The emerging role of Rituximab in the treatment of large granular lymphocytic leukemia associated with rheumatoid arthritis: a single center experience

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Abstract

Large Granular Lymphocytic (LGL) leukemia is a rare lymphoproliferative disorder with a peculiar association with Rheumatoid Arthritis (RA). The most common feature is neutropenia and patients can have splenomegaly, resembling Felty's Syndrome. These diseases have similar clinical and laboratory abnormalities, but the diagnosis of T-cell LGL (T-LGL) leukemia requires evidence of clonality. Even though T-LGL leukemia is indolent in most cases, inadequate treatment when it is indicated can lead to significant morbidity and mortality, mainly associated with recurrent infections. We present two clinical cases that emphasize the emerging role of Rituximab as an effective therapeutic option in patients with T-LGL and RA.

Keywords: Lymphocytes; Immunosuppressants; Felty's syndrome; Rheumatoid arthritis; Rituximab; T cells.

Introduction

Large Granular Lymphocytic (LGL) leukemia is a rare lymphoproliferative disorder with chronic proliferation of T or natural killer cells. T-cell LGL (T-LGL) leukemia can occur concomitantly with autoimmune diseases and its association with Rheumatoid Arthritis (RA) is frequent, occurring in up to 30% of patients[1,2]. The most common feature is neutropenia and patients can have splenomegaly, resembling Felty's Syndrome (FS)[3]. These diseases have similar clinical and laboratory abnormalities, but the diagnosis of T-LGL leukemia requires evidence of clonality[3, 4]. Treatment is indicated depending on the clinical features and immunosuppressive drugs such as methotrexate, cyclosporine A or cyclophosphamide are classically used, with complete hematological response obtained only in half of patients, which is far from ideal[5]. Recently, rituximab (RTX) emerged as a potential treatment, with sporadic case reports demonstrating its efficacy[6-10].

We present two cases of T-LGL associated with RA from a single center, highlighting the role of rituximab in the treatment of this disorder.

Case Report

Case 1

A 51-year-old woman had a three-year history of polyarthritis of small joints and was diagnosed with RA in 2017. She was RF positive and ACPA negative and had moderate disease activity with a disease activity assessment-28 C-reactive protein (DAS 28-CRP) of 4.41. MTX was started but was soon stopped due to hepatic toxicity with concomitant treatment with isoniazid for latent tuberculosis (LT).

Six months after, she developed leucopenia with neutropenia (490/mm^3). Toxic etiology was ruled out after stopping rifampicin, which she was taking at that time to treat LT. She had no fever and no history of recent infections.

Full blood count showed a hemoglobin concentration of 13.2 g/dL and a platelet count of 150x10^9/L. Total protein electrophoresis was normal. Viral serologies for human immunodeficiency virus (HIV), hepatitis virus B and C (HBV and HCV), Epstein-bar virus (EBV) and cytomegalovirus (CMV) were negative. The abdominal ultrasound did not reveal hepatomegaly or splenomegaly. LGL count in peripheral blood smear was 0.59x10^9/L and neutrophils went down to as low as 90/mm^3, requiring treatment with GCS-F.

Lymphocyte surface markers on flow cytometry of peripheral blood smear reflected a constitutively activated T-cell phenotype (55% of lymphocytes were positive for CD3+/CD8+ and the majority of these had LGL features: CD57+, CD5+ diminished, CD45 R0+, CD28-, CD16-). Flow cytometry analysis of the TCR variable region allowed for the presumption of monoclonality.

Since the previous hepatic toxicity was mainly attributed to the treatment with isoniazid, treatment with MTX 20mg/week was started and there was a complete normalization of blood count after two months, with no intercurrences. Two years later, increased RA disease activity with polyarthritis prompted initiation of a biologic disease modifying antirheumatic drug (bDMARD).

Given the concomitant diagnosis of T-LGL and RA, RTX was started (two 1000mg infusions, two weeks apart every 6 months), achieving disease remission and sustained control of blood counts for the last 3 years.

Case 2

A 55-year-old man with a diagnosis of seropositive RA for 10 years was admitted in the rheumatology department in 2023 due to newly identified pancytopenia and sustained fever. He was taking leflunomide 10mg/day and presented a low disease activity (DAS28-CRP 3.01)

Blood analysis on admission showed anemia (hemoglobin 11.4 g/dL), leucopenia ($1.86x10^9$/L leucocytes) with neutropenia ($630/mm^3$), thrombocytopenia ($124x10^9$/L) and elevation of c-reactive protein (43.2 mg/L). He was currently taking leflunomide 10 mg/day for RA and the wash out with cholestyramine (8 grams three times a day for 11 days) had no improvement in full blood count.

