

EXTENDED REPORT

Serum immunoglobulin free light chain assessment in rheumatoid arthritis and primary Sjögren's syndrome

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Background: B cell activation may result in an increased secretion of immunoglobulin free light chains (FLCs) in autoimmune diseases.

Objective: To analyse serum FLC levels in patients with rheumatoid arthritis and in those with primary Sjögren's syndrome (pSS).

Patients and methods: Blood samples were collected from 80 healthy blood donors, 50 patients with rheumatoid arthritis and 139 patients with pSS. Serum FLC level was measured using a new quantitative immunoassay.

Results: Mean (standard error (SE)) serum κ and λ FLC levels were significantly higher in patients with rheumatoid arthritis and in those with pSS than in controls (κ : 18.9 (1.1) and 16.3 (1.4) v 10.5 (0.4) mg/l, $p < 0.001$ and $p = 0.001$, respectively; λ : 16.7 (1.2) and 19.3 (1.5) v 11.6 (0.6) mg/l, $p < 0.001$ for both). 18 (36%) patients with rheumatoid arthritis and 31 (22.3%) patients with pSS had abnormal serum FLC levels (increased κ or λ levels and abnormal ratio of κ : λ). Serum κ and λ levels were correlated with other B cell activation markers in both diseases. FLC levels increased with disease activity, because, unlike total gammaglobulin and immunoglobulin G levels, they were significantly correlated with Disease Activity Score 28 in patients with rheumatoid arthritis ($p = 0.004$ for κ , $p = 0.05$ for λ) and with extraglandular involvement in pSS ($p = 0.01$ for κ , $p = 0.04$ for λ).

Conclusion: FLC levels are increased and correlate with disease activity in patients with rheumatoid arthritis and in those with pSS, two diseases in which increased risk of lymphoma could result from persistent B cell activation and disease activity. Further studies are required to determine whether FLC assessment could represent a relevant biomarker for response to treatment (especially B cell depletion) and for the risk of lymphoma in autoimmune diseases.

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Immunoglobulin light chains and heavy chains are combined together during the synthesis of immunoglobulins; however, more light chains than heavy chains are produced. Thus, light chains that are not bound to intact immunoglobulins can be detected as circulating free light chains (FLCs) under physiological conditions. Increased FLC levels have been reported in several immunopathological conditions but until very recently, serum immunoassays required the separation of FLCs from intact immunoglobulins and were impractical for routine use. A new automated immunoassay now allows for sensitive and specific FLC assessment using antibodies directed against the "hidden" epitopes of FLC molecules, located at the interface between the light and heavy chains of intact immunoglobulins.^{1–3} To date, this assay has essentially been used to assess the excess of one light chain over another, using κ : λ ratio as a surrogate for clonal expansion. Thus, assessment of quantitative FLC levels already represents a major breakthrough in the routine monitoring of non-secretory myeloma,³ light-chain myeloma,⁴ primary amyloidosis⁵ and monoclonal gammopathy of undetermined significance (MGUS).⁶ However, assessment of serum FLC levels might also prove useful in autoimmune diseases. The interest in B cell activation markers has undergone a renaissance over the past few years, given the pivotal role of B cells in the pathogenesis of autoimmune diseases⁷ and the proved efficacy of B cell-targeted treatment in patients with rheumatoid arthritis.⁸ We therefore investigated FLC levels in patients with rheumatoid arthritis and in those with primary Sjögren's syndrome (pSS), two diseases in which the pathogenic role of B cell activation has been shown well.^{9–11}

PATIENTS AND METHODS

Patients

Blood samples were collected from 80 healthy blood donors (mean age 45 years), from 50 patients with rheumatoid arthritis according to the American College of Rheumatology criteria and from 139 Caucasian patients with pSS as defined by the American–European consensus group criteria (including a focus score ≥ 1 on labial salivary gland biopsy or the presence of anti-SSA/Ro or anti-SSB/La antibodies).¹² The patients successively attended the Department of Rheumatology, Hôpital de Bicêtre, Le Kremlin Bicêtre, France, and the Department of Rheumatology, Hôpital de Hautepierre, Strasbourg, France. Informed consent was obtained from all patients, and ethics committees of the two hospitals approved the study.

