Associations of the Levels of C4d-bearing Reticulocytes and High-avidity Anti-dsDNA Antibodies with Disease Activity in Systemic Lupus Erythematosus

Claudia Mora, Jorge Medina-Rosas, Ana Maria Santos, Diego A. Jaimes, Ana M. Arbeláez, Consuelo Romero, Annie Cortes, and John Londono

ABSTRACT. ***Objective.*** There are no laboratory tools that detect early flares in systemic lupus erythematosus (SLE). Our aim was to validate in our population the previous findings of the association of C4d-bearing reticulocytes (R-C4d) compared to anti-dsDNA antibodies, with disease activity assessed by the Safety of Estrogens in Lupus Erythematosus National Assessment–Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) and the British Isles Lupus Assessment Group (BILAG) 2004 scales.

Methods. All patients who met the 1987 American College of Rheumatology classification criteria and were seen consecutively in 2013 at a specialized SLE care clinic were included. Disease activity was established by the SELENA-SLEDAI and BILAG 2004. Anti-dsDNA and R-C4d were quantified in peripheral blood. Comparisons were made between values of active and inactive patients, and the correlations between the SELENA-SLEDAI and serum levels of anti-dsDNA and R-C4d were measured.

Results. Sixty-two patients (83.9% women) were included. A total of 32.3% had active disease according to the SELENA-SLEDAI. There was a significant statistical difference ($p = 0.0001$) in the distribution of R-C4d between disease activity groups. The correlation coefficient between R-C4d and the SELENA-SLEDAI score was $r_s = 0.738$ ($p = 0.0001$). R-C4d differed between patients with and without activity in the BILAG 2004 constitutional, mucocutaneous, gastrointestinal, renal, and hematological domains.

Conclusion. R-C4d showed a higher correlation with SLE activity measured by the SELENA-SLEDAI and BILAG 2004 than anti-dsDNA did, suggesting a possible involvement in diagnosing disease activity. Prospective studies that confirm these findings and evaluate its involvement in followup are needed. (First Release May 1 2016; J Rheumatol 2016;43:1657–64; doi:10.3899/jrheum.150486)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS
DISEASE ACTIVITY
SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE ACTIVITY INDEX
SLEDAI
CD ANTIGENS
BIOLOGICAL MARKERS

Systemic lupus erythematosus (SLE) is an autoimmune disease with a worldwide prevalence estimated at 20–240 cases per 100,000 and an incidence of 1–10 cases per 100,000 person-years[1]. Its incidence continues to increase. By 2012, about 450,000 new cases were diagnosed in First World countries[2]. Functional impairment of the immune system, a characteristic of the disease, induces antibody production, immune-complex deposition in tissues, and excessive complement activation[3], leading to inflammation, injury, and nonfunctional healing. Because of its pathogenesis, presentation, and course variables, SLE is still a major challenge for the physician. Clinical and serological markers that are currently relied upon do not evaluate activity appropriately or predict recurrence of disease. Current measures enable limited reduction of organ damage[4]. All of these factors produce a large economic burden; the annual cost of treatment for patients on average is US $19,718.00[5], without considering work absenteeism and indirect costs from comprehensive treatment of the disease and its complications[6,7].

Traditionally, complement factor 3 (C3) and C4 have been measured in clinical monitoring of patients with SLE. During

From the Department of Rheumatology and Immunology, University of La Sabana, Chía; and the Laboratory of Immunology, Hospital Militar Central, Bogotá, Colombia.

Supported by the Universidad de La Sabana and Asociación Colombiana de Reumatología.

C. Mora, MD, MSc, University of La Sabana; J. Medina-Rosas, MD, MSc, University of La Sabana; A.M. Santos, PhD, University of La Sabana; D.A. Jaimes, MD, MSc, University of La Sabana; A.M. Arbeláez, MD, MSc, University of La Sabana; C. Romero, PhD, Hospital Militar Central; A. Cortes, MsC, University of La Sabana; J. Londono, MD, MSc, PhD, University of La Sabana.

Address correspondence to Dr. J. Londono, Universidad de La Sabana, Campus del puente del común, Km 7 autopista norte de Bogotá C.P. 250005, Chía, Cundinamarca, Colombia. E-mail: johnlp@unisabana.edu.co

Accepted for publication March 1, 2016.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2016. All rights reserved.

Downloaded on April 18, 2024 from www.jrheum.org

inflammatory states, complement system activation takes place and the proteins of the system are consumed in proportion to the disease activity. Therefore, measuring complement activation may be useful for diagnosis, evaluation, and determination of the clinical stage[8]. Complement activation generates proteolytic fragments such as C3a, C4a, and C5a anaphylatoxins and chemoattractants that are leading to the release of many inflammatory mediators[9]. It has been shown that incubation of erythrocytes of patients with SLE, unlike healthy controls, leads to the deposition of C4d fragments on the cell surface. Then these red blood cells cause a significant decrease in the deformability of the cell surface membrane, with altered phosphorylation of cytoskeletal proteins that ultimately leads to the production of nitric oxide. This phenomenon affects tissue oxygen delivery[10].