An extensive investigation was performed with no abnormalities detected in the thoracic radiography, electrocardiogram and urine analysis. Similarly, viral serologies for HIV, hepatitis virus A/B/C, EBV and CMV were negative, as well as the bacterial and mycological cultures and PCR and cultures for mycobacteria. Abdominal ultrasound revealed splenomegaly. A computed tomography scan of the thorax and abdomen was performed, showing a small pulmonary nodule (5mm), stable when compared with previous exams, and homogeneous splenomegaly.

Bone marrow biopsy showed hypercellularity and infiltration with cytotoxic T-cells with features of LGL. The peripheral blood smear had a T-LGL count of $0.53x10^9$/L with an immunophenotype analysis suggesting T-LGL leukemia (62% of the lymphocytes were CD8+ compatible with LGL features: CD57+, CD5+ diminished, CD28-, CD45R0-, CD16-). Flow cytometry analysis of the TCR favored monoclonality but further assessment was performed with PCR evaluation of TCR. The analysis revealed clonal TCR Vb chain rearrangements, confirming clonality.

MTX was chosen as a first-line treatment, but the starting was delayed due to several nosocomial infections during the prolonged hospitalization. Two weeks after MTX initiation, severe neutropenia persisted (20/mm3) which required maintenance of GCS-F treatment. RTX was then initiated as a second-line therapy (two 1000mg infusions, two weeks apart). After 5 months, a complete hematologic response was obtained, defined as the normalization of blood counts (hemoglobin >12 g/dL; platelets >150 x 10^9/L; absolute neutrophil count > $1.5x10^9$/L and lymphocytes <$4x10^9$/L) and circulating LGL in the normal range ($0.25x10^9$/L). Simultaneously, there was no recrudescence of fever nor infectious intercurrences.

Discussion

The etiology of LGL leukemia is still not completely understood, but the current model includes an antigen as an initial stimulus for cytotoxic T-cell expansion, further characterized by clonal T-cell receptor rearrangements, secretion of inflammatory cytokines including Fas ligand (Fas-L) and mutations that activate JAK/STAT signaling and impact survival of LGL cells[1].

Neutropenia is the most common feature in LGL leukemia, occurring in up to 80% of patients[11]. Proposed mechanisms include bone marrow infiltration by LGL, Fas-L induced apoptosis (Fas-L is produced by LGLs and binds to neutrophils) and antibodies produced against neutrophils (found in 20-40% patients with LGL leukemia)[4, 12].

RA is a chronic inflammatory immune mediated disease with primary joint involvement, due to synovial inflammation and progressive joint destruction, but also with systemic involvement including several extra-articular manifestations[13]. RA is the most common disease associated with T-LGL leukemia, usually precedes the development of T-LGL leukemia and patients are commonly seropositive. An RA disease duration before T-LGL leukemia ranging from 0-36 years, with a median of 6 years, has been described[12, 14].

Even though RA patients do not usually develop neutropenia unless they have LGL leukemia, Felty Syndrome or associated with medication side effects or secondary comorbidities, it has been shown that neutrophil lysis is involved in citrullination, a key recognized mechanism in RA immunopathogenesis[15]. The presence of an expansion of CD8+ T cells in some asymptomatic RA patients has also been shown[16]. Whether this proliferation represents the early stages of LGL leukemia is still unknown, but it is interesting that there may be a common mechanism for the pathogenesis of RA and LGL-T leukemia neutropenia.

The presence of splenomegaly is a hallmark of FS but is also seen in some T-LGL leukemia patients, probably due to spleen infiltration of LGL cells[3]. Of importance, the degree of splenomegaly has no implication in the severity of the hematologic abnormalities in both diseases[3, 17].

Most patients with T-LGL leukemia are asymptomatic and have an indolent course. However, infections secondary to neutropenia are not rare, and are the most common cause for prompt

treatment initiation[17]. Particularly in the second case, severe neutropenia led to infectious intercurrences with hospitalization and antibiotic therapy and the need for supportive treatment with GCS-F.

The diagnosis of T-LGL leukemia is currently considered in the consistent clinical context with typical hematologic features (namely leucopenia). The presence of a T-LGL peripheral blood count greater than $0.5x10^9$/L is also required, and the LGL immunophenotypic subtype is identified through surface markers in flow cytometry. The great majority of cases have a T-LGL population that show a CD3+, CD8+, TCR-alfabeta+, CD4-, CD5 diminished, CD27-, CD28-, CD45R0-, CD57+/CD16+ phenotype. The confirmation of the diagnosis is made with the evidence of a clonal expansion of T-LGL. Clonality of T-LGL is preferentially demonstrated with PCR analysis of TCR, which highlights the TCR gene rearrangement. Alternatively, flow cytometry can show a restricted population of the TCR variable beta (Vb) chain that allows for the presumption of clonality[5, 18].