Patients with rheumatoid arthritis had a mean (standard error (SE)) age of 53 (14) years and a disease duration of 15 (9) years. In two patients, rheumatoid arthritis was associated with Sjögren's syndrome. Patients with rheumatoid arthritis were treated with methotrexate ($n = 11$), anti-tumour necrosis factors (adalimumab, $n = 7$; infliximab, $n = 17$; etanercept, $n = 8$), or other disease-modifying antirheumatic drugs ($n = 7$).

Patients with pSS had been previously included in a study evaluating B cell activation markers.¹³

Table 1 summarises the clinical and immunological features of the patients with pSS (mean (SE) age 56 (12.5) years, disease

Abbreviations: CCP, cyclic citrullinated peptide; DAS, Disease Activity Score; FLC, free light chain; MGUS, monoclonal gammopathy of undetermined significance; pSS, primary Sjögren's syndrome

Table 1 Clinical and immunological features of 139 patients with primary Sjögren's syndrome

Enlarged parotid glands	45 (32.4)
Raynaud's phenomenon	49 (36.2)
Extraglandular involvement	62 (44.6)
Purpura	9 (6.4)
Synovitis	21 (15.1)
Myositis	3 (2.1)
Lung involvement	15 (10.8)
CNS involvement/peripheral neuropathy	1 (0.7)/9 (6.4)
Previous lymphoma*	4 (2.8)
Medium-size vessel vasculitis/lymphadenopathy	1 (0.7)/3 (2.1)
κ FLC serum level, mg/l†	16.3 (1.4)
λ FLC serum level, mg/l†	19.3 (1.5)
κ : λ ratio†	1 (0.3)
Positive RF results (>20 IU/ml)	82 (58.9)
RF, IU/ml†	165 (42)
β 2-microglobulin value, mg/l, n = 129†	1.8 (0.2)
BAFF, ng/ml, n = 107†	5.7 (0.9)
Gammaglobulin serum level, g/l†	13.6 (1)
IgG serum level, g/l†	16 (1.3)
IgA serum level, g/l†	2.7 (0.3)
IgM serum level, g/l†	2.4 (0.6)
No anti-SSA or anti-SSB antibodies	43 (30.9)
Anti-SSA antibody only	47 (33.8)
Anti-SSA and anti-SSB antibodies	49 (35.2)
Focus score \geq 1 on labial salivary gland biopsy (n = 125)	112 (89.6)
Serum creatinine level, μ mol/l†	85.6 (3.2)

BAFF, B cell-activating factor; CNS, central nervous system; FLC, free light chain; RF, rheumatoid factor.

Values are n (%) unless otherwise mentioned.

*All patients with lymphoma were in remission and had normal FLC levels.

†Values are mean (SE).

duration 14 (8.6) years). Extraglandular involvement was defined as the presence or confirmed records of purpura, lung or neurological involvement, synovitis, myositis, vasculitis, lymphadenopathy, enlarged spleen or previous lymphoma during the evolution of the disease. Extraglandular involvement was present in 62 (44.6%) patients.

Laboratory analysis

Serum FLC levels were measured using a latex-enhanced immunoassay (Freelite, The Binding Site, Birmingham, UK) using a Behring nephelometric analyzer (Dade Behring, Deerfield, Illinois, USA). The immunoassay consisted of two separate measurements, one to detect free κ (normal range: 3.3–19.4 mg/l) and the other to detect free λ (normal range: 5.7–26.3 mg/l). The diagnostic ranges had been previously established by the manufacturer to include 100% of a reference population of 282 serum samples.² A ratio of κ : λ <0.26 or >1.65 is abnormal, according to the manufacturer's recommendations.² An abnormal FLC level was defined as an increased κ or an λ level or abnormal κ : λ ratio.

In patients with rheumatoid arthritis, levels of rheumatoid factor and of anticyclic citrullinated peptide (anti-CCP) antibodies were assessed using nephelometry and ELISA (anti-CCP2 kit; Immunoscan RA, Arnhem, The Netherlands, Eurodiagnostica, Arnhem, The Netherlands), respectively. Serum samples were diluted 1:50 and were considered to be positive for anti-CCP if the antibody titre was >50 AU, as determined by dilution of a positive standard serum.