However, there is no consensus on using the levels of C3 and C4 involvement as biomarkers, for different reasons[11,12,13,14,15,16]. The intact protein levels as part of the acute-phase response increase in the synthesis of C3 and C4, a change that can compensate for consumption and partial C4 deficiency. This is common in the general population and in patients with SLE. Additionally, some groups of studies have found that the levels of CH50, C3, and C4 correlate with low renal activity and other systemic disease cases[11,12,13], but others have not obtained the same results[14,15,16]. Possibly measuring complement-activation products (CAP) results in a better alternative as a biomarker. The activation products resulting from the classical pathway (C1rs-C1 complex inhibitor, C4a, and C4d) of the alternative pathway (Bb and C3bBbP), the lectin pathway (C4a and C4d), and products common to all channels (C3a, iC3b, C3d, C5a, and SC5b-9) can be measured in clinical immunology laboratories[17]. Soluble CAP are readily hydrolyzed and therefore have a very short life. Because complement proteins are abundant in the circulation, they permanently interact with circulating blood cells. CAP have the ability to covalently bind to cell surface components accompanying the cell throughout the life cycle and thus persist longer and are more easily detectable in plasma than soluble CAP. Erythrocytes and platelets are the most abundant circulating cells and they are potential targets for the binding of complement proteins generated locally or systemically.

Several groups are investigating biomarkers of SLE and have emphasized the importance of CAP[18]. C4d-bearing cells are one of the most promising. C4d has been studied in soluble form or bound to cells (erythrocytes, reticulocytes, lymphocytes, or platelets). C4d measurement has been compared to validated activity scales such as the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) version of the Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) and the Systemic Lupus Activity Measure (SLAM)[19]. Kalunian, *et al* showed in a multicenter study that levels of C4d-bearing erythrocytes (E-C4d), C4d-bearing B cells (B-C4d), and C4d-bearing platelets (P-C4d) were higher, and a panel with anti-dsDNA, antinuclear antibodies (ANA), E-C4d, B-C4d, and anti-mutated citrullinated vimentin antibody (anti-MCV) was sensitive and specific for the diagnosis of SLE[20]. Given the limited life of reticulocytes in peripheral blood (48 h), assessment of C4d bound to these cells would allow more accurate tracking of changes in SLE activity[21].

In 2004, Liu, *et al* began studying C4d-bearing reticulocytes (R-C4d), showing that the measurement of R-C4d correlates with the SELENA-SLEDAI ($r = 0.45$, $p < 0.00001$), but not with the SLAM score ($r = 0.23$, $p < 0.003$)[21]. In more recent years, Liu, *et al* and others have investigated the involvement of blood cells bearing CAP, distinct from the erythroid lineage, focusing on the lymphoid series[22] and P-C4d. They have a specificity of 100% for SLE compared with healthy volunteers and 98% for patients with other autoimmune diseases ($p < 0.0001$)[23].

In South America, Sardeto, *et al* studied antinucleosome antibodies (ANC) and its association with disease activity measured by the SLEDAI, showing a higher mean SLEDAI for patients with positive ANC compared with negative ANC (mean SLEDAI 8, range 3–16 vs mean SLEDAI 1, range 0–10, $p < 0.0001$)[24]. However, to our knowledge, there are no studies on C4d-bearing cells done on a Latin American population. In a previous study from Mora, *et al*, a trend toward association between SLE activity and R-C4d value was found[25].

We aimed to validate previous findings from other cohorts[21,26] on the association of R-C4d with SLE disease activity scales (SELENA-SLEDAI and BILAG 2004) and compare it with the behavior of a classic biomarker (anti-dsDNA) in patients with SLE followed at a high-complexity referral center in Bogotá, Colombia.

MATERIALS AND METHODS

Study participants. Patients over 18 years with a diagnosis of SLE who fulfilled the 1982 criteria of the American College of Rheumatology (ACR)[27] with the 1997 amendments[28] and who attended the outpatient SLE clinic of the Central Military Hospital of Bogotá (HMC in Spanish) from November 2013 to February 2014 were included. Patients were excluded if they had active infection, ongoing pregnancy, demonstrated neoplasia (current or previous), or another autoimmune disease (other than Sjögren syndrome or antiphospholipid syndrome). The day after the intervention, all patients were interviewed using a previously validated, structured form detailing sociodemographic data, background associated with disease, degree of disease activity, and medications used. The physical examination was performed by one of the authors (JEM, AIO, or HC), who was blinded to the R-C4d results. Disease activity was calculated by the SELENA-SLEDAI[29] and the BILAG 2004[30]. For the SELENA-SLEDAI, disease activity was defined in 2 ways[31]: (1) overall activity ("active" meant a SELENA-SLEDAI score ≥ 4, while any lower score was considered inactive); and (2) degree of activity [scored as follows: inactive disease (SELENA-SLEDAI of 0), mild activity (SELENA-SLEDAI between 1 and 3), moderate activity (SELENA-SLEDAI between 4 and 7), and severe activity (SELENA-SLEDAI ≥ 8)]. For the BILAG 2004, each active domain was divided into active (categories A, B, or C) or inactive (categories D or E).

Processing of anti-dsDNA: Immunoassay method. A peripheral blood sample

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2016. All rights reserved.

Downloaded on April 18, 2024 from www.jrheum.org

was taken, and the serum was separated by centrifugation and kept frozen at –80°C until processing. ELISA with an Inova kit was used to measure high-avidity dsDNA as an antigen; purified calf thymus attached to ELISA microplate wells was used. Following instructions from the manufacturer that other authors have reported[32], a dilution of patient sera was made and added to each of the wells. After incubation for 30 min at room temperature, the wells were washed and human anti-IgG conjugate labeled with horseradish peroxidase was added to each well, followed by another 30-min incubation. After washing to remove unbound conjugate, a specific substrate for the enzymes used in determination was added and incubated for 30 min. Then we determined the enzymatic activity present in the well based on color intensity in a photometer at a wavelength of 450 nm. The experimental results were compared to commercial controls and calibrators. The results are expressed in IU/ml. There were positive and negative commercial controls for each determination.

