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Features Associated With, and the Impact of, Hemolytic Anemia in Patients With Systemic Lupus Erythematosus: LX, Results From a Multiethnic Cohort

SERGIO DURÁN, MD¹, MANDAR APTE, MD, MSPH¹, GRACIELA S. ALARCÓN, MD, MPH¹, MIRANDA C. MARION, MA², JEFFREY C. EDBERG, PhD¹, ROBERT P. KIMBERLY, MD¹, JIE ZHANG, MPH¹, CARL D. LANGEFELD, PhD², LUIS M. VILÁ, MD³, and JOHN D. REVEILLE, MD⁴ for the LUMINA STUDY GROUP

¹University of Alabama at Birmingham

²Wake Forest University School of Medicine, Winston-Salem, North Carolina

³University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico

⁴University of Texas Health Science Center at Houston.

Abstract

Objective—To examine the clinical and genetic correlates of hemolytic anemia and its impact on damage accrual and mortality in systemic lupus erythematosus (SLE) patients.

Methods—SLE patients (American College of Rheumatology [ACR] criteria) of Hispanic (Texan or Puerto Rican), African American, and Caucasian ethnicity from the LUMINA (LUpus in MInorities, NAture versus nurture) cohort were studied. Hemolytic anemia was defined as anemia with reticulocytosis (ACR criterion). The association between degrees of hemolytic anemia and socioeconomic/demographic, clinical, pharmacologic, immunologic, psychological, and behavioral variables was examined by univariable and multivariable (proportional odds model) analyses. Genetic variables (*FCGR* and Fas/Fas ligand polymorphisms) were examined by 2 degrees of freedom test of association and Cochran-Armitage trend tests. The impact of hemolytic anemia on damage accrual and mortality was examined by multivariable linear and Cox regression analyses, respectively.

Results—Of 628 patients studied, 90% were women, 19% were Texan Hispanic, 16% were Puerto Rican Hispanic, 37% were African American, and 28% were Caucasian. Sixty-five (10%) patients developed hemolytic anemia at some time during the disease course, 83% at or before diagnosis. Variables independently associated with degrees of hemolytic anemia were African American ethnicity, thrombocytopenia, and the use of azathioprine. Hemolytic anemia was associated with damage accrual after adjusting for variables known to affect this outcome; however, hemolytic anemia was not associated with mortality.

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Address correspondence to Graciela S. Alarcón, MD, MPH, 830 Faculty Office Tower, 510 20th Street South, Birmingham, AL 35294-3408. E-mail: graciela.alarcon@ccc.uab.edu..

AUTHOR CONTRIBUTIONS

Dr. Alarcón had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Durán, Alarcón, Kimberly, Reveille.

Acquisition of data. Durán, Alarcón, Kimberly, Vilá, Reveille.

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Conclusion—The association of hemolytic anemia with thrombocytopenia suggests a common mechanism in their pathophysiology. Hemolytic anemia is an early disease manifestation and is associated with African American ethnicity and the use of azathioprine; it appears to exert an impact on damage but not on mortality.

INTRODUCTION

Hematologic abnormalities are common in patients with systemic lupus erythematosus (SLE); nearly all SLE patients develop some hematologic manifestation during the course of the disease. After lymphopenia, anemia is one of the most common hematologic disturbances (1), with almost 50% of SLE patients developing it at some point, attributed in the majority of cases to anemia of (normocytic, normochromic) chronic disease.

Hemolytic anemia occurs in 5–10% of patients with SLE (2–6), more frequently in men than in women (19.5% versus 10.9%) (7). It may be the single presenting manifestation of the disease and may precede the appearance of SLE by several months or even years (8). Autoimmune hemolytic anemia also occurs in the primary antiphospholipid syndrome (APS) (9); however, in lupus it does not necessarily occur in the context of secondary APS (10).

Autoimmune hemolytic anemia results from the binding of autoantibodies (IgG or IgM) directed against erythrocytes; the Fc gamma receptors (*FCGR*) play a predominant role in the clearance of these immune complexes by phagocytes of the reticuloendothelial system, whereas the Fas/Fas ligand (*Fas/FasL*)—mediated pathway is associated with the production of autoantibodies (11–14). Therefore, we hypothesized that *FCGR* and *Fas/FasL* polymorphisms could be associated with the presence of hemolytic anemia in SLE patients.

