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Brief communication

Anti-C1s autoantibodies as complementary serologic biomarker in lupus nephritis

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ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by the presence of circulating autoantibodies. Autoantibodies targeting C1 complex proteins, particularly C1q, have already been described in lupus nephritis (LN). However, autoantibodies targeting the C1s protease remain poorly studied. We determined the prevalence of anti-C1s autoantibodies in serum of SLE patients, and evaluated their presence in relation to clinical conditions. For this purpose, sera from 187 SLE patients with different disease activity were selected and anti-C1s autoantibodies were measured by ELISA. We observed that patients with LN had significantly higher levels of anti-C1s autoantibodies than SLE patients with other flare types. Anti-C1s autoantibodies recognised mainly the C1s N-terminal part. Interestingly, the combination of anti-C1s, anti-DNA and anti-C1q autoantibodies showed high specificity (94.6 %) and a significant positive predictive value of 80 %. These results suggest the potential interest of anti-C1s autoantibodies as a complementary serological biomarker in the early screening for LN.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects around 3 million people worldwide, mainly women who account for 90 % of cases [1]. The evolution of this inflammatory disease is highly uncertain, with patients going through phases of active disease and phases of remission. These flares are difficult to predict and can affect different organs such as skin, joints, nervous system and kidney. Their diversity and unpredictability make the diagnosis and monitoring of patients difficult. SLE is characterised by the production of a large number of circulating autoantibodies directed against autoantigens, such as nuclear antigens and double-stranded DNA (dsDNA) [1,2]. These autoantibodies form immune complexes that activate the complement system, leading to inflammation and damage in numerous organs. Diagnosis is currently based on a combination of clinical examination and measurement of various biomarkers, such as the presence of

autoantibodies and assessment of the activity of the complement system [1,3].

The complement system, a key component of innate immunity, plays an essential role in host defence against pathogens and the maintenance of homeostasis. Although used as a diagnostic tool, the involvement of the complement system in the pathogenesis of SLE is complex. It is generally accepted that SLE is associated with hypocomplementaemia as a result of complement hyperactivity [3]. Such an excessive and non-specific response contributes to tissue damage. Moreover, mutations leading to an innate deficiency of the classical complement components (C1q, C1r, C1s, C4 and C2) are important risk factors for the development of the disease, illustrating the protective role of this system [3–8]. This highlights the complex role of complement in maintaining immune balance and preventing autoimmunity.

The classical complement pathway is particularly involved in the recognition and elimination of immune complexes [9]. Their

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recognition by C1q triggers successive activation of the C1r and C1s proteins. The serine protease C1s is crucial in this cascade, cleaving C4 and C2 to form the C3 convertase, a key event in complement activation [10]. In the context of SLE, excessive C1s activity could intensify inflammation and lead to tissue damage at the site of immune complex deposits [11], whereas C1 deficiency would prevent adequate clearance of immune complexes and apoptotic cells and contribute to the persistence of autoimmunity and worsening of symptoms [2,12].

Lupus nephritis (LN) is one of the most serious clinical manifestations of SLE affecting the kidney, with significantly high morbidity and mortality. It affects around 30 to 60 % of lupus patients throughout life, and only half of them show complete remission after treatment [13,14]. Clinical presentation of LN is highly heterogeneous, ranging from asymptomatic renal lesions to acute renal dysfunction [15]. Between 10 % and 30 % of patients develop end-stage renal disease [13]. Early detection of this damage is essential to enable better patients management and to preserve renal function in the long term. Renal biopsy is the gold standard for diagnosing and monitoring LN, but it is an invasive procedure. Other non-invasive investigations such as evaluation of proteinuria, elevated anti-dsDNA antibody titre and reduced complement levels are useful markers of the overall activity of SLE and LN. However, there is a major need for more sensitive and specific biomarkers for detecting ongoing disease activity.

Anti-C1q autoantibodies, which have a high specificity up to 97 % for the development of LN, are commonly used to monitor this flare [16]. The presence of autoantibodies targeting other complement molecules such as ficolin-3, ficolin-2, C1r and C1s has also been reported [16–19] but remains poorly explored and is not commonly used in diagnosis and patients follow-up. These autoantibodies can lead to the acquired deficiency of their target protein and participate in complement dysregulation.

