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Relationship between Clinical Manifestations and Serological Profile in Patients Affected by Systemic Lupus Erythematosus

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Abstract: Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterized by a variety of both signs and symptoms; it mainly affects women of childbearing age, with an estimated prevalence of 24/100,000 people in Europe and North America. SLE is often described as an antibodies-driven disease as its clinical manifestations are usually associated with the presence or the absence of specific antibodies. Objectives: To evaluate clinical manifestations in patients with SLE and to assess the relationship with the presence of specific antibodies by using real-world data. Methods: A retrospective study was performed; the 2019 EULAR/ACR Classification Criteria for Systemic Lupus Erythematosus were used to classify patients with SLE. Data concerning serological profiles (which included Antinuclear antibodies – ANA, anti dsDNA, anti-Ro/SS-A, anti-La/SS-B, anti-Sm) were gathered along with medical records of clinical manifestations. Complement levels were also tested for possible clinical correlations. χ^2 or Fisher's exact tests were utilized to establish associations between autoantibodies and symptoms. The odds ratios (OR) and their 95% confidence intervals (CI) were computed. No correction was made for multiple testing; only a p-value 0.01 ≤ was considered significant. Results: One-hundred and twenty-seven patients (n=127, mean age 53.43 ±14.02) were enrolled in this study. Anti-dsDNA antibodies were found to be statistically significant for both malar rash and proteinuria; anti-Ro/SSA antibodies showed an association with photosensitivity and pericarditis; furthermore, a strong association was found between anti-Ro antibodies and proteinuria, but only if anti-dsDNA antibodies were present as well. Patients who tested positive for anti-La/SSB antibodies correlated with a threefold increase in the risk of developing pericarditis. Lastly, anti-Smith appeared to be associated with NPSLE as well as an increased risk for both autoimmune haemolytic anemia and thrombocytopenia. Conclusions: In our study, many associations confirmed those found in previous studies; however, new relationships between antibodies and clinical manifestations were found thus indicating the need for additional evaluations to assess these correlations further.

Keywords: systemic lupus erythematosus; cytokines; antibodies; clinical manifestations; serological profile

INTRODUCTION

Background

Systemic lupus erythematosus, also known as SLE, is a chronic autoimmune disease characterized by various signs and symptoms [1].

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To date, the aetiology of the disease has not been thoroughly assessed; however, a multifactorial participation has been established since it incorporates epigenetic, genetic, ecological, and environmental components [2–4].

Epidemiology

In accordance with the estimates provided by the worldwide epidemiological community, the proportion of females to males is nine to one (F:M 9:1), with the age range of 15 to 44 years old being the highest. SLE is most likely to affect women of childbearing age; nevertheless, males encounter a more fast and severe form of the disease, which offers them a less favourable prognosis [5].

It has been estimated that the incidence of Systemic Lupus Erythematosus is 5.14 (1.4; 15.13) per 100 thousand person-years; however, this number can vary greatly depending on the region of interest, ranging from 1.18 (0.16; 3.68) per 100 thousand person-years in central Asia to 13.74 (3.2; 31.82) per 100 thousand person-years in central Europe. However, Poland had the highest estimates of SLE incidence (81.84 per 100,000 person-years), whereas Kazakhstan had the lowest value worldwide (0.57, 0.17 to 1.24 per 100,000 person-years) [6]. Poland had the highest estimates of SLE incidence.

To this day, it is estimated that the prevalence of SLE across the globe is roughly 43.7 (15.87 to 108.92) per 100,000 individuals. However, the prevalence can widely vary depending on the country that is being considered. Indeed, it varies everywhere from 15.9 (3.29 to 45.85) per 100,000 people in southern Asia to 110.85 (26.74 to 314.1) per 100,000 people in tropical Latin America [7,8]. On the other hand, the populations of the United Arab Emirates and Barbados have been shown to have the highest prevalence rates, with a quantity of 166.92 per 100 000 and 163.31 per 100 000, respectively [9].

As concerns ethnicity, Systemic Lupus Erythematosus appears to be significantly more frequent in African-American individuals when compared to Caucasians.

Numerous studies conducted in the USA from the 1980s to the present time have frequently supported this conclusion [10,11]; similar results were reported in UK studies from Birmingham and Nottingham [12]. These studies showed a 5-to 9-fold increase in incidence and a 5- to 10-fold increase in the prevalence of SLE in Afro-Caribbeans individuals, as well as a 1.2- to 6-fold increase in the incidence and a 2- to 2.4-fold increase in the prevalence in South Asian people when compared with Caucasians.

