


SHORT REPORT

Large granular lymphocyte expansions
in primary Sjögren's syndrome:
characteristics and outcomesAlistair Baber ¹, Gaetane Nocturne,^{1,2} Roman Krzysiek,³ Julien Henry,¹
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Primary Sjögren's syndrome (pSS) is a systemic auto-immune disease (AID) that mainly causes mucosal dryness, asthenia, arthralgia and, sometimes, severe organ involvement requiring immunosuppressive treatment. Despite negative randomised controlled trials, rituximab (RTX) is frequently used in case of systemic complications.¹

Large granular lymphocyte (LGL) disorders are characterised by proliferation of LGL cytotoxic lymphocytes of either T-cell (mainly CD3+TCRαβ+CD8+CD57+CD56+/-CD16+/-, rarely CD4+CD8+/-) (T-LGL) or less frequently NK-cell (CD3-CD2+CD16+CD56+CD57+/-) (NK-LGL) origin. The spectrum of LGL disorders extends from reactive LGL expansions (LGLe), which are usually asymptomatic, transitory and have polyclonal or oligoclonal profile, to LGL leukaemias, which are monoclonal proliferations associated with cytopenias and somatic activating mutations in STAT3.² T-LGL expansions (T-LGLe) have frequently been described in several AIDs,² especially rheumatoid arthritis (RA). In this context, they could exhibit either polyclonal, oligoclonal or monoclonal profile, and could be accompanied by neutropenia irrespective of their clonal status.³ However, LGLe have been exceptionally reported in pSS. Thus, we here report eight cases of LGLe in patients with pSS.

In our study, all patients from the Paris-Sud hospital, national reference centre for pSS, diagnosed with pSS and having a LGLe were retrospectively included between September 2016 and March 2018. Diagnosis of pSS was based on ACR/EULAR 2016 criteria.⁴ Three of our eight patients did not fulfil these criteria, but diagnosis was validated by experts from our reference centre based on anti-SSA antibodies (patient 4), focal sialadenitis (patient 3) and/or associated specific

Key messages

What is already known about this subject?

- ▶ Large granular lymphocyte expansion (LGLe) have frequently been described in association with rheumatoid arthritis, but rarely with primary Sjögren's syndrome (pSS).
- ▶ The role of LGLe in neutropenia following treatment with rituximab (RTX) has already been suggested in patients with lymphoma.

What does this study add?

- ▶ Our study suggests for the first time that LGLe associated neutropenia could be a side effect of RTX in patients with pSS.
- ▶ Methotrexate could be useful in case of severe neutropenia.

How might this impact on clinical practice?

- ▶ RTX being more and more used in systemic autoimmune diseases, this possible effect must be known.

systemic complications (patients 3 and 7) (table 1). Collected data included: clinical and biological characteristics, treatments received for LGLe or pSS and patients' outcome. Disease activity was measured using the EULAR Sjögren Syndrome Disease Activity Index (ESSDAI),⁵ with active disease defined as an ESSDAI ≥5. Since neutropenia was considered as a feature of LGLe, it was not included in the calculation of the ESSDAI. LGLe were defined on flow cytometry results, as monoclonal, oligoclonal or polyclonal, expansions of T-cells or NK-cells harbouring an LGL phenotype (different combinations of CD57, CD56 and CD16 markers), representing ≥10% of peripheral lymphocytes. In patients with T-LGLe, Vβ repertoire analysis of the T-cell receptor was performed to assess clonal rearrangement. When possible, STAT3 mutational status of LGL cells by direct sequencing was also performed.

