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The complement system is linked to insulin resistance in patients with systemic lupus erythematosus

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Abstract

Objective

Systemic lupus erythematosus (SLE) patients more commonly have insulin resistance (IR) than control subjects. Recent studies have revealed that the complement (C) system is not only a mediator of the immune system but is also related to the pathogenesis of atherosclerosis in the general population. Given that the C alteration is a characteristic of SLE, in the present work we set out to analyse if there is a relationship between the C system and IR in patients with SLE.

Methods

New generation functional assays of the three pathways of the C system were performed in 225 non-diabetic patients with SLE. In addition, the serum levels of inactive (C1q, C2, C3, C4, factor D), activated (C3a) and regulators (C1 inhibitor and factor H) molecules of the C system were evaluated. Insulin and C-peptide serum levels were measured, and insulin resistance and indices of beta cell function were calculated using the homeostatic model assessment (HOMA). Metabolic syndrome criteria fulfillments were applied. Multivariable linear regression analysis was performed to assess the relationship between C system and IR indices and the presence of metabolic syndrome.

Results

After adjusting for covariates that included traditional cardiovascular risk factors associated with IR and prednisone, serum C3a and factor H levels were positively related to higher levels of the HOMA2-IR index. Besides, in the multivariable analysis, after adjustment for covariates, serum levels of C1q and C3 associated with a higher odds ratio for the presence of metabolic syndrome.

Conclusion

IR and metabolic syndrome are positively and independently related to higher serum levels of some serum C elements in patients with SLE with a predominant role of the alternative pathway elements.

Key words

insulin resistance, complement system, systemic lupus erythematosus.

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Introduction

Insulin resistance (IR) is defined as the inability of a known amount of endogenous insulin to increase glucose uptake and utilisation in an individual as much as it does in a normal population. IR occurs as part of a group of cardiovascular and metabolic abnormalities commonly known as the metabolic syndrome. This cluster of abnormalities may lead to the development of type 2 diabetes or accelerated atherosclerosis. Chronic inflammation has been described as a key component in the pathogenesis of IR and metabolic syndrome. In this sense, many studies have focused on the role of inflammation as a common mediator that relates IR to both the pathogenesis of diabetes (1) and atherosclerosis (2). Pro-inflammatory cytokines can cause IR in adipose tissue, skeletal muscle, and liver by inhibiting insulin signal transduction (3). Supporting this notion, IR is augmented in patients with inflammatory diseases like systemic lupus erythematosus (SLE) (4-7) and rheumatoid arthritis (8-10) compared with control subjects.

An increasing body of evidence supports a functional role for complement system (C) activation in the pathogenesis of IR, diabetes, and cardiovascular disease through pleiotropic effects on endothelial and haematopoietic cell function and homeostasis (11). For example, C proteins contribute to the development of type 1 diabetes by enhancing the underlying organ-specific autoimmune processes while complement upregulation appears to be an important feature of IR and type 2 diabetes (12). This has been supported by prospective and case control studies that confirmed strong relationships between several C components and cardiovascular outcomes (13, 14). Moreover, *in vitro* studies and animal models supported this dysfunctional role (15).

Patients with SLE exhibit disruption of the C system. Low serum C components occur in about 50 percent of patients with SLE, reflecting in most cases activation of the classical complement pathway by immune complexes. In the present study we have evaluated the three pathways of the C system in a large group of patients with SLE by means of functional assays and the measurement of the individual C components belonging to these three pathways. We assessed if there is a relationship between the C system and IR in patients with SLE.

Material and methods

Study participants

This was a cross-sectional study that included 225 patients with SLE. All patients were 18 years or older, had a clinical diagnosis of SLE, and met ≥4 American College of Rheumatology (ACR) classification criteria (16). They had been diagnosed by rheumatologists and were regularly followed up in rheumatology outpatient clinics. Patients were excluded if they had a history of cancer, chronic liver and/or renal failure, evidence of acute and/or chronic active infection, and/or any other chronic autoimmune disease other than a condition such as antiphospholipid and/or Sjögren's syndrome associated with SLE. Patients with diabetes mellitus were excluded. All patients had to have a fasting blood glucose <110 mg/dl, and none of them was being treated with hypoglycaemic drugs or insulin. Research was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Ethics Committees of the Hospital Universitario de Canarias and the Hospital Universitario Doctor Negrín (both in Spain), and all subjects provided informed written consent (approval number 2015_84).