R-C4d processing: Flow cytometry. R-C4d was measured using flow cytometry on a 3-ml sample of venous blood collected in tubes containing EDTA that was processed on the day of collection, as reported[21,23]. Blood cells were washed and processed with a buffer (phosphate saline), and then the mouse monoclonal antibody specific for human C4d was added to the cell suspension. Then anti-mouse IgG F(ab′)2 bound to phycoerythrin (10 ug/ml) was added to label the antibody, followed by incubation with thiazole orange (Retic-Count). Labeled cells were analyzed in the Becton Dickinson FACSCanto II flow cytometer. Reticulocytes were selected based on cytometry properties and staining with Retic-Count. The results are expressed in units of mean fluorescence intensity (MFI), which was calculated as the MFI of R-C4d minus the MFI of the commercial control. Every day, the flow cytometer was calibrated and samples were compared with healthy controls to maintain the reliability of C4d measurements.

Statistical analysis. Nonprobabilistic sampling was performed consecutively. A power of 80% and a confidence level of 95% was chosen based on the previous findings by our group[25]. Frequency distributions for categorical variables and descriptive statistics, including measures for central tendency and dispersion, were determined. Differences in the levels of anti-dsDNA and R-C4d between groups of active SLE versus inactive SLE by the SELENA-SLEDAI and between active and inactive status for each of the domains of the BILAG 2004 were analyzed with the Mann-Whitney U test. The Spearman correlation coefficient was used to determine the relationship between anti-dsDNA, R-C4d, and quartiles of SLE activity measured by the SELENA-SLEDAI. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed using the statistical package IBM SPSS 22.

Ethical aspects. The study was conducted according to international standards for human research (Helsinki Declaration). The study was approved by the ethics committee of the University of La Sabana and the Military Hospital of Bogotá. All patients gave written informed consent. Confidentiality was strictly maintained.

RESULTS

Patient characteristics. Sixty-two patients with SLE were studied, mostly women (83.9%), with a mean age at diagnosis of 33.3 ± 13.5 years. Forty-eight percent of patients had lupus nephritis according to 2012 ACR criteria (Table 1).

Disease activity by SELENA-SLEDAI. Of the patients, 32.3% had active disease. Anti-dsDNA and R-C4d values were compared in patients with and without disease activity. A statistically significant difference was found for the R-C4d value between active and inactive status [median (interquartile range) active: 12.7 (4.5–22.3) MFI; inactive 2.4 (0.1–4.1) MFI, p = 0.0001]. There was no difference in anti-dsDNA between groups (Figure 1).

Degree of disease activity. Of the patients, 8.1% had no disease activity during assessment. Of the group of active individuals (91.8%), 59.7% showed mild disease activity. Anti-dsDNA and R-C4d medians were compared for these 4 groups of disease activity, and no significant difference was found for anti-dsDNA (chi-square = 4.98, p = 0.173; Figure 1).

A significant difference was found for R-C4d between disease activity groups (chi-square = 28.0, p = 0.0001). When making a pairwise comparison between the groups of disease activity, a significant difference was found between the inactive and the severe activity group and between the mild and moderate groups. The Jonckheere-Terpstra test was conducted on medians of the 2 biomarkers for the groups of degree of disease activity. It showed a significant difference in R-C4d (p = 0.0001), but not for anti-dsDNA (p = 0.535). R-C4d progressively increased from quartile to quartile.

The Spearman correlation coefficient between anti-dsDNA and the SELENA-SLEDAI score was $r_s = -0.008$ (p = 0.701). The correlation coefficient between R-C4d and SELENA-SLEDAI score was $r_s = 0.738$ (p = 0.0001; Figure 2).

BILAG 2004. The Mann-Whitney U test was used to compare medians of anti-dsDNA and R-C4d in active (A, B, C) and inactive (D, E) patients for each of the 9 different domains of the BILAG 2004. Table 2 shows the findings for each domain and the median comparison results between active and inactive patients for each biomarker. Differences were found in the median of anti-dsDNA in patients with or without activity only in the musculoskeletal domain. When comparing R-C4d medians between patients with versus without activity, significant differences were found for the constitutional (Z = –2.43, p = 0.015), mucocutaneous (Z = –3.34, p = 0.01), gastrointestinal (Z = –2.28, p = 0.017), renal (Z = –2.412, p = 0.016), and hematological domains (Z = –2.876, p = 0.04). When comparing medians of

Table 1. Sociodemographic characteristics of the population. Values are % unless otherwise specified.

Characteristic	Value
Current age, yrs, mean ± SD	40.2 ± 14.9
Age at diagnosis, yrs, mean ± SD	33.3 ± 13.5
Female/male	83.9/16.1
Marital status	
Single	40.3
Married	59.7
Education level	
Primary	58
Secondary	35.5
University	6.5
Socioeconomic strata	
Low	27.4
Middle	61.3
High	11.3
Occupation	
Unemployed	14.5
Employed	59.7
Homemaker	25.8

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2016. All rights reserved.

