Analysis of a French cohort of patients with large granular lymphocyte leukemia: a report on 229 cases

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ABSTRACT

Background
Large granular lymphocyte leukemia is a rare lymphoproliferative disorder associated with autoimmune diseases and impaired hematopoiesis. This study describes the clinical and biological characteristics of 229 patients with T-cell or NK-cell large granular lymphocyte leukemia.

Design and Methods
The diagnosis was based on a large granular lymphocyte expansion (> 0.5×10^9/L) lasting more than 6 months. Monoclonal T-cell receptor γ gene rearrangement was detected in all the cases of T-cell large granular lymphocyte leukemia. Patients with chronic NK-cell lymphocytosis had an indolent disease, while those with multiorgan large granular lymphocyte infiltration and an aggressive clinical disease were considered to have NK-cell large granular lymphocyte leukemia.

Results
The diagnosis of T-cell large granular lymphocyte leukemia was confirmed in 201 cases, chronic NK-cell lymphocytosis in 27 cases and NK-cell large granular lymphocyte leukemia in one case. Associated auto-immune diseases or other neoplasms were present in 74 and 32 cases, respectively. One hundred patients (44%) required treatment, mainly for neutropenia-associated infections (n=45), symptomatic auto-immune diseases (n =24), transfusion-dependant anemia (n=18), and other causes (n=13). Patients were treated with steroids (n= 33), methotrexate (n=62), cytoxan (n=32), or cyclosporine (n=24) either as first-, second-, third- or fourth-line therapy. The overall response rate at 3 months and complete response rate for the various treatments were as follows: steroids (12% and 3%), methotrexate (55% and 21%), cytoxan (66% and 47%), cyclosporine (21% and 4%), respectively. Four out of 13 patients responded to splenectomy. Eleven out of 15 patients responded to cytoxan after methotrexate treatment had failed. The mean number of treatments was 3.4 (range, 1-7). There were 15 large granular lymphocyte leukemia-related deaths.

Conclusions
Patients with T-cell large granular lymphocyte leukemia and chronic NK-cell lymphocytosis have similar clinical and biological features and responses to treatment. First-line therapy with cytoxan should be tested in a prospective trial.

Key words: large granular lymphocyte leukemia, LGL leukemia, NK lymphocytosis.

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Introduction

Large granular lymphocyte (LGL) leukemia is a rare lymphoproliferative disorder associated with autoimmune diseases and impaired hematopoiesis.[1-11] The World Health Organization (WHO) classification has recognized LGL leukemia as a specific entity among mature peripheral T-cell neoplasms, including $CD3^+$ T-cell LGL (T-LGL) and $CD3^-$ natural killer (NK)-LGL leukemia subtypes.[12] The diagnosis of LGL leukemia is based on the presence of chronic (>6 months) and expanded circulating LGL ($>0.5\times10^9$/L), which usually infiltrate the bone marrow. T-LGL leukemia has a $CD3^+/CD8^+/CD45RA^+/CD57^+/CD62L$ negative phenotype compatible with a terminal effector memory T-cell expansion due to antigen-driven T-cell activation, along with increased cell survival. A T-cell receptor (TCR)-β/γ rearrangement underlines the monoclonal nature of the T-LGL expansion. NK-LGL leukemias include chronic NK-LGL lymphocytosis, usually an indolent disease, and aggressive NK-LGL leukemia. While the monoclonality of the NK cell subtypes is difficult to assess, phenotypic analysis shows a preferential $CD3^-/CD8^+/CD16^+/CD56^+$ pattern of surface markers with dysregulation of killer cell immunoglobulin-like receptor (KIR) function and expression.[6,11,13]

The real incidence of LGL leukemia has not been determined with precision but these proliferations could amount to 2% to 5% of chronic lymphoproliferative diseases, thus representing the most frequent T-cell/NK cell lymphoproliferative disorders.[14] While LGL leukemia is usually described as an indolent disease, more than half of the patients require treatment because of cytopenias such as anemia, and life-threatening infections associated with neutropenia.[11] Most publications have only included case reports or studied small numbers of patients. Furthermore, very few series have reported extended clinical and biological features relating to more than 50 patients. It is difficult to provide an accurate clinical description and comprehensive therapeutic recommendations given the variability of the diagnostic criteria, differences in the ethnic origin of patients, and the variety of treatments used.

This study describes the clinical and biological characteristics of 229 patients with LGL leukemia included in the French registry from 1999 to 2007.

Design and Methods

Population of patients

In 1999, a French national LGL proliferation registry was set up at Rennes University Hospital. From 1999 to 2007, the clinical and biological data regarding 250 patients suspected of having LGL leukemia from 35 medical centers were collected. This study was approved by the French Hematology Society and the Rennes Hospital Institutional Review Board. All patients gave their informed consent to the use of their medical records for the benefit of research.