The aim of our study was to investigate the presence of anti-C1s autoantibodies in SLE patients in remission and in various types of flares and to assess their potential as biomarkers for LN. In addition, the C1s domains targeted by these autoantibodies were identified.

2. Materials and methods

2.1. Study population

This study is a retrospective, non-interventional study conducted in the Grenoble Alpes University Hospital and Public Assistance Hospital of Marseille, as well as Leiden University Medical Centre (Netherlands). It was approved by the Committee for the Protection of Individuals (38RC21.0388) and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. SLE patient samples ($n = 187$) were obtained from different declared biobanks, (DC-2014-2268, DC-2012-1704). This study has been registered on clinicaltrials.gov (NCT05193591).

Diagnosis of SLE was based on the American College of Rheumatology's revised 1997 classification criteria for SLE. The characteristics of the 187 SLE patients are detailed in Table S1. The median age of the patients was 50 years (range 23 to 92 years) and 88.6 % were female.

For SLE patients, SLE Disease Activity Index (SLEDAI) score, as well as clinical and biological characteristics, were recorded at the time of sampling [20]. Patients were divided into two groups: a group with quiescent disease characterised by a SLEDAI ≤ 5 ($n = 67$) and a group with active disease characterised by a SLEDAI > 5 ($n = 120$).

Joint involvement ($n = 39$), defined as the presence of at least three painful and inflamed joints, was determined by the clinician in charge. Patients with LN ($n = 39$) showed renal involvement defined as significant proteinuria (≥ 0.5 g/day) with or without haematuria, aseptic leukocyturia or urinary cylinders. LN was characterised by renal biopsy immediately after sampling (classes III, IV or V of the International Society of Nephrology/Renal Pathology Society classification) (Table S1). Active proliferative LN was defined as class III or IV LN with

activity, +/- class V, as opposed to nonproliferative LN (class I, II, or isolated V). SLE patients presenting with a neuropsychiatric (NP) exacerbation ($n = 42$) were selected with a multidisciplinary assessment as presented previously [21]. Samples from healthy blood donors ($n = 48$) were obtained from the *Etablissement Français du Sang*.

2.2. Protein purification and production

C1s was purified from human plasma using the procedure published previously [22]. It was quantified using a molecular weight of 79,800 Da and a A1%, 1 cm at 280 nm of 14.5.

C1s fragments were produced by transient transfection of Expi293F cells (Gibco, New York, USA) with pcDNA3.1 expression vectors encoding the CUB1-EGF-CUB2-CCP1 region with a C-terminal His₆ tag (C1s-Nt) and the catalytic CCP1-CCP2-SP domain with a C-terminal Twin Strep tag (C1s-Ct). The pcDNA3.1 C1s-Ct was purchased from Thermo Fisher Scientific (Illkirch, France). The pcDNA3.1 C1s N-ter was obtained by deletion from a pcDNA3.1 C1sWT-Flag [23] by site-directed mutagenesis using the QuickChange II XL kit (Agilent Technologies, Les Ulis, France) and was confirmed by DNA sequencing analysis (Genewiz/Azenta, Leipzig, Germany). The concentration of the C1s-Nt and C1s-Ct fragments was determined using molecular masses calculated by PROTPARAM program on the Expsy Server (<http://web.expsy.org/p/rotparam/>), of respectively 39,452 Da and 46,885 Da and A1%, 1 cm at 280 nm of 12.37 and 18.84.

The C1s-Nt fragment was purified by a two steps procedure. The C1s-Nt Expi293F supernatant dialysed against 50 mM Na₂HPO₄, 300 mM NaCl, pH 7.4 was first applied on a 1 mL HiTrap TALON Crude resin at a flow rate of 1 mL/min (Cytiva, Marlborough, USA). Elution was carried out with an imidazole gradient (0 to 0.15 M). After dialysis in 50 mM Tris, 150 mM NaCl, pH 7.4, the sample was applied on a Q-Sepharose resin (6 mL - Cytiva) and eluted by a NaCl gradient (0.15 to 1 M) at a flow rate of 2.5 mL/min.

