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Free light chains and autoimmunity☆

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ABSTRACT

The study of free light chains (FLCs) has grown as area of enormous interest for many clinicians with the aim of disclosing the exact biological role and potential use of FLCs in the clinical routine. Moreover, the attention given to immunological functions of FLCs has sparked a new light into their pathogenic contribution in different chronic autoimmune-based inflammatory diseases. The release of intracellular antigens following cell death or ineffective clearance of apoptotic debris, modification of self-antigens, and molecular mimicry may trigger the production of immunoglobulins after activation and polyclonal expansion of B cells, by which FLCs are released. The discovery of polyclonal FLCs as potential biomarkers started with the observation of their increased concentrations in a variety of biological fluids related to patients with autoimmune diseases. This review deals with the use of polyclonal FLCs for identifying severity and monitoring outcome after treatment in some autoimmune diseases, namely systemic lupus erythematosus, myasthenia gravis, systemic sclerosis, rheumatoid arthritis and Sjögren's syndrome, as supported by the fact that levels of FLCs correlate with both B cell activation markers and other specific markers of disease activity. In a near future, following the evidence shown, FLCs might probably work as early prognostic markers of severity and also as indicators of response to treatment or early assessment of relapse in selected autoimmune diseases.

1. Introduction

Breaking of the immunological tolerance towards one or more "self"-antigens is the cornerstone of an autoimmune disease: the result of lost tolerance is the formation of different autoantibodies and/or autoreactive T cells that might determine tissue or organ damage of different severity and a frank status of disease [1,2]. In organ-specific autoimmune disorders the response is directed against multiple organ antigens, while systemic autoimmune disorders usually affect a host of organs and are distinctly associated with a response directed against self-molecules distributed in all affected organs [3]. The release of intracellular antigens due to cell death or ineffective clearance of apoptotic debris, modification of self-antigens and molecular mimicry contribute to the initiation of immunoglobulin (Ig) production after activation and polyclonal expansion of B cells [4]. Hence, autoimmune diseases arise from chronic inflammation in which autoantibodies, genetic factors, and potential environmental triggers (viruses, bacteria or protozoa, vitamin D levels, ultraviolet radiation, etc.) are intertwined with specific subsets of lymphocytes acting like main pathogenetic actors [5–7].

It is well-known that immunoglobulins have a tetrameric structure composed of two heavy and two light chains linked by noncovalent forces and disulphide bonds [8]: two light chain isotypes are known, kappa (κ) and lambda (λ) (see Fig. 1). The production of antibodies is accompanied by a slight excess of synthesis of κ and λ light chains, which may be around 500 mg per day [9,10], that are secreted into circulation as polyclonal free light chains (FLCs). FLCs are a mixture of proteins having all approximately the same weight, but have a different

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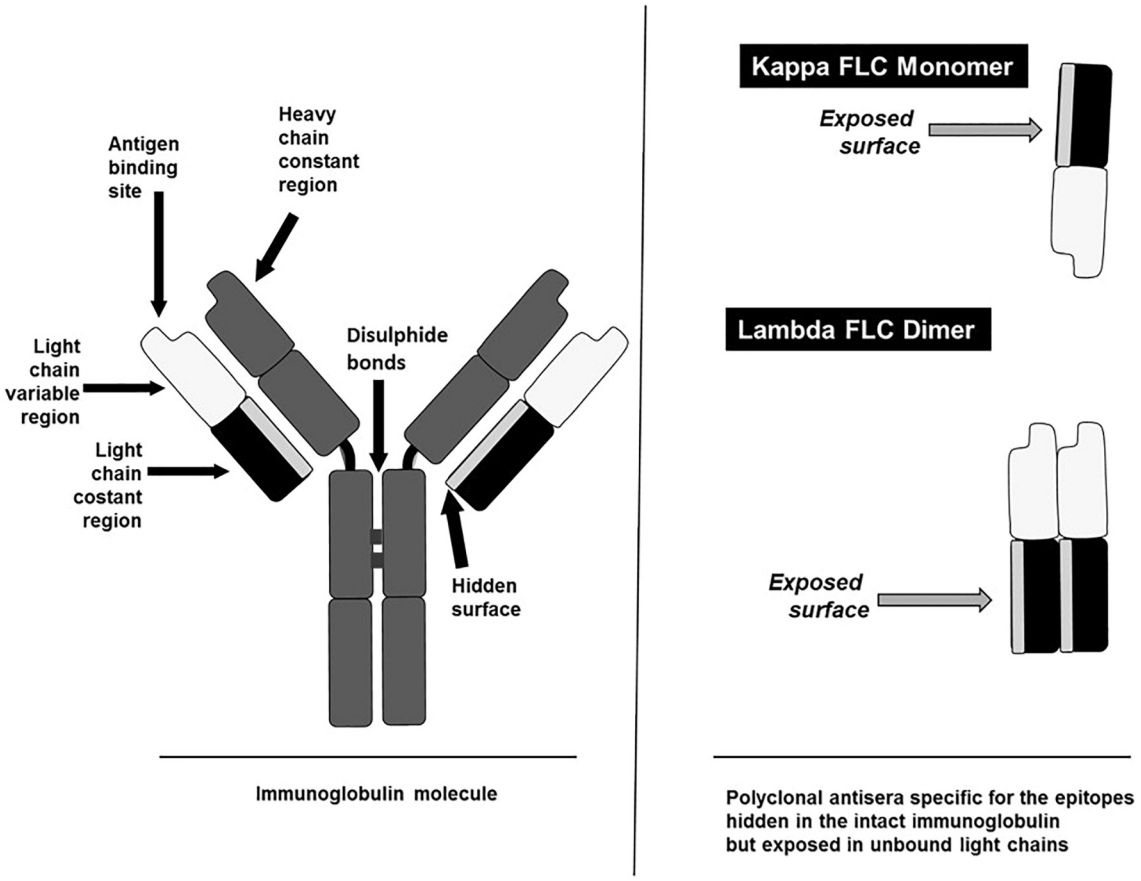