Diagnostic criteria

Common criteria for LGL leukemia

The diagnosis was based on a LGL peripheral expansion ($>0.5\times10^9$/L; normal value $<0.3\times10.^9$/L) lasting more than 6 months, excluding transient or reactive LGL proliferations. In the case of a circulating LGL count less than 0.5×10^9/L, the diagnosis was confirmed if patients exhibited typical clinical or hematologic presentations: concomitant autoimmune diseases such as rheumatoid arthritis, splenomegaly, or cytopenias associated with a clonal LGL marrow infiltration.

Specific criteria for T-LGL leukemia

The diagnosis of T-LGL was based on the expression of LGL surface markers compatible with an activated T-cell (commonly $CD3^+/CD8^+/CD57^+$ and/or $CD16^+/CD56^+$ phenotype) and assessment of clonal rearrangement of TCR-γ gene according to previously described methods.

Specific criteria for NK-LGL leukemia and NK-LGL lymphocytosis.

The diagnosis of the subforms of NK-LGL was based on the expression of LGL surface markers compatible with a NK-cell phenotype (commonly $CD3^-/CD8^+/CD16^+$). The term chronic NK-LGL lymphocytosis was used for patients with paucisymptomatic disease, while patients with tissue LGL infiltration of the spleen, liver or bone marrow presenting with aggressive clinical disease were considered as having NK-LGL leukemia.

Flow cytometric analysis was carried out on samples from all the patients using at least the following markers, CD3/CD4/CD8/CD16/CD57/CD56, and LGL counts were performed. TCR-γ gene rearrangement was assessed in all cases suspected of a T-LGL proliferation.

Complementary phenotypic analysis

To complete the assessment of $CD3^+$ LGL leukemia monoclonality, the Vβ repertoire was investigated in 43 cases using specific Vβ monoclonal antibodies (IOTest Beta Mark TCR Vβ repertoire kit, Beckman Coulter, Miami, FL, USA).

Assessment of treatment response

Response to treatment was determined by periodic clinical assessments and blood counts. The primary response criterion was defined using blood count results at 3 months. Hematologic complete response was defined as the complete normalization of blood counts (i.e., hemoglobin >12g/dL, platelets $>150\times10^9$/L, absolute neutrophil count $>1.5\times10^9$/L, and lymphocytosis $<4\times10^9$/L), and a circulating LGL count of less than 0.5×10^9/L. A hematologic partial response was defined as an improvement in blood counts (hemoglobin >8 g/dL, platelets $>50\times10^9$/L, and neutrophils $>0.5\times10^9$/L), as well as the absence of transfusion requirements. Phenotypic and molecular responses could not be assessed in this retrospective and multicenter analysis. Treatment failure was defined as any response not meeting the above criteria within 3 months after the beginning of the treatment.

Statistical analysis

All statistical analyses were performed using Statistical Analysis System software version 9.1. The characteristics of patients with T-cell or NK-cell subtype disease were compared. The significance of the differences observed was tested using the χ^2-test and Fisher's exact test, when appropriate. The response rates were evaluated as the proportion of complete plus partial responses after treatment (steroids, methotrexate, cytoxan, and cyclosporine) had been administered to at least ten patients. The overall response rate was evaluated after first and second-line treatments. Initial characteristics were investigated for their correlation to the overall response rate using the χ^2-test and a logistic regression model. The Kaplan-Meier method was used to estimate survival rates, based on comparisons between T-LGL and NK-LGL leukemia subtypes using the two-sided log-rank test.

Results

The clinical records, phenotypic patterns, and molecular analysis of TCR-γ rearrangement were reviewed for 250 patients presenting with a LGL proliferation. The diagnosis of T-LGL leukemia was confirmed in 201 cases based on the criteria described in the Design and Methods section (all patients had TCR-γ gene rearrangement), NK-LGL lymphocytosis in 27 cases, and NK-LGL leukemia in one case. Twenty-one cases were excluded and considered to have reactive transient or polyclonal LGL proliferations.

Patients' characteristics

The clinical and biological characteristics of the patients are summarized in Table 1, along with those of five, previously published series including more than 50 patients.[3,7,10,13,15]

A diagnosis of CD3$^+$ T-LGL leukemia was confirmed in 201 patients, with no specific predilection for either men or women. At the time of diagnosis, the median age of the patients was 59 years (range, 12-87 years). Only 52 patients (26%) were younger than 50 years old, and four patients were 18 or under. Most of the patients were symptomatic at the time of presentation. Splenomegaly was observed in 24% of the cases, whereas hepatomegaly, lymphadenopathy, and B symptoms were rare (10%, 6%, and 7%, respectively). Rheumatoid arthritis was present in 38 cases (mostly diagnosed prior to the onset of LGL leukemia).