Downloaded on April 18, 2024 from www.jrheum.org

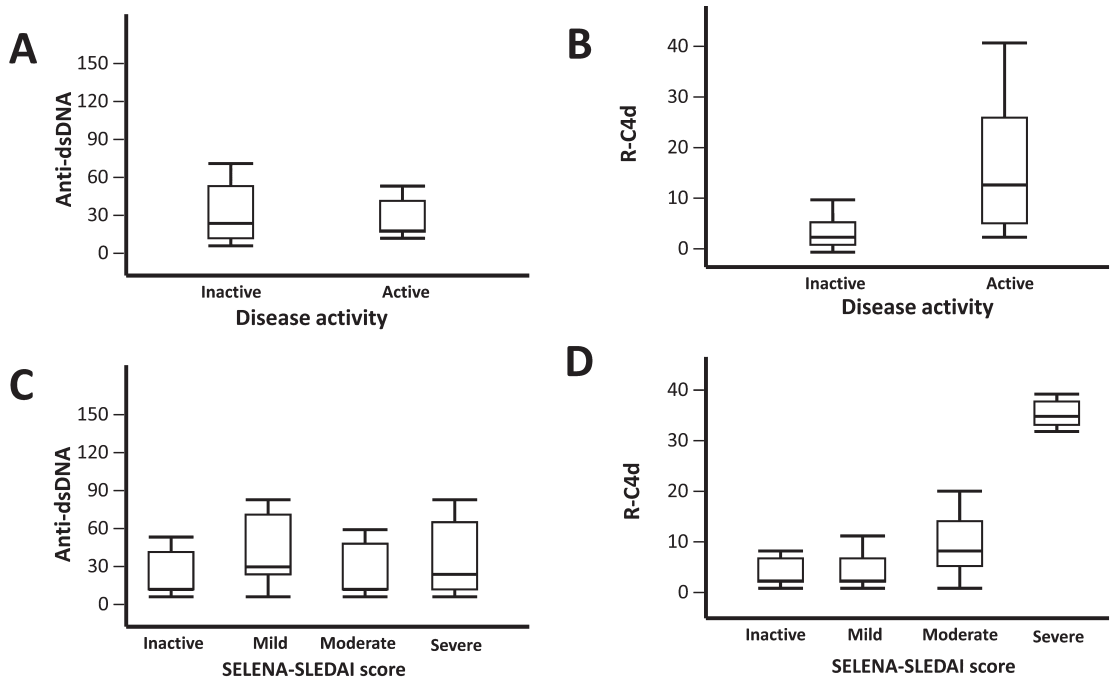

Figure 1. Comparison of anti-dsDNA and R-C4d medians by disease activity according to the SELENA-SLEDAI score between active and inactive patients and between categories of activity. R-C4d: C4d-bearing reticulocytes; SELENA-SLEDAI: Safety of Estrogens in Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index.

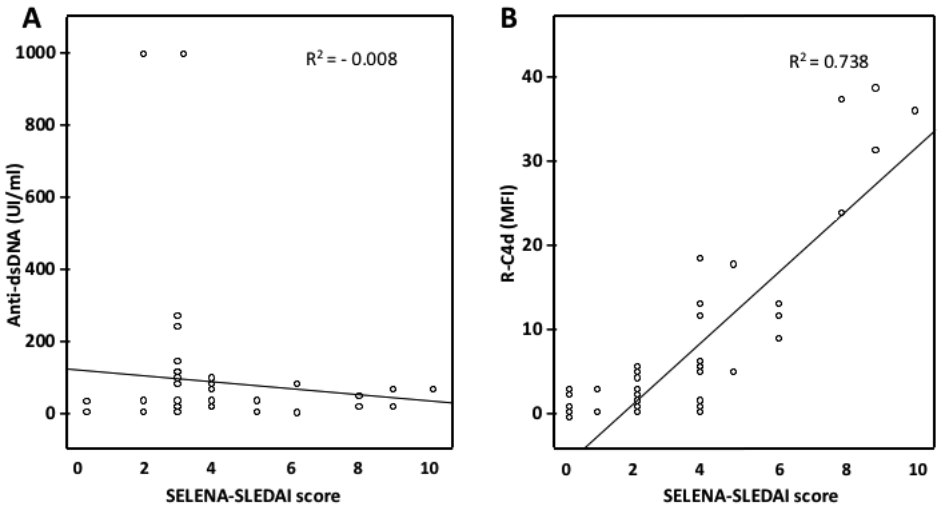

Figure 2. Correlation between anti-dsDNA, R-C4d, and the total SELENA-SLEDAI score. R-C4d: C4d-bearing reticulocytes; SELENA-SLEDAI: Safety of Estrogens in Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index; MFI: mean fluorescence intensity.

anti-dsDNA and R-C4d in patients with disease activity in each domain of the BILAG 2004, large variability was found. However, there were domains in which the number of active patients did not exceed 3 per group (2 in the neuropsychiatric group, 1 cardiorespiratory, 3 gastrointestinal, and 1 ophthalmic); thus, no statistical analysis was performed to determine differences between them. A possible association between R-C4d values with age, sex, duration of disease, and the BILAG 2004 domains was analyzed by fitting a regression model (multivariate analysis). No differences were found in the regression model or in the bivariate analysis (adjusted $R^2 = 0.4096$, $R^2 = 0.3790$, p values not significant).

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2016. All rights reserved.