Fig. 1. Immunoglobulin and general structure of free light chains (FLCs).
Each immunoglobulin is composed of two heavy chains and two light chains linked by disulphide bonds. There are two types of light chain: lambda (λ), released as dimers, and kappa (κ), relased as either monomers or dimers. The immunoassay studying serum FLCs uses sheep polyclonal antisera specific for the epitopes found on the constant domain of light chains: these epitopes are hidden in the intact immunoglobulin, but exposed in unbound light chains. Therefore, serum FLC assay is specific for κ or λ FLCs.

primary structure leading to different antigenicity and avidity towards protean cellular targets. Generally, λ FLC are released as dimers and κ FLC as either monomers or dimers [10]. FLCs in the blood stream have a half-life of 2–6 h, more precisely of 2–4 h for κ FLC and 3–6 h for λ FLC, following a rapid renal clearance [11,12]. Usually, FLCs are quickly filtered by kidney glomeruli and almost completely reabsorbed from cells of the proximal convoluted tubule, resulting in traces in the urine (10–30 mg/day) [13,14].

2. General properties of free light chains

Much of the understanding of immunological properties and structure of light chains came from the study of doctor Henry Bence Jones (1813–1873) [15], which also allowed the development of highly specific serological tests to identify and quantify FLCs, paving the way to their application in the clinical practice [11]. For years the interest in FLCs was exclusively related to the diagnosis and monitoring of plasma cell dyscrasia, such as gammopathies and non-Hodgkin lymphoma, in which the circulating FLCs have the characteristic of being "monoclonal": monoclonal FLCs are produced by a malignant proliferation of a single clone of B cells, while "polyclonal" FLCs are the product of multiple B cell clones [12,16,17] (see Table 1).

A turbidimetric immunoassay for detection and quantification of plasma FLCs is regularly used *via* specific polyclonal antibodies capable to selectively bind FLCs through the identification of their surface that is hidden in intact immunoglobulin molecules [18,19] (Fig. 1). The only method useful to highlight protein monoclonal morphology is electrophoresis, while the κ/λ ratio can be used to confirm the monoclonal origin of FLCs. In fact, the main difference between polyclonal and monoclonal FLCs is the absence of significant alterations of the κ/λ ratio [18,19]. The use of κ/λ FLC ratio improves the diagnostic interpretation of malignant plasma cell disorders [20,21]. Moreover, an abnormal serum FLC ratio is a risk factor for progression to plasma cell disorders in patients with monoclonal gammopathy of undetermined significance [22].

A clear distinction between polyclonal and monoclonal FLCs may be useful to better understand the pathogenic role of FLCs in determining renal impairment and damage. Under normal conditions the synthesis of light chains is slightly unbalanced, originating a small excess of combined polyclonal FLCs (cFLC = κ FLC *plus* λ FLC) in serum. These cFLC are readily filtered through the glomerulus and then almost completely reabsorbed by proximal tubular cells. As a result, only a low amount of polyclonal cFLC appears in the urine of healthy subjects [23].

Serum concentrations of FLCs are dependent on the balance between FLC production (from plasma cells and their progenitors) and FLC renal clearance. Circulating λ and κ chains may increase from 10 to 20 times following the overproduction of polyclonal Ig and/or increased renal damage [11,16]. Overproduction of FLCs may occur following an excess of antibody production by B cells, usually as a result of chronic immune stimulation, and for this reason the measurement of FLCs has been proposed as a biomarker of B cell activity in a number of autoimmune and chronic inflammatory conditions [12,24] (Fig. 2).

Different studies have demonstrated that an excess of FLCs released into the circulation is synthesized *de novo* by B cells that are matured beyond the pre-B cell phase, proving that there is a structural and

Table 1
Main differences between monoclonal and polyclonal free light chains (FLCs).

	Monoclonal FLCs	Polyclonal FLCs
Production	By a malignant proliferation of single clones of B cells	By multiple B cell clones
Features	Each clone of FLCs has the same physical-chemical properties	Each clone of FLCs has distinct physical-chemical properties
Significant alterations of the ratio κ/λ	Yes	No
Clinical significance	FLC ratio is a predictor of disease severity and a marker useful in the follow-up	Marker for monitoring activity of the disease
Disorders characterized by Increased FLCs	Plasma cell dyscrasias, including monoclonal gammopathies and non-Hodgkin lymphoma	Pathologies characterized by activation of the B cell line, such as autoimmune diseases and chronic inflammatory disorders