Twenty-seven patients were diagnosed with NK-LGL lymphocytosis. No significant differences in clinical and biological features were observed between T-LGL and NK-LGL lymphocytosis, except for the rate of mild thrombocytopenia, which was more frequent in T-LGL leukemia. Only one patient presented with aggressive NK-LGL leukemia with organomegaly, B symptoms, and a high LGL count. He died of progressive disease 4 months after diagnosis.

Recurrent infections were mostly related to neutropenia,

Table 1. Clinical and biological features of LGL leukemia from the five main series and the French registry.

	Pandolfi (1990)	Loughran (1993)		Semenzato (1997)	Dhodapkar (1994)	Neben (2003)		French registry (2009)	
T-LGL or NK-LGL subtype	NS*	T	NK#	T	T	T	NK	T	NK##
Number of patients	151	129	11	162	68	44	14	201	28
Median age	55	57	39	59	61	63	67	59	58
Sex ratio (M/F)	86/65	57/71	6/5	71/91	34/34	22/22	7/7	90/111	14/14
Symptomatic	72%	-	-	-	69%	73%	50%	82%	75%
Splenomegaly	50%	50%	91%	50%	19%	35%	0	24%	25%
Hepatomegaly	34%	23%	64%	32%	1%			10%	14%
Adenopathy	13%	1%	27%	13%	3%	5%	0	6%	7%
B symptoms	-	-	-	-	12%	-	-	7%	10%
Infections	38%	39%	-	56%	15%	-	-	23%	18%
Rheumatoid arthritis	12%	28%	0	36%	26%	20%	14%	17%	11%
Autoimmune cytopenia	-	-	-	9%	PRCA 7%	PRCA 5%	PRCA 8%	7%	11%
					AIHA 9%	5%	8%		
Need for treatment	30%	73%	100%	33%	69%	80%	64%	44%	39%
LGL-related deaths	14%	36%	82%	27%	8%	-	-	14/201	1/28
Lymphocytosis (x10^9/L)	-	7.8 (1-49)	>4 (90%)	-	>5 (29%)	2.8 (0.5-13)	2.9 (0.5-15)	>4 (51%)	>4 (54%)
LGL >4x10^9/L	52%	52%	90%	-	**	-	-	14%	21%
LGL >1 and <4x10^9/L	38%	40%	10%	-	**	-	-	50%	32%
LGL <1x10^9/L	10%	8%	-	7%	**	-	-	36%	47%
Neutropenia <1.5x10^9/L	64%	84%	64%	-	74%	52%	69%	61%	48%
Neutropenia <0.5x10^9/L	7%	48%	18%	37%	40%	41%	46%	26%	16%
Anemia	25%	49%	100%	26%	51%	89%	77%	24%	28%
Anemia Hb <8 g/dL	37% (<8.9)				19%	36%	31%	7%	4%
Thrombocytopenia	9%	19%	75%	29%	20%	36%	15%	19%	8%
LGL marrow infiltration	67%	88%	100%	76%	***	83%	82%	72% (53/73)	70% (7/10)
Polyclonal hypergammaglobulinemia	-	45%	-	43%	5%	-	-	35% (38/108)	56% (9/16)
Monoclonal gammapathy	-	45%	-	-	8%			10% (11/108)	12% (2/16)
Rheumatoid factor	-	57%	-	43%	61%	48%	38%	41% (30/72)	43% (3/7)
Antinuclear antibodies	-	38%	-	38%	44%	48%	33%	48% (34/70)	63% (5/8)

*#NK-LGL leukemia with aggressive clinical outcome; ##One case had an aggressive clinical outcome, and 27 had chronic NK-LGL lymphocytosis; *16/136 patients had less than 33% CD3$^+$, suggesting a CD3$^-$NK$^+$ phenotype; **13/61 (21%) patients had an LGL count <0.5x10^9/L (median 1.3; range 0.1-10); ***59% of patients had increased lymphoid elements. AIHA: autoimmune hemolytic anemia; PRCA: pure red cell aplasia.*

affecting 46 patients with T-LGL (23%) and five of these with NK-LGL (18%). The majority of infections involved the oral cavity (58%), skin (14%), and respiratory tract (14%). Two patients suffered from a Gram-negative bacillus septicemia. Eleven patients with T-LGL leukemia had viral infections (cytomegalovirus n=7, Epstein-Barr virus and cytomegalovirus co-infection n=2, hepatitis C virus n=2).

The hematologic features of LGL leukemia are listed in Table 1. Hyperlymphocytosis (>4×10^9/L) was observed in 51% of the patients, and the mean absolute circulating LGL count for the whole series was 1.71×10^9/L. Anemia and thrombocytopenia were less frequent than neutropenia and seldom severe: only 14 (6%) patients required red blood cell transfusions. Rheumatoid factor and antinuclear antibodies were detected in 33/79 cases (42%) and 39/78 cases (50%), respectively. Serum protein electrophoresis showed polyclonal hypergammaglobulinemia (38%), whereas monoclonal gammapathy was less frequent (10%).