Systemic Lupus Erythematosus is known to be more frequent in patients with a positive family history for the disease, as the heritability is about 66% [13]. This element is strongly suggestive of a significant genetic connection; in fact, in monozygotic twins, the concordance rate for Systemic Lupus Erythematosus is 24%, while in dizygotic twins it drops to 2% [14]. Furthermore, the introduction of the genome-wide association studies (GWAS) allowed researchers to find more than 52 genetic loci with significant evidence of connection with SLE susceptibility [15].

Many studies evaluated the role of the human leukocyte antigen complex (HLA) in the pathogenesis of Systemic Lupus Erythematosus; HLA-DR4, DR11, and DR14 were shown to be protective factors against SLE. On the other hand, HLA-DR3, DR9, and DR15 have been positively associated with an increased risk of disease; moreover, while DR4 and 11 significantly reduced the risk of lupus nephritis, the presence of either DR3 or DR15 strongly increase the risk of renal involvement [16,17].

leukocyte antigen complex (HLA) in the pathogenesis of SLE. On the other hand, HLA-DR3, DR9, and DR15 have been found to be positively related with an elevated risk of disease. Furthermore, DR4 and 11 were found to greatly lower the risk of lupus nephritis, whilst the presence of either DR3 or DR15 was found to significantly increased the chance of a renal involvement [16,17].

Pathogenesis and autoantibodies

To date, the underlying causes of Systemic Lupus Erythematosus have not been thoroughly identified. It is a complex mechanism that includes the interaction between the genome and the

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environment. This interaction may lead to changes in gene expression, known as epigenetic alterations, which play a role in the development of the disease [18]

B-cells are crucial to the development and progression of SLE. B-cell activating factor (BAFF) is a biomolecule generated by activated T cells that promotes their survival Individuals with systemic lupus erythematosus (SLE) exhibit elevated concentrations of BAFF, which prevent the elimination of self-reactive B cells. This might lead to the formation of germinal centers and the production of autoantibodies [19].

Furthermore, immune complexes carrying self-DNA and RNA can stimulate the production of excessive amounts of type 1 interferon (IFN) by plasmacytoid dendritic cells via Toll-like receptors seven and nine (TLR7 and TLR9). Type I IFN binds to these receptors and initiates the activation of the tyrosine kinase TIK2 and Janus kinase (JAK)1/STAT 1 or JAK1/ STAT 2 signal transducers and interferon regulatory factors. As such, this mechanism can differentiate naïve CD 4^+ T cells and produce CD 8^+ memory T cells. Moreover, it promotes Th17 cell development and suppress Treg function thus allowing autoreactive T cells to increase their number [20,21]. Type I IFN also affects B-cell activity as it promotes enhanced survival and activation, which includes differentiation, class-switch recombination and can results in the formation of autoantibodies [22].

Ultraviolets (UV) deserves a special mention for their mechanism of damage. In fact, epidermal keratinocytes exposed to UV light exhibit were observed to increase both the synthesis and the expression of the Ro antigen; as such, anti-Ro antibodies might form and bind to the antigens on the cell surface; this complex can the get recognized by lymphocytes which lastly results in keratinocyte death. Moreover, the expressions of Ro and La antigens are mostly found on the surface of cardiac myocytes, which can lead to the severe manifestations of pericarditis in patients with Systemic Lupus Erythematosus. Functional investigation showed that the anti La/SSB autoantibodies had several effects on human polymorphonucleates, such as increased IL-8 production, faster apoptosis, and reduced phagocytosis; these elements can neutropenia and functional impairment of PMN [23,24].

On account of the fact that the clinical manifestations of systemic lupus erythematosus are typically connected with the presence or absence of particular antibodies, the disease is frequently referred to as an antibody-driven disease. In the past, a few studies attempted to investigate the connection between the presence of these antibodies and the clinical manifestations that were observed in patients. However, these studies either concentrated on particular antibodies or were updated more than a decade ago and thus used outdated criteria for the classification of SLE [25,26]. These limitations may impact the correlations observed in real life because the cohorts are either not comparable or cannot be classified as Systemic Lupus Erythematosus according to our current criteria.