Hemolytic anemia has recently been found to be a risk factor for decreased survival in lupus (15); it has also been found to be associated with serious nonhematologic features, particularly renal and central nervous system involvement (3). We therefore decided to examine the role of hemolytic anemia in terms of disease manifestations, damage accrual, and mortality in patients with SLE from the LUMINA (LUpus in Minorities, NAture versus nurture) cohort. We hypothesize that more serious disease manifestations, damage accrual, and mortality will be associated with the more severe forms of hemolytic anemia.

PATIENTS AND METHODS

Patients

As has been previously described (16), LUMINA is a longitudinal study of outcome of SLE patients from 3 ethnic groups (Hispanic, African American, and Caucasian) living in 3 distinct geographic areas of the US (Texas, Alabama, and Puerto Rico). The eligibility and enrollment of the patients, patients' evaluation and followup, and data collection have been previously described (16). Briefly, patients who meet the American College of Rheumatology (ACR) criteria for the classification of SLE (17), have a disease duration ≤ 5 years, are ≥ 16 years of age at the time of enrollment into the cohort, are of a defined ethnicity (all 4 grandparents of the same ethnicity as the patient), and live in the geographic catchment areas of the participating institutions are eligible to participate. The Institutional Review Board of each center approved the LUMINA study; written informed consent was obtained from each patient according to the Declaration of Helsinki.

Prior to enrollment, all medical records are reviewed. This is done to confirm the patient's eligibility and to gather information about the patient's socioeconomic/demographic and clinical features before disease onset and the enrollment visit. Every patient has a baseline visit (TO); followup visits are conducted every 6 months for the first year and yearly thereafter until

the last visit (TL). Each LUMINA study visit consists of an interview, a physical examination, and laboratory tests. Data for missed study visits are obtained whenever possible by review of all available medical records.

Variables

As previously reported (18), the LUMINA database includes variables from the following domains: socioeconomic/demographic, clinical, immunologic, genetic, behavioral, and psychological. These variables are measured at T0 and at every subsequent visit except for genetic variables, which are obtained only at T0. Only the variables included in the present study are described here.

Variables from the socioeconomic/demographic domain include age, sex, ethnicity, education, poverty (as defined by the US Federal Government, adjusted for the number of subjects in the household) (19), health insurance, and marital status. Time variables are defined as follows: time of diagnosis of SLE (TD) as the time at which a patient meets 4 ACR criteria, disease duration at T0 as the interval between TD and T0, followup time as the time elapsing between T0 and TL, and total disease duration as the interval between TD and TL.

Hemolytic anemia secondary to lupus is defined, per the corresponding ACR criterion, as anemia with reticulocytosis (hemoglobin level <13 gm/dl in men or <11 gm/dl in women and corrected reticulocyte count $>3\%$); patients were categorized as having severe hemolytic anemia if their hemoglobin level was ≤ 7 gm/dl and mild/moderate if >7 gm/dl. For the purpose of these analyses, the first episode of hemolytic anemia, which is usually the most severe, was the one categorized for those patients who had experienced >1 episode of hemolytic anemia.

Clinical variables included the number of ACR criteria at TD, disease manifestations, disease activity, damage accrual, laboratory tests, medications, and death. Cumulative organ system manifestations were recorded by categories as follows: neurologic involvement (seizures, psychosis, headaches, cerebrovascular accidents, neuropathies, transverse myelitis, myelopathy, and/or radiculopathy), renal involvement (nephritis by histopathology [World Health Organization class II–VI] and/or 2 and 3+ proteinuria), hematologic involvement (thrombocytopenia [$<100,000/\text{mm}^3$], leucopenia [$<4,000/\text{mm}^3$], and/or lymphopenia [$<1,500/\text{mm}^3$]), cardiopulmonary involvement (pericarditis, endocarditis, cardiomyopathy, congestive heart failure, pneumonitis, pulmonary hemorrhage, pulmonary hypertension, and/or interstitial lung disease), arterial thrombosis (myocardial infarction, definite or classic angina, and/or vascular procedure for myocardial infarction [coronary artery bypass graft], stroke, intermittent claudication, and/or peripheral arterial thrombosis), and venous thrombosis (peripheral and/or visceral).