An LGL count lower than 1×10^9/L (with these counts ranging from 0.4×10^9/L to 1×10^9/L) was observed in 39% of patients (n=85) of whom 72 had T-LGL and 13 the NK-LGL subtype. All these 85 patients had specific LGL marker expression of T or NK lineage (see *Design and Methods* section) according to the flow cytometry analysis. The T-LGL patients were considered to have T-LGL leukemia as they all had monoclonal TCR-γ rearrangements (n=72) along with either neutropenia (n=52) or anemia (n=20), autoimmune disease (n=20), or bone marrow infliltrated by LGL (n=46). Using Vβ flow cytometry analysis, 19 of these T-LGL patients were analyzed and found to have monoclonal Vβ expression (see below). NK lymphocytosis was assessed on the combination of a slightly but significantly increased LGL count and association with neutropenia (n=4), anemia (n=2), thrombocytopenia (n=2), autoimmune diseases (n=4), and bone marrow LGL infiltration (n=4).

Eighty-three out of the 229 patients underwent bone marrow aspiration, with 72% (n=60) showing bone marrow LGL infiltration (53/73 and 7/10 patients with T or NK proliferation, respectively). Of 20 bone marrow biopsies performed, 11 (55%) showed a specific infiltration by interstitial clusters of CD8 cytolytic lymphocytes in 10/16 T-LGL and 1/4 NK-LGL cases: immunochemistry studies were not always carried out.

Autoimmune anemia or thrombocytopenia occurred in 17 cases. Associated autoimmune diseases or other neoplasms were present in 74 and 32 cases, respectively (Table 2). Rheumatoid arthritis appeared to be the most frequent autoimmune disease (17%) and accounted for more than half of the autoimmune manifestations associated with LGL disorders. Other autoimmune diseases included vasculitis, autoimmune endocrinopathy, chronic inflammatory bowel disease, and Gougerot-Sjogren syndrome. B-lymphoproliferative diseases were the most frequently associated neoplasms; 12 cases were described (non-Hodgkin's lymphomas n=8; multiple myeloma, n=3; chronic lymphocytic leukemia, n=1), whereas eight cases of myelodysplasia were noted. LGL leukemia coexisted with solid tumors in ten patients (2 with breast cancer, 2 with prostate cancer, 2 with lung cancer, 1 each with renal cancer, basocellular skin cancer, anal cancer, and thymoma). All these neoplasms were diagnosed 6 to 16 years before the diagnosis of the LGL proliferation.

Two patients had pulmonary artery hypertension together with T-LGL leukemia. Neither had any other cause of pulmonary hypertension and the elevated pulmonary artery pressure improved with treatment of the LGL leukemia. These findings and previous descriptions in the literature[16,17] lead us to consider that the pulmonary artery hypertension resulted from the LGL proliferation.

The classical phenotype of T-LGL leukemia was CD3$^+$/TCR-αβ$^+$/CD4$^-$/CD8$^+$/CD57$^+$/CD16$^+$. Most of the T-LGL proliferations expressed TCRαβ (83%), CD8$^+$ (90%), and CD57$^+$ (57%). Furthermore, γδ$^+$ T-LGL leukemias accounted for 17% of the T-LGL leukemias. With the exception of the 35% patients with a double-negative CD4$^-$/CD8$^-$ phenotype, the common phenotype for the γδ$^+$ T-LGL leukemia group was similar (CD3$^+$/CD4$^-$/CD8$^+$/CD57$^+$) to that of the αβ subgroup. The group of patients with NK-LGL leukemia predominantly expressed a CD3$^-$/CD16$^+$/CD56$^+$ phenotype (60% of cases). In the 43 cases in which the Vβ repertoire was analyzed, monoclonal Vβ expression was found in 39 cases (particularly Vβ14, n=7; Vβ8, n=5; Vβ7.1, n=4), a potential defective repertoire in two cases, and two monoclonal clones in three cases. However, no specific restricted Vβ subset was observed (Figure 1).

Table 2. Diseases associated with LGL leukemia in n=229 patients from the French registry.