In light of this, our objective was to assess and evaluate the correlation between the presence of autoantibodies and the clinical manifestations as defined by the current sets of criteria. This was done in order to verify any possible differences with the studies that came beforehand and to assist clinicians in predicting possible outcomes of the disease by taking into account these components. As such, the findings from this study might help to establish specific courses of action when assessing a therapeutic regime for patients with SLE based on both clinical manifestations and their current serological profile.

Diagnosis

The most recent classification criteria were edited in 2019 by the "European League Against Rheumatism/American College of Rheumatology" (ACR/EULAR) as to include patients in an early stage of disease [27]; in fact, their sensitivity is 93% (95% CI, 0.83-0.98), which is higher than the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria (83%, 95% CI, 0.72-0.91)[28]. However, the newest criteria did not improve specificity as they show a comparable percentage (75%, 95% CI, 0.61-0.85 for the EULAR/ACR VS 73%, 95% CI, 0.59-0.83 for SLICC).

Unlike the previous ones, the presence of an entry criterion (the presence of antinuclear antibodies -ANA) is now mandatory; furthermore, items are now divided into clinical and

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immunological domains; patients are considered affected if the score is at least 10, but only the item with the highest score from each section is to be considered.

Treatment

The recent update of the recommendations (2023 ACR/EULAR Recommendations on the management of Systemic Lupus Erythematosus) changed the therapeutic approaches for patients with SLE [29]; if renal dysfunction is not present and there are no other contraindications, antimalarials are usually well tolerated; as such, HCQ should be considered in the first line of therapy at a dosage of no more than 5 mg/kg. Prednisone or equivalent is also required for chronic maintenance; however, numerous studies managed to underline the systemic effects of long courses of administration. Therefore, if possible, its dosage should not exceed 7.5 mg per day and, wherever feasible, should be stopped if not required.

In addition, an immunosuppressant should be taken into consideration in individuals who are refractory to HCQ therapy (with or without glucocorticoids or too high dosages thereof). In this situation, methotrexate and azathioprine are feasible options; immunosuppressants may be used even in the early phases of systemic lupus erythematosus therapy for individuals who have already had organ involvement.

When the aforementioned forms of therapy fail or there are recurrent flare-ups, it may be essential and beneficial to turn to biological therapies; among these, Belimumab (anti-Blys) can be considered in cases of inadequate control by first-line treatments (including the combination of HCQ, prednisone, and immunosuppressive agents) with an inability to reduce the daily dose of glucocorticoids to acceptable levels (i.e., maximum 7.5 mg/day); furthermore, Belimumab has recently been approved for the treatment of patients with Lupus Nephritis since the BLISS-LN trial managed to show its effectiveness in these patients [30].

Anifrolumab is a fully human IgG1κ that targets IFNAR1 and inhibits signaling from all type I IFNs; phase 2 and 3 trials (MUSE, TULIP-1, TULIP-2) demonstrated its efficacy in non-renal active SLE, with TULIP-2 reporting a significant increase in the BICLA response in patients receiving this therapeutic agent [31,32]. Furthermore, patients with high disease activity (e.g., SLEDAI > 10), prednisone dose >7.5 mg/day, and serological activity (low C3/C4 titers, high anti-dsDNA titers) are most likely to show a better response to these lines of therapies when compared to seronegative patients and/or to patients with nominal levels of complement system.

Mycophenolate or a calcineurin inhibitor may be administered to the patient in situations when they are resistant to the aforementioned medication or have severe forms. Cyclophosphamide or, lastly, Rituximab (a biological medication that blocks CD20) may be used in the event of subsequent failure or for very resistant types.

Cyclophosphamide (CYC) may be considered if a severe form of SLE is observed and/or after the failure of other forms of treatment; however, CYC should still be used with caution in women and men of reproductive age due to its gonadotoxic effects. Rituximab (RTX) is used off-label in severe, refractory SLE to other immunosuppressive agents or belimumab, or in patients with contraindications to these drugs. Early use of RTX is instead to be considered in severe autoimmune thrombocytopenia and hemolytic anemia, where RTX has shown efficacy.

Unlike the previous versions of the recommendations, a specific set of indications for Lupus Nephritis (LN) has been included. Among these, Voclosporin, a new therapeutic agent for the treatment of LN, has been added; it inhibits CNI-related immune responses thus inducing an immunomodulatory effect on T-cells; moreover, it helps stabilizing the podocyte by blocking the phosphorylation of the synaptopodin within the podocyte, thereby preserving the cytoskeleton's stabilizing function and decreasing proteinuria [33].