The catalytic C1s-Ct fragment was also purified using two steps after initial dialysis of the cell supernatant in buffer A (50 mM Tris, 150 mM NaCl, pH 7.4). The dialysed extract was loaded on a 2 mL Strep-Tactin Sepharose resin (IBA Lifesciences, Göttingen, Germany). Elution was carried out according to the manufacturer's instructions at a flow rate of 10 mL/h. After dialysis in buffer A, the eluate was further purified by size exclusion chromatography (HiLoad 16/600 Superdex 75 pg - Cytiva) at a flow rate of 1 mL/min.

2.3. Anti-C1s antibodies assessment

Detection of anti-C1s autoantibodies and assessment of their C1s fragment specificity were performed using an ELISA method adapted from tests previously described for the measurement of anti-ficolin-3 antibodies [17].

96-well microtitre plates were coated overnight at 4 °C with 52 pmol/mL purified plasma C1s or recombinant C1s-Nt or C1s-Ct fragments in bicarbonate buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, pH 9.6). The wells were then washed three times with phosphate-buffered saline (PBS) containing 0.1 % (w/v) Tween-20 (PBS-T) and then saturated with PBS-T supplemented with 1 % (w/v) bovine serum albumin (BSA) for 1 h at 37 °C. After washing as described above, the serum samples were added (1:50 dilution - PBS-T-BSA 0.1 %) and incubated for 2 h at room temperature (RT). After 4 washes, horseradish peroxidase-conjugated polyclonal goat anti-human IgG (Abliance, Compiègne, France) was added (1:25,000 dilution - PBS-T-BSA 0.1 %) and incubated for 1 h at RT. After final washes, the substrate 3,3',5,5'-tetramethylbenzidine was added and the reaction was stopped by the addition of 1 M H₂SO₄. Optical densities (OD) were measured at 450 nm.

The antibody rate was defined as follows: $OD_{\text{sample}} / OD_{\text{calibrator}} \times 100$. The cut-off value of 24 arbitrary units (AU) was determined by calculating the 98th percentile of the healthy control group, defining a positivity threshold.

2.4. Complement proteins assessment

Plasma C1s concentration in serum of SLE patients was measured by ELISA (Invitrogen, Carlsbad, USA) following manufacturer recommendations. Sera samples were diluted 1:500 in PBS. Complement C3 and C4 levels were measured by nephelometry following the manufacturer instructions (BNII, Siemens, Munich, Germany).

2.5. Anti-dsDNA and anti-C1q antibodies assessment

Anti-dsDNA antibody levels were measured by electrochemiluminescence, QUANTA Flash dsDNA (Werfen, Barcelona, Spain). The level of anti-C1q antibodies was determined as described previously by ELISA [12] or using the Orgentec kit (Mainz, Germany).

2.6. Statistics

Data analysis was performed using RStudio software (version 2024.09.1 + 394) coupled with R (version 4.4.1). Kolmogorov-Smirnov test was used to compare continuous variables between the healthy blood donors/SLE and remission/flare subgroups. Bonferroni-Dunn tests were used to compare flares with each other. Correlations between continuous variables were assessed using Spearman rank correlation coefficients. Comparisons of categorical variables were made using the Chi² test. Statistical significance was determined by considering p-values <0.05.