Associated diseases	T-LGL leukemia patients n=201	NK-LGL leukemia patients n=28
Neoplasms	9 (4%)	1 (4%)
Autoimmune cytopenia	14 (7%)	3 (11%)
Pure red cell aplasia	6	-
Autoimmune hemolytic anemia	7	3
Idiopathic thrombocytopenic purpura	1	-
Evans' syndrome	-	1
B-cell lymphoid neoplasms	10 (5%)	2 (7%)
Non-Hodgkin's lymphoma	8	-
Low-grade non-Hodgkin's lymphoma	7	-
Diffuse large B-cell lymphoma	1	-
Multiple myeloma	2	1
Chronic lymphocytic leukemia	-	1
Autoimmune diseases	67 (33%)*	7 (25%)*
Rheumatoid arthritis	35 (17%)	3 (11%)
Vasculitis	6	1
Endocrinopathy	3	-
APECED	1	-
Type I MEN	1	-
Hashimoto	1	-
Chronic inflammatory bowel disease	9	-
Celiac disease	1	-
Gougerot-Sjogren syndrome	4	-
Glomurolenephritis	4	-
Polymyositis	-	1
Rhizomelic pseudopolyarthritis	-	1
Inflammatory arthritis	3	1
(unclassified)	12	2
Poly/multineuritis	3	1
Myelodysplasia	6 (17.8%)	2 (7%)
Acute myeloid leukemia	2	-
Pulmonary hypertension	2	-

*APECED: autoimmune polyendocrinopathy-candidosis-ectodermal dystrophy; MEN: multiple endocrine neoplasia; *Some patients presented with more than one auto-immune associated disease.*

Treatment

One hundred patients (44%) required treatment; of these, 89 suffered from T-LGL leukemia and 11 from NK-LGL leukemia. The indications for treatment were as follows: (i) LGL leukemia (n=66): recurrent infections due to severe neutropenia (n=45), transfusion-dependent anemia (n=18) or severe thrombocytopenia (n=3); (ii) autoimmune associated diseases (n=24): rheumatoid arthritis or arthralgia (n=18), vasculitis (n=4) and inflammatory bowel diseases (n=2).

The onset of treatment was at diagnosis (43%), within 6 months of diagnosis (15%), between 6 and 12 months after diagnosis (16%), and over 1 year after diagnosis (26%). One patient was treated 14 years after diagnosis.

The main treatments and their results are detailed in Figure 1 and Table 3. Steroid therapy was used alone as first-line therapy for 22 patients. This treatment was effective (complete or partial response) in only two patients, and the response diminished when the doses were tapered. Steroids partly reduced joint pain or B symptoms but were ineffective at correcting neutropenia and reducing LGL clones. Methotrexate (7.5 mg/m^2 per week) was administered to 62 patients, of whom nine had failed to respond to steroid therapy while 36 patients received the drug as first-line therapy. The main indications were neutropenia (n=36), anemia (n=6) and associated autoimmune diseases (n=17). The overall response rate was 55%, with 21% of the patients obtaining a complete response. Thirty-two patients were treated with oral low-dose cytoxan (100 mg/day), which was administered as first-line therapy (n=4), second-line therapy (n=12), or third-line therapy (n=16): neutropenia (n=17) and anemia (n=10) were the main reasons for treatment. The overall response rate was 66%, with 47% of patients achieving a complete response. Fifteen patients failed to respond to methotrexate and were subsequently treated with cytoxan: 11 responded favorably (Figure 2). Grade 2 or higher toxicity was observed for either treatment. Twenty-seven patients were evaluated for their long-term response. The mean duration of response was 21 months (range, 3-72 months) after methotrexate and 31 months (range, 12-60 months) after cytoxan. Among 18 patients responding to methotrexate, 12 relapsed during treatment, while four maintained their response after completing treatment. Six patients receiving cytoxan were still in complete response 12 to 48 months following drug cessation, while the three other patients are still undergoing treatment. Cytoxan was not administered for more than 1 year, while methotrexate was generally continued in responders.

Twenty-four patients were given cyclosporine (2-3 mg/Kg/day) for a median period of 12.2 months (range, 2-65 months) as a first-, second- or third-line option, mainly for neutropenia (n=15) and/or anemia (n=7). The overall response rate was 21%, with only one patient having a complete response. Six patients had to stop treatment due to renal insufficiency, cramps, or hypertension. Very few patients (n=16) received steroids concomitantly with one of the three above treatments. Indeed, prednisone was rapidly stopped in the case of either treatment failure or efficacy.

After failure or relapse with methotrexate, cytoxan, or cyclosporine, 31 patients received the following treatments: a polychemotherapy regimen (n=9), a purine analog (n=6), alemtuzumab (n=4), antithymocyte globulin (n=2), a combination of chlorambucil, danatrol, and splenic irradiation (n=1), and other treatments (n=7). Given the small number of patients, no conclusions can be drawn on the efficacy of these treatments.