conformational difference between light chains newly synthesized compared to those secreted [25–27]. This confirms that FLCs are not an unimportant or a waste of Ig synthesis, as initially thought, but active molecules *de novo* synthesized following activation of B cells, with immunological properties. The study of FLCs is now an area of growing interest in order to disclose their biological role and a possible clinical use. Moreover, the attention on their function has sparked new interest in their potential role in different chronic inflammatory and autoimmune diseases [12,19]. Several experimental works on animals and *in vitro* studies have highlighted the ability of FLCs to interact with mast cells and neutrophils, with the potential of promoting inflammation [28,29]. When an antigen binds to mast cells FLCs bind themselves to the mast cell-antigen complex, prompting the sensitization of an immune-competent cell that is activated to release inflammatory mediators [30–32]. Therefore, the concentrations of polyclonal FLCs reflect the inflammatory status independently from well-known acute phase reactants, such as C-reactive protein, as commonly found in autoinflammatory disorders [33,34]. The study of the relationship between FLCs and C-reactive protein in different chronic diseases has demonstrated the presence of a weak correlation between the two and differences in their kinetics of response [35]. The interest in polyclonal FLCs as biomarkers started with the observation of increased concentrations in a variety of biological fluids, including blood, cerebrospinal fluid, synovial fluid, salivary and bronchoalveolar lavage [24]. Different studies published in the medical literature have shown increased serum and/or urinary levels of FLCs in patients with autoimmune diseases compared to healthy subjects, and have shown that there is a relationship between FLC concentrations and disease activity. All these data suggest that FLCs might act as pathogenic mini-autoantibodies. This review deals with polyclonal FLCs and their possible use as biomarkers for monitoring treatment outcome for some autoimmune diseases, *i.e.* systemic lupus erythematosus (SLE), myasthenia gravis (MG), systemic sclerosis (SSc), rheumatoid arthritis (RA) and Sjögren's syndrome (SjS).

3. Free light chains and systemic lupus erythematosus

SLE is a chronic multisystem autoimmune disease primarily affecting women in the reproductive age of African-American descent [36,37]. Both genetic and environmental factors cooperate to induce SLE as well as to exacerbate the disease and its symptoms [38,39]. Disease course is featured by flares alternating with remission phases.

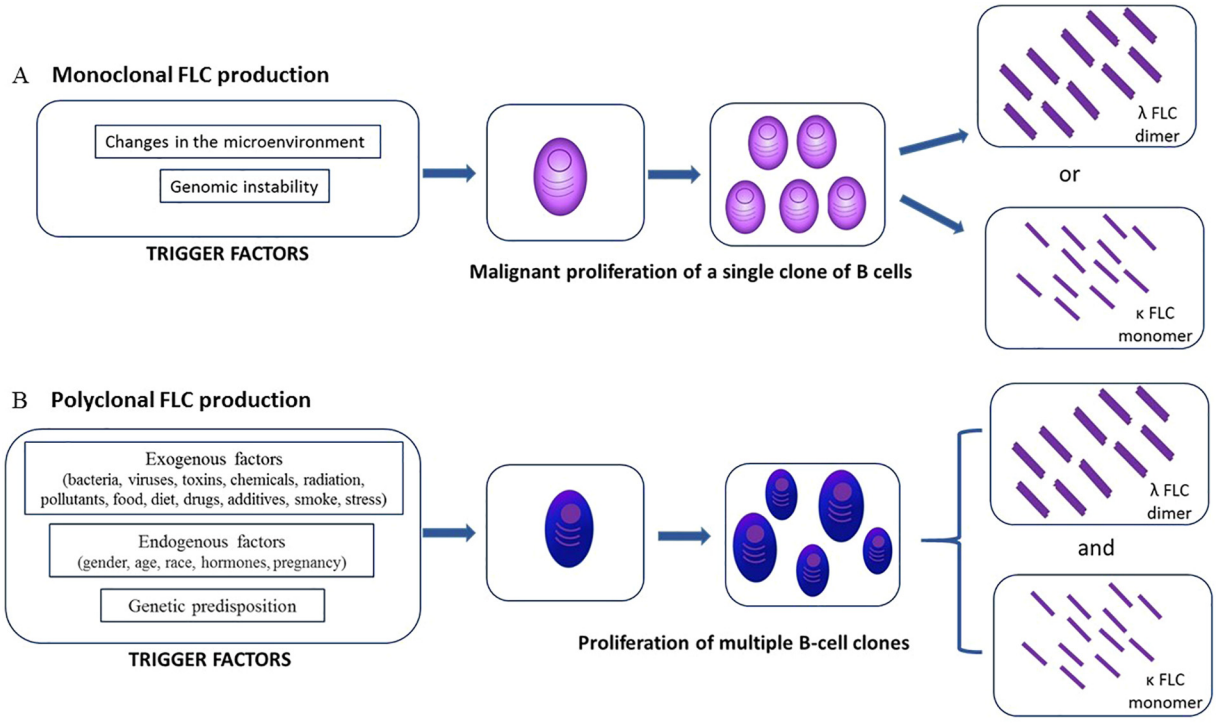

Fig. 2. Production of free light chains (FLCs).
Monoclonal FLCs are produced following the activation of a malignant plasma cell clone, caused by triggering factors such as changes in the microenvironment and/or genomic instability. The result is the release of the FLC clone with identical physical-chemical properties in the blood stream.
Polyclonal FLCs are produced following the activation of multiple B cell clones, caused by several triggering factors such as exogenous/endogenous noxae and/or peculiar genetic predisposition. The result is the release of the FLC clone with distinct physical-chemical properties in the blood stream.

Currently, a set of SLE-associated autoantibodies and conventional clinical parameters are used to measure disease activity and predict complications in the clinical practice, such as creatinine, proteinuria, serum complement or autoantibodies against cellular components, including double-stranded DNA, histones, Ro52, Ro60, La and Sm antigens [37–42]. However, these markers are not always sufficiently sensitive or specific to detect an ongoing disease, because an active SLE is usually identified through evidence of frank clinical manifestations [43]. B cell activation has a seminal role in the pathogenesis of SLE: an extensive polyclonal B cell hyperactivity is usually observed during active disease, followed by paramount polyclonal Ig synthesis with subsequent abundant release of FLCs [37,42].

