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Haemolytic anaemia in a multi-ethnic cohort of lupus patients: a clinical and serological perspective

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Abstract

Systemic lupus erythematosus is a chronic autoimmune disease that can be associated with a variety of haematological manifestations. We identified 76 patients with haemolytic anaemia in a cohort of 1251 unrelated female lupus patients enrolled in our studies. The presence of the various American College of Rheumatology clinical criteria for lupus and serological specificities were determined in lupus patients with haemolytic anaemia and compared with a group of race-matched control lupus patients without haemolytic anaemia. Clinical data were obtained from medical records, and serological specificities were determined in our clinical immunology laboratory at OMRF. The presence of haemolytic anaemia in lupus patients was associated with a higher frequency of proteinuria (OR = 2.70, $P = 0.000031$), urinary cellular casts (OR = 2.83, $P = 0.000062$), seizures (OR = 2.96, $P = 0.00024$), pericarditis (OR = 2.21, $P = 0.0019$), pleuritis (OR = 1.72, $P = 0.028$) and lymphopenia (OR = 1.79, $P = 0.015$). These findings were independent of the presence of thrombocytopenia, which was approximately five times more common in lupus patients with haemolytic anaemia. Lupus patients with haemolytic anaemia were about 8 years younger than lupus patients without haemolytic anaemia at the time of disease onset ($P = 0.000001$). In the absence of thrombocytopenia, lupus patients with haemolytic anaemia were approximately two times more likely to have anti-dsDNA antibodies ($P = 0.024$). The presence of haemolytic anaemia is associated with a subset of lupus characterized by a younger age of disease onset, and a more severe disease with a higher likelihood of renal involvement, seizures, serositis and other cytopenias.

Keywords

anaemia; haemolysis; haemolytic; lupus

Introduction

Systemic lupus erythematosus is a chronic, relapsing autoimmune disease associated with a number of autoantibodies targeted to nuclear components.¹ Although a number of factors contribute to the pathogenesis of lupus, a complete picture of the disease aetiology remains elusive. Lupus can affect multiple organ systems, and is associated with significant morbidity and mortality. Haematological manifestations in lupus are common and include anaemia, leucopenia, lymphopenia and thrombocytopenia. Anaemia is present in about 50% of lupus patients.² Gradation of anaemia over the disease course is a marker of disease activity in lupus patients.³ While anaemia of chronic disease is the most common cause of anaemia in lupus,² haemolytic anaemia is a common complication of the disease, and is included in the American College of Rheumatology classification criteria for lupus.^{4,5} The prevalence of haemolytic anaemia in lupus is 8–28%.^{6,7} Autoimmune haemolytic anaemia in lupus, typically detected by a positive direct anti-globulin test, is usually mediated by the warm-IgG type anti-erythrocyte antibodies.^{2,8} The exact specificity of anti-erythrocyte autoantibodies in most lupus patients with autoimmune haemolytic anaemia remains unclear.² Haemolytic anaemia can be regarded as a *forme fruste* of lupus, and can be present in isolation before lupus is diagnosed.⁹

In this study, we evaluated the clinical and serological manifestations in patients with haemolytic anaemia in a large multi-ethnic cohort of lupus patients. We confirm the previously reported association between haemolytic anaemia and renal involvement, and thrombocytopenia.¹⁰ We also describe an association between the presence of haemolytic anaemia and seizures, pericarditis, pleuritis and lymphopenia in lupus patients. These findings imply that haemolytic anaemia is associated with a more severe subset of lupus, which requires close monitoring for organ involvement.

Materials and methods

Patients and clinical data

We studied a multi-ethnic group of 1251 unrelated female lupus patients enrolled in the Lupus Family Registry and Repository (LFRR) at OMRF. All patients met the American College of Rheumatology (ACR) lupus classification criteria.^{4,5} Haemolytic anaemia was defined according to the American College of Rheumatology criteria for the classification of SLE.⁴ We identified 76 patients with evidence of haemolytic anaemia in our cohort. These patients included 28 (36.8%) European-Americans, 27 (35.5%) African-Americans, 12 (15.8%) Hispanics, 5 (6.6%) Gullah African-American patients, and 4 (5.3%) American-Indians. Each lupus patient with haemolytic anaemia was matched to eleven randomly selected race-matched female lupus patient controls without haemolytic anaemia. Clinical data used in this study were extracted from the medical records, and the presence or absence of the various ACR lupus classification criteria were verified by either a physician or a

physician assistant in our research group. The study protocols were approved by our Institutional Review Boards.