Twenty-seven patients with anemia or neutropenia received growth factors (erythropoietin, n=7; granulocyte colony-stimulating factor, n=14), with a poor or transient response noted when these agents were used alone. Splenectomy was performed in 13 cases, and was consid-

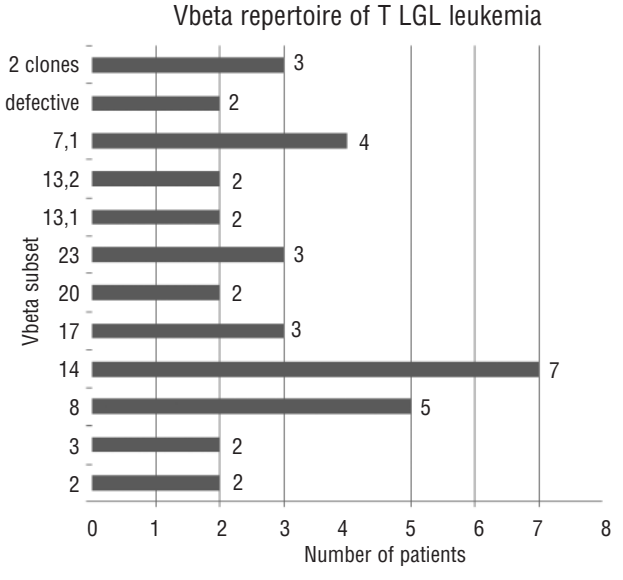

Figure 1. Vβ CMF analysis in T-LGL leukemia showing the Vβ subsets expressed in more than one case (n=43).

Table 3. Response to treatment.

	Response rate to initial therapy			Response to second-line therapy			Overall response rate		
	N.	ORR (%)	CR	N.	ORR (%)	CR	N.	ORR (%)	CR
Steroids	22	2 (9%)	0	7	1 (14%)	1 (14%)	33	4 (12%)	1 (3%)
Methotrexate	36	16 (44%)	5 (14%)	13	9 (69%)	4 (31%)	62	34 (55%)	13 (21%)
Cytoxan	4	3 (75%)	2 (50%)	12	11 (92%)	6 (50%)	32	21 (66%)	15 (47%)
Cyclosporine	6	1 (17%)	0	12	2 (17%)	0	24	5 (21%)	1 (4%)
Splenectomy	10	4 (40%)	1 (10%)	2	1 (50%)	0	13	4 (31%)	1 (8%)
Other treatments	2	4 (18%)	2 (9%)	10	2 (20%)	1 (10%)	50	10 (20%)	5 (10%)

ORR : overall response rate, CR : complete response.

ered to have failed in nine of those cases. Fifty-four patients received more than two lines of treatment.

In univariate and multivariate analyses, none of the patients' initial characteristics correlated with either overall response rate or overall survival. Fifteen patients with LGL leukemia died, ten due to infections and the remaining because of disease progression. With a median follow-up of 58 months, the 5-year overall survival was 89% for patients with T-LGL leukemia and 95% for those with a NK cell proliferation (Figure 3). There was no difference in overall survival between T-LGL and NK-LGL leukemias.

Discussion

We reported on a series of 229 patients with LGL leukemia from the French registry. This is the largest series published so far, providing complementary data on clinical and biological features of the disease and useful information on the response to treatment.

For the purposes of this report, the updated criteria of LGL proliferation were used.[13] Initially, diagnostic criteria for T-LGL leukemia required an absolute LGL count greater than 2×10^9/L.[7,10] Recent studies revealed that 25% to 30% of newly diagnosed patients presented with an absolute LGL count below 0.5×10^9/L. Since then, the advent of flow cytometry as well as progress in immunohistochemistry and molecular clonality studies have allowed physicians to detect much smaller populations of clonal LGL, given a background of normal polyclonal hematopoiesis. Detection of a minor but clonal LGL population in patients with systemic symptoms or cytopenias, commonly found in LGL, is sufficient to establish the diagnosis, and a 6-month waiting period is not required.[11]

Our data were compared with those of the five main series published between 1993 and 2003, including more than 50 patients.[3,7,10,13,15] With regards to T-LGL leukemia, we observed both similarities and discrepancies when comparing the findings of our study with those of the other five. This may be explained by the selection of patients and inclusion criteria. Indeed, in the first large study published 20 years ago, the diagnostic criteria used were less stringent, especially in terms of molecular and phenotypic analyses.[10] In the study by Semenzato,[13] patients with low LGL counts were included in the analysis. This may have contributed to extending the LGL leukemia criteria, based on monoclonal LGL populations rather than LGL counts.[18] We observed that the median age, sex ratio, and low proportion of patients with hepatomegaly, B symptoms, or adenopathy were disease features in common to all the series. The presence of splenomegaly varied and ranged from 19% to 50%. The main difference noted was the LGL count: 39% of patients in the French registry had a count below 1×10^9/L, as compared to 10% and 21% in the two series reported by Loughran *et al.* and Dhodhakpar *et al.*[3,7] Nevertheless, these patients had an authentic T-LGL leukemia attested by a monoclonal LGL expansion and associated diseases, and