Over the years different studies have been performed on serum and urinary FLCs of patients with SLE, demonstrating an intriguing association between the concentration of FLCs and overall SLE activity. Preliminary studies had suggested that measurement of urinary FLC levels in patients with SLE could be used to identify or monitor *in vivo* polyclonal B cell activation and predict disease relapses [44,45]. Hopper et al. observed a significant increase of urinary FLCs 4–8 weeks prior to the onset of the first signs of acute SLE relapses, suggesting the possible existence of a considerable time frame between the onset of immunopathological stimulation of B cells, tissue damage induced by acute inflammation, and start of consequent symptoms [44]. In another study the same group found that the level of urinary FLCs was dramatically increased during active phases, widely oscillating during therapy, and reaching normal values only after remission [46]. Therefore, these results upheld the hypothesis that urine levels of FLCs might be used to monitor disease-related B cell activity in SLE.

Moreover, SLE is often associated with infections which may be difficult to distinguish, and indeed urinary levels of FLCs have been evaluated to differentiate these two conditions. Mastroianni-Kirsztajn et al. determined the concentration of FLCs in patients with lupus nephritis (LN), with or without a concomitant infection. They reported significantly different urinary levels of FLCs between healthy subjects, patients with active SLE, and those with non-active SLE, but could not distinguish patients with infection from those without. Therefore, using urinary FLCs allows the detection of SLE activity but neither the presence, nor absence of infections [47]. The increase of FLCs in the sera of patients with SLE was observed for the first time in 1966 by Epstein et al. The authors observed increased FLCs in 90% of SLE patients compared to healthy subjects regardless of the presence or absence of renal failure, with an equal presence of the two isotypes of FLCs [45]. Cooper and Bluestone studied the levels of FLCs in a large group of patients with autoimmune disorders and connective tissue diseases: in patients with SLE they observed high serum FLC levels with a simultaneous increase of FLCs in urine. However, some patients had high urinary FLCs in the absence of detectable serum FLCs. Results of their study have shown no correlation between the increase of serum or urinary FLCs and occurrence or severity of renal disease, but revealed also a correlation between FLCs and disease severity or disease activity. In addition, the purification of FLCs from urine of a single SLE patient showed for the first time that FLCs had an heterogeneous structure as commonly observed in the light chains of intact immunoglobulins [48].

Afterwards, many other studies have confirmed that patients with SLE have high serum levels of FLCs even in comparison with healthy subjects. Aggarwal et al., in fact, determined serum levels of FLCs in patients with rheumatologic diseases, such as SLE and RA, observing that both κ and λ concentrations were elevated respect to healthy individuals, whilst κ/λ ratio was normal, showing the polyclonal nature of FLCs. Furthermore, FLC levels were significantly higher in patients with SLE than in patients with RA, and the authors also noticed a strong correlation between FLCs and disease activity measured by physician global assessment as well as by the Safety of Estrogens in Systemic Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) score [40,43]. These results can be explained by the strong relationship between B cell hyperactivity and disease activity of SLE [43].

Accordingly, Chiche et al. found that 8 out of 11 SLE patients had elevated concentrations of serum FLCs (mainly κ) with normal κ/λ ratios. In addition, they investigated how FLC concentrations change on the basis of therapies by determining the concentration of serum IgG, IgA, IgM and serum FLCs before or after rituximab (RTX) treatment: in particular, they observed that κ and λ FLC concentrations decreased significantly after RTX therapy, while total IgG, IgA and IgM levels decreased though remaining in a normal range [49]. This study confirmed a strong correlation between κ FLC levels and C3 consumption, that is characteristic of active SLE, proving that this correlation is preserved even after B cell depletion. The fast turnover of FLCs, particularly of κ chains (less than 6 h compared to 20–25 days for total IgG), justifies the interest in their use as markers of response to RTX [19,47,49].

A study performed in 2015 showed that the increase of FLCs in patients with SLE correlates with several serologic parameters of SLE, unlike IgG levels, because the secretion of FLCs is independent from antibody production [37]. Furthermore, a strong significant difference between SLE patients and healthy controls was found after adjusting FLC levels for total Ig concentrations, indicating that elevated FLC concentrations do not reflect an altered Ig production [37]. Moreover, serum FLCs correlated with anti-dsDNA antibody titers and total serum IgG and IgA, confirming the strong relationship between plasma cell productivity and SLE disease activity [37]. The association between FLCs and antibodies against Epstein-Barr virus was also evaluated: FLC concentrations correlated with total IgA and total IgG, but not with total IgM concentrations in SLE patients and healthy controls. The increase of antibodies against Epstein-Barr virus in patients with SLE has not been associated with high levels of FLCs, suggesting that high FLC concentrations might reflect a reactivation of the virus [37]. A concurrent measurement of Epstein-Barr virus load in the peripheral blood mononuclear cells and serum FLC concentrations in longitudinal samples from SLE patients could clarify this hypothesis.

Levels of FLCs have also been studied in relationship with specific SLE manifestations, *i.e.* central nervous system (CNS) involvement (CNS-SLE) [50] and LN [42,51]. Hirohata and Miyamoto found an increased intrathecal synthesis of FLCs in 4 out of 15 patients with CNS-SLE in addition to Ig-bound light chains [50]. Furthermore, cerebrospinal fluid levels of κ and λ chains correlated with cerebrospinal fluid Ig/albumin ratio and oligoclonal banding, and strikingly decreased when CNS manifestations subsided after treatment [50].

Conversely, the composition of proteins in urine was analyzed and compared in patients with LN and in patients with other forms of nephritis, finding that soluble interleukin-2 (IL-2) receptors and FLCs were increased and that urinary presence of the cytokine might reflect the inflammation severity [51]. In addition, Hanaoka et al. demonstrated that urinary levels of FLCs were elevated and associated with B cell infiltration in SLE patients with class III/IV LN according to the International Society of Nephrology/Renal Pathology Society [42].