In patients with pSS, the results of the following biological markers of B cell activation have been previously reported: anti-SSA and anti-SSB antibodies, rheumatoid factor, serum levels of immunoglobulin (Ig) A, IgG and IgM, serum B cell-activating factor level in 107 patients and serum β 2-microglobulin value in 129 patients.¹³

The presence of monoclonal gammopathy, analysed in all patients by serum and urine electrophoresis together with

immunofixation, and in controls by serum electrophoresis and immunofixation, was observed in three patients with rheumatoid arthritis and in 15 patients with pSS. The clonality status of blood B cell populations was analysed in 13 patients with pSS by amplification of VH-(DH)-JH junctional regions by polymerase chain reaction using the VH framework region 3 (FR3) and JH FR4 consensus primers. Polymerase chain reaction products were analysed on an ethidium bromide-stained polyacrylamide gel after high-resolution electrophoresis.

Statistical analysis

Continuous values are shown as mean (SE) and qualitative values as n (%). The χ^2 test (with Yates' correction, when appropriate) was used to assess the differences in frequencies for qualitative values. Analysis of variance was used to compare FLC levels between controls, patients with rheumatoid arthritis and those with pSS. Correlation was evaluated using Spearman's test. Data were analysed using SPSS V.11.5 program.

RESULTS

Serum FLC assessment in patients with rheumatoid arthritis or pSS

No healthy participant had increased κ or λ levels, or abnormal κ : λ ratio.

In the 50 patients with rheumatoid arthritis, mean (SE) serum κ and λ FLC levels were significantly higher than in controls (κ : 18.9 (1.1) v 10.5 (0.4) mg/l, $p < 0.001$; λ : 16.7 (1.2) v 11.6 (0.6) mg/l, $p < 0.001$; figs 1, 2). The κ : λ ratio was not significantly different between patients and controls (mean (SE) 1.4 (0.2) v 1.1 (0.1), $p = 0.4$). In all, 18 (36%) patients had abnormal FLC serum levels: 15 (30%) had increased κ levels (including 3 (6%) patients with increased κ : λ ratio) and 3 (6%) had increased λ levels.

In the 139 patients with pSS, mean (SE) serum κ and λ FLC levels were significantly higher than in controls (κ : 16.3 (1.4) v 10.5 (0.4) mg/l, $p = 0.001$; λ : 19.3 (1.5) v 11.6 (0.6) mg/l, $p < 0.001$; table 1; figs 1, 2). The κ : λ ratio was not significantly different between patients and controls (1 (0.3) v 1.14 (0.1), $p = 0.15$). In all, 31 (22.3%) patients had abnormal FLC serum levels, including 17 (12.2%) patients with increased κ levels (5/

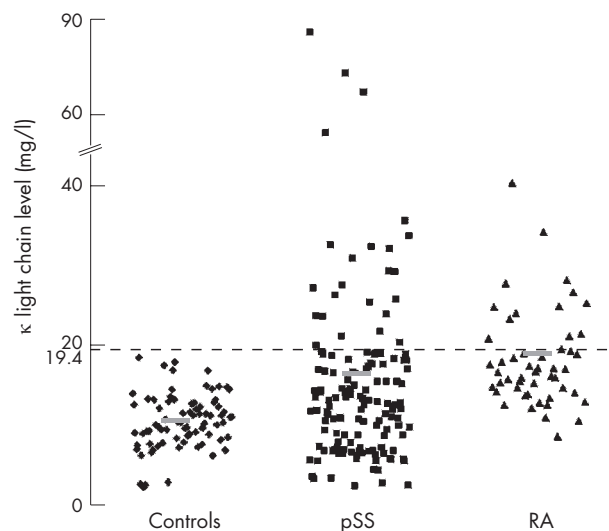


Figure 1 Serum κ levels in patients with rheumatoid arthritis (RA) or primary Sjögren's syndrome (pSS) and in healthy controls. Bars represent mean values. Increased κ levels are defined by values >19.4 mg/l.

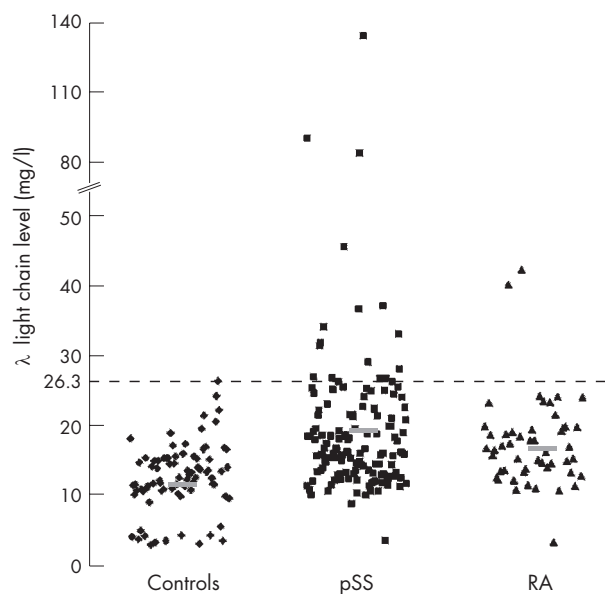


Figure 2 Serum λ levels in patients with rheumatoid arthritis (RA) or primary Sjögren's syndrome (pSS) and in healthy controls. Bars represent mean values. Increased λ levels are defined by values >26.3 mg/l.