Data collection

Patients included in the study completed a medication use questionnaire and underwent a physical examination. Medical records were reviewed to verify specific diagnoses and medications. SLE disease activity and damage were assessed using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (17) and the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index -SDI- (18), respectively. For the present study, the SLEDAI-2K index was divided into none (0 points), mild (1-5 points), moderate (6-10 points), high (11-19 points),

and very high activity (>20 points) as previously described (19). The severity of the disease was measured using the Katz index (20). The presence of metabolic syndrome was determined using the National Cholesterol Education Program (NCEP/ATPIII) criteria (21). According to the NCEP ATP III definition, metabolic syndrome is present if three or more of the following five criteria are met: waist circumference over 102 cm (men) or 88 cm (women), blood pressure over 130/85 mmHg, fasting triglyceride level over 150 mg/dl, fasting high-density lipoprotein (HDL) cholesterol level less than 40 mg/dl (men) or 50 mg/dl (women) and fasting blood sugar over 100 mg/dl.

Laboratory assessments

The homeostatic model assessment (HOMA) method was performed to determine IR. Briefly, the HOMA model enabled an estimate of insulin sensitivity (%S) and β-cell function (%B) from fasting plasma insulin, C peptide, and glucose concentrations. In this study we used HOMA2, the updated-computer HOMA model (22). This model can be used to assess insulin sensitivity and β-cell function from paired fasting plasma glucose and specific insulin, or C peptide, concentrations across a range of 1–2,200 pmol/l for insulin and 1-25 mmol/l for glucose. C peptide better estimates β-cell function since it is a marker of secretion; and insulin data is preferable when calculating %S since HOMA-%S is derived from glucose disposal as a function of insulin concentration. In our study, IR and %S were calculated using insulin serum levels. Otherwise, %B was calculated using C-peptide serum levels. The computer model provided a value for insulin sensitivity expressed as HOMA2-%S (in which 100% is normal). HOMA2-IR (insulin resistance index) is simply the reciprocal of %S. Insulin (Architect Abbott, 2000I) and C peptide (Immulite 2000, Siemens) were determined by chemiluminescent immunometric assays. Additionally, standard techniques were used to measure plasma glucose, C-reactive protein, and serum lipids.

The SVAR functional C assays under the Wieslab® brand (Sweden) were used to assess the activity of the classical (CL), alternative (AL) and lectin (LE) pathways. These tests combine principles of the haemolytic assay for C function with the use of labelled antibodies specific for the neoantigen produced as the result of C activation. The amount of neoantigen generated is proportional to the functional activity of C pathways. Microtitre strip wells are coated with CL, LE, or AL pathway-specific activators. The patient's serum is diluted in a diluent containing a specific blocker to ensure that only the studied pathway is activated. During the incubation of the diluted patient serum in the wells, the specific coating activates C. The wells are then washed, and C5b-9 is detected with an alkaline phosphatase labeled specific antibody against the neoantigen expressed during membrane attack complex formation. After an additional washing step, detection of specific antibodies is obtained by incubation with alkaline phosphatase substrate solution. The amount of C activation correlates with the intensity of the color and is measured in terms of absorbance (optical density). The amount of formed membrane attack complex (neo-epitope) reflects the activity of the C cascade. The result is expressed semi-quantitatively using the optical density ratio between a positive control and the sample. Wieslab® has validated these functional assays by studying their correlation and concordance with the classical CH50 and AH50 haemolytic tests (https://www.svarlifescience.com/). C2, C3, C3a, C4 and C1q were analysed by turbidimetry (Roche), C1-inhibitor -C1-inh- was analysed through nephelometry (Siemens) whereas factor D and factor H were assessed by enzyme linked immunosorbent assay (ELISA Duoset, R&D). Both intra and inter-coefficients of variability were <10% for these assays. Fasting serum samples were collected and frozen at -80°C until analysis of C system.