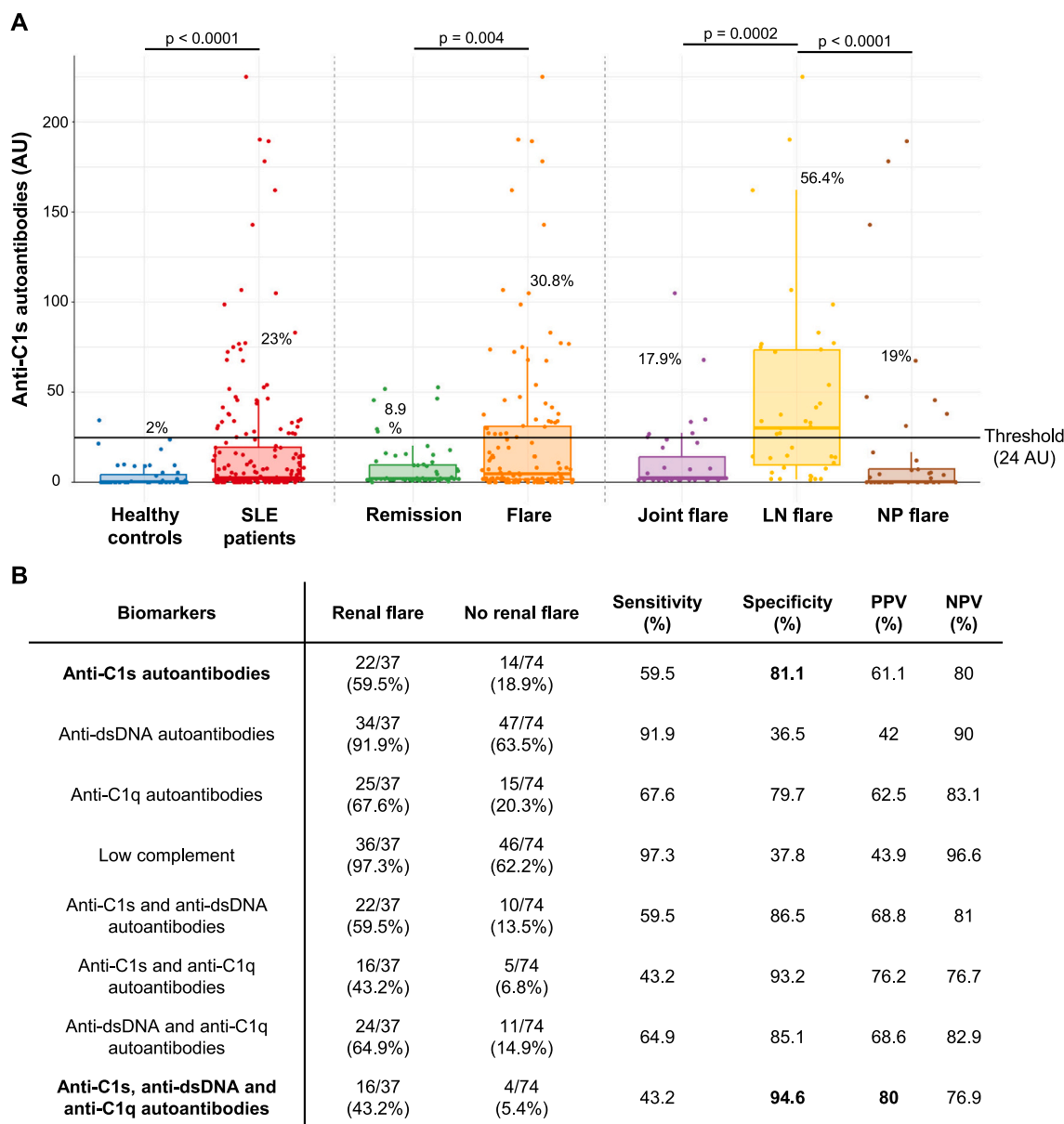


Fig. 1. Anti-C1s autoantibodies in lupus nephritis.

(A) Anti-C1s autoantibodies titres in serum samples. Anti-C1s autoantibodies were measured in serum samples from 48 healthy controls and 187 patients with Systemic Lupus Erythematosus (SLE), including cases in remission (n = 67) and various flare types (n = 120): joint (n = 39), lupus nephritis (LN) (n = 39), and neuropsychiatric (NP) (n = 42). Prevalences are given for each group. Kolmogorov-Smirnov tests assessed differences between Control/SLE and Remission/Flare groups, while Bonferroni-Dunn tests compared the different flare types. The significant p-values obtained are indicated for each test. AU: Arbitrary units.

(B) Diagnostic performance of anti-C1s autoantibodies in lupus nephritis. PPV: positive predictive value, NPV: negative predictive value.

3. Results

3.1. Detection of anti-C1s autoantibodies in patients with SLE and association with lupus activity

Anti-C1s autoantibodies were quantified in the serum samples of 48 healthy controls and 187 patients with SLE. Among them, 67 were in quiescent phase and 120 were in active phase. Patients with flare mainly presented joint, kidney or NP involvement (2.1 Materials and Methods, Table S1).