4. Free light chains and myasthenia gravis

MG is an autoimmune neuromuscular disease caused by antibodies directed against the post-synaptic muscle membrane, targeting the acetylcholine receptor (AChR) or the muscle-specific tyrosine kinase (MuSK), which induce muscle weakness and excessive fatigability with variable overall severity. In a recent paper, levels of FLCs were evaluated in 34 sera from 17 AChR-positive and 13 MuSK-positive patients, in comparison with 20 sera from patients with other rheumatologic diseases and 18 from healthy blood donors (HBD), along with titers of specific autoantibodies and IgG subclass distribution [52]. Eleven and 4 out of 17 samples related to AChR-positive patients revealed free κ and free λ chains above the range of normality, respectively, while 1/17 had a λ value below the range of normality. Conversely, 8 out of 13 samples related to MuSK-positive patients displayed a free κ value

above the range of normality, while 2/13 had a value of free λ above and 2/13 below the range of normality [52]. The statistical analysis revealed significant differences among AChR-positive and MuSK-positive groups and HBD: the mean values of free κ chains were above the cut-off and significantly higher in both MG subgroups and in other rheumatologic diseases as compared to HBD. The mean value of free λ chain levels was significantly different only in AChR-positive patients when compared to HBD [52]. Conversely, free λ chains were increased only in the AChR-positive group and in patients with rheumatologic diseases. A significant reduction of both κ and λ FLCs was observed in MuSK-MG patients treated with RTX at least after two months from the first infusion, suggesting that serum FLCs may represent a new marker of B cell activation in MG, probably useful to monitor response to treatment [52].

5. Free light chains and systemic sclerosis

SSc is a chronic disease of the connective tissue, mainly characterized by protean immune system abnormalities leading to endothelial dysfunction and progressive accumulation of collagen in the skin and different internal organs [53]. Patients with SSc fulfilling the diagnostic criteria proposed by the American College of Rheumatology were classified according to the LeRoy classification [54,55]. Although patients with anti-nuclear, anti-topoisomerase I, anti-centromere antibody positivity and a scleroderma-pattern at the nailfold videocapillaroscopy could be easily managed over time, there is still no agreement on which are the predictors that can help identify the subset of patients who will evolve towards a defined form of SSc. Recent studies on animal models of SSc indicate a potential role for B cells in the pathogenesis [56,57]: B cell activation is associated with exaggerated polyclonal synthesis of immunoglobulins, causing an increase of FLCs in the circulation, which are also responsible for mast cell activation and release of different inflammatory mediators [24,31,32].

Up to now only two studies investigated the role of FLCs in SSc. One of them was conducted by our research group: we found that the mean serum levels of total FLCs were significantly higher in patients with SSc if compared with healthy controls. In particular, serum κ chains and κ/λ ratio were significantly higher when compared with healthy subjects, while serum λ chains were similar (these results were also confirmed after correction for age and creatinine concentrations). Moreover, direct correlations emerged between disease activity markers and both κ FLCs and λ FLCs, but not with severity scores, while no differences were noted for patients with active and inactive disease. We also found a correlation between FLCs and IL-6, C-reactive protein and erythrocyte sedimentation rate in our cohort of SSc patients [57], accordingly with previous results by Lanteri et al., which correlated with the Rodnan skin score, showing also an independent association with lung disease and overall disease severity [57,58].

6. Free light chains and rheumatoid arthritis

Patients with RA had significantly higher mean concentrations of total, κ and λ FLCs as compared to healthy subjects: indeed, Deng et al. found that 5 years before RA onset FLCs increased 1% per year and remained high during follow-up. Furthermore, they observed that serum levels of FLCs might also predict a higher mortality in patients with RA [59].

Gottenberg et al. in 2007 analyzed serum FLCs in 50 patients with RA, observing that serum κ and λ chains were significantly higher than in controls, whilst the k/λ ratio was not significantly different between patients and controls: 36% of patients had increased serum FLCs, 30% increased κ chains (including 6% of patients with increased κ/λ ratio) and 6% increased λ chains [60]. A strong correlation was observed between FLCs and specific markers of B cell activation, such as immunoglobulins, IgG, and rheumatoid factor-IgM, but not with anti-cyclic citrullinated peptide antibodies. Interestingly, FLC levels markedly increased along with the Disease Activity Score 28 (DAS28), supporting a potential relationship between B cell activation and overall disease activity in RA [60]. The increase of FLCs in close relationship with the DAS28 demonstrates that serum κ and λ light chains are important inflammatory indexes which could be used as indicators of disease activity in RA [61]. The predictive value of FLCs in urine of patients with RA was evaluated by Bramlage et al., who reported an elevation of both κ and λ light chains, but not for the κ/λ ratio. Moreover, FLC concentrations correlated with inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate, demonstrating their potential predictive role in measuring disease activity [62].

The usefulness of FLCs in monitoring the efficacy of RA treatment has been investigated by different studies. Abatacept, a soluble glycosylated fusion protein which links the extracellular domain of human cytotoxic T lymphocyte-associated antigen 4 to the Fc portion of a human immunoglobulin G1, having activity as a selective co-stimulation modulator with inhibitory activity on T lymphocytes, can also reduce signs of polyclonal B cell activation, inducing a trend towards normalization of serum levels of different classes of Ig and FLCs, reducing titers of anti-citrullinated protein antibodies and rheumatoid factor, and also decreasing circulating post-switch memory B cells [63].