Disease activity was assessed using the revised Systemic Lupus Activity Measure (SLAM-R) (20) at all visits. A weighted average SLAM-R score was calculated, from TD to TL as a measure of disease activity over time, by multiplying the SLAM-R score at each individual visit by the number of months in the preceding interval; these scores were then summed and averaged over the total disease duration. This measure was chosen in order to better capture the undulant course of the disease. Because hemolytic anemia is one of the parameters included in the SLAM-R, it was excluded from the total SLAM-R score so that no activity could be attributed to it. Quantification of disease activity was also assessed at all study visits using the physician's and the patient's global assessments. To this end, 0–10-cm visual analog scales were used, where 0 represents completely inactive disease and 10 represents most disease activity possible. Damage was measured with the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SDI) (21) at T0 (or first computed for those patients with disease duration <6 months at T0) and TL.

Cumulative exposure to glucocorticoids (as prednisone equivalent), hydroxychloroquine, azathioprine, cyclophosphamide, methotrexate, aspirin, nonsteroidal anti-inflammatory drugs, hormones (oral contraceptives and hormone replacement therapy), and warfarin were ascertained. The average dose of glucocorticoids was computed at each study visit, taking into account the dose taken every month; a weighted average from T0 to TL was then calculated by multiplying the average glucocorticoid dose for each individual visit by the number of months in the interval between visits and dividing it by the total followup time (T0 to TL).

The presence of autoantibodies (obtained at T0) included anti—double-stranded DNA (anti-dsDNA; by immunofluorescence against *Crithidia luciliae* [normal titer <1:10]), anti-Sm, anti-Ro, and anti-La (by counterimmunoelectrophoresis against human spleen and calf thymus extract) (22,23), IgG and/or IgM antiphospholipid antibodies (aPL; abnormal value >13 IgG phospholipid units/ml and/or >13 IgM phospholipid units/ml, respectively) by enzyme-linked immunosorbent assay technique (24), and the lupus anticoagulant (StaClot test; Diagnostica Stago 92600, Asnières-Sur-Seine, France) (25).

In the genetic domain, *FCGR* functionally important promoter single-nucleotide polymorphisms [*SNPs*; *FCGR2A-H131/R131* [rs1801274], *FCGR2B-I131T* [rs1050501], *FCGR2B-120* [rs34701572], *FCGR2B-386* [rs3219018], *FCGR3A-F176V* [rs396991], *FCGR3B-NA1/NA2*, and *FCRH3-169* [rs7528684]] and select promoter SNPs in *Fas* and *FasL* (*Fas-670* [rs1800682], *Fas-690* [rs2234768], *FasL-205* [no rs number currently assigned], and *FasL-844* [rs763110]) were included. Genomic DNA was extracted using the PureGene kit (Gentra Systems, Minneapolis, MN) following the manufacturer's recommendations. *FCGR* and *Fas/FasL* SNPs were genotyped using Pyrosequencing (Biotage, Charlottesville, VA) as previously described (26,27). Primer sequences are available from the authors.

Self-reported physical and mental functioning were assessed using the Medical Outcomes Study Short Form 36 (SF-36) (28), a 36-item questionnaire with 8 scales that can be aggregated into physical and mental component summary measures. Higher scores on the SF-36 indicate better functioning.

Psychosocial and behavioral domain variables include social support (ascertained with the Interpersonal Support Evaluation List, where higher scores indicate better social support) (29), learned helplessness (assessed with the Rheumatology Attitudes Index) (30,31), and abnormal illness-related behaviors (ascertained with the Illness Behavior Questionnaire [32], where higher scores indicate either greater degree of helplessness or of abnormal illness-related behaviors).

Statistical analyses

Allele and genotype frequencies from *FCGR*, *Fas*, and *FasL* were calculated for all patients and for those with and without hemolytic anemia. Each marker was tested for departure from Hardy-Weinberg equilibrium expectations using a chi-square goodness-of-fit test. To identify any association between the individual polymorphism and hemolytic anemia status, we performed a 2 degrees of freedom (3 genotypes) test of association, tests of the dominant, additive, and recessive genetic models (Cochran-Armitage trend tests), and the corresponding test for lack of fit to additivity. Ethnic-specific analyses were computed with careful attention to potential population substructure.