The level of anti-C1s autoantibodies was significantly higher in patients with SLE compared to healthy controls ($p < 0.0001$, Kolmogorov-Smirnov test). We observed that 43 SLE patients (23 %) were positive for anti-C1s autoantibodies, with a positivity threshold of 24 AU, determined from healthy controls (Fig. 1A). Anti-C1s autoantibody positivity was reported in 37 patients with flare, representing 30.8 % of this group. The level of anti-C1s autoantibodies was significantly higher in patients in flare than in those in remission ($p = 0.004$, Kolmogorov-Smirnov test) (Fig. 1A).

In SLE patients, anti-C1s autoantibody levels correlated significantly with the SLEDAI score ($r = 0.27$, $p < 0.001$, Spearman test) and inversely correlated with serum C1s protein levels ($r = -0.21$, $p = 0.004$, Spearman test) (Figure S1). In addition, a significant association was observed between anti-C1s autoantibody positivity and that of anti-dsDNA and anti-C1q autoantibodies, as well as with low complement levels ($p < 0.001$, Chi-2 tests).

3.2. High prevalence of anti-C1s autoantibodies in patients with lupus nephritis

Interestingly, 22 patients out of 39 with LN (56.4 %) presented anti-C1s autoantibodies. As shown in Fig. 1A, anti-C1s autoantibody levels were significantly higher in this subgroup than in patients with articular or neuropsychiatric flares ($p = 0.0002$ and $p < 0.0001$, Bonferroni-Dunn tests). When patients with LN are compared with the group without renal relapse, the difference remains particularly significant (Figure S1C).

Given the high prevalence of anti-C1s autoantibodies in LN, we assessed their diagnostic performance for renal involvement in our cohort, alone or in combination with other SLE biomarkers (Fig. 1B). The sensitivity and specificity of anti-C1s autoantibodies for LN were 59.5 % and 81.1 % respectively. The positive predictive value was 61.1 %, while the negative predictive value was 80 %. Although low levels of C3 and/or C4 and anti-dsDNA autoantibodies showed better sensitivities, anti-C1s autoantibodies showed better specificity. Interestingly, the combination of anti-C1s, anti-C1q and anti-dsDNA autoantibodies increased specificity to 94.6 % for LN, with a positive predictive value (PPV) at 80 %, which is also the highest score (Fig. 1B). We further wanted to investigate the possible link between the presence of anti-C1s autoantibodies and immunohistologic characteristics of the kidney biopsies of the patients with active LN. However, we do not have in our cohort any patients with non proliferative LN. We nevertheless observed that patients with active proliferative LN classes IV +/- V had more positive anti-C1s autoantibodies than those with active proliferative LN classes III +/- V (74 % (14/19) and 40 % (8/20) respectively, data not shown).

3.3. Anti-C1s autoantibodies mainly target the C1s N-terminal fragment

As it is known that the C1s molecule is composed of several modules with different functions [23], we explored which part of the molecule is recognised by SLE anti-C1s autoantibodies. To this end, two C1s fragments were produced, the C1s-Ct catalytic region of C1s composed of CCP1-CCP2-SP and C1s-Nt comprising the interaction region of C1s, CUB1-EGF-CUB2, and CCP1. The specificity of anti-C1s autoantibodies to these two fragments was assessed in 39 SLE patients (6 in remission, 5

in joint flare, 20 in LN flare and 8 in NP flare) with autoantibody levels above 24 AU (Fig. 2).

The two C1s fragments tested showed that a majority of patients (77 %) had autoantibodies directed against C1s-Nt. Of these, 13 % (5/39) also showed a signal against the C1s-Ct fragments. A significant proportion of patients (25/39) showed a signal only against the N-terminal fragment, underlining the immunogenic potential of this region of C1s.

4. Discussion

To our knowledge, this study is the first which investigates the prevalence of anti-C1s autoantibodies in different types of SLE flares, particularly in NP patients. Only two previous studies have reported the presence of these autoantibodies in SLE and LN patients with divergent prevalence. He and Li observed the presence of anti-C1s autoantibodies in 7 out of 15 SLE patients [24]. More recently, another study reported the low presence of anti-C1s autoantibodies in patients with LN (5/72) [16]. Our cohort showed a moderate prevalence of almost 23 %, with 43 of 187 SLE patients presenting with these autoantibodies (Fig. 1A). However, an association between their level and the clinical and immunological parameters of the disease suggests a more important pathological role than previously reported. As a major interest, it should be noted that the prevalence of anti-C1s autoantibodies was particularly high (56.4 %) in the group of patients with LN. This was not observed in the other two types of exacerbation studied, joint and NP flare, with prevalence rates of 17.9 % and 19 % respectively. As a sharp contrast, all patients without LN presented particularly low levels of anti-C1s autoantibodies.