In the first studies exploring the relationship between RA and FLCs an increased amount of FLCs was found not only in sera and urine of patients, but also in the synovial fluid of inflamed joints. Only 3 out of 41 patients contained detectable FLCs in the serum, but none of these were high enough to be quantitated. In contrast, FLCs were found in the urine of 17 out of 36 patients tested. Moreover, synovial aspirates of knee effusions were obtained from 27 patients with RA, and 22 of these contained FLCs: in 19 cases FLCs were present in a sufficient amount to be quantitated [48]. Furthermore, no correlation emerged between the amount of FLCs and rheumatoid factor measured in the synovial fluid. Difference in concentrations between serum and synovial fluid suggested the presence of a concentration gradient maintained by the synovial membrane [48,49]. To date, we do not know if FLCs are synthesized *de novo* in the synovial membrane resulting from an immunologic event within the joint or are selectively concentrated from the serum into the synovial fluid.

High concentrations of FLCs in the synovial fluid from inflamed joints were also found in another study by Kormelink et al., who noted a positive correlation with serum FLC amounts. Furthermore, serum concentrations of FLCs significantly correlated with disease activity, erythrocyte sedimentation rate and C-reactive protein [64]. Changes in FLCs correlated with clinical improvement after treatment with RTX, and the effect of treatment on FLC concentrations discriminated clinical responders from non-responders [64]. These studies indicated that FLCs could be used as early prognostic biomarkers to monitor treatment response in patients with RA.

7. Free light chains and Sjögren's syndrome

SjS is a chronic autoimmune disease most frequently showing a progressive course in women during the fifth decade of life [65], also in association with other autoimmune diseases such as SLE and RA [66]. This systemic disease is characterized by lymphocyte infiltration that affects the epithelium of exocrine glands, leading to decreased production of tears and saliva, with risk of disease transformation into lymphoma [67]. Recent studies have shown that there are subgroups of patients with different clinical manifestations, histological patterns, cytokine profile, and prognosis of SjS [68,69].

Different studies analyzed serum levels of FLCs in patients with SjS since they are sensitive indicators of B cell proliferation and antibody formation. Gottenberg et al. found that 22.3% patients had abnormal serum levels of FLCs, including 12.2% of patients with increased κ levels (5/17 had abnormal κ/λ ratio), 35.3% increased λ levels (5/6 with abnormal k/λ ratio), 5% increased κ and λ levels, and 0.7% with only

abnormal FLC ratio. High levels of FLCs with normal FLC ratio were more often detected in patients with concomitant monoclonal gammopathy of undetermined significance compared to patients with only primary SjS [60]. Moreover, other biological markers of B cell activation have been reported to be correlated with FLCs in patients with primary SjS, such as anti-SSA and anti-SSB autoantibodies, rheumatoid factor, serum levels of IgA, IgG and IgM, serum B cell-activating factor and serum β_2-microglobulin [60,70].

In particular, patients with anti-SSB antibodies have shown higher serum levels of FLCs than patients with anti-SSA antibody alone or patients without autoantibodies with no correlation between κ or λ levels and age, serum creatinine level, and disease duration. Increased levels of FLCs were also correlated with overall SjS activity [60]. Serum levels of FLCs were higher in patients with primary SjS displaying systemic features than in those with only glandular involvement. Extraglandular involvement was associated with abnormal levels of FLCs and also anti-SSB antibodies [60].

As the salivary gland is the most relevant disease site in SjS, it is most likely that saliva itself early reflects the disease impact in comparison with serum [71]. In particular, Sandhya et al. observed that salivary κ chain was elevated in 66.67% of patients and in 26.67% of controls, whilst salivary λ chain was elevated in 11/15 (73.33%) patients and 1/15 controls (6.67%), respectively. As regards serum, κ chain was abnormal in 92.3% of patients and in 26.67% of controls, while serum λ chain was abnormal in 11/13 patients and 5/15 controls, respectively [71]. Previous reports have shown that minor salivary glands of patients with primary SjS have monoclonal plasma cells containing the same monoclonal isotype found in serum [72]. For these reasons FLCs have been evaluated in SjS patients' saliva. Salivary and serum κ and λ chains were higher in primary SjS as compared to controls. Moreover, salivary λ and serum λ chains were associated with ocular signs and symptoms, whereas salivary λ chains had the highest specificity and serum κ chains the highest sensitivity for the diagnosis of primary SjS. A further combination of salivary λ FLC and serum κ FLC improved the sensitivity of the assay until 100% [71,73].

A lacking concordance between salivary and serum FLCs implies that elevated production of FLCs in the saliva of patients with primary SjS may not be due to their diffusion from serum. It is likely that salivary FLCs are a reflection of local B cell proliferation and local Ig production, being representative of ectopic germinal center formation in the salivary glands [73]. Therefore, salivary FLCs have the potential to emerge as useful markers, which might replace minor salivary gland biopsy mostly in serologically negative patients with SjS.