In lupus nephritis, RTX is usually considered after the failure of first-line treatments or in the case of disease relapse but is, to date, off-label for this usage.

The stated objective for SLE patients with LN is to achieve a decrease of at least 25% in their proteinuria (UPr) within three months, a reduction of at least 50% in UPr at six months, and a level

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of proteinuria that is less than three grams per day in the range between twelve to twenty-four months from the onset of the disease.

MATERIALS AND METHODS

Study design and population

This retrospective monocentric cohort observational study enrolled outpatients with a confirmed diagnosis of SLE according to the 2019 ACR/EULAR classification criteria with systemic lupus erythematosus [27].

To ensure the quality of the data, patients with inadequate or incomplete medical documentation were excluded from the study. We also applied a range of exclusion criteria, including active neoplasia, diagnosis of primary or secondary immunodeficiency, and substance abuse.

Data collection

Data concerning age, sex, smoking habits, ongoing and previous treatments, as well as comorbidities were collected.

Serological evaluation

Data on the serological profiles of patients were collected. These data include antinuclear antibodies (ANA, HEp-2 IFA by Euroimmun S.r.L, Italy), anti-double-stranded DNA antibodies (anti-dsDNA, evaluated on fluorescent enzyme immunoassays - FEIAs - by ThermoFisher, Waltham, MA and IFA on Chrithidia luciliae -Euroimmun S.r.L. for positive findings in order to confirm the result), anti-Ro/SS-A antibodies (divided, where available, into 52 kDa and 60 kDa, evaluated on fluorescent enzyme immunoassays - FEIAs by ThermoFisher), anti-La/SS-B and anti-Smith (anti-Sm, FEIAs byThermoFisher) antibodies [34–36].

Complement levels, namely C3 and C4, were also evaluated with blood tests to determine their trend and possible correlation with clinical manifestations.

Systemic and organ involvement

Organ involvement was assessed by using the definitions of 2019 ACR/EULAR for Systemic Lupus Erythematosus (supplementary material, Table 1 of the classification criteria) and the Safety of Estrogen in Lupus National Assessment - Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) [37].

The presence of hematological manifestations (namely thrombocytopenia, lymphopenia, and hemolytic anemia) was evaluated with seriated blood tests with a focus on platelets, hemoglobin, WBC, and lymphocytes, haptoglobin, Lactate Dehydrogenase (LDH), direct and indirect bilirubin levels; direct Coombs tests were also performed. Joint manifestations (including arthralgia, Jaccoud's arthritis, non-deforming non-erosive arthritis, and erosive arthritis) were assessed by the presence of either two or more joints with pain and signs of inflammation (tenderness, swelling or effusion) or US of the joints with PWD.

Cutaneous manifestations were defined as acute lupus, discoid lupus alopecia, presence of oral ulcers or malar rash; renal involvement was evaluated by the presence of glomerulonephritis (established with a kidney biopsy) or the presence of proteinuria >0.5 g/L in a 24-hour urine collection.

Neuropsychiatric involvement was assessed in the presence of encephalitis, psychosis, epilepsy, or depression, which could not be related to other plausible causes; pericarditis or pleuritis with or without pericardial or pleural effusion were investigated as per 2019 ACR/EULAR definitions on Systemic Lupus Erythematosus [27,38,39]

Statistical analysis

The information that was gathered was analyzed with the help of STATA SE 18.0 (1985-2023 StataCorp LLC, College Station, Texas, United States of America).

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For the purpose of establishing connections between autoantibodies and symptoms, either the $\chi 2$ or Fisher's exact tests were applied. Both the odds ratios (OR) and the confidence intervals (CI) for each of them were computed 95% There were no adjustments made to account for multiple testing; as such, the only p value that was considered significant was 0.01 or less.

Ethical approval and acceptance to participate are required.

All of the subjects gave their consent after being fully informed. All of the procedures were carried out in accordance with the pertinent rules, in accordance with the Declaration of Helsinki from 1964, and in accordance with the regulations that were imposed by the legislative body.

RESULTS

One-hundred and twenty-seven patients (n=127) were enrolled in this study as per inclusion criteria; demographics and records can be found in Table n.1, 2 and 3.