Statistical analysis

Demographic and clinical characteristics were described as mean ± standard deviation (SD) or percentages for categorical variables. For non-normally distributed continuous variables, data were expressed as median and interquartile range (IQR). The relationship of circulating C system molecules and pathways functional tests with IR indices and the presence of metabolic syndrome was assessed through multivariable linear and logistic regression analysis. Confounders were selected if they had a significant relationship with exposure or outcome with a *p*-value less than 0.20 and were not on the causal pathway between both variables. All the analyses used a 5% two-sided significance level and were performed using Stata software, v. 17/SE (StataCorp, College Station, TX, USA). *p*-values <0.05 were considered statistically significant.

Results

Demographic and disease-related data of patients with systemic lupus erythematosus

Demographic and disease-related characteristics of patients with SLE are shown in Table I. Most of them were women (92%) and the mean age ± SD was 49±12 years. The age of diagnosis was 34±13 years, and the duration of the disease was 15±10 years. At the time of recruitment, 66% of the patients were positive for anti-DNA and 70% for extractable nuclear antigens -ENA-, with anti-SSA being the most frequently found antibody (35%). Sixteen percent of patients met the definition of associated antiphospholipid syndrome, and 35% had at least one positive antiphospholipid antibody. Most patients with SLE were in the categories of no activity (39%) or mild-moderate activity (56%) as shown by the SLEDAI-2K score. SDI and Katz indexes were 1 (IQR 0–2) and 2 (IQR 1–4), respectively. Sixty-three percent of the patients had an SDI score equal to or higher than 1. Regarding treatments at the time of assessment, half of the patients (48%) were taking glucocorticoids and the median equivalent daily dose of prednisone was 5 mg/day (IQR 4.25-7.5 mg). Seventy percent of the patients were taking hydroxychloroquine when the study was performed. Other less commonly used drugs were methotrexate (11%) and azathioprine (16%). Table I shows additional information on the data related to SLE.

Table I. Demographics and disease-related data of SLE patients.

	SLE (n=225)
Age, years	49 ± 12
Female, n (%)	207 (92)
SLE related data	
Age at diagnosis, years	34 ± 13
Disease duration, years	15 ± 10
Antiphospholipid syndrome, n (%)	35 (16)
Auto-antibody profile	
Anti-DNA positive, n (%)	113 (66)
ENA positive, n (%)	133 (70)
Anti-Sm	15 (8)
Anti-ribosome	11 (8)
Anti-nucleosome	27 (20)
Anti-histone	18 (13)
Anti-RNP	46 (25)
Anti-SSA	50 (35)
Anti-SSB	5 (4)
Antiphospholipid antibodies, n (%)	39 (35)
ACA IgM	16 (10)
ACA IgG	29 (19)
Anti beta2 glycoprotein IgM	13 (9)
Anti beta2 glycoprotein IgG	24 (16)
Disease scores	
Median SLEDAI-2K	2 (0-4)
SLEDAI-2K categories	
No activity, n (%)	84 (39)
Mild, n (%)	83 (39)
Moderate, n (%)	36 (17)
High or Very High, n (%)	11 (5)
Median SDI	1 (0-2)
SDI ≥ 1, n (%)	141 (63)
Katz Index	2 (1-4)
Katz ≥ 3, n (%)	100 (44)
Comorbidity	
Smoking, n (%)	57 (25)
Hypertension, n (%)	76 (34)
Dyslipidaemia, n (%)	168 (75)
Abdominal circumference, cm	92 ± 14
Body mass index, kg/m^2	27 ± 6
Obesity, n (%)	63 (28)
Metabolic syndrome, n (%)	75 (33)
Treatment at the time of the visit	
Statins use, n (%)	48 (21)
Aspirin use, n (%)	63 (29)
Glucocorticoids, n (%)	107 (48)
Prednisone equivalent daily dose, mg	5 (4.25-7.5)
Antimalarials drugs, n (%)	155 (70)
Methotrexate, n (%)	24 (11)
Azathioprine, n (%)	35 (16)
Mycophenolate mofetil, n (%)	26 (12)
Belimumab, n (%)	7 (3)
Rituximab, n (%)	6 (3)
Glucose homeostasis molecules and IR indices	
Glucose, mg/dl	91 ± 9
Insulin, μU/ml	6.8 (4.4-9.9)
C-peptide, ng/ml	2.2 (1.5-3.4)
HOMA2-IR	0.88 (0.60-1.30)
HOMA2-S%	115 (78-171)
HOMA2-B%-C-peptide	134 (99-180)
Functional complement assays, %	
Classical pathway	80 ± 40
Lectin pathway	9 (1-40)
Alternative pathway	35 (7-71)
Individual complement components	
C1q, mg/dl	34 ± 11
C2, mg/dl	2.4 ± 1.2
C4, mg/dl	20 ± 12
Factor D, ng/ml	2517 ± 1808
C3, mg/dl	128 ± 36
C3a, mg/dl	38 ± 10
C1 inhibitor, mg/dl	32 ± 8
Factor H, ng/ml x10e-3	587 (173-923)