Serology

A single serum sample was obtained from each lupus patient at the time of enrolment into LFRR. Sera were available to us for all but three lupus patients included in this study. Serological tests were performed at the clinical immunology laboratory at OMRF. Tests for extractable nuclear antigen autoantibodies were performed using Oüchterlony immunodiffusion assays.¹¹ Anti-dsDNA antibodies were detected by indirect immunofluorescence using *Crithidia luciliae* (Inova Diagnostics, San Diego, California, USA). ELISA was used to determine the levels of IgM, IgG and IgA anti-cardiolipin antibodies (anti-ACL). Cardiolipin was purchased from Sigma-Aldrich (St Louis, Missouri, USA).

Statistical analysis

Logistic regression was used to identify association between haemolytic anaemia and lupus clinical manifestations and serological specificities. VassarStats online clinical research calculators¹² were used to calculate Pearson Chi-Squares and *P*-values. *P*-values were calculated by Fisher's exact two-tailed probability test when a Pearson Chi-Square could not be calculated due to small number of observations. *P*-values <0.05 were considered suggestive, while those <0.002 remained significant after correction for multiple testing and are considered robust. Student's *t*-test was used to calculate the difference in the age of disease onset in lupus patients with and without haemolytic anaemia.

Results

The frequency of various ACR clinical manifestations and autoantibody specificities in lupus patients with and without haemolytic anaemia were determined (Tables 1 and 2). Lupus patients with haemolytic anaemia had more pericarditis (OR = 2.21, *P* = 0.0019) and pleuritis (OR = 1.72, *P* = 0.028) compared with lupus patients who did not have haemolytic anaemia. In addition, lupus nephritis was more common in patients with haemolytic anaemia. Evidence of proteinuria was present in 61% of lupus patients with haemolytic anaemia compared with 36% of lupus patients without haemolytic anaemia (OR = 2.70, *P* = 0.000031). Similarly, cellular casts were detected in 30% of lupus patients with haemolytic anaemia compared with 13% in control lupus patients (OR = 2.83, *P* = 0.000062). Furthermore, we observed a strong association between the presence of haemolytic anaemia and seizures in lupus patients (21% vs. 8%, OR = 2.96, *P* = 0.00024). The presence of haemolytic anaemia was also associated with other cytopenias in lupus patients. The odds of having thrombocytopenia were approximately five times higher (OR = 4.70, *P* = 1.3×10^{-10}), and the odds of lymphopenia were almost two times higher in lupus patients with haemolytic anaemia than in lupus patients without haemolytic anaemia (OR = 1.79, *P* = 0.015). When autoantibody specificities were examined, we found a trend towards an association of haemolytic anaemia with anti-dsDNA (OR = 1.63, *P* = 0.051) (Table 2).

The age of onset of lupus in patients with haemolytic anaemia was almost 8 years younger compared with race-matched control lupus patients with no evidence of haemolytic anaemia [28.57 ± 1.47 vs. 36.87 ± 0.46 , (mean age of onset (years) $\pm$ SEM), $t = 5.06$, $P = 0.000001$]. There was no difference in the presence of other autoimmune diseases (rheumatoid arthritis, Sjogren's syndrome, scleroderma, Addison's disease and thyroid disease) between lupus patients with and without haemolytic anaemia (Table 3). The mean duration of disease at the time of enrollment in our study was 8.91 ± 0.93 versus 6.73 ± 0.25 (years $\pm$ SEM) in patients with and without hemolytic anemia, respectively ($P=0.015$).

Thrombocytopenia is known to be associated with severe lupus and both renal and neuropsychiatric features in lupus.¹³ Therefore, it is necessary to determine whether the lupus manifestations that we detected in association with haemolytic anaemia were independent of the concurrent presence of thrombocytopenia. We determined the frequency of proteinuria, cellular casts, seizures, pericarditis, pleuritis, lymphopenia and anti-dsDNA antibodies, in lupus patients with and without haemolytic anaemia, in the absence of thrombocytopenia (Table 4). Our results indicate that the higher frequency of renal involvement, seizures, serositis, lymphopenia and anti-dsDNA antibodies seen in lupus patients with haemolytic anaemia, were independent of the presence of thrombocytopenia.