Such a specific prevalence of anti-C1s autoantibodies in the case of LN lead us to further assess their potential as biomarker. Indeed, a high priority remains on finding more sensitive and specific biomarkers for diagnosis and detecting ongoing disease activity. Interestingly, in our cohort, anti-C1s autoantibodies showed high specificity for LN, almost equivalent to that of anti-C1q. Moreover, the combination of anti-C1s autoantibodies with anti-dsDNA and anti-C1q showed greater specificity, reaching 94.6 % and a positive predictive value of 80 %. Consistently, it is important to note that in our study, 46 % of patients with LN without anti-C1q autoantibodies presented instead anti-C1s antibodies. These results highlight the interest of anti-C1s autoantibodies as new biomarker in the diagnosis of LN. Some discrepancy between studies may partly relate to the type of cohort, here with a bias towards active nephritis classes III, IV and V. Due to our patients cohort with only proliferative active nephritis, it was not possible in our study to evaluate the relationship between anti-C1s autoantibodies and all the types of LN, proliferative and non proliferative active LN. Therefore, larger cohorts with multicentric approach and longitudinal studies would help to further explore the diagnostic and prognostic potential of anti-C1s autoantibodies.

Although the causal relationship between anti-C1s autoantibodies and the development of LN remains to be studied, the implications of these autoantibodies in the pathogenesis of lupus is a key question. A classical hypothesis is that immune complexes deposited in the kidneys induce local tissue lesions. This is relevant for anti-C1s autoantibodies, since C1s is constitutively expressed in the kidney. More specifically, these autoantibodies could affect the functions of C1s and classical pathway activation. On the one hand, they could overactivate C1s, as previously observed [24]. This overactivation would contribute to the excessive consumption of C4 and C3 observed in lupus patients. On the other hand, autoantibodies could inhibit C1s functions or induce C1s deficiency, as supported in our study by the negative correlation between the level of serum C1s and anti-C1s autoantibodies (Figure S1). Identifying the specific domain of C1s targeted by these autoantibodies could help to decipher their functional impact. To this end, autoantibodies binding to two C1s fragments was analysed. We observed that the majority of anti-C1s autoantibodies in lupus patients (64 %) target the N-terminal part of C1s, composed of the CUB1, EGF and CUB2 modules.