Data represent mean ± SD or median (interquartile range) when data were not normally distributed.
SLEDAI categories were defined as: 0 no activity; 1-5 mild; 6-10 moderate; >10 high activity.
ENA: extractable nuclear antibodies; ACA: anticardiolipin antibodies;
SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index;
HOMA2-IR: Insulin resistance index through homeostatic model assessment (calculated with glucose and insulin serum levels). HOMA2-S%: Insulin sensitivity index through homeostatic model assessment (calculated with glucose and insulin serum levels). HOMA2-B%-C-peptide: β-cell function index through homeostatic model assessment (calculated with glucose and C-peptide serum levels).

Functional C assays of the CL, AL and LE pathways were 80±40%, 35 (IQR 7–71)% and 9 (IQR 1–40)%, respectively. Besides, single C components C1q, C2, C3, C4 and factor D (inactivated zimogens), activated molecule C3a, and regulators (C1-inh and and factor H) serum values are shown in Table I. HOMA2-IR was 0.88 (IQR 0.60–1.30) and HOMA2-B%-C peptide had a value of 134 (IQR 99–180). Thirty-three percent of the patients met the definition of metabolic syndrome (Table I).

Univariable associations of demographics and disease related data with HOMA2-IR and HOMA2-B% are shown in Supplementary Table S1. Traditional IR risk factors such as hypertension, obesity, dyslipidaemia, body mass index (BMI), and waist circumference were highly and significantly associated with higher values of both IR and β-cell function. Regarding SLE related data, disease damage (SDI) and severity (Katz) scores were significantly associated with higher values of β-cell function. Similarly, the presence of ENA, and, specifically, positivity to anti-SSB, were also significantly related to a greater HOMA2-B%.

Association of complement system pathways with insulin resistance indices

C system pathways functional tests and individual elements showed significant relationships with IR indices in the univariable and multivariable analysis (Table II). In this regard, after multivariable analysis that included age, hypertension, dyslipidaemia, BMI and the use of statins and prednisone, serum C3a and factor H values were associated with higher HOMA2-IR levels. In contrast, after adjusting for covariates, no relationship was found between system C and the HOMA2-B% index expressing β-cell function.

Differences of C system pathways between patients with and without metabolic syndrome

Seventy-five (33%) patients fulfilled the definition of metabolic syndrome using the NCEP ATP III criteria. The differences between SLE patients with and without metabolic syndrome are shown in

Table II. Relationship of complement system to insulin resistance and beta cell function indices.