Table 1 Characteristics of patients and LGL expansions

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Characteristics of patients								
Sex/age (years)	F/55	M/62	F/82	F/80	F/80	F/52	F/67	F/67
pSS disease duration (years)	7	1	2	5	1	9	14	2
Subjective dryness	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Schirmer test (mm)	3	3	15	NA	0	4	NA	0
USF (mL/min)	0.3	0.2	0.2	NA	0.15	0.2	NA*	0.08
Sialadenitis: focus score	3.4	1.6	1.71	NA	2.6	2.1	0	3.2
Past or present pSS systemic manifestations	Arthralgia, Interstitial nephritis, Lymphoma (remission at LGLe diagnosis)	No (in particular, no signs or symptoms of RA)	Auto-immune thrombopenia	Arthralgia	Polyarthritis, Peripheral neuropathy, Myositis	Arthralgia, ILD, glomerulonephritis, Cryoglobulinemia	Arthralgia, Pulmonary AL amyloidosis, Cryoglobulinemia	Peripheral neuropathy, Cryoglobulinemia
Anti-SSA/anti-SSB	Yes/No	Yes/Yes	No/No	Yes/No	No/No	No/No	No/No	Yes/Yes
Anti-CCP/RF	No/Yes	Yes/Yes	No/No	No/No	No/Yes	No/Yes	No/Yes	No/Yes
ESSDAI (highest ever/at LGLe diagnosis)	52/6	20/14	8/8	3/3	35/35	51/11	10/10	22/22
Treatment received for pSS	HCC RTX and Bendamustin (for lymphoma)	None	None	None	Prednisone MTX, RTX	Prednisone RTX	RTX	RTX
Characteristics of LGLe								
Total lymphocytes count (/mm ³)	848	3081	2430	7250	890	850	3300	960
LGL on blood smear	No	No	Yes	No	No	No	No	No
LGL type (% of total CD8+, CD4+ orNK cells)	T CD8+ (72%) and CD4+ (21%)	T CD8+ (38%) and T CD8+ (72%) and CD4+ (21%)	LGL T CD8+ (% of CD8 cells not available)	NK (95%)	Prednisone MTX, RTX	T CD8+ (69%) and CD4+ (26%)	T CD8+ (37%) and T CD8+ (60%)	T CD8+ (60%)
LGL cell count, /mm ³ (% of total lymphocyte count)	288 (34%)	627 (20%)	440 (18%)	6000 (86%)	180 (20%)	750 (64%)	1770 (57%)	163 (17%)
Clonality	T CD8+: oligoclonal	T CD8+: oligoclonal	NA	NA	T CD8+: oligoclonal T CD4: polyclonal	T CD8+: monoclonal T CD4+: oligoclonal	T CD8+: monoclonal	T CD8+: oligoclonal

Continued

Table 1 Continued

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
STAT3 mutation	No	No	NA	NA	NA	NA	NA	No
Time from first RTX injection to LGLe diagnosis	4 months				15 days	7 months	7 months	12 months
Neutrophil count (/mm ³):								
Before RTX	2680	NR	NR	NR	3800	2100	1750	5000
At LGLe diagnosis	224	100	470	3200	4340	870	980	700
Nadir/time from first RTX injection (years)	10/4 months	68/NR	470/NR	2850/NR	3370/NR	870/7 months	980/7 months	700/12 months
Infections	Recurrent sinusitis	No	No	No	Pneumocystosis	No	No	No
Treatment of neutropenia	MTX, G-CSF	MTX, G-CSF	None	None	None	None	None	None
Therapeutic response, outcome	Remission of neutropenia after 3 months	Improvement of neutropenia (500–1000/mm ³) after 3 months	Persistent neutropenia (500–1000/mm ³) after 2 months	Persistent neutropenia (500–1000/mm ³) after 2 months	Remission of neutropenia after 2 months	Remission of neutropenia after 2 months	Persistent neutropenia (1000–1500/mm ³)	Persistent neutropenia (500–1000/mm ³)

*Salivary gland scintigraphy showed a decrease in secretory function of parotid and submandibular gland. ESSDAI, EULAR Sjögren's syndrome disease activity index ; F, female ; HCC, hydroxychloroquine ; ILD, interstitial lung disease ;LGL, large granular lymphocyte; M, male ; MTX, methotrexate ; NA, not available ;NR, not relevant; pSS, primary Sjögren's syndrome ; RA, rheumatoid arthritis ; RF, rheumatoid factor ; RTX, rituximab; USF, unstimulated salivary flow.

LGLe were identified in eight pSS patients. Their main characteristics are reported in table 1. All but one had active disease at the time of LGLe diagnosis (median ESSDAI: 11 (range: 3–35)) and a history of systemic manifestations. Circumstances of LGLe diagnosis were neutropenia (n=6), hyperlymphocytosis (n=1) and incidental (n=1). Seven patients (87.5%) had a T-LGLe, all having a predominant T-CD8+ population, but three patients had additional LGL-type T-cell populations: CD4+CD8+ in one and CD4+CD8 in two. In these three patients, each LGL population displayed expansions of different TCR V β families. T-LGLe were monoclonal in two patients and oligoclonal in four patients; clonality has not been assessed in one. One patient had NK-LGL expansion whose clonality could not be determined. STAT3 mutation was negative in the three patients tested.