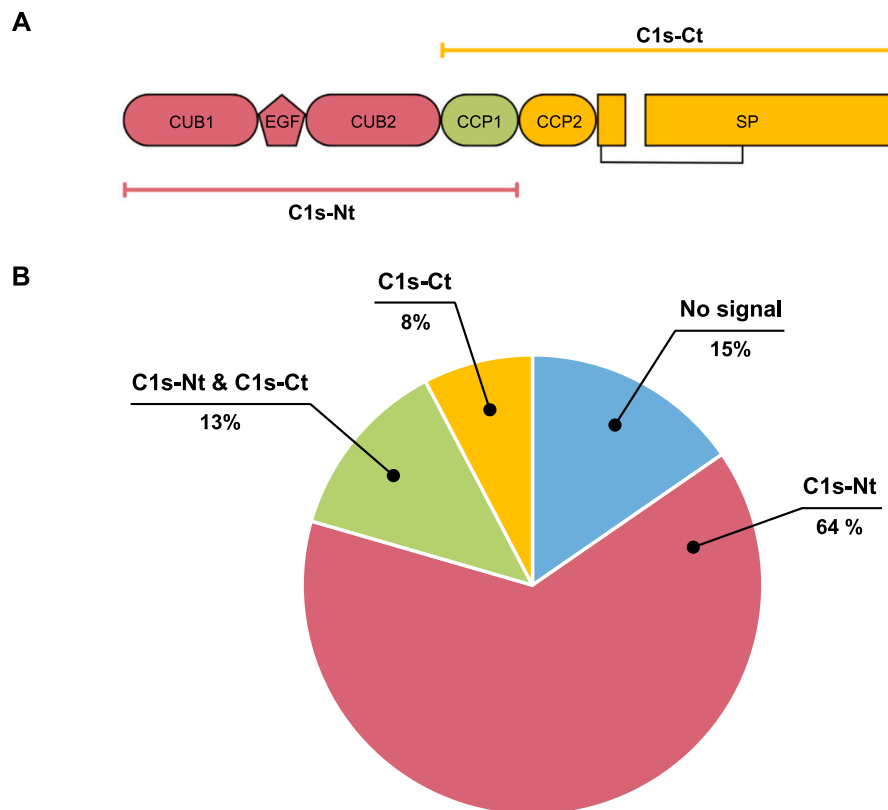


Fig. 2. Anti-C1s autoantibody epitopes in SLE.

(A) C1s complement protease modular structure showing its N- and C-terminal fragments produced for the study. The CCP1 module is common to both fragments. (B) Targets of anti-C1s autoantibodies in SLE patients (n = 39: 6 in remission, 5 in joint flare, 20 in LN and 8 in NP flare). Four groups emerged from this analysis: one group with no recognition signal (blue), two groups targeting only one of the fragments tested and one group both fragments (green). The majority of patients recognise the C1s-Nt fragment, shown in red.

These modules constitute the protease interaction domain and are involved in C1s binding to its various partners. In particular, the N-terminal part of C1s is essential for the formation of the C1r₂C1s₂ tetramer, and therefore for the activation of proenzyme C1s. Autoantibodies targeting these domains could prevent the formation of this tetramer, the assembly of the C1 complex and thus affect the cleavage of its canonical substrates C4 and C2. Indeed, it has been reported that a mouse anti-C1s monoclonal antibody targeting the CUB2 module induced a reduction in the complement activation by preventing C1 assembly [25]. Inactivation of the classical complement pathway would impair immune complexes removal, leading to their accumulation. Whether they lead to inhibition or overactivation of C1s, anti-C1s autoantibodies would contribute to the complement imbalance frequently observed in SLE. In addition, anti-C1s autoantibodies could affect the non-canonical functions of C1s, in particular its ability to cleave nuclear antigens [26,27], which are particularly present in SLE. Further studies are needed to determine the functional impact of anti-C1s autoantibodies in lupus. Detailed molecular studies on human anti-C1s monoclonal antibodies from SLE patients, just as recently performed for human anti-C1q autoantibodies [28], would provide important insight into the molecular properties and pathological impact.

In conclusion, this study reports for the first time the high prevalence of anti-C1s autoantibodies in patients with LN, in contrast to other flares affecting joint or neuropsychiatric systems. Our results suggest the potential interest of these autoantibodies as serological biomarkers of this lupus renal disease. Particularly, we demonstrated the high specificity of anti-C1s autoantibodies in combination with other biomarkers as a potential tool in the early screening for LN. However, additional studies are needed to validate the diagnostic and/or prognostic role of this new SLE parameter.

CRediT authorship contribution statement

Jeanne Vigne: Formal analysis, Investigation, Resources, Writing – Original Draft, Visualisation. **Nolwenn Haut:** Resources, Writing – Review & Editing. **Giovanna Clavarino:** Resources, Writing – Review & Editing. **Noémie Jourde-Chiche:** Resources, Writing – Review & Editing. **Françoise Sarrot-Reynaud:** Resources, Writing – Review & Editing. **Leendert A. Trouw:** Resources, Writing – Review & Editing. **Federica Defendi:** Resources, Writing – Review & Editing. **Nicole M. Thielens:** Resources, Writing – Original Draft, Writing – Review & Editing. **Christine Gaboriaud:** Conceptualisation, Methodology, Validation, Resources, Writing – Original Draft, Writing – Review & Editing, Visualisation, Supervision, Project administration, Funding acquisition. **Véronique Rossi:** Conceptualisation, Methodology, Validation, Investigation, Resources, Writing – Original Draft, Writing – Review & Editing, Visualisation, Supervision, Project administration, Funding acquisition. **Chantal Dumestre-Pérard:** Conceptualisation, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing – Original Draft, Writing – Review & Editing, Visualisation, Supervision, Project administration, Funding acquisition.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clim.2025.110487>.

Data availability

Data will be made available on request.

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