	HOMA2-IR x 100				HOMA2-B%-C-peptide			
	Beta coef. (95%), *p*							
	Univariable		Multivariable		Univariable		Multivariable	
Classical pathway								
Functional test, %	0.3 (-0.05-0.6)	0.094	0.1 (-0.2-0.5)	0.36	0.08 (-0.2-0.4)	0.61	-0.03 (-0.3-0.3)	0.83
C1q, mg/dl	0.05 (-0.8-2)	0.45	0.03 (-1-1)	0.96	**1 (0.2-2)**	**0.023**	0.9 (-0.2-2)	0.099
Lectin pathway								
Functional test, %	0.1 (-0.2-0.4)	0.46	0.07 (-0.2-0.4)	0.66	-0.09 (-0.4-0.2)	0.53	-0.1 (-0.4-0.1)	0.35
Common elements of the classical and lectin pathways								
C2, mg/dl	-4 (-15-8)	0.52	-8 (-20-3)	0.13	8 (-2-18)	0.13	5 (-5-15)	0.31
C4, mg/dl	0.6 (-0.6-2)	0.34	0.4 (-0.9-2)	0.56	0.5 (-0.5-2)	0.33	0.4 (-0.6-1)	0.99
C1 inhibitor, mg/dl	2 (0.06-4)	0.043	0.8 (-1-3)	0.44	**2 (0.03-3)**	**0.018**	1 (-0.6-3)	0.22
Alternative pathway								
Functional test, %	**0.4 (0.03-0.8)**	**0.032**	0.3 (-0.04-0.7)	0.082	-0.07 (-0.4-0.3)	0.68	-0.1 (-0.5-0.2)	0.37
Factor D, ng/ml	0.003 (-0.006-0.01)	0.57	-0.0003 (-0.009-0.009)	0.94	-0.0005 (-0.008-0.007)	0.90	-0.0005 (-0.008-0.007)	0.90
Common elements of the three pathways								
C3, mg/dl	0.4 (-0.05-0.7)	0.083	0.2 (-0.3-0.6)	0.48	0.1 (-0.2-0.5)	0.41	0.06 (-0.3-0.4)	0.73
C3a, mg/dl	**3 (1-4)**	**<0.001**	**2 (0.2-3)**	**0.030**	**2 (0.7-3)**	**0.003**	1 (-0.1-2)	0.081
Factor H, ng/ml x10e-3	**0.02 (0.002-0.04)**	**0.033**	**0.02 (0.003-0.04)**	**0.019**	0.005 (-0.01-0.02)	0.51	0.006 (-0.008-0.02)	0.39

Beta coefficients are expressed using HOMA2-IR and HOMA2-B% as the dependent variables.
Linear regression analysis is adjusted for age, hypertension, dyslipidaemia, body mass index and the use of statins and prednisone.
HOMA2-IR is calculated using insulin and glucose serum levels; HOMA2-B% is calculated with circulating C peptide and glucose.
Significant *p*-values are depicted in bold.

Table III. Differences of C system pathways between patients with and without metabolic syndrome.