Downloaded on April 18, 2024 from www.jrheum.org

Table 2. Percentages of patients with activity for each BILAG 2004 domain and comparison of medians between patients with active and inactive disease for anti-dsDNA and R-C4d. Values are median (interquartile range) unless otherwise specified.

Domain	n	DNA	R-C4d	p
Constitutional				
Active	17	26 (12–83)	13.2 (1.4–23.7)	0.015
Inactive	45	22 (12–66)	3.1 (1.4–4.6)	
Mucocutaneous				
Active	17	57 (12–98)	13.2 (3.8–17.5)	0.01
Inactive	45	15 (12–54)	3 (1.2–4.5)	
Hematological				
Active	27	42 (12–93)	5.3 (2.9–16.7)	0.04
Inactive	35	13 (12–54)	2.6 (1.2–4.5)	
Renal				
Active	28	21 (12–68)	4.5 (1.9–13.5)	0.016
Inactive	34	26 (12–68)	3 (1.1–5.1)	
Ophthalmic				
Active	1	35	2.9	
Inactive	61	22 (12–68)	3.8 (1.4–8.5)	
Gastrointestinal				
Active	3	83 (12–1000)	34.6 (5.1–36.4)	0.017
Inactive	59	22 (12–66)	3.5 (1.3–8)	
Neuropsychiatric				
Active	2	87 (83–92)	19.5 (4.4–34.6)	
Inactive	60	21 (12–65)	3.6 (1.4–8.3)	
Cardiorespiratory				
Active	1	83	34.6	
Inactive	61	22 (12–66)	3.6 (1.4–8)	
Musculoskeletal				
Active	19	53 (12–98)	3.1 (1.4–23.9)	0.015
Inactive	43	13 (12–59)	3.9 (1.3–6.3)	

BILAG: British Isles Lupus Assessment Group index.

DISCUSSION

There is an urgent need to find new SLE biomarkers. Despite major advances in the understanding of the pathophysiology of the disease, few biomarkers have been validated, and those known for over 20 years continue to be used even though they do not provide good diagnostic performance[21]. With the advent of new laboratory technologies, it has been possible to find promising biomarkers of some characteristic of the disease, such as its diagnosis, activity, injury, or prognosis[18], but few have been validated[7]. While some groups have focused on genetic markers, looking for variants that predispose to susceptibility to disease or lupus nephritis or using proteomics[33], others have focused on the search for markers of disease activity to prevent damage and minimize healthcare costs[4]. This search has led researchers to emphasize the need for treatment based on measures with appropriate performance[34]. Some groups have focused on disease activity and damage markers using CAP bound to cells and tissues, with promising results. Putterman, *et al* showed that E-C4d and B-C4d have higher sensitivity than anti-dsDNA and the complement proteins C3 and C4 for the diagnosis of SLE, and that the diagnosis performance is maximized when a panel with these biomarkers and others such as ANA, anti-dsDNA, and anti-MCV are used[35]. We decided to use R-C4d in our population based on observations made by other authors[36]: (1) R-C4d is an activation product and not a substrate of the classic pathway and the lectin component, providing more reliable disease activity, (2) the covalent bond of R-C4d to the surfaces of blood cells, tissues, and endothelium enables its use as a disease activity biomarker in different substrates, and (3) R-C4d does not vary with age, sex, or ethnicity[26].

Our group of patients showed diverse sociodemographic characteristics, as would be expected given the large variability in the spectrum of disease presentation. We found a higher prevalence of SLE in women, with an age at diagnosis similar to that reported in North American populations[1], England[37], and in Colombian patients in the assessment previously conducted by our group[38]. The low education level and low socioeconomic status of a significant portion of the patients should be noted because these are risk factors for the development of disease and poor prognosis[1], possibly because of their relationship with limited access to health systems. These factors could explain why more than 90% of patients showed some degree of disease activity. Of these active patients, most had mild activity. Only 8.1% of

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2016. All rights reserved.

Downloaded on April 18, 2024 from www.jrheum.org

patients had severe disease activity, lower than previous data that estimated this figure at up to 15%[39]. Although we did not find any explanation for this finding, it could be associated with the health system of these patients that would facilitate early intervention once disease activity is found.

The behavior of anti-dsDNA regarding disease activity groups by the SELENA-SLEDAI in our population confirms its limited use in differentiating patients with a flare of the disease compared with healthy patients or for early differentiation of different degrees of activity[40].

The lack of correlation between subgroups of SLE activity and anti-dsDNA value, according to data published by other authors[41], could be explained by the SELENA-SLEDAI scale itself, which was designed to assess disease activity in the last 10 days, while different studies have demonstrated that anti-dsDNA could be elevated up to 3 months before an SLE flare is present. This would support the presence of elevated anti-dsDNA in patients without clinical signs of disease activity[40].

The findings of Liu, *et al* showed a good correlation between R-C4d and the SELENA-SLEDAI ($r = 0.45$, $p < 0.00001$). Our correlation was better ($r = 0.725$), confirming the potential use of this marker in diagnosing disease activity in patients with similar characteristics to our population[21].

Regarding the correlation between R-C4d and the SELENA-SLEDAI score, there is a biological explanation given the average life of reticulocytes in blood (48 h) and the time evaluated by the clinimetric instrument. In addition, we showed that R-C4d allows us to differentiate mild versus moderate disease activity and the absence of activity versus severe activity of the disease. This could have an effect on defining the most appropriate therapy in patients according to their individual status. R-C4d seems to be a promising biomarker of early diagnosis of SLE activity, a conclusion that agrees with the literature on this topic[36].