17 had abnormal $\kappa:\lambda$ ratio), 6 (35.3%) patients with increased λ levels (5/6 with abnormal $\kappa:\lambda$ ratio), 7 (5%) patients with increased κ and λ levels and 1 (0.7%) patient with only an abnormal $\kappa:\lambda$ ratio.

Abnormal FLC levels were more often detected in the 15 patients with pSS and MGUS compared with patients with pSS without monoclonal gammopathy (33% *v* 20%, $p = 0.2$).

Analysis of IgH gene rearrangements by high-resolution gene electrophoresis did not show a difference between the five patients with pSS without monoclonal gammopathy who had an abnormal $\kappa:\lambda$ ratio and eight patients, randomly selected from those with normal FLCs, who all had a polyclonal pattern. Thus, no blood monoclonal B cell populations could be detected in patients with pSS with abnormal $\kappa:\lambda$ ratio.

We found no significant difference between patients with rheumatoid arthritis and patients with pSS regarding the frequency of abnormal FLCs and levels of κ and λ light chains.

Correlation between FLC levels and immunological features

In patients with rheumatoid arthritis, κ and λ FLC levels were correlated with serum levels of gammaglobulin (κ : $r = 0.38$, $p = 0.04$; λ : $r = 0.4$, $p = 0.03$) and IgG (κ : $r = 0.37$, $p = 0.04$; λ : $r = 0.38$, $p = 0.04$). Moreover, a significant correlation was observed between κ and rheumatoid factor levels ($r = 0.3$, $p = 0.03$) but not between λ and rheumatoid factor levels ($r = 0.21$, $p = 0.17$). Anti-CCP levels were not correlated with FLC levels (κ : $r = 0.23$, $p = 0.2$; λ : $r = 0.12$, $p = 0.7$).

In patients with pSS, κ and λ FLC levels were correlated with serum levels of gammaglobulin (κ : $r = 0.37$, $p < 0.002$; λ : $r = 0.28$, $p = 0.001$); IgG (κ : $r = 0.40$, $p < 0.001$; λ : $r = 0.28$, $p = 0.001$); rheumatoid factor (κ : $r = 0.24$, $p = 0.008$; λ : $r = 0.28$, $p = 0.002$); β_2 -microglobulin (κ : $r = 0.4$, $p < 0.001$; λ : $r = 0.4$, $p < 0.001$) and B cell-activating factor levels (κ : $r = 0.17$, $p = 0.08$; λ : $r = 0.27$, $p = 0.005$). Mean serum FLC concentrations were higher in patients with autoantibodies. Thus, patients with anti-SSB had higher mean (SE) serum FLC levels (κ : 22.3 (3.1) mg/l; λ : 24.7 (2.8) mg/l) than patients with anti-SSA antibody alone (κ : 14 (1) mg/l; λ : 17.1 (0.8) mg/l,

$p = 0.002$ and $p = 0.04$, respectively) and than patients without autoantibodies (κ : 12 (2) mg/l; λ : 17.3 (1.9) mg/l, $p = 0.003$ and $p = 0.09$, respectively). Likewise, the frequency of abnormal FLC levels was higher in patients with anti-SSB (36%) than in patients with anti-SSA antibody alone (11.2%) or in patients without autoantibodies (10.1%; $p = 0.01$). We found no correlation between κ or λ levels and age, serum creatinine level or disease duration (data not shown).