	Metabolic syndrome			Univariable		Multivariable	
	No=148	Yes=75	*p*	Odds ratio, 95%(CI)	*p*	Odds ratio, 95%(CI)	*p*
Classical pathway							
Functional test, %	**85 ± 41**	**98 ± 36**	**0.036**	**1.01 (1.00-1.02)**	**0.038**	1.00 (0.99-1.01)	0.26
C1q, mg/dl	**32 ± 9**	**37 ± 13**	**0.004**	**1.04 (1.01-1.07)**	**0.005**	**1.04 (1.01-1.07)**	**0.011**
Lectin pathway							
Functional test, %	8 (1-41)	9 (1-39)	0.76	1.00 (0.99-1.01)	0.76	-	
Common elements of the classical and lectin pathways							
C2, mg/dl	2.3 ± 1.1	2.6 ± 1.3	0.060	1.26 (0.99-1.60)	0.062	1.28 (0.98-1.66)	0.070
C4, mg/dl	20 ± 11	22 ± 13	0.14	1.02 (0.99-1.04)	0.15	1.01 (0.99-1.04)	0.36
C1 inhibitor, mg/dl	**31 ± 9**	**34 ± 7**	**0.047**	1.04 (1.00-1.08)	0.050	1.02 (0.98-1.07)	0.31
Alternative pathway							
Functional test, %	35 (6-67)	42 (10-80)	0.13	1.01 (1.00-1.01)	0.13	1.01 (0.99-1.02)	0.19
Factor D, ng/ml	**2238 ± 1617**	**3055 ± 2041**	**0.004**	**1.00 (1.00-1.00)**	**0.010**	1.00 (0.99-1.00)	0.064
Common elements of the three pathways							
C3, mg/dl	**122 ± 30**	**141 ± 42**	**<0.001**	**1.02 (1.01-1.03)**	**0.000**	**1.02 (1.01-1.03)**	**0.001**
C3a, mg/dl	**37 ± 8**	**40 ± 12**	**0.033**	**1.03 (1.00-1.06)**	**0.036**	1.03 (0.99-1.06)	0.12
Factor H, ng/ml x10e-3	385 (284-550)	389 (291-614)	0.51	1 (0.99-1)	0.52	-	

According to the NCEP ATP III definition, metabolic syndrome is present if three or more of the following five criteria are met: waist circumference over 102 cm (men) or 88 cm (women), blood pressure over 130/85 mmHg, fasting triglyceride (TG) level over 150 mg/dl, fasting high-density lipoprotein (HDL) cholesterol level less than 40 mg/dl (men) or 50 mg/dl (women) and fasting blood sugar over 100 mg/dl.
Metabolic syndrome is considered the dependent variable in this analysis. NCEP ATP III criteria for metabolic syndrome could not be calculated in 2 of 225 patients. Multivariable analysis is adjusted for age, prednisone and hydroxychloroquine intake.
Significant *p*-values are depicted in bold.

Supplementary Table S2. In this regard, as expected, patients with metabolic syndrome were significantly older, used statins and antihypertensive treatment more frequently, suffered more commonly obesity and dyslipidaemia, and had higher values of BMI and abdominal circumference. Regarding disease-related data, in the univariable analysis, patients with metabolic syndrome had more accrual damage measured through the SDI score, and less frequently were taking hydroxychloroquine when the study was performed (Suppl. Table S2). Besides, SLE patients with metabolic syndrome had some differences in C system routes functional assays and inactive and active individual C elements. With respect to this, in the univariable analysis, patients with metabolic syndrome exhibited higher serum levels of C1q, factor D, C3, C3a and C1-inh (Table III). Besides, in the multivariable analysis, after adjustment for covariates, serum levels of C1q and C3 were associated with a higher odds ratio for the presence of metabolic syndrome (Table III).

Discussion

The present study evaluated for the first time the relationship between a complete characterisation of the C system and IR in patients with SLE. According to our results, both processes seem to be linked independently. Given that the key

phenomenon in SLE pathophysiology is the presence of a dysfunctional C system, our findings suggest that alteration of the C pathways may influence the changes in insulin sensitivity that these patients present. However, the interplay between C and IR observed in our study points to a predominant role of the AL pathway elements, rather than plasmatic activation of the CL pathway.

Previous studies have shown that IR is increased in patients with SLE compared with control subjects, that C-peptide levels are up-regulated in these patients (23, 24), and that IR is related to the disease damage accrual and may accelerate subclinical atherosclerosis (4). The patients with SLE in our series presented IR levels around one. This means that they did not have high IR levels. However, β-cell function was upregulated. This is probably consistent with the fact that the patients had a pre-insulin resistant state. It is known that the first mechanism to be altered in the process leading to IR is β-cell function, raising C-peptide and the corresponding indices of β-cell function. Subsequently, as the process progresses, the development of a peripheral phenomenon occurs that will eventually lead to hyperglycaemia (23). We believe this is the case in our SLE population in which β-cell dysfunction was evidenced, but IR indices remained within the normal range. We understand this is likely because the patients were relatively young and had low to moderate disease activity at enrolment. Despite this, in our series, SDI and Katz Severity Index were associated with IR. This has been described previously and supports the notion that the pathophysiological mechanisms of the disease lead to the alteration in hydrocarbon metabolism (25).

