Original Study

Single-center Series of Bone Marrow Biopsy-Defined Large Granular Lymphocyte Leukemia: High Rates of Sustained Response to Oral Methotrexate

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Abstract

A total of 39 patients were diagnosed with large granular lymphocyte leukemia using stringent diagnostic criteria. Of the 39 patients, 15 (38%) were untreated. Of the remaining 24 patients, 13 were initially treated with prednisolone. The overall response rate (ORR) was 84.6%, and the median duration of response (DOR) was 13.5 months. For the 9 patients who received oral methotrexate, the ORR was 89% and the median DOR was 132.7 months. Five patients received methotrexate after prednisolone failure, with a treatment response in all 5. Single-agent oral methotrexate results in long responses with minimal toxicity.

Introduction: Large granular lymphocyte (LGL) leukemia is a rare chronic lymphoproliferative disorder, with few large series reported to date. Series using stringent diagnostic criteria incorporating bone marrow biopsy (BMB), immunophenotyping, and T-cell receptor rearrangements are even scarcer. **Patients and Methods:** The present study was a single-center series of 39 patients with LGL leukemia diagnosed using immunohistochemical analysis of BMB samples and flow cytometric and molecular data. **Results:** With a median follow-up of 3.2 years (range, 1.0-15.1 years), 15 patients (38%) never required treatment. Of the remaining 24 patients requiring treatment, 13 were initially treated with prednisolone, for an overall response rate (ORR) of 84.6% and a median duration of response (DOR) of 13.5 months (range, 5.7-70.3 months). Of the 24 patients, 9 received oral low-dose weekly methotrexate as first-line therapy, with 8 (89%) achieving a hematologic response and a median DOR of 132.7 months (range, 6.7-180.5 months). Another 5 patients received methotrexate after prednisolone failure; all 5 responded, with a median DOR of 14 months (range, 4-96 months). Only 2 patients developed progression during methotrexate therapy, and 4 patients experienced responses lasting $\geq$ 5 years. **Conclusion:** Single-agent oral methotrexate appears to be highly efficacious, resulting in long response durations and minimal toxicity.

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Introduction

Large granular lymphocyte (LGL) leukemia is a rare, chronic lymphoproliferation of activated cytotoxic CD8+ T cells or occasionally natural killer (NK) cells.[1-5] Reported series incorporating $\geq$ 10 patients are scarce, with differences in diagnostic definitions limiting interstudy comparisons. To date, investigators have chiefly relied on peripheral blood samples for the diagnosis, typically defined as a persistent excess ($> 0.5 \times 10^9$/L) of LGLs, associated with monoclonal T-cell receptor (TCR) gene rearrangements by polymerase chain reaction (PCR). However, it has been increasingly appreciated that well-characterized cases of LGL leukemia can initially present with lower circulating LGL numbers.[6,7] Moreover, benign monoclonal CD8+ T-cell expansions, phenotypically indistinguishable from T-LGL, are well recognized in healthy elderly individuals and those with autoimmune disease or viral infections.[8-11] Correlative bone marrow

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Table 1 Patient Characteristics

Characteristic	n (%)
Patients	39 (100)
Age (y)	
Median	66
Range	21-90
Sex	
Male	20 (51)
Female	19 (49)
Splenomegaly	2 (5)
Hepatomegaly	2 (5)
Lymphadenopathy	1 (2.5)
Recurrent infection	8 (21)
Rheumatoid arthritis	6 (15)
Autoimmune disease	11 (28)
Pure red blood cell aplasia	2 (5)
LGL-related death	3 (8)
Malignancy-related death	2 (5)
Total deaths	9 (23)
Median lymphocytosis ($\times 10^9$/L)	3 (8)
LGLs $>4 \times 10^9$/L	14 (36)
LGLs $<4 \times 10^9$/L	25 (64)
Neutropenia $<0.5 \times 10^9$/L	10 (26)
Anemia <8 g/L	4 (10)
Thrombocytopenia $<100 \times 10^9$/L	7 (18)
Polyclonal hypergammaglobulinemia	8 (21)
Monoclonal gammopathy	3 (8)
Rheumatoid factor	11 (28)
Antinuclear antibody	6 (15)

Data presented as n (%).
Abbreviation: LGL = large granular lymphocyte.

biopsy (BMB) data within these larger series have been restricted to a few patients, presumably owing to the heterogeneity of clinical practice across multiple treatment centers. Although the World Health Organization classification of lymphoid neoplasms incorporates BMB findings into the diagnostic algorithm for LGL disease, this has not been mandated for diagnosis.[12] Reliable identification of an intrasinusoidal and interstitial infiltration of LGLs is not possible solely by morphologic review and requires a panel of antibodies directed against T-cell and NK-cell lineage markers and cytotoxic effector proteins such as TIA1, granzyme B, and perforin.[13]

Currently, no standard of treatment is available for LGL disease.[14,15] A watch-and-wait approach is applicable for patients with indolent asymptomatic LGL leukemia. For symptomatic patients, the first-line regimen is typically immunosuppressive therapy in the form of single-agent methotrexate (MTX), cyclosporine A (CYA), or cyclophosphamide.[7,14] The response is assessed at 4 to 6 months, with current guidelines recommending stopping treatment at 4 months for nonresponsive or progressive disease.[14,16] Most previous studies (summarized by Lamy et al[14]) have included < 10 patients, and many did not report the duration of treatment and/or the duration of response (DOR) to MTX. The optimal duration of immunosuppressive therapy has not been defined. In the recently published Eastern Cooperative Oncology Group 5998 protocol, patients achieving complete remission (CR) with MTX were scheduled to stop therapy 1 month after documentation of the CR, and the maximum therapy duration for patients with partial remission (PR) was 1 year.[15] Cyclophosphamide is typically used for those without a response to MTX, with a recommended duration of 6 to 12 months owing to the potential mutagenic effects.

To distinguish bona fide LGL leukemia from monoclonal T-cell populations of other etiologies, the standard practice within our institution has been to routinely perform BMB with immunohistochemistry (IHC) and flow cytometry and TCR gene rearrangement studies at the initial diagnosis. We studied the disease characteristics and clinical outcomes of a consecutive cohort of patients with BMB-defined LGL leukemia from a single center with a sufficient follow-up duration. In contrast to previously published data, we found high rates of durable responses to single-agent MTX.

Patients and Methods

All patients were diagnosed with LGL leukemia at Nottingham University Hospitals National Health Services Trust from 1990 to 2011. In each case, the diagnosis was established using a combination of flow cytometry for either activated T-LGL (CD3+/CD8+/CD57+) or NK-LGL (CD16+/CD56+) surface markers and assessment for clonal rearrangements of the *TCR*γ gene for T-LGLs. BMBs were performed routinely at diagnosis, with IHC analysis for T cells, NK cells, and the cytotoxic effector proteins TIA1, granzyme B, and perforin. The diagnosis was confirmed by 2 expert hematopathologists in all cases.

The indications for treatment of LGL disease were consistent with published guidelines[14] and included persistent severe neutropenia ($< 0.5 \times 10^9$/L), neutropenia associated with recurrent infections, and symptomatic anemia. The responses were

Figure 1 Bone Marrow Trephine Section Demonstrating Intravascular Linear Arrays of Large Granular Lymphocytes Expressing Granzyme B