Table n.1. Demographic characteristics.

	no. of patients	%	note
Demographics			
patients	127	100,00	
F	114	89,76	
M	13	10,24	
age (mean, SD)	53.43	(14.02)	
F:M	8.77:1		

Table 2. Distribution of autoantibodies.

Antibodies				
ANA	AC-1	**84**	66.14	AC-1 only
	AC-4	**36***	28,35	*36 out of 36 also showed AC-1
	AC-8	**3***	2,36	*7 out 8 also showed AC-1

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	AC-14	**2***	1,57	*1 out of 2 also showed AC-8
	AC-21	**2***	1,57	*2 out of 2 also showed AC-1
Anti dsDNA		77	60,63	
Anti-Ro/SSA		49	38,58	
Anti-La/SSB		18	14,17	
Anti-Sm		16	12,60	

Table 3. Distribution of clinical manifestations.

Clinical manifestations		
Malar rash	67	52,76
Photosensitivity	79	62,20
Proteinuria	33	25,98
Pericarditis	29	22,83
Pleuritis	24	18,90
NPSLE	14	11,02
AIHIA	13	10,24
Thrombocytopenia	42	33,07
Leukopenia	24	18,90

As shown in the tables, the demographic characteristics of the enrolled patients are comparable to those found in literature.

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Alas, different patterns of Antinuclear antibodies (ANA) failed to demonstrate a significant association with specific clinical manifestations.

As concerns the other types of autoantibodies, the results are summarized and shown in Table 4. Anti dsDNA antibodies were found to be statistically significant for both malar rash (OR 2.35, CI 95% 1.13; 4.87, p=0.01) and proteinuria (OR 3.13, CI 95% 1.24; 7.92, p=0.008).

Table 4. Relationship between clinical manifestations and antibodies that reached statistical significance.

Antibody	Clinical manifestation	OR (95% CI)	p-value	no of patients with the clinical manifestation (%)
Anti dsDNA	Malar rash	2.35 (1.13; 4.87)	**0.010**	**67 (52.75)**
	Proteinuria	3.13 (1.24; 7.92)	**0.008**	**33 (25.98)**
Anti Ro/SSA	Photosensitivity	2.64 (1.20; 5.82)	**0.007**	**79 (62.20)**
	Pericarditis	2.92 (1.25; 6.85)	**0.006**	**29 (22.83)**
Anti Ro/SSA AND Anti dsDNA	Proteinuria	7.13 (1.39; 36.66)	**0.010**	**14 (11.02)**
Anti La/SSB	Pericarditis	3.56 (1.25; 10.16)	**0.009**	**28 (22.05)**
Anti Sm	NPSLE	5.15 (1.46; 18.12)	**0.005**	**14 (11.02)**
	AIHA	5.85 (1.63; 21.02)	**0.003**	**13 (10.24)**
	Thrombocytopenia	5.68 (1.82; 17.67)	**<0.001**	**42 (33.07)**

Anti-Ro/SSA antibodies showed an association with photosensitivity (OR 2.64, CI 95% 1.20; 5.82, p=0.007) and pericarditis (OR 2.92, CI 95% 1.25; 6.85, p=0.006). A strong association was found between anti Ro antibodies and proteinuria, but only in the cohort of patients who also tested positive for anti dsDNA; in fact, this association appeared to be related with a sevenfold increased risk for patients in case of double positivity (OR 7.13, CI 95% 1.39, 36.66, p=0.010).

A different scenario was ascertained in patients who tested positive for anti La/SSB antibodies; in our cohort these antibodies were found to be related with a threefold increase in the risk of pericarditis (OR 3.56, CI 95% 1.25, 10.16, p=0.009). Lastly, anti-Smith antibodies were evaluated; its presence appeared to positively correlate with NPSLE (OR 5.15, CI 95% 1.46, 18.12, p=0.005); as for the hematological manifestations, anti-Smith antibodies increased the risk for both autoimmune haemolytic anemia (OR 5.85, CI 95% 1.63, 21.02, p= 0.003) and thrombocytopenia (OR 5.68, CI 95% 1.82, 17.67, p< 0.001).

Lastly, the relationship between clinical manifestations and complement levels were tested; as showed in Table 5, reduced levels of complement were statistically significant for the development of proteinuria (OR 3.40, CI 95% 1.39, 8.31, p=0.007).

