LGL leukemia is known to have an indolent course than CD8+ counterpart and is usually not associated with cytopenia [12].

Immunomodulation by agents like prednisolone, methotrexate and cyclosporine is the mainstay treatment [1]. In this study, cyclosporine was the most common drug used; which is in collaboration with other studies [1, 4]. Prednisolone is less commonly used as it requires long term therapy and risk of worsening infection along with other side effects. Ojusi et al had described 23 patients treated with cyclosporine and 8 patients treated with methotrexate and found overall response rate of 78% and 85%, respectively [13].

Bone marrow remission has been reported in very few studies, as mostly it is not performed in patients who achieve clinical remission. In study by Aribi A. et al, bone marrow examination was performed in 3 out of 7 patients who achieved clinical remission and they found that two patients had persistent foci of T-LGL cells while one patient had clinical remission on bone marrow. In the two cases with persistent foci of T-LGL cells, PCR and flow-cytometry was also performed on bone marrow and both of them showed positive results [1].

In our study, T-LGL leukemia represented as an indolent disease with relatively long overall survival as reported earlier [1, 3]. Two patients had died, but their death was attributed due to severe infection and not as a result of disease.

In conclusion, the frequency of T-LGL leukemia in our study was 2.9 % of total CLPD, which is from a large Indian cohort with data on the frequency and charactersistics of T-LGL. Careful peripheral blood smear examination is required for its diagnosis as many patients are asymptomatic at presentation with persistent lymphocytosis and neutropenia. Immunophenotyping is essential for its diagnosis. It has an indolent course and responds well to treatment. Asymptomatic patients should be under regular follow-up with wait-and- watch approach. Treatment in early stage should be restricted to supportive management and only few drugs are reserved for symptomatic disease. Role of targeted therapy and immunotherapy need to be studied in future. Long term follow-up also needs to be studied.

Limitation

TCR gamma chain gene rearrangement study by PCR was not performed. This could have helped in further confirmation of T-LGL leukemia diagnosis.

Acknowledgments

Statement of Transparency and Principals:

- Author declares no conflict of interest
- Study was approved by Research Ethic Committee of author affiliated Institute.
- Study's data is available upon a reasonable request.
- All authors have contributed to implementation of this research.

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