Similarly, because our data show a trend towards an association with anti-dsDNA antibody in lupus patients with haemolytic anaemia, and because the presence of anti-dsDNA antibody is known to be associated with lupus nephritis,¹ we determined the frequency of both proteinuria and cellular casts in lupus patients with and without haemolytic anaemia in the absence of anti-dsDNA antibody. We had 48 lupus patients with haemolytic anaemia and without antidsDNA antibody, and 613 lupus patients without haemolytic anaemia and without anti-dsDNA antibody. Both proteinuria and cellular casts were more frequent in lupus patients with haemolytic anaemia compared with lupus patients without haemolytic anaemia, in the absence of anti-dsDNA antibody (proteinuria: 54% vs. 34%, OR = 2.27, 95% confidence interval = 1.26–4.10, $P = 0.0056$; cellular casts: 29% vs. 12%, OR = 2.91, 95% confidence interval = 1.49–5.67, $P = 0.0011$).

Discussion

This study determines the prevalence of clinical and serological features in a large cohort of lupus patients with and without haemolytic anaemia. We find that lupus patients with haemolytic anaemia are significantly younger at the onset of lupus compared with lupus patients who do not have haemolytic anaemia. We show that the presence of haemolytic anaemia identifies a subset of lupus patients characterized by higher likelihood of renal involvement, seizures, pericarditis, pleuritis, lymphopenia and thrombocytopenia.

Importantly, these associations are independent of the presence of thrombocytopenia, which is known to predispose to severe lupus with renal and neuropsychiatric involvement (Table 4).¹³ The association between haemolytic anaemia and renal involvement in lupus has been reported previously in patient populations from Greece and the United Kingdom.^{10,14} Our findings confirm this observation in a large multi-ethnic cohort of lupus patients as we find increased frequency of both proteinuria and cellular casts in lupus patients with haemolytic

anaemia. Renal involvement in lupus is associated with the presence of anti-dsDNA antibodies in lupus.¹ The presence of anti-dsDNA antibody is higher in our lupus patients with haemolytic anaemia, although the association did not quite reach statistical significance ($P = 0.051$). In the absence of thrombocytopenia, we find that lupus patients with haemolytic anaemia are more likely to have anti-dsDNA antibodies (OR = 1.98, $P = 0.025$). However, our subset analysis suggests that patients with haemolytic anaemia have higher odds of renal involvement, independent of the presence of anti-dsDNA antibody. Contrary to previous reports,^{10,14} we did not find a difference in the presence of anti-cardiolipin antibodies (IgG, IgM or IgA) between lupus patients with and without haemolytic anaemia.

In conclusion, the presence of haemolytic anaemia defines a subgroup of lupus patients who are younger at the age of disease onset, and who are likely to develop life-threatening lupus features, such as renal involvement, seizures and serositis. Therefore, the presence of haemolytic anaemia is a laboratory marker that indicates a more aggressive course of lupus.

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The frequency of lupus manifestations in female lupus patients with haemolytic anaemia when compared with race-matched control female lupus patients without haemolytic anaemia

Table 1

Clinical features	Haemolytic anaemia present (<i>n</i> = 76)	Haemolytic anaemia absent (<i>n</i> = 836)	Odds ratio	95% confidence interval	χ^2	<i>P</i> -value
Malar rash	30 (39%)	336 (40%)	0.97		0.01	0.92
Discoid rash	11 (14%)	110 (13%)	1.12		0.1	0.75
Photosensitivity	19 (25%)	286 (34%)	0.64		2.66	0.1
Oral ulcers	20 (26%)	234 (28%)	0.92		0.1	0.75
Arthritis	48 (63%)	598 (72%)	0.68		2.36	0.12
Pericarditis	25 (33%)	152 (18%)	2.21	(1.32–3.67)	9.64	0.0019
Pleuritis	29 (38%)	221 (26%)	1.72	(1.05–2.80)	4.81	0.028
Proteinuria	46 (61%)	303 (36%)	2.70	(1.67–4.36)	17.39	0.000031
Cellular casts	23 (30%)	111 (13%)	2.83	(1.67–4.81)	16.04	0.000062
Seizures	16 (21%)	69 (8%)	2.96	(1.62–5.42)	13.5	0.00024
Psychosis	6 (8%)	29 (3%)	2.39		—	0.063*
Leucopenia	31 (41%)	284 (34%)	1.34		1.43	0.26
Lymphopenia	41 (54%)	331 (40%)	1.79	(1.14–2.86)	5.94	0.015
Thrombocytopenia	29 (38%)	97 (12%)	4.70	(2.83–7.82)	41.26	1.3×10^{-10}