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Table 5. Relationship between clinical manifestations and complement levels.

Complement	Clinical manifestation	OR (95% CI)	p-value	no of patients with the clinical manifestation (%)
C3, C4	Proteinuria	3.40 (1.39; 8.31)	0.007	**33 (25.98)**

DISCUSSION

Systemic Lupus Erythematosus is a disease characterized by a combination of clinical manifestations that can widely vary among affected patients; as such, it might be helpful to use real-world data to assess possible correlations between antibodies and clinical manifestations.

The population described in our study is rather comparable to the cohorts described in the literature for both sex distribution and age [40,41].

As mentioned in the results, diverse patterns of ANA were not statistically significant for different clinical manifestations among patients with Systemic Lupus Erythematosus. This result might be explained by the usage of a set of criteria which require ANA to be present as the main inclusion condition; unlike studies from other countries who used ACR criteria (in which differences could be observed due to the heterogeneity of the cohort), our patients were fairly similar and all of them were positive for the presence of ANA. Moreover, the great majority of the patients showed an AC-1 pattern, thus reducing the chance to observe any statistically significant difference.

Patients with anti-dsDNA antibodies saw a statistically significant association with the presence of proteinuria and malar rash, which appear to be in agreement with previous studies [25,42,43]; however, unlike other evaluations, pleuritis, alopecia and lymphopenia did not reach a significant correlation [44].

Proteinuria was also observed in patients with a double positivity for both anti Ro/SS-A antibodies and anti dsDNA as their copresence appeared to be significant for a sevenfold risk of its development. Additionally, anti-Ro/SSA antibodies correlated with increased photosensitivity for patients who tested positive, which has been assessed in previous studies as well [45,46]; however, unlike other reports, serositis failed to show a strong correlation with anti-SSA antibodies [47,48].

Our work managed to confirm the relationship between anti-La/SSB antibodies and pericarditis, which was observed in previous studies [35,49] but did not show a significant correlation with renal involvement nor with other manifestations of Systemic Lupus Erythematosus.

This element might be explained by the difference between the cohorts; in the study of Novak and colleagues, patients only needed to fulfil the American College of Rheumatology (ACR) definition of SLE, which does not require an entry criterion (namely, the presence of ANA); moreover, they needed to have a diagnosis of Systemic Lupus Erythematosus before 18 years of age. Childhood-onset SLE (cSLE) usually shows, in fact, a more severe combination of constitutional signs and symptoms when compared to patients with a later onset [50]; lastly, it has already been established that patients from different ethnicities might develop non-identical manifestations of SLE as cohorts from South America tend to manifest a renal involvement more frequently than Caucasian patients [51].

In our cohort of patients, anti-Smith antibodies were highly associated with hematological pictures, namely lymphopenia and AIHA as well as NPSLE, in agreement with previous reports [18,52]. Interestingly, we didn't find any significant association between anti-Smith antibodies and the presence of proteinuria, renal involvement, lymphopenia, or cardiac involvement, which was observed in other studies [53,54]. Similarly to before, this difference might be explained by taking into account the epidemiological elements on which these studies have been conceived.

The main work on this topic comes from the Genetic Profile Predicting the Phenotype study (PROFILE), which is a well-characterized multi-ethnic cohort of SLE patients constituted in 1998; not

only was the study based on the 1997 American College of Rheumatology (ACR) revised criteria, but it also enrolled patients who were either Latin-American, Hispanic or African-American. As such, different clinical manifestations are to be expected as this cohort appears to be rather specific and poorly comparable to the one we analyzed in this study.

We also checked for possible correlations between low levels of complement and clinical manifestations in our sample; in agreement with previous studies, our work confirmed that patients with reduced C3 and/or C4 levels might develop proteinuria [55,56].

In conclusion, relationships between clinical manifestations and antibodies have been widely assessed in the past but different definitions of disease and new laboratory methods changed the cohorts of patients in which we can verify such associations. Among these, many relationships have confirmed previous reports, whilst others have diverged or described new possible relationships which might benefit from further investigations.

Author Contributions: All the listed authors have contributed substantially to this work. First co-authors have also chosen the data set, the statistical analysis and supervised the final version of this study which has been approved by all the authors.

Data Availability Statement: The clinical data reported in this manuscript are available if requested to any of the listed authors.

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Conflicts of Interest: The authors declare no conflict of interest.

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