To compare features from the nongenetic domains between patients with none, mild/moderate, and severe hemolytic anemia (3 groups), standard statistical tests, a oneway analysis of variance for continuous variables, and the Mantel-Haenszel chi-square test for categorical variables were used. Variables with a *P* value less than or equal to 0.10 in the univariable analyses were

included in multivariable analyses of association; age, sex, and ethnicity were entered into this model regardless of their level of significance in the univariable analyses. A proportional odds model was used to evaluate factors potentially associated with degrees of hemolytic anemia. The proportional odds model assumes that the odds ratio (OR) associated with severe hemolytic anemia versus none or mild/moderate is the same as the OR associated with severe and mild/moderate hemolytic anemia versus none. To assess the validity of the underlying assumption, score tests were used.

The role of hemolytic anemia in damage accrual, as measured by the SDI at TL, was examined by a multivariable linear regression analysis, adjusting for those variables previously found to be associated with damage accrual (age, sex, ethnicity, poverty, health insurance, total disease duration, disease activity over time, damage first computed, and glucocorticoid weighted average) (33). The contribution of hemolytic anemia to mortality was examined by multivariable Cox proportional hazards regression model, also adjusting for variables previously found to be associated with mortality (age, sex, ethnicity, poverty, disease activity, and damage at T0) (34). Statistical significance in all cases was defined as a *P* value less than or equal to 0.05. Statistical analyses were performed using the SPSS program, version 15.0 (SPSS, Chicago, IL) or SAS, version 9.1 (SAS Institute, Cary, NC); statistical analyses of the genotype data were done with SNPGWA software (Research IT Services, Winston-Salem, NC).

RESULTS

At the time this study was conducted, 628 patients comprised the LUMINA cohort. Ninety percent of the patients were women. The ethnic groups were represented as follows: 117 (18.6%) Hispanics from Texas, 102 (16.2%) Hispanics from Puerto Rico, 232 (36.9%) African Americans, and 177 (28.2%) Caucasians. Sixty-five (10.4%) patients had hemolytic anemia at any time during the course of SLE, of which 93.8% were women; 54 (83%) of these 65 patients had developed it at or before TD. Thirteen (20%) patients had ≥ 2 hemolytic episodes (6 patients had 2 episodes, another 6 patients had 3, and 1 patient had 4). In all 13 patients, the subsequent episodes fell within the same severity category ($n = 12$) or the second was less severe ($n = 1$). Of the 65 patients with hemolytic anemia, 50 (77%) were classified as mild/moderate and 15 (23%) as severe.

Univariable analyses

Socioeconomic/demographic and clinical features are depicted in Tables 1 and 2, respectively. Age, sex, education, poverty, insurance, and marital status were comparable in all groups (none, mild/moderate, and severe hemolytic anemia). A higher proportion of African American patients had hemolytic anemia (mild/moderate and severe), whereas Puerto Rican Hispanics exhibited it infrequently ($P = 0.004$).

Patients with either mild/moderate or severe hemolytic anemia tended to have more serious clinical manifestations and higher frequency of other hematologic abnormalities taken together, but particularly thrombocytopenia. Patients with hemolytic anemia (in both categories) had greater levels of damage accrual at T0 (or first computed) and at TL and exhibited higher levels of disease activity, whether measured at TL or averaged over time. The proportion of deceased patients was higher among those patients with hemolytic anemia (11.2% none, 18.0% mild/moderate, 26.7% severe; $P = 0.025$).

Anti-dsDNA antibodies were more frequent among patients with hemolytic anemia (55.1% none, 60.0% mild/moderate, 86.7% severe; $P = 0.026$); the frequency of anti-Sm, anti-Ro, anti-La, aPL antibodies, and lupus anticoagulant were similar in all groups.

In the genetic domain, *FCGR2B-I131T* (dominant model), *FasL-205* (dominant model), and *FasL-844* (recessive model) polymorphisms were associated with hemolytic anemia for all ethnic groups taken together (Table 3). Within the ethnic groups, *FasL-844* was associated with hemolytic anemia in African Americans, but the association was in the opposite direction for Caucasians. Furthermore, it was noted that 1 Caucasian subject who had the TT allele was responsible for the association observed in Caucasians and drove the overall association in all of the patients. Finally, the frequency of hemolytic anemia was different among the different ethnic groups and the frequency of *FasL-844* allele varied among them (Table 4).