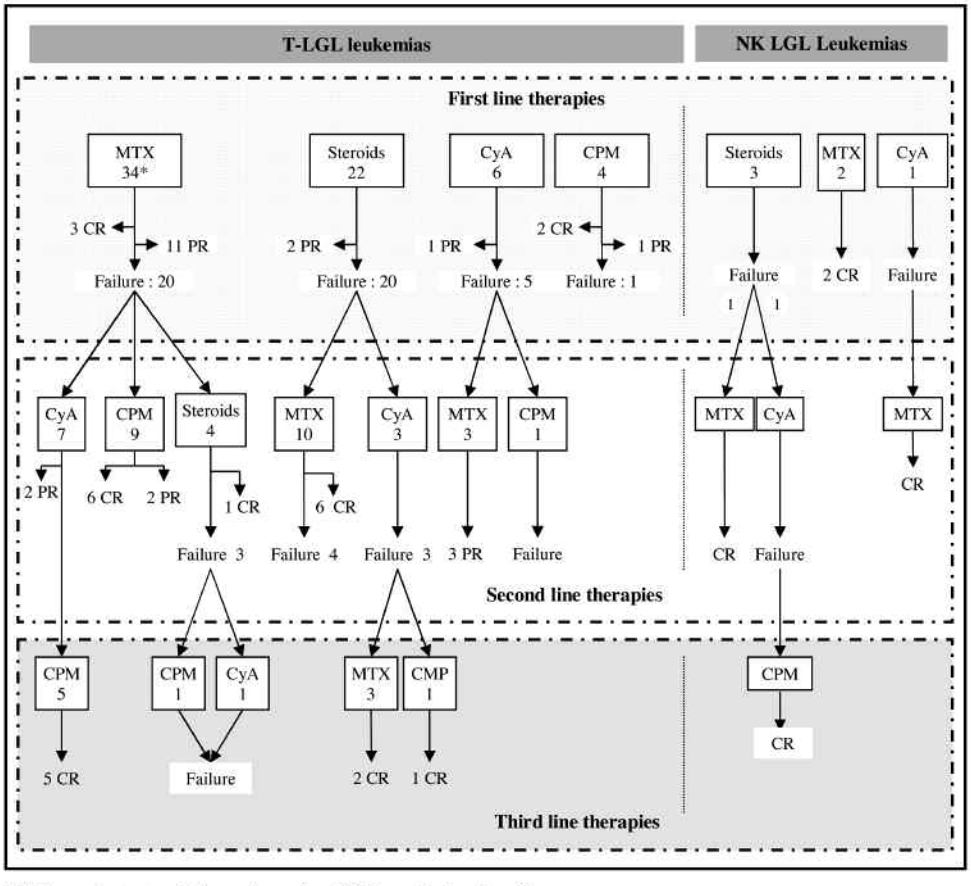

Figure 2. Algorithm of treatment outcome for 70 patients with T- or NK-LGL leukemia.

MTX : methotrexate, CyA : cyclosporine, CPM : cyclophosphamide
CR : complete response, PR : partial response
*Numbers correspond to the numbers of patients (in the box)

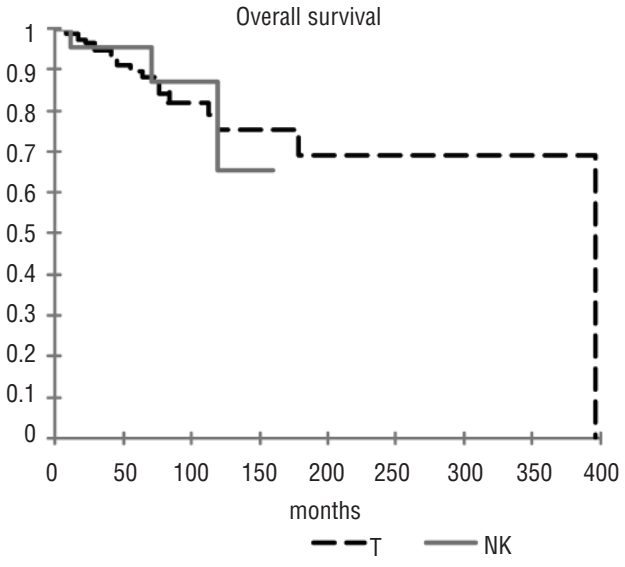

Figure 3. Overall survival of the 228 patients (the patient with the aggressive form of NK-LGL leukemia was excluded).

58% required treatment. The incidence of rheumatoid factor/antinuclear antibodies was relatively similar (41% to 51%), and the proportion of patients with rheumatoid arthritis ranged from 17% to 28%. There were notable variations in the degree of anemia, and less than 30% of patients required red blood cell transfusion. Thrombocytopenia was usually mild. The rate of infections was lower in the French cohort than in the series reported by Pandolfi, Semenzato and Loughran *et al.*, as a consequence of the lower rate of severe neutropenia in the former (26% *versus* more than 40%).