* *P*-value calculated by Fisher's exact two-tailed probability test.

The frequency of serological specificities in female lupus patients with haemolytic anaemia when compared with race-matched control female lupus patients without haemolytic anaemia

Table 2

Autoantibodies	Haemolytic anaemia present (<i>n</i> = 76)	Haemolytic anaemia absent (<i>n</i> = 833)	Odds ratio (95% confidence interval)	χ^2	<i>P</i> -value
Anti-dsDNA	28 (37%)	220 (26%)	1.63 (1.00–2.66)	3.82	0.051
Anti-Sm	6 (8%)	70 (8%)	0.93 (0.39–2.23)	0.02	0.89
Anti-nRNP	16 (21%)	195 (23%)	0.87 (0.49–1.55)	0.22	0.64
Anti-Ro	18 (24%)	191 (23%)	1.04 (0.60–1.81)	0.02	0.89
Anti-La	2 (3%)	53 (6%)	0.40 (0.10–1.67)	—	0.22*
Anti-ribosomal P	2 (3%)	13 (2%)	1.71 (0.38–7.70)	—	0.63*
ACL IgG	28 (37%)	244 (29%)	1.41 (0.86–2.30)	1.89	0.17
ACL IgM	10 (13%)	69 (8%)	1.68 (0.83–3.41)	2.09	0.15
ACL IgA	0 (0%)	0 (0%)	—	—	1*

* *P*-value calculated by Fisher's exact two-tailed probability test.

The frequency of various other associated autoimmune conditions in lupus patients with and without haemolytic anaemia

Table 3

Other autoimmune disorders	Haemolytic anaemia present (<i>n</i> = 29)	Haemolytic anaemia absent (<i>n</i> = 234)	Odds ratio	χ^2	<i>P</i> -value
Rheumatoid arthritis	12 (41%)	70 (30%)	1.65	1.58	0.21
Sjogren's syndrome	0 (0%)	24 (10%)	—	—	0.087*
Scleroderma	2 (7%)	19 (8%)	0.84	—	1.0*
Addison's disease	1 (3%)	2 (1%)	4.14	—	0.30*
Thyroid disease	9 (31%)	79 (34%)	0.88	0.09	0.76

* *P*-value calculated by Fisher's exact two-tailed probability test.

Table 4

The frequency of proteinuria, cellular casts, seizures, pericarditis, pleuritis, lymphopenia and anti-dsDNA antibody in lupus patients with and without haemolytic anaemia and in the absence of thrombocytopenia

	Haemolytic anaemia present (<i>n</i> = 47)	Haemolytic anaemia absent (<i>n</i> = 739)	Odds ratio (95% confidence interval)	χ^2	<i>P</i> -value
Proteinuria	30 (64%)	262 (35%)	3.21 (1.74–5.94)	15.24	0.000095
Cellular casts	14 (30%)	103 (14%)	2.62 (1.36–5.06)	8.76	0.0031
Seizures	10 (21%)	58 (8%)	3.17 (1.50–6.71)	—	0.0047*
Pericarditis	16 (34%)	130 (18%)	2.42 (1.28–4.55)	7.91	0.0049
Pleuritis	20 (43%)	197 (27%)	2.04 (1.12–3.72)	5.59	0.018
Lymphopenia	25 (53%)	279 (38%)	1.87 (1.04–3.39)	4.44	0.035
Anti-dsDNA	19 (40%)	188 (25%)	1.99 (1.09–3.64)	5.12	0.024

* *P*-value calculated by Fisher's exact two-tailed probability test.