When analyzing the domains of activity by the BILAG 2004, it became evident that the renal and hematological domains were the most affected (45.2% and 43.5%), similar to other studies of disease activity incidence in which renal involvement was between 39% and 53% and hematological involvement between 20% and 59%[42,43,44]. Activity in these domains has been associated with increased morbidity and mortality[45]. This may explain in part why a worse prognosis has been found in the Latin population compared with the white population[46]. This could be an argument for the need to treat patients differently depending on ethno-racial characteristics.

When assessing anti-dsDNA with each domain of the BILAG 2004, a difference in medians was only found between active and inactive status for the musculoskeletal domain (about one-third of patients). However, no prior results were found that specifically supported this assumption, so it is necessary to conduct prospective studies that evaluate it. Although we found differences in patients with active and inactive disease in the R-C4d medians in 5 domains of the BILAG 2004, it was not possible to determine whether there were differences between medians of active patients in each domain (for anti-dsDNA or R-C4d), given the low number of active patients in some domains (such as neuropsychiatric, cardiorespiratory, and ophthalmic). Because no studies have compared the BILAG 2004 and R-C4d, it is necessary to conduct prospective studies with a larger number of active patients in each domain to assess whether R-C4d behaves as a specific damage marker of a system in patients with SLE. Interestingly, in the renal domain, although there is a significant percentage of patients with active disease, no differences were found in the medians of anti-dsDNA between patients with and without activity, while for R-C4d, there was a statistically significant difference. We consider this finding significant, given the prevalence of renal involvement in patients with SLE and the morbidity and mortality of this complication[36]. Other authors have described the lack of a relationship between R-C4d and the SLAM scale[21,47], which might suggest a weak association with clinimetric measures different from those of the SELENA-SLEDAI. Kao, *et al* studied serum samples and renal biopsies, showing a poor correlation of C3 and C4 with activity in the renal pathology, while E-C4d was correlated with SLE activity measures[26]. Further, in 3 patients with proliferative nephritis, anti-dsDNA was negative while E-C4d was elevated. Our findings do not support the conclusion that R-C4d is associated more closely with the renal domain, given the small number of patients with disease activity in the domains of the BILAG 2004. This result needs to be clarified against the ability to discriminate SLE with versus without nephritis[48]. However, it can be stated that anti-dsDNA is unhelpful for determining disease activity in the BILAG 2004 groups, a finding that highlights the need to continue the search for new biomarkers to determine the involvement of a system or specific organ.

SLE behavior is difficult to predict, and validated tools do not provide the most appropriate performance for clinical practice. Thus, researchers continue to search for new biomarkers to provide patients better care and to prevent complications from their disease. Validation of these biomarkers is a process that takes time, with multiple patients from different centers and in different studies. R-C4d has proven useful as a marker of activity, both overall and in renal tissue samples. To our knowledge, ours is the first such study conducted in our country, and its results confirm what has been found in other countries[21,47] with different populations. Given the large diversity of SLE manifestations, it is possible that a single biomarker does not encompass all the needs of the clinic treating a patient with disease activity, so it is likely that in the future, as has been proposed[34], doctors will rely on panels of biomarkers that include R-C4d and provide better diagnostic performance for disease activity. In our country, other groups are working on different biomarkers

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2016. All rights reserved.

Downloaded on April 18, 2024 from www.jrheum.org

for SLE and lupus nephritis[49], with positive results that could be useful for validating a panel of biomarkers of our population that overcomes the shortcomings in organic specificity that R-C4d could present.

There are 2 important limitations of this study. First, it was conducted in a single center and with a small number of patients, which could prevent the results from being validated for our population and generalized to other populations. However, the studied patients belonged to every social and cultural stratum, and in all patients, all the necessary data were obtained to carry out our analysis, indicating that the sample could be representative of our country. Second, the cross-sectional design of our study does not allow us to define whether R-C4d could be useful in monitoring patients for assessing recurrence and adapting management, but it provides a starting point for studies of this design. The small number of patients with disease activity in some BILAG 2004 domains prevented us from defining whether R-C4d was more specific for an involved organ. More prospective information will be needed with a greater number of patients to determine whether this domain specificity exists.

Our study shows a population with characteristics similar to those described in other series: difficulty in accessing health systems and one-third of the group having disease activity. R-C4d may be valid as a biomarker of disease activity, both globally and for different domains, contrary to what was observed with anti-dsDNA, which is a traditional biomarker. Prospective studies are needed with more patients to confirm these findings and validate the use of these biomarkers globally for monitoring disease activity and determining whether there is an association with a specific BILAG 2004 domain.