Neutropenia (<1500/mm³) was observed in 6 (75.0%) patients and was <500/mm³ in 3 (37.5%) of them. Lymphocyte phenotyping showed an inverted CD4/CD8 ratio (<1) in four patients with absolute CD4+lymphopenia (<500/mm³) in three patients and CD8+lymphocytosis (>1000/mm³) in three patients; CD19+cell counts were low or undetectable in all the patients previously treated by RTX, normal in others. Five patients had received RTX (one for lymphoma, four for pSS systemic manifestations of cryoglobulinemia). The median time between LGLe diagnosis and first RTX injection was 7 months (range 15 days to 12 months). In all patients who received RTX, a lymphocyte immunophenotyping was performed prior to RTX administration and did not reveal any LGLe. Neutropenia occurred in 4/5 patients having received RTX and in 2/3 in patients who did not receive RTX. Patient 8 was retreated with RTX for a cryoglobulinemia relapse: neutropenia occurred after the first cycle of RTX (1200/mm³) and worsened after the second (700/mm³).

In two patients, severe neutropenia required treatment by Granulocyte-Colony Stimulating Factor (G-CSF) which only led to transient efficacy. Methotrexate was added in these two patients; it allowed neutrophil count normalisation in one case and improvement of neutropenia in the other (figure 1). Among the four remaining neutropenic patients, spontaneous remission occurred in one and non-severe neutropenia persisted in the three others.

Contrarily to RA, there are only a few reports of LGLe in pSS patients. Friedman *et al*⁶ reported that pSS was present in 27% of patients in a case series of 48 patients presenting with T-LGL leukaemia. However, the other studies having explored the prevalence of systemic AIDs in LGL disorders did not underline a specific association with pSS.⁷

Since all pSS patients with LGLe had an active disease, the hypothesis of a relationship between pSS activity and occurrence of LGLe could be raised. Nevertheless, since 5/8 patients developed LGLe after treatment with RTX, LGLe may be linked to RTX treatment rather than to pSS itself. Neutropenia is a well-known adverse effect of RTX, particularly late onset neutropenia (occurring >4 weeks after treatment). Its frequency is estimated around 7%–27% in lymphoma,⁸ 1.3%–3% in RA and 2.3% in other AIDs.⁹ Of note, in this context, LGLe may not necessarily be monoclonal.¹⁰ Several pathogenic hypotheses have been raised and expansion of T-LGL cells is one of them. Indeed, LGLe could induce immune-mediated neutropenia via Fas/Fas-ligand dependent pathways, antineutrophil antibodies production and/or myelosuppression.⁸

Mechanisms by which RTX could induce T-LGLe remains unclear. One can wonder if RTX could lead to depletion of regulatory B-cells implicated in immune surveillance against T-cell clones. Alternatively, CD4+T cell depletion has been described after RTX in RA patients.¹¹ If some

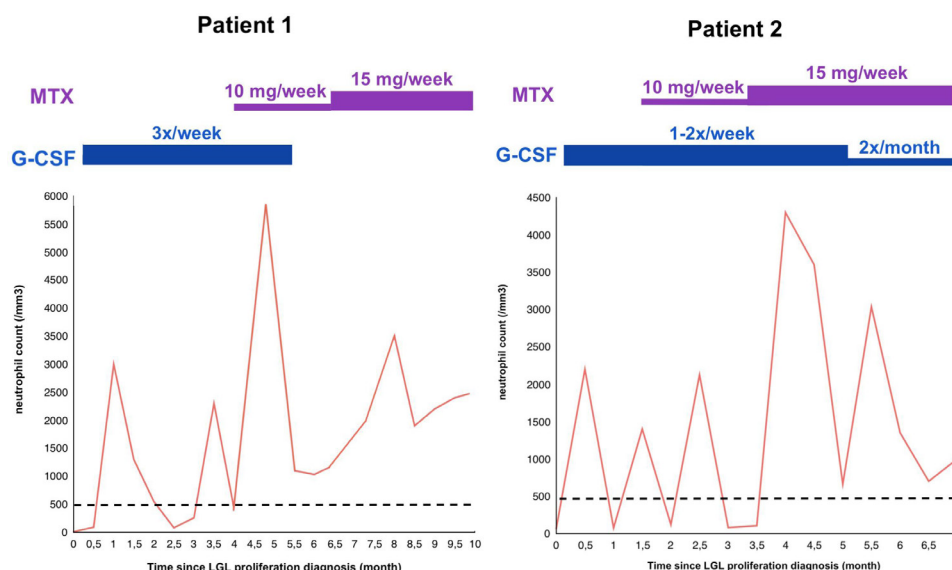


Figure 1 Treatments and Changes in neutrophil counts in patient 1 and 2. LGL, large granular lymphocyte; MTX, methotrexate.

of these T-cells are regulatory, their depletion might promote expansion of LGL cells. Among our patients who received RTX, three presented an inverted CD4/CD8 ratio and three presented absolute CD4+lymphopenia. An increased production of STAT3-activating cytokines, such as interleukin 6, triggered by RTX administration could be another hypothesis.