In these two RA patients, the development of neutropenia prompted firstly exclusion of infectious and toxic etiologies, and the diagnosis of T-LGL leukemia was made following the recommended algorithm (Table 1). Given the typical clinical and analytic picture in the first patient, the PCR analysis of TCR was not pursued and the flow cytometry analysis results were considered sufficient for the diagnosis.

Acknowledging the frequent indolent course of T-LGL leukemia, a conservative approach is often chosen, and it is estimated that up to 20% of patients will not need treatment during the entire course of the disease[18]. However, severe neutropenia ($<0.5x10^9$/L), infections associated with neutropenia and transfusion-dependent anemia are common treatment indications[5].

Treatment options for T-LGL are mainly based in case series. Immunosuppressive therapy is recommended, with methotrexate, cyclophosphamide and cyclosporine A being the most classic treatment options[5]. RTX is a monoclonal anti-CD20 antibody, which is expressed on the surface of B-lymphocytes. It is associated with reduced T-cell activation and T-cell responsiveness to antigen-presenting cells. Additionally, it impairs antigen presentation due to B-cell depletion. All these mechanisms contribute to its possible effect in T-LGL leukemia[19]. Recently, a retrospective study involving 14 patients with T-LGL associated with RA showed that RTX is an effective therapeutic option either as an initial treatment or after starting conventional treatment,

achieving complete hematologic response in 11 out of 14 patients, and partial response in the remaining patients[8].

In these cases, RTX treatment was started during the course of the disease. Importantly, in the first case, the purpose of RTX treatment was to control RA disease activity, since the complete hematological response was previously obtained with MTX. However, it is still relevant that after 3 years of treatment with RTX the patient had no disease relapses, emphasizing the possible role of RTX in preventing relapses in these patients.

On the other hand, initiation of treatment with RTX in the second patient was essential, as severe neutropenia and infections were persistent despite treatment with MTX and complete hematologic response was achieved only with RTX.

RTX is being increasingly reported as an effective treatment in T-LGL leukemia associated with RA and exhibits a good safety profile. Although T-LGL leukemia is indolent in most cases, inadequate treatment when it is indicated can lead to significant morbidity and mortality, mainly associated with recurrent infections, as seen in the first presented case.

These clinical cases highlight the importance of having RTX as a therapeutic option and contributes to the accumulating evidence of its beneficial effect in treatment of T-LGL and RA.

Tables and Figures

Table I – Relevant clinical and analytical data from the patients

	Case 1	Case 2
Duration of RA (years)	<1	10
RF positive *RF titer (IU/mL)*	Yes 300	Yes 320
ACPA positive *ACPA titer (U/mL)*	No	Yes 351
DAS28-CRP value	4.41	3.01
Erosive disease	No	No
Splenomegaly	No	Yes
Hematologic features		
Hemoglobin (g/dL) Ref. values 13.0-18.0 g/dL	13.2	11.4
Platelets (x10^9/L) Ref. values: 150-400x10^9/L	150	124
Leucocytes (x10^9/L) Ref. values: 4,0-11,0 x10^9/L	2.1	1.5
Lymphocytes (x10^9/L)	1,2	0.9
Neutrophils (x10^9/L)	0.49	0.12
T-LGL (x10^9/L)	0.59	0.53
Immunophenotypic characteristics of cytotoxic (CD3+ CD8+) T-lymphocytes	Peripheral blood smear: CD57+, CD5+ diminished, CD45 R0+, T-Cell Receptor alpha/beta + CD28-, CD16-	Peripheral blood smear: CD57+, CD5+ diminished, T-Cell Receptor alpha/beta + CD28-, CD45R0-, CD16-
Evidence of monoclonality	Flow cytometry analysis of TCR variable region	Flow cytometry analysis of TCR variable region TCR genes PCR rearrangement analysis
Treatment of T-LGL associated with RA	Methotrexate Rituximab	Methotrexate Rituximab
Response to treatment	Hematologic Complete Response	Hematologic Complete Response

RA: Rheumatoid Arthritis; RF: Rheumatoid Factor; ACPA: anti-cyclic-citrullinated peptides antibodies; DAS-28 CRP: Disease Activity Score-28 C- reactive protein; T-LGL: T-Cell Large Granular Lymphocytes; TCR: T-Cell Receptor; PCR: polymerase chain reaction.

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