REFERENCES

1. Pons-Estel GJ, Alarcón GS, Scofield L, Reinlib L, Cooper GS. Understanding the epidemiology and progression of systemic lupus erythematosus. Semin Arthritis Rheum 2010;39:257-68.
2. Koutsokeras T, Healy T. PharmaPoint: systemic lupus erythematosus and lupus nephritis - global drug forecast and market analysis to 2022. [Internet. Accessed March 17, 2016.] Available from: www.reportstack.com/product/144291/pharmapoint-systemic-lupus-erythematosus-and-lupus-nephritis-global-drug-forecast-and-market-analysis-to-2022.html
3. Tsokos GC. Systemic lupus erythematosus. N Engl J Med 2011;365:2110-21.
4. Illei G, Tackey E, Lapteva L, Lipsky P. Biomarkers in systemic lupus erythematosus. I. General overview of biomarkers and their applicability. Arthritis Rheum 2004;50:1709-20.
5. Furst DE, Clarke A, Fernandes AW, Bancroft T, Gajria K, Greth W, et al. Resource utilization and direct medical costs in adult systemic lupus erythematosus patients from a commercially insured population. Lupus 2013;22:268-78.
6. Cho JH, Chang SH, Shin NH, Choi BY, Oh HJ, Yoon MJ, et al. Costs of illness and quality of life in patients with systemic lupus erythematosus in South Korea. Lupus 2014;23:949-57.
7. Illei GG, Tackey E, Lapteva L, Lipsky PE. Biomarkers in systemic lupus erythematosus: II. Markers of disease activity. Arthritis Rheum 2004;50:2048-65.
8. Liu CC, Manzi S, Danchenko N, Ahearn JM. New advances in measurement of complement activation: lessons of systemic lupus erythematosus. Curr Rheumatol Rep 2004;6:375-81.
9. Liu CC, Manzi S, Kao AH, Navratil JS, Ahearn JM. Cell-bound complement biomarkers for systemic lupus erythematosus: from benchtop to bedside. Rheum Dis Clin North Am 2010;36:161-72.
10. Ghiran IC, Zeidel ML, Shevkoplyas SS, Burns JM, Tsokos GC, Kyttaris VC. Systemic lupus erythematosus serum deposits C4d on red blood cells, decreases red blood cell membrane deformability, and promotes nitric oxide production. Arthritis Rheum 2011;63:503-12.
11. Valentijn RM, van Overhagen H, Hazevoet HM, Hermans J, Cats A, Daha MR, et al. The value of complement and immune complex determinations in monitoring disease activity in patients with systemic lupus erythematosus. Arthritis Rheum 1985;28:904-13.
12. Lloyd W, Schur PH. Immune complexes, complement, and anti-DNA in exacerbations of systemic lupus erythematosus (SLE). Medicine 1981;60:208-17.
13. Ricker DM, Hebert LA, Rohde R, Sedmak DD, Lewis EJ, Clough JD. Serum C3 levels are diagnostically more sensitive and specific for systemic lupus erythematosus activity than are serum C4 levels. The Lupus Nephritis Collaborative Study Group. Am J Kidney Dis 1991;18:678-85.
14. Morrow WJ, Isenberg DA, Todd-Pokropek A, Parry HF, Snaith ML. Useful laboratory measurements in the management of systemic lupus erythematosus. Q J Med 1982;51:125-38.
15. Esdaile JM, Abrahamowicz M, Joseph L, MacKenzie T, Li Y, Danoff D. Laboratory tests as predictors of disease exacerbations in systemic lupus erythematosus. Why some tests fail. Arthritis Rheum 1996;39:370-8.
16. Esdaile JM, Joseph L, Abrahamowicz M, Li Y, Danoff D, Clarke AE. Routine immunologic tests in systemic lupus erythematosus: is there a need for more studies? J Rheumatol 1996;23:1891-6.
17. Calvo-Alén J, Bastian HM, Straaton KV, Burgard SL, Mikhail IS, Alarcón GS. Identification of patient subsets among those presumptively diagnosed with, referred, and/or followed up for systemic lupus erythematosus at a large tertiary care center. Arthritis Rheum 1995;38:1475-84.
18. Ahearn JM, Liu CC, Kao AH, Manzi S. Biomarkers for systemic lupus erythematosus. Transl Res 2012;159:326-42.
19. Buyon J, Tamerius J, Belmont HM, Abramson SB. Assessment of disease activity and impending flare in patients with systemic lupus erythematosus. Comparison of the use of complement split products and conventional measurements of complement. Arthritis Rheum 1992;35:1028-37.
20. Kalunian KC, Chatham WW, Massarotti EM, Reyes-Thomas J, Harris C, Furie RA, et al. Measurement of cell-bound complement activation products enhances diagnostic performance in systemic lupus erythematosus. Arthritis Rheum 2012;64:4040-7.
21. Liu CC, Manzi S, Kao AH, Navratil JS, Ruffing MJ, Ahearn JM. Reticulocytes bearing C4d as biomarkers of disease activity for systemic lupus erythematosus. Arthritis Rheum 2005;52:3087-99.
22. Liu CC, Kao AH, Hawkins DM, Manzi S, Sattar A, Wilson N, et al. Lymphocyte-bound complement activation products as biomarkers for diagnosis of systemic lupus erythematosus. Clin Transl Sci 2009;2:300-8.
23. Navratil JS, Manzi S, Kao AH, Krishnaswami S, Liu CC, Ruffing MJ, et al. Platelet C4d is highly specific for systemic lupus erythematosus. Arthritis Rheum 2006;54:670-4.
24. Sardeto GA, Simas LM, Skare TS, Nisihara RM, Utiyama SR. Antinucleosome in systemic lupus erythematosus. A study in a Brazilian population. Clin Rheumatol 2012;31:553-6.
25. Mora C, Arbelaez A, Romero-Sanchez C, Londono J, Valencia Toro PA, Muñoz D, et al. Behavior of reticulocyte-bound C4d in Colombian patients with systemic lupus erythematosus [abstract]. Lupus 2013;22 Suppl 1:126.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2016. All rights reserved.