Association between FLC level and disease activity

In patients with rheumatoid arthritis, a significant correlation was observed between disease activity assessed by the Disease Activity Score 28 (DAS28) and κ ($r = 0.51$, $p = 0.004$; fig 3) or λ levels ($r = 0.31$, $p = 0.05$). Patients with abnormal κ levels had a higher DAS28 than those with normal λ levels (mean (SE) 6.2 (0.4) *v* 4.2 (0.2), $p = 0.006$). No correlation was observed between DAS28 and serum gammaglobulin or IgG levels ($r = 0.2$, $p = 0.2$ and $r = 0.18$, $p = 0.36$, respectively). The mean C reactive protein value was 18.2 (SE 3) mg/l. κ and λ levels were significantly correlated with C reactive protein levels (κ : $r = 0.34$, $p = 0.03$; λ : $r = 0.3$, $p = 0.04$). FLC levels were not significantly different between patients treated with methotrexate and those treated with anti-tumour necrosis factor (data not shown).

In pSS, mean (SE) serum FLC levels were higher in patients with systemic features than in patients with only glandular involvement (κ : 19 (1.8) *v* 13 (1.1) mg/l, $p = 0.01$; λ : 21.6 (2.5) *v* 16.7 (0.6) mg/l, $p = 0.04$). Likewise, the frequency of abnormal FLC levels was significantly higher in patients with extraglandular involvement than in those without (20/62 (32.2%) patients with extraglandular involvement *v* 11/77 (14.3%) patients with no extraglandular involvement, $p = 0.01$). Among the 124 patients without MGUS, extraglandular involvement remained associated with abnormal FLC levels (31.3% *v* 12.3% in patients without extraglandular involvement, $p = 0.02$). Extraglandular involvement was associated with abnormal FLC levels and also with anti-SSB (58.3% *v* 36% in patients without anti-SSB, $p = 0.02$). This association between anti-SSB and extraglandular involvement was not observed in patients with increased FLC levels (59% in patients with anti-SSB *v* 56% in patients without anti-SSB, $p = 0.7$). In

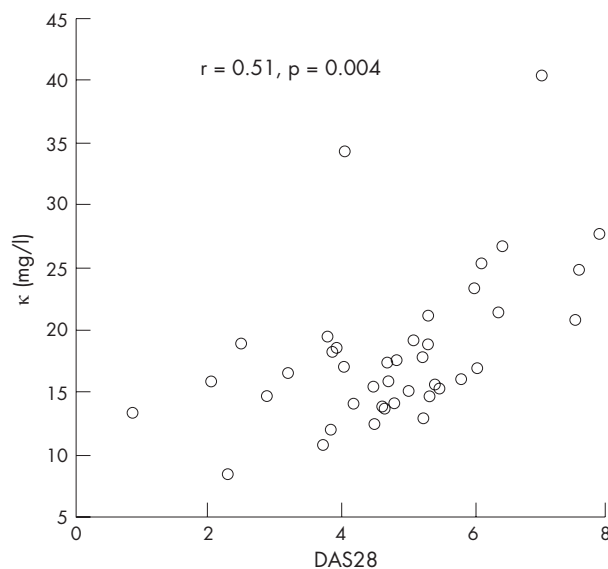


Figure 3 Correlation between κ levels and Disease Activity Score (DAS)28 in patients with rheumatoid arthritis.

anti-SSB-negative patients, increased FLC levels remained significantly associated with extraglandular involvement (29.4% *v* 8.5% in patients without extraglandular involvement, *p* = 0.02). As previously reported,¹³ the mean (SE) serum IgG (16.8 (7) *v* 14.2 (6) g/l, in patients with and without extraglandular involvement, respectively, *p* = 0.3) and gammaglobulin levels (13.9 (7.1) *v* 12.9 (5.5) g/l in patients with and without extraglandular involvement, respectively, *p* = 0.8) were not significantly associated with extraglandular involvement.

DISCUSSION

Using a new quantitative immunoassay, we showed that one third of patients with rheumatoid arthritis and one fifth of those with pSS had abnormal FLC levels. Interestingly, patients with increased FLC levels had considerably higher disease activity in rheumatoid arthritis and higher frequency of extraglandular involvement in pSS.

Our study is, to our knowledge the first to evaluate the level of FLCs in patients with rheumatoid arthritis and in those with pSS, and does not deal with their clonality directly. Previous studies on autoimmune diseases used older qualitative immunoassay methods (high-resolution electrophoresis or isoelectric focusing, followed by immunofixation) to detect monoclonal immunoglobulin FLCs. In patients with rheumatoid arthritis, monoclonal κ and λ chains were detected in the serum of patients and were associated with increased disease activity.¹⁴ In studies on a small number of patients with pSS, 25–40% had serum monoclonal light chains and had a more frequent extraglandular involvement than patients without serum monoclonal light chains.^{15–17}

A strong correlation was observed between FLCs and other markers of B cell activation. FLCs were correlated with gammaglobulin, IgG and rheumatoid factor levels, but not with anti-CCP in patients with rheumatoid arthritis. This might be related to the fact that gammaglobulin, IgG and rheumatoid factor levels reflect polyclonal B cell activation rather than a specific B cell response such as anti-CCP antibodies. Likewise in pSS, FLC levels were correlated with B cell-activating factor and non-specific markers of B cell activation, such as β 2-microglobulin, gammaglobulin and IgG.

