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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 $\leq$ 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 AND BACKGROUND

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

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].

The global epidemiological estimates show that the ratio of females to males is nine to one (F:M 9:1), with a peak age between 15 and 44 years old. Women who are of childbearing age are the most likely to be affected by SLE; however, males experience a more rapid and severe form of the disease, which gives them a less favourable prognosis [5].

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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 [6,7]. 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.

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 [8].

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 [9–11].

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 S1 of the classification criteria) and the Safety of Estrogen in Lupus National Assessment - Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) [12].

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

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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 [8,13,14]

Statistical analysis

Collected data have been analyzed using STATA SE 18.0 (1985-2023 StataCorp LLC, College Station, Texas, USA).

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 $0.01 \leq$ was considered significant.

Ethical approval and consent to participate

Informed consent was obtained from all subjects. All methods were carried out in accordance with relevant guidelines and according to the Declaration of Helsinki of 1964 and following legislative regulations.

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 1

Table 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		
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
	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	

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Anti-La/SSB	18	14,17
Anti-Sm	16	12,60
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 Table 1, the demographic characteristics of the enrolled patients are comparable to those found in literature.

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 2. 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).

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).

Table 2. 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)

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	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)

Table 3. 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 [15,16].

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 [6,17,18]; however, unlike other evaluations, pleuritis, alopecia and lymphopenia did not reach a significant correlation [18,19].

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 [20,21]; however, unlike other reports, serositis failed to show a strong correlation with anti-SSA antibodies [22,23].

Our work managed to confirm the relationship between anti-La/SSB antibodies and pericarditis, which was observed in previous studies [10,24] 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 [25]; 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 [26].

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 [11,27]. 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

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observed in other studies [28,29]. 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 [30,31].

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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