The type of associated diseases, more particularly autoimmune diseases, and the other hematologic malignancies observed in our study were similar to those of the published series. The findings highlight that LGL leukemia may be observed in the context of B-cell lymphoproliferative disorders and myeloid malignancies such as myelodysplasia. This raises the question of the pathogenesis of LGL expansion in this setting, and whether or not it represents an aberrant immune response to an underlying malignant disease. The link between LGL expansion and solid tumors was not evident in all cases since most patients were over 60 years old and thus, had a higher risk of developing another malignancy.

Monoclonality was routinely assessed by examining TCRβ/γ gene rearrangements. All the patients with T-LGL leukemia in our series had a monoclonal TCRγ gene rearrangement. Several authors reported the potential involvement of the Vβ repertoire.[19-22] We confirmed the usefulness of these analyses as all of our 43 patients with T-LGL leukemia in whom the Vβ repertoire was analyzed had a monoclonal proliferation with a unique Vβ (n=38), biclonal (n=2), or defective subset (n=3) also suggestive of monoclonal LGL expansion.

We then compared the clinical and biological features of T-LGL and NK-LGL cell leukemias. The WHO classification has divided NK-LGL proliferations into two categories:[12] (i) NK-LGL leukemia with massive LGL marrow infiltration and an aggressive clinical behavior, mainly found in Asia and associated with Epstein-Barr virus infection[23] (these criteria applied to the 11 cases presented in Loughran's initial series);[7] and (ii) chronic NK-LGL lymphocytosis, an indolent disease described in series reported by Neben and the French registry (except one case).[15] We noted that NK-LGL lymphocytosis and T-LGL leukemia have very similar clinical and biological features (Tables 1 and 2). The immunological patterns as well as the association of autoimmune diseases were comparable in T-LGL and NK-LGL subtypes. Furthermore, the percentages of patients with NK-LGL or T-LGL requiring therapy were identical. The only difference observed was the phenotype, with lower CD8/CD57 and higher CD16/CD56 expression in NK-LGL lymphocytosis.

Across the six series, the proportion of patients with T-LGL leukemia who were treated ranged from 30% to 80%. All patients with aggressive NK-LGL leukemia required treatment, but they were usually refractory and died of disease progression.[23] Very limited data are available in the literature, as most series reported therapeutic results for fewer than 20 patients, except the series by Dhodapkar which included 47 patients. A consensus on therapeutic options has not been reached.[1,3,6,10,24-26] In the French series, 100 patients were treated for a median duration of 4 months. Methotrexate and cytoxan remain the first lines of treatment. Given the small numbers of patients and the absence of control studies, no definitive conclusions can be drawn. The cytoxan response rate compared favorably with that of methotrexate, with overall response rates of 66% and 55%, respectively. Eleven out of 15 patients responded to cytoxan after having failed to respond to methotrexate. Our data suggest that cytoxan may be proposed as a first-line therapy instead of methotrexate for the following reasons: (i) the duration of response persisting after cessation of treatment is longer with cytoxan; (ii) the duration of treatment is shorter; and (iii) the overall response rate is the same or higher. In our series, response and tolerance to cyclosporine compared unfavorably to those previously reported. A good response was mainly found in patients with an HLA DR4 phenotype.[3,27-31] Cyclosporine may be proposed as first-line therapy because of its lack of myelotoxicity. Responses usually occur without eradication of the abnormal LGL clone.

Only five of 13 patients who underwent a splenectomy showed a clinical improvement. The main complications were exacerbation of symptoms and infections. Recently, Subbiah *et al.* reported positive results in a series of 15 splenectomized patients with pancytopenia. However, two patients died, and the post-operative analysis showed persistence of the LGL expansion.[32] As concluded by the authors, splenectomy is a valid therapeutic option in cases of T-LGL leukemia with splenomegaly and refractory cytopenia and careful prophylaxis against infections should be given.[32] We did not observe any clear, prolonged response to hematopoietic factors (erythropoietin or granulocyte colony-stimulating factor). However, good responses to erythropoietin have been reported in anemic patients and this treatment may postpone the decision to start cytotoxic or immunosuppressive therapy.

Finally, the good long-term clinical outcome was confirmed as 70% of our patients are still alive 10 years after diagnosis. We observed that T-LGL leukemia and NK-LGL lymphocytosis had very similar clinical and biological features and that response to treatment was identical in both subtypes. Current progress in the understanding of the

pathogenesis of LGL leukemia should help to determine the optimal therapeutic strategy.

Authorship and Disclosures

BB and TL designed the research, collected the data and wrote the paper. JR, MH, JD, OR, NS, OT, MR and TF provided patients' data and corrected the paper. MR and TF performed part of the biological analyses. JM and BB performed the statistical analysis.

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