Downloaded on April 18, 2024 from www.jrheum.org

26. Kao AH, Navratil JS, Ruffing MJ, Liu CC, Hawkins D, McKinnon KM, et al. Erythrocyte C3d and C4d for monitoring disease activity in systemic lupus erythematosus. Arthritis Rheum 2010;62:837-44.
27. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
28. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.
29. Petri M, Kim MY, Kalunian KC, Grossman J, Hahn BH, Sammaritano LR, et al; OC-SELENA Trial. Combined oral contraceptives in women with systemic lupus erythematosus. N Engl J Med 2005;353:2550-8.
30. Isenberg DA, Rahman A, Allen E, Farewell V, Akil M, Bruce IN, et al. BILAG 2004. Development and initial validation of an updated version of the British Isles Lupus Assessment Group's disease activity index for patients with systemic lupus erythematosus. Rheumatology 2005;44:902-6.
31. Thanou A, Chakravarty E, James JA, Merrill JT. How should lupus flares be measured? Deconstruction of the safety of estrogen in lupus erythematosus national assessment-systemic lupus erythematosus disease activity index flare index. Rheumatology 2014;53:2175-81.
32. Andrejevic S, Jeremic I, Sefik-Bukilica M, Nikolic M, Stojimirovic B, Bonaci-Nikolic B. Immunoserological parameters in SLE: high-avidity anti-dsDNA detected by ELISA are the most closely associated with the disease activity. Clin Rheumatol 2013; 32:1619-26.
33. Li Y, Fang X, Li QZ. Biomarker profiling for lupus nephritis. Genomics Proteomics Bioinformatics 2013;11:158-65.
34. Liu CC, Kao AH, Manzi S, Ahearn JM. Biomarkers in systemic lupus erythematosus: challenges and prospects for the future. Ther Adv Musculoskelet Dis 2013;5:210-33.
35. Putterman C, Furie R, Ramsey-Goldman R, Askanase A, Buyon J, Kalunian K, et al. Cell-bound complement activation products in systemic lupus erythematosus: comparison with anti-double-stranded DNA and standard complement measurements. Lupus Sci Med 2014;1: e000056.
36. Batal I, Liang K, Bastacky S, Kiss LP, McHale T, Wilson NL, et al. Prospective assessment of C4d deposits on circulating cells and renal tissues in lupus nephritis: a pilot study. Lupus 2012;21:13-26.
37. Somers EC, Thomas SL, Smeeth L, Schoonen WM, Hall AJ. Incidence of systemic lupus erythematosus in the United Kingdom, 1990-1999. Arthritis Rheum 2007;57:612-8.
38. Medina JE, Mora C, Jaimes DA, Arbeláez AM, Valencia Toro PA, Salazar RE, et al. [Rating activity, chronic damage and impaired quality of life in a cohort of Colombian patients with systemic lupus erythematosus by SELENA-SLEDAI, BILAG 2004, SLICC/ACR and SF-36]. [Article in Spanish] Rev Col Reumatol 2013;20:211-7.
39. Koutsokeras T, Healy T. Systemic lupus erythematosus and lupus nephritis. Nat Rev Drug Discov 2014;13:173-4.
40. Bootsma H, Spronk PE, Ter Borg EJ, Hummel EJ, de Boer G, Limburg PC, et al. The predictive value of fluctuations in IgM and IgG class anti-dsDNA antibodies for relapses in systemic lupus erythematosus. A prospective long-term observation. Ann Rheum Dis 1997;56:661-6.
41. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology 2007;46:1052-6.
42. Dubois EL, Tuffanelli DL. Clinical manifestations of systemic lupus erythematosus. Computer analysis of 520 cases. JAMA 1964;190:104-11.
43. Estes D, Christian CL. The natural history of systemic lupus erythematosus by prospective analysis. Medicine 1971;50:85-95.
44. Hochberg MC, Boyd RE, Ahearn JM, Arnett FC, Bias WB, Provost TT, et al. Systemic lupus erythematosus: a review of clinico-laboratory features and immunogenetic markers in 150 patients with emphasis on demographic subsets. Medicine 1985;64:285-95.
45. Zubair A, Frieri M. Lupus nephritis: review of the literature. Curr Allergy Asthma Rep 2013;13:580-6.
46. Alarcón GS, Roseman J, Bartolucci AA, Friedman AW, Moulds JM, Goel N, et al. Systemic lupus erythematosus in three ethnic groups: II. Features predictive of disease activity early in its course. LUMINA Study Group. Lupus in minority populations, nature versus nurture. Arthritis Rheum 1998;41:1173-80.
47. Manzi S, Navratil JS, Ruffing MJ, Liu CC, Danchenko N, Nilson SE, et al. Measurement of erythrocyte C4d and complement receptor 1 in systemic lupus erythematosus. Arthritis Rheum 2004;50:3596-604.
48. Dhir V. Is cellular C4d a good biomarker for SLE nephritis? Lupus 2012;21:1036.
49. López YP, González LA, Restrepo M, Rodríguez LM, León AL, Severiche D, et al. [Anti-C1q antibodies as markers of renal involvement in patients with systemic lupus erythematosus]. [Article in Spanish] Rev Col Reumatol 2013;20:195-201.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2016. All rights reserved.

Downloaded on April 18, 2024 from www.jrheum.org