Multivariable analyses

Variables associated with hemolytic anemia—Although *FasL-844* polymorphisms were associated with hemolytic anemia in the Cochran-Armitage trend analyses for the combined ethnicities, the apparent statistical association is likely due to sparse data within the individual ethnic groups and differential hemolytic anemia and allele frequencies (i.e., population structure). Therefore, *FasL-844* was not included in the multivariable analyses.

Variables independently associated with degrees of hemolytic anemia over the course of the disease are shown in Table 5 and include African American ethnicity (OR 4.21, 95% confidence interval [95% CI] 1.12–15.81, $P = 0.033$), thrombocytopenia (OR 2.38, 95% CI 1.34–4.01, $P = 0.003$), and the use of azathioprine (OR 2.25, 95% CI 1.26–4.01, $P = 0.001$).

Hemolytic anemia and damage accrual—At TL and after adjusting for pertinent variables as noted before, severe hemolytic anemia was associated with damage accrual (t value 2.31, $P = 0.021$) (Table 6).

Hemolytic anemia and mortality—By multivariable Cox proportional hazards regression analyses, hemolytic anemia was not associated with the occurrence of mortality after adjusting for those variables known to influence it. Even after removing poverty from the model, a strong predictor of this outcome, hemolytic anemia remained not significant (data not shown).

DISCUSSION

Very few authors have focused on the features associated with the occurrence of hemolytic anemia in SLE patients; likewise, whether it exerts an impact on the course and outcome of the disease has been studied only in a limited manner, with more attention being placed on other hematologic manifestations like thrombocytopenia (35-37) and lymphopenia (1). We have now examined not only the factors associated with and the impact of hemolytic anemia, but also the relationship between the severity of hemolytic anemia and disease manifestations, damage accrual, and mortality, utilizing the extensive database of the LUMINA cohort. We considered the first episode of hemolytic anemia to categorize our patients into mild/moderate versus severe, given that in all patients the subsequent episodes were of comparable severity (12 patients) or were more severe than the others (1 patient). SLE patients with hemolytic anemia tend to have not only more serious clinical manifestations but also in higher proportions; therefore, damage may be related to involvement of other organ systems. For the most part, a trend was observed so that the most serious clinical manifestations or correlates were observed in higher proportions (or higher mean values) among those patients with severe hemolytic anemia than in those with mild/moderate or no hemolytic anemia; that was the case for cardiopulmonary, neurologic, renal, and hematologic (thrombocytopenia, in particular) manifestations, arterial and venous thrombosis, disease activity, damage, and anti-DNA antibody positivity. Likewise, the proportion of deceased patients was higher among the severe hemolytic anemia cases than the mild/moderate and none. In the multivariable analyses, however, only African American ethnicity, thrombocytopenia, and the use of azathioprine were

found to be independently associated with the occurrence and severity of hemolytic anemia. Of note, Sultan et al have reported the association of renal and central nervous system involvement with hemolytic anemia, but in that study the severity of hemolytic anemia was not assessed (3).

Hemolytic anemia appears to be an early manifestation in the common autoimmune pathogenesis that leads to SLE, and as such, may suggest a higher likelihood of developing other autoimmune manifestations and more severe disease later on. Hematologic abnormalities are common initial manifestations of SLE (38); in fact, it has been reported that almost two-thirds of patients with SLE have their first episode of hemolysis at disease onset (10,39). We have corroborated this observation, because 83% of our patients developed hemolytic anemia at or before the diagnosis of SLE, with very few patients developing it much later. Recurrences of clinically important autoimmune hemolysis at some point during the course of the disease occurred in approximately 20% of our patients, which is consistent with the 17% to 40% reported in the literature (3,10).