In a recent paper, serum κ and λ chains were abnormal in 58% and 44% of patients with primary SjS, respectively [74], whereas these percentages were considerably higher than in the previous study by Gottenberg et al. [60]. Moreover, they observed that high FLC levels were more pronounced in anti-SSA-positive patients compared with negative ones with primary SjS, suggesting that patients with anti-SSA antibodies exhibit higher B cell activity and produce more FLCs. Verstappen et al. evaluated the κ/λ ratio in addition with the absolute levels of FLCs, observing a small but significant increase in patients with primary SjS: they found that abnormal FLC κ/λ ratios could be indicative for the presence of mucosa-associated lymphoid tissue lymphoma, confirming that abnormal FLC levels in primary SjS are associated with higher disease systemic activity [74]. The correlation between FLC levels with the EULAR SS DAI (ESSDAI: European League Against Rheumatism Sjögren's Syndrome Disease Activity Index) and clinESSDAI scores (a version of the ESSDAI without the biological domain, for assessing the potential eligibility and outcomes for clinical trials in patients with primary SjS) showed that κ chain was significantly and more strongly correlated with both scores than λ chain [74–76]. Therefore, increased disease activity of primary SjS is not only associated with increased polyclonal B cell activity, but remarkably also with preferential increase of the κ chain clone, as confirmed by other studies [60,77]. This study further showed the correlation among biological drugs and FLCs. FLC levels rapidly decreased following treatment with RTX or abatacept, and these levels were associated with systemic disease activity at baseline and longitudinally to response with treatment [74].

8. Conclusive remarks

Many studies have confirmed the importance of early detection of autoantibodies as biomarkers in the mosaic of autoimmunity, as their presence can predict the development of a full-blown autoimmune disease [78,79], and this is still important in the era of personalized medicine for disease prevention or for mitigating the clinical impact of a future disease [80]. In this review the pathogenic role of FLCs in selected autoimmune diseases has been listed and shown. To diagnose and monitor these pathologies it is useful to evaluate overall B cell activity through specific biomarkers and also through polyclonal FLCs, which have demonstrated a direct correlation with multiple autoantibodies and are now recognized as biomarkers for some specific autoimmune diseases [37,43,52,60].

FLCs represent a family of proteins with combinational diversity accounting for substantial variable region diversity and sharing only the constant part of the molecule. Diversity arises from the many different combinations of heavy and light chains, which are produced by somatic hypermutation occurring in variable region genes of mature activated B cells. The amino acid sequence of the variable region of light chains is unique to each FLC, and the number of amino acid residues in this region can differ contributing to the diversity and heterogeneity of FLCs. This is likely to be involved at least in part in the activation of B cell clones producing FLCs which work as mini-antibodies against tissue-specific target antigens. We suppose that FLCs involved in autoimmune disorders are expression of different B cell inflammatory clones with specific autoreactivity, and that they might be used as early prognostic markers of disease severity and/or assessment of disease relapses [46,49,52,63]. Furthermore, FLCs could be used to monitor disease activity along with therapies administered, due to the strong relationship between B cell and overall disease activity [37,43,49,60,74] (Fig. 3).

A further molecular clarification of the role of FLCs in so different autoimmune diseases will provide a better understanding of their pathogenesis and indicate a more specific treatment of the self-directed inflammation operating in such complex disorders Table 2.

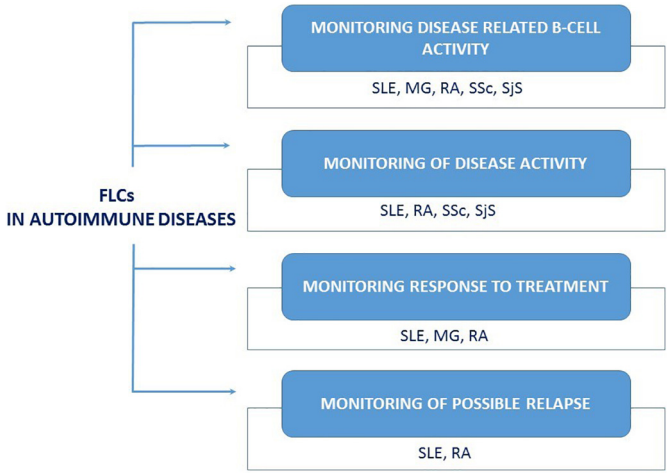

Fig. 3. Role of polyclonal free light chains (FLCs) in the management of autoimmune diseases.
Abbreviations: SLE: systemic lupus erythematosus, MG: myasthenia gravis, RA: rheumatoid arthritis, SSc: systemic sclerosis, SjS: Sjögren's syndrome.

Table 2
Summary of all clinical studies evaluating free light chains (FLCs) in several autoimmune diseases.