Surprisingly, Schwaneck *et al* did not identify RTX as a risk factor for LGLe in RA.¹² Also, RTX has been used in the treatment of LGL leukaemia associated with RA, sometimes with success.¹³ This opposite effects of RTX suggest different mechanisms underlying LGLe: changes in regulatory cells/cytokine profile induced by RTX treatment on the one hand, or beneficial effect of RTX on persistent antigenic stimulation due to an active AID on the other hand.

In our study, the number of LGL cells was lower than the usual threshold used to define LGL leukaemia ($\geq 500/\text{mm}^3$) in 4/8 patients. However, LGLe represented $\geq 10\%$ of total lymphocytes, a threshold recently proposed in RA.¹² In fact, these four patients had a global lymphopenia ($< 1000/\text{mm}^3$) due to disease activity and/or associated treatments. Thus, the relative threshold of $\geq 10\%$, seems to be more appropriate in the context of AIDs, where peripheral lymphopenia is a hallmark of the disease.

Finally, we hypothesise that, even small, these LGLe could be involved the pathogenesis of neutropenia. Nevertheless, although T-LGLe have been previously reported in post-RTX neutropenia in patients with lymphoma, our study lacks power to affirm with certainty the existence of a link between RTX administration and LGLe. However, the simultaneous occurrence of LGLe and neutropenia made this link likely. Bone marrow examination, anti-neutrophil antibody testing and measurement of soluble Fas ligand might have been useful to determine the exact mechanism of neutropenia but have not been performed.

In conclusion, these results suggest for the first time that post-RTX neutropenia could be linked to LGLe in pSS patients, as already observed in lymphoma but rarely in RA. Also, methotrexate could be a valid therapeutic option in severe cases. While RTX is frequently used in different systemic AIDs, this possible effect must be known by clinicians. However, larger scale studies are needed to confirm this relationship between RTX administration, LGLe and neutropenia in patients with AIDs.

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REFERENCES

- Mariette X, Criswell LA. Primary Sjögren's syndrome. *N Engl J Med* 2018;378:931–9.
- Lamy T, Moignet A, Loughran TP. Lgl leukemia: from pathogenesis to treatment. *Blood* 2017;129:1082–94.
- Prochorec-Sobieszek M, Rymkiewicz G, Makuch-Łasica H, *et al*. Characteristics of T-cell large granular lymphocyte proliferations associated with neutropenia and inflammatory arthropathy. *Arthritis Res Ther* 2008;10.
- Shiboski CH, Shiboski SC, Seror R, *et al*. 2016 American College of Rheumatology/European League against rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Arthritis Rheumatol* 2017;69:35–45.
- Seror R, Ravaud P, Bowman SJ, *et al*. EULAR Sjogren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjogren's syndrome. *Ann Rheum Dis* 2010;69:1103–9.
- Friedman J, Schattner A, Shvidel L, *et al*. Characterization of T-cell large granular lymphocyte leukemia associated with Sjogren's syndrome—an important but under-recognized association. *Semin Arthritis Rheum* 2006;35:306–11.
- Bareau B, Rey J, Hamidou M, *et al*. Analysis of a French cohort of patients with large granular lymphocyte leukemia: a report on 229 cases. *Haematologica* 2010;95:1534–41.
- Wolach O, Bairey O, Lahav M. Late-onset neutropenia after rituximab treatment: case series and comprehensive review of the literature. *Medicine* 2010;89:308–18.
- Salmon JH, Cacoub P, Combe B, *et al*. Late-onset neutropenia after treatment with rituximab for rheumatoid arthritis and other autoimmune diseases: data from the autoimmunity and rituximab registry. *RMD Open* 2015;1:e000034.
- Stamatopoulos K, Papadaki T, Pontikoglou C, *et al*. Lymphocyte subpopulation imbalances, bone marrow hematopoiesis and histopathology in rituximab-treated lymphoma patients with late-onset neutropenia. *Leukemia* 2008;22:1446–9.
- Lavielle M, Mulleman D, Goupille P, *et al*. Repeated decrease of CD4+ T-cell counts in patients with rheumatoid arthritis over multiple cycles of rituximab treatment. *Arthritis Res Ther* 2016;18.
- Schwaneck EC, Renner R, Junker L, *et al*. Prevalence and characteristics of persistent clonal T cell large granular lymphocyte expansions in rheumatoid arthritis: a comprehensive analysis of 529 patients. *Arthritis Rheumatol* 2018;70.
- Cornec D, Devauchelle-Pensec V, Jousse-Joulin S, *et al*. Long-term remission of T-cell large granular lymphocyte leukemia associated with rheumatoid arthritis after rituximab therapy. *Blood* 2013;122:1583–6.