Hemolytic anemia and immune thrombocytopenia are caused by production of self-reactive antibodies against either platelet antigens or erythrocyte antigens. The association of hemolytic anemia with thrombocytopenia suggests a common mechanism in the pathophysiology of these 2 hematologic manifestations (3); however, the relationship between specific autoantibodies and hemolytic anemia in SLE has not been well-established. Although anti-dsDNA antibodies were not associated with hemolytic anemia in the multivariable analyses, they were more frequent among patients with mild/moderate and severe hemolytic anemia, adding clinical information to the assessment of SLE patients. To and Petri (40) observed a negative association between the anti-Sm/anti-RNP cluster of autoantibodies and hemolytic anemia in SLE patients, whereas Hoffman et al (41) found antibodies to ribosomal P to be associated with hemolytic anemia. Other investigators have reported a strong association between hemolytic anemia and IgM (42) or IgG aPL antibodies (10); however, others have found only medium-high titers of IgM aPL antibodies to be associated with hemolytic anemia and cerebrovascular accidents (43). We failed to confirm the association between hemolytic anemia and any of the aPL antibodies.

SLE patients of African American and Hispanic (Texan) ethnicities tend to experience, by and large, more severe disease manifestations and less favorable intermediate and long-term outcomes than patients of other ethnicities (16,33,44,45); in fact, the highest proportions of patients with hemolytic anemia (and its severe form) were found in these patients. In the multivariable analysis, however, the association remained significant only for African Americans.

Our patients with hemolytic anemia had greater levels of damage accrual at both T0 (or first computed) and TL compared with patients without it; moreover, more severe anemia was correlated with a higher SDI score. In fact, severe hemolytic anemia was found to be an independent contributor to the occurrence of damage when examined as a continuous variable. These data support the notion that although hemolytic anemia is an acute event, severe episodes exert impact in the intermediate outcome of the disease.

Patients with hemolytic anemia were more frequent users of cyclophosphamide and azathioprine but less frequent users of hydroxychloroquine, probably reflecting the use of these agents as steroid-sparing agents. In the multivariable analyses, the use of azathioprine was associated with hemolytic anemia; in most cases, hemolytic anemia preceded the use of azathioprine, therefore no causal relationship can be attributed to its use, being more a reflection of the severity of the disease among these patients. Although glucocorticoids are considered the first line of treatment in hemolytic anemia, we did not find any difference in the use of

glucocorticoids (weighted average and highest dose), probably because most of the patients received these treatments for reasons other than hemolytic anemia or because the dose was not examined concomitant with the episode of hemolysis.

Although we found an increased proportion of deceased patients among the hemolytic anemia groups, it did not appear to have an independent effect on mortality, even when poverty, a known prognostic factor, was removed from the analyses, although the hazard ratio was in the right direction. These results contrast with the data reported by Kasitanon et al (15), who studied 1,378 SLE patients from the Hopkins Lupus Cohort and found hemolytic anemia to be associated with increased risk of mortality even after adjusting for demographic, economic, and other clinical features. The mortality rate in patients with hemolytic anemia among the Hopkins cohort patients and ours are quite comparable (23% and 20%, respectively); therefore, we do not have a good explanation for our discrepant results, albeit one possibility is that the effect of hemolytic anemia may have been masked by the inclusion of disease activity in our analyses and not in theirs.

Our study is not without limitations. First, we were not able to examine the association between the titers of IgG and IgM aPL antibodies and hemolytic anemia because these data were not available. Second, we may have missed other causes of hemolysis in our patients, such as G6PDH deficiency, hereditary spherocytosis, sickle cell anemia, and conditions associated with increased cell fragmentation or microangiopathy (disseminated intravascular coagulation; hemolysis, elevated liver enzymes, and low platelets syndrome; thrombotic thrombocytopenic purpura; and malignant hypertension), given that these conditions were not systematically examined. Finally, we did not include the use of medications associated with hemolysis such as penicillin and quinine derivatives, because these data are not part of the LUMINA database.

In summary, hemolytic anemia occurred in 10% of our entire cohort, but it was more frequent among African Americans. Although hemolytic anemia does not seem to exert a direct effect on mortality, patients with hemolytic anemia tend to have more severe disease, particularly thrombocytopenia, and as such need to be carefully monitored.