Study	Type of sample	Results
Systemic lupus erythematosus		
Epstein et al. [45]	Serum	≻ Increased FLC levels regardless of the presence or absence of renal failure
Cooper et al. [48]	Serum	≻ No correlation between increase of serum or urinary FLCs and occurrence or severity of renal disease
	Urine	≻ Correlation between the presence of FLCs and both severity and disease activity
Hopper et al. [44]	Urine	≻ Increased FLC levels during asymptomatic intervals 4–8 weeks before the onset of the first signs of acute relapses
Hopper et al. [46]	Urine	≻ Levels of FLCs increase during the active phase of the disease and reach normal values only during remission phases
Aggarwal et al. [43]	Serum	≻ FLC concentrations are significantly higher than in healthy controls with a normal κ/λ ratio
		≻ Strong correlation between FLC levels and disease activity scores (PGA and SLEDAI score)
Chiche et al. [49]	Serum	≻ SLE patients have elevated concentrations of serum FLCs (mainly κ chains) with normal κ/λ ratio
		≻ After rituximab FLC concentrations decrease significantly, while total IgG, IgA and IgM levels remain in a normal range
		≻ Strong correlation between κ chain levels and C3 decrease
Draborg et al. [37]	Serum	≻ Increased levels of FLCs correlate with several serologic parameters of SLE, unlike IgG levels
		≻ Strong difference between patients and healthy controls are still observed after adjusting FLC levels for total Ig concentrations
Systemic lupus erythematosus with involvement of the central nervous system		
Hirohata et al. [50]	Cerebrospinal fluid	≻ Increased synthesis of intrathecal FLCs
		≻ CSF κ and λ chains are correlated with many disease activity markers
		≻ κ and λ chains decrease along with treatment
Lupus nephritis		
Tsai et al. [51]	Urine	≻ Increased excretion of sIL-2R and FLCs directly reflect the severity of inflammation
Hanaoka et al.[51]	Urine	≻ FLCs significantly higher in the subset of patients with class III/IV LN than in those with non-III/IV LN
		≻ λ chain levels are correlated with urinary protein/creatinine ratio in the class III/IV LN
		≻ FLC levels are correlated with the number of $CD138^+$ cells in the kidney
		≻ λ chains decrease along with treatment
Rheumatoid arthritis		
Deng et al. [59]	Serum	≻ FLC levels increase 1% per year 5 years before RA onset and remain elevated during follow-up
Gottenberg et al. [60]	Serum	≻ Strong correlation between FLC levels and markers of B cell activation
		≻ FLC levels are markedly increased in relationship with the DAS28
Ye et al. [61]	Serum	≻ FLC increase in relationship with the DAS28
Bramlage et al. [62]	Urine	≻ Elevation of FLC levels in patients with RA
		≻ No elevation of the κ/λ chain ratio
		≻ FLC concentrations correlate with inflammatory markers
Scarsi et al. [63]	Serum	≻ Decreased FLC levels after treatment with abatacept
Kormelink et al. [64]	Synovial fluid	≻ High FLC concentrations in the synovial fluid from inflamed joints
	Serum	≻ Serum FLC concentrations correlate with disease activity and inflammatory markers
Epstein et al. [45]	Synovial fluid	≻ No correlation between FLCs and rheumatoid factor
	Serum	
Cooper et al. [48]	Synovial fluid	≻ Higher FLC concentrations in the synovial fluid
	Serum	
	Urine	
		≻ No correlation of FLC amounts among biological fluids
Systemic sclerosis		
Bosello et al. [57]	Serum	≻ κ chains and FLC ratio are significantly higher in comparison with healthy subjects
		≻ Correlation between disease activity markers and FLCs
		≻ Correlation between FLCs and IL-6, C-reactive protein and erythrocyte sedimentation rate
Lantieri et al. [58]	Serum	≻ FLC levels correlate with the Rodnan skin score and are independently associated with lung disease and its severity
Myasthenia gravis		
Basile et al. [52]	Serum	≻ Statistically significant increase of κ chains in AChR and MuSK-positive patients, when compared to healthy subjects, while λ chain levels are increased only in the AChR-positive group
		≻ Significant reduction of both κ and λ chains in MuSK-positive patients two months after rituximab
Sjögren's syndrome		
Sandhya et al. [71]	Serum	≻ Salivary and serum FLC levels are higher in primary Sjögren's syndrome if compared to controls
	Saliva	≻ Salivary κ chain has the highest specificity and positive predictive value, whereas serum λ chain has the highest sensitivity and negative predictive value
Gottenberg et al. [60]	Serum	≻ FLC levels are significantly higher in comparison with controls
		≻ 22.3% of patients have abnormal serum levels of FLCs, including 12.2% who showed increased κ and 35.3% increased λ chains
		≻ Serum FLC levels are correlated with B cell activation markers
Verstappen et al. [74]	Serum	≻ κ FLC and λ FLC levels are significantly higher in primary SjS compared with non-SjS sicca patients
		≻ κ FLC and λ FLC levels are abnormal in 58% and 44% of primary SjS patients, respectively
		≻ The κ/λ chain ratio is abnormal in 11% of patients with primary SjS
		≻ FLC levels are significantly correlated with systemic disease activity, as assessed by ESSDAI and clinESSDAI
		≻ FLC levels rapidly decrease following treatment with rituximab or abatacept

PGA: Physician Global Assessment; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SLE: Systemic Lupus Erythematosus; CSF: Cerebrospinal Fluid; sIL-2R: Soluble Interleukin-2 Receptor; LN: Lupus Nephritis; DAS28: Disease Activity Score 28; IL: Interleukin; AChR: Acetylcholine receptor; MuSK: Muscle-Specific Tyrosine Kinase; ESSDAI: EULAR SS Disease Activity Index; clinESSDAI version of the ESSDAI without the biological domain.

9. Take-home messages

- The role of free light chains in autoimmune diseases is only partially elucidated.
- Different studies have been performed on serum and urinary free light chains in patients with systemic lupus erythematosus and have demonstrated an intriguing association between their concentration and overall disease activity.

- In particular, serum free light chains are correlated with anti-dsDNA antibody titers, total serum IgG and IgA levels in patients with systemic lupus erythematosus.
- Free light chains have been tested as markers of response to rituximab in patients with systemic lupus erythematosus.
- The reduction of free light chains following treatment with rituximab in patients with myasthenia gravis suggests their potential use as predictors of therapeutic efficacy.
- Mean serum levels of total free light chains are significantly higher in patients with systemic sclerosis, in particular both serum κ free light chains and κ/λ ratio.
- The level of free light chains tends to increase along with disease activity scores in patients with rheumatoid arthritis.
- Free light chains have been found in the synovial aspirates of knee effusions from patients with rheumatoid arthritis, though no correlation has emerged between their amount in the synovial fluid and rheumatoid factor.
- The use of salivary free light chains is emerging as a diagnostic marker for patients with Sjogren's syndrome who are negative at the autoantibody screening.
- In summary, polyclonal free light chains could be used as biomarkers indicating short term-prognosis and outcome in selected autoimmune diseases.
- Future studies are needed to exactly identify benefits deriving from the clinical use of free light chains in the real-life scenery of autoimmune diseases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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