Interestingly, FLC levels markedly increased with DAS28, which supports the potential relationship between B cell and disease activity in patients with rheumatoid arthritis. Interestingly, no major correlation was observed between DAS28 and serum immunoglobulin or IgG, markers of B cell activation, which have a much longer half life (20–25 days) than FLCs (2–4 h).⁴ The faster turnover of FLCs might account for their predominant potential as disease activity markers, which suggests that they might be a good and early surrogate marker for response to treatments. Thus, evaluation of the role of FLCs as prognostic markers for response to treatments (particularly to B cell depletion, such as rituximab) and of structural evolution deserves further investigation.

Increased FLC levels were associated with extraglandular involvement in patients with pSS. Regarding disease activity, serum FLC levels might add a definite association to routine measurements, such as erythrocyte sedimentation rate, quantitative immunoglobulin values¹³ or detection of monoclonal immunoglobulin, which were not markedly associated with extraglandular involvement either in our study or in a recent survey of 237 patients with pSS (except for monoclonal immunoglobulin and pulmonary involvement in the survey).¹⁸ Our results confirm the increase in FLC levels in one third of patients with MGUS.⁶ Serum FLC levels are associated with extraglandular involvement, especially in patients without anti-SSB, and could be a potentially useful marker of systemic complications in this subgroup of patients. Our results reinforce

the hypothesis that extraglandular involvement in patients with pSS might be the result of more intense stimulation of B cells, suggested by the reported association of anti-SSB and increased β 2-microglobulin with extraglandular involvement in patients with pSS.^{13, 19}

In systemic lupus erythematosus, increased FLC levels have also been reported to be associated with disease activity, as a strong correlation was observed between FLC levels and lupus disease activity assessed by the European Consensus Lupus Activity Measure score.²⁰

The increase in FLC levels with disease activity may passively result from polyclonal B cell activation, but FLCs could also have an intrinsic pathogenic role. Indeed, there is compelling evidence that FLCs do have the ability to bind antigen, albeit generally with lower affinities. IgM expressed by CD5 cells binds FLCs, but no information is available on intracellular signalling after binding.²¹ Moreover, FLCs induced mast-cell activation and elicited allergic asthma in a murine experimental model.²² Interestingly, mast cells could have a key role in the early phases of autoimmune disorders, notably in patients with rheumatoid arthritis.²³

Patients with rheumatoid arthritis,²⁴ pSS,²⁵ or systemic lupus erythematosus²⁶ share an increased risk of developing lymphoma as a possible result of a persistent B cell activation and disease activity. To date, no biological marker is available to evaluate the individual risk for lymphoma in these patients. Of note, FLC levels are increased in these three diseases and correlate with disease activity. An abnormal κ : λ ratio indicates a loss of control over the proportion of heavy and light chains synthesised, and it was recently shown to be a relevant clinical marker of malignant evolution in MGUS.⁶ Among the five patients with pSS without MGUS who had abnormal κ : λ ratio, one had purpura, two had decreased C4 levels, which are two known risk factors for lymphoma. No clonal B cell populations could be detected in the blood of these patients, which suggests that an abnormal κ : λ ratio could be a more sensitive marker of clonality that could be restricted initially to the site of autoimmunity.²⁷ The predictive value of abnormal κ : λ ratio regarding the occurrence of lymphoma deserves to be investigated in a longitudinal study on patients with rheumatoid arthritis, pSS or systemic lupus erythematosus.

Conclusion

Increased FLC levels is a common feature of autoimmune diseases and correlates with disease activity, which supports the relationship between B cell activation and autoimmune disease activity. Prospective longitudinal studies are necessary to determine the potential utility of FLC assessment as a biomarker of response to treatment (especially B cell depletion). Lastly, because of the possible relationship of persistent B cell activation and disease activity with lymphoma, further studies are required to determine whether FLC assessment could represent a relevant biomarker for the risk of lymphoma in autoimmune diseases.

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