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Table 1

Socioeconomic/demographic variables associated with degrees of hemolytic anemia in the course of systemic lupus erythematosus in LUMINA patients by univariable analyses*

Variable	Hemolytic anemia			P [†]
	None (n = 563)	Mild/moderate (n = 50)	Severe (n = 15)	
Age, mean ± SD years	36.8 ± 12.6	36.1 ± 13.6	32.0 ± 10.8	
Women	89.2	94.0	93.3	
Ethnicity				
Texan Hispanic	18.5	20.0	20.0	0.004
Puerto Rican Hispanic	17.6	4.0	6.7	0.004
African American	34.6	56.0	60.0	0.004
Caucasian	29.3	20.0	13.3	0.004
Education, mean ± SD years	13.1 ± 3.1	12.5 ± 3.0	12.4 ± 2.2	
Poverty [‡]	32.7	37.5	41.7	
Lack of insurance	19.1	29.2	26.7	
Married	52.0	46.0	53.3	

* Values are the percentage unless otherwise indicated. LUMINA = LUpus in Minorities, NAture vs. nurture.

[†] Only P values ≤0.10 are noted.

[‡] As defined by the US Federal Government (19).

Clinical variables associated with degrees of hemolytic anemia in the course of systemic lupus erythematosus in LUMINA patients by univariable analyses*

Table 2

Variable	Hemolytic anemia			P [†]
	None (n = 563)	Mild/moderate (n = 50)	Severe (n = 15)	
Organ system involvement, cumulative %				
Integument	90.4	86.0	86.7	
Musculoskeletal	97.9	95.0	93.3	
Cardiopulmonary	59.5	72.0	80.0	0.022
Neurologic	66.4	82.0	80.0	0.023
Renal	43.9	62.0	86.7	< 0.001
Hematologic	73.8	88.0	93.3	0.037
Thrombocytopenia	19.7	42.0	46.7	< 0.001
Leucopenia	38.9	50.0	46.7	
Arterial thrombosis	10.7	26.0	13.3	0.022
Venous thrombosis	9.41	14.0	20.0	0.010
SLAM-R score at baseline	8.8 ± 5.2	9.8 ± 6.3	9.9 ± 5.3	
SLAM-R score at last visit	6.8 ± 4.9	8.1 ± 5.8	9.6 ± 6.3	0.032
SLAM-R score over time	7.2 ± 4.2	8.7 ± 5.7	8.9 ± 3.7	0.031
Physician's global assessment at baseline [‡]	2.4 ± 2.1	2.7 ± 2.0	3.4 ± 2.8	0.098
Physician's global assessment at last visit [‡]	1.7 ± 1.8	2.5 ± 2.2	2.1 ± 2.2	0.011
SDI score first computed	0.67 ± 1.10	1.10 ± 1.60	1.73 ± 2.00	< 0.001
SDI score at last visit	1.69 ± 2.10	2.53 ± 2.30	3.73 ± 3.90	< 0.001
Autoantibody reactivity, %				
Anti—double-stranded DNA	55.1	60.0	86.7	0.026
Anti-Sm	31.3	46.0	33.3	
Antiphospholipid	23.5	26.0	26.7	
Lupus anticoagulant	4.5	0	14.3	
Treatments received, % [§]				
Glucocorticoids [¶]	79.2	88.0	93.3	
Hydroxychloroquine	86.2	76.0	80.0	0.083

Variable	Hemolytic anemia			<i>P</i> [†]
	None (n = 563)	Mild/moderate (n = 50)	Severe (n = 15)	
Azathioprine	26.6	52.0	40.0	0.001
Cyclophosphamide	22.4	36.0	60.0	<0.001
Methotrexate	16.0	22.0	33.3	
Deceased, %	11.2	18.0	26.7	0.025

* Values are the mean ± SD unless otherwise indicated. LUMINA = LUpus in MInorities, NAture vs. nurture; SLAM-R = revised Systemic Lupus Activity Measure; SDI = Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

[†] Only *P* values ≤0.10 are noted; Mantel-Haenszel chi-square test for categorical variables.

[‡] Measured on a visual analog scale.

[§] Cumulative exposure.

[¶] As prednisone equivalent.