There are no studies in the literature that have studied the relationship between a complete analysis of the C system, which includes the evaluation of its three pathways and activated and non-activated components, and the presence of IR. Remarkably, factor H and C3a, which are common elements of the three pathways, showed an independent relationship to HOMA2-IR. This fact is not expected in a plasmatic C activation scenario, where factor H should be consumed. On the other hand, C3a levels were also positively associated, even though plasmatic C activation measured through functional assays was not apparent. Similarly, C1q and C3 were positively related to the presence of metabolic syndrome after adjustment for confounders. Interestingly, other studies have also found positive relations between IR and C3 in non SLE patients (26), and between IR and C1q in murine models and *in vitro* experiments (11) .

Besides, positive relationships between C and IR and metabolic syndrome in our cohort occurred even though SLE pathophysiology is characterised by activation of the classical pathway by immune complexes, which usually leads to hypocomplementaemia. However, it is known that the majority of the C elements are acute phase reactants, and they rise in inflammatory states (27). Non-canonical activation mechanisms of the alternative pathway through innate and adaptative immune cells (28), and the presence of autoantibodies and mutations that interfere with factor H function (29), could have also contributed to these positive associations between C system parameters and IR related variables.

Thirty-three percent of the patients in our series met the definition of metabolic syndrome. This is consistent with the fact that the prevalence of metabolic syndrome in SLE has been reported to range from 18% to 30% depending on the definitions of the syndrome, the size of the sample, the age of the patients, the distribution by gender, and bias in patient selection (30). In a previous study of 50 women with SLE, patients with metabolic syndrome had significantly higher serum concentrations of C3 and C4 (31). Similarly, in another study of 114 patients with SLE in whom twenty-three (16%) met the criteria for metabolic syndrome, higher serum levels of C3, but not C4, were related to the presence of metabolic syndrome (32). In contrast, in a report of 200 women with SLE, the presence of low C3 levels (as a binary variable) in 10 subjects was associated with a higher odds ratio for the presence of metabolic syndrome (33). Compared with previous reports, our study has recruited a larger number of patients, the characterisation of the complement system was more exhaustive, and we performed a multivariable analysis.

In our work we found that patients with metabolic syndrome took hydroxychloroquine less frequently. This speaks in favor of a protective role of this drug for the development of metabolic syndrome. The protective effect on the prevalence of metabolic syndrome in SLE patients has been described before. For example, in a report of 104 SLE patients, chloroquine was less frequently used in metabolic syndrome-SLE patients (34). It is believed that the anti-hyperglycaemic, anti-hyperlipidaemic, cardioprotective, anti-hypertensive, and anti-obesity effects of antimalarials might be elicited through reduction of inflammatory response and oxidative stress, improvement of endothelial function, activation of insulin signalling pathway, inhibition of lipogenesis and autophagy, as well as regulation of adipokines and apoptosis (35).

We also recognise some limitations. For example, our study has a cross-sectional design and therefore it cannot be inferred how disruption of C might affect IR in SLE patients over time. In addition, the euglycemic hyperinsulinaemic clamp test is considered the gold standard for IR measurement. However, it is technically complex and cannot be performed in a large number of patients like those recruited in our work. For this reason, we used the HOMA2 index which compares favourably with the clamp method (22).

The link between C system and IR has some potential avenues of research that will need to be pursued. This includes biology approaches, such as transcriptomics, proteomics, and metabolomics, to comprehensively analyse the changes induced by C activation in insulin-resistant states; to explore the potential of C-targeted therapies in improving insulin sensitivity and glycaemic control; or conducting observational and interventional studies in human populations to establish correlations between C activation, IR, and metabolic disorders.

In conclusion, C system is linked to IR and metabolic syndrome in SLE patients, with a predominant role of the alternative pathway elements.

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