Table 3

Genotype association of selected Fc gamma receptor (*FCGR*), *Fas*, and *Fas* ligand (*FasL*) polymorphisms with hemolytic anemia in LUMINA (LUpus in Minorities, NAture vs. nurture) patients by 2 degrees of freedom and Cochran-Armitage trend tests

Polymorphism	Minor allele frequencies		2 degrees of freedom, <i>P</i>	Cochran-Armitage trend tests, <i>P</i>		
	Cases, %	Controls, %		Dominant test	Additive test	Recessive test
<i>FCGR2A</i>	46.1	40.4	0.138	0.073	0.268	0.821
<i>FCGR2B-1/3/IT</i>	20.8	15.7	0.019	0.029	0.167	0.431
<i>FCGR2B-120</i>	4.3	6.0	0.388	0.594	0.906	0.319
<i>FCGR2B-386</i>	4.9	7.3	0.254	0.445	0.762	0.249
<i>FCGR3A</i>	25.5	31.7	0.359	0.445	0.226	0.164
<i>FCGR3B</i>	42.2	43.5	0.595	0.467	0.798	0.701
<i>FCGRH3</i>	30.6	38.2	0.374	0.184	0.167	0.353
<i>Fas-670</i>	40.8	41.2	0.956	0.835	0.944	0.896
<i>Fas-690</i>	6.1	9.5	0.688	0.419	0.469	0.885
<i>FasL-205</i>	10.2	5.6	0.123	0.050	0.040	0.236
<i>FasL-844</i>	42.9	47.6	0.002	0.277	0.422	0.011
						0.098
						0.014
						0.170
						0.103
						0.446
						0.324
						0.813
						0.771
						0.636
						0.979
						0.001

Genotype association of *FoxL-844* with hemolytic anemia in all LUMINA (LUpus in Minorities, NATure vs. nurture) patients and by ethnic groups by 2 degrees of freedom and Cochran-Armitage trend tests

Table 4

Ethnic group	C allele frequencies		2 degrees of freedom, <i>P</i>	Cochran-Armitage trend tests, odds ratio (95% confidence interval), <i>P</i>		
	Cases, %	Controls, %		Dominant test (TT)	Additive test (CT)	Recessive test (CC)
All patients	42.9	47.6	0.002	1.44 (0.74–2.81), 0.277	0.86 (0.58–1.25), 0.422	0.34 (0.14–0.81), 0.011
Texan Hispanic	70.8	75.4	0.344	2.20 (0.11–42.27), 0.592	0.74 (0.30–1.80), 0.498	0.51 (0.16–1.66), 0.258
Puerto Rican Hispanic	66.7	48.4	0.724	3.14 (0.16–61.44), 0.426	1.63 (0.43–6.19), 0.470	1.49 (0.20–11.15), 0.696
African American	26.9	17.7	0.027	2.60 (1.13–5.96), 0.021	1.77 (0.91–3.45), 0.086	0.39 (0.02–7.08), 0.507
Caucasian	43.8	65.1	0.037	0.96 (0.16–5.88), 0.967	0.47 (0.19–1.16), 0.091	0.07 (0.01–1.19), 0.016

Table 5

Variables independently associated with degrees of hemolytic anemia in the course of systemic lupus erythematosus in LUMINA patients by multivariable analyses (proportional odds model)^{*}

Variables	OR (95% CI)	<i>P</i> [†]
Ethnicity [‡]		
Texan Hispanic	2.71 (0.65–11.27)	0.033
African American	4.21 (1.12–15.81)	
Caucasian	2.03 (0.50–8.20)	
Thrombocytopenia	2.38 (1.34–4.01)	0.003
Use of azathioprine	2.25 (1.26–4.01)	0.001

^{*} Accounts for the degrees of hemolytic anemia OR = odds ratio; 95% CI = 95% confidence interval; LUMINA = LUpus in MInorities, NAture vs. nurture.

[†] Only *P* values ≤0.05 are noted.

[‡] Hispanics from Puerto Rico are the reference group.

Table 6

Hemolytic anemia and damage accrual in LUMINA patients by multivariable linear regression*

Hemolytic anemia	t value	P
Mild/moderate	0.90	0.368
Severe	2.31	0.021

* Adjusted for age, sex, ethnicity, poverty, health insurance, revised Systemic Lupus Activity Measure (average), glucocorticoid dose (average), and damage (first computed); patients without hemolytic anemia were the reference group LUMINA = LUpus in MInorities, NAture vs. nurture.