Elevated Subclinical Double-Stranded DNA Antibodies and Future Proliferative Lupus Nephritis

Stephen W. Olson,† Jessica J. Lee,*† Lisa K. Prince,*† Thomas P. Baker,*† Patricia Papadopoulos,†‡ Jess Edison,*† and Kevin C. Abbott*†*

*Walter Reed National Military Medical Center, and †Uniformed Service University of the Health Sciences, Bethesda, Maryland; and ‡Madigan Army Medical Center, Tacoma, Washington

Correspondence: Dr. Stephen Olson, 7010 Tilden Lane, Rockville, MD 20852. Email: Stephen.w.olson@health.mil

Summary

Background and objectives Elevated anti–double-stranded DNA (dsDNA) antibody and C-reactive protein are associated with proliferative lupus nephritis (PLN). Progression of quantitative anti-dsDNA antibody in patients with PLN has not been compared with that in patients with systemic lupus erythematosus (SLE) without LN before diagnosis. The temporal relationship between anti-dsDNA antibody and C-reactive protein elevation has also not been evaluated.

Design, setting, participants, & measurements This case-control Department of Defense Serum Repository (established in 1985) study compared longitudinal prediagnostic quantitative anti-dsDNA antibody and C-reactive protein levels in 23 patients with biopsy-proven PLN (Walter Reed Army Medical Center, 1993–2009) with levels in 21 controls with SLE but without LN matched for patient age, sex, race, and age of serum sample. The oldest (median, 2601 days; 25%, 1245 days, 75%, 3075 days), the second to last (368; 212, 635 days), and the last (180; 135, 477 days) serum sample before diagnosis were analyzed.

Results More patients with PLN had an elevated anti-dsDNA antibody level than did the matched controls at any point (78% versus 5%; $P<0.001$), <1 year (82% versus 8%; $P<0.001$), 1–4 years (53% versus 0%; $P<0.001$), and >4 years (33% versus 0%; $P=0.04$) before diagnosis. A rate of increase >1 IU/ml per year (70% versus 0%; $P<0.001$) was most specific for PLN. The anti-dsDNA antibody levels increased before C-reactive protein did in most patients with an antecedent elevation (92% versus 8%; $P<0.001$).

Conclusions Elevated anti-dsDNA antibody usually precedes both clinical and subclinical evidence of proliferative LN, which suggests direct pathogenicity. Absolute anti-dsDNA antibody level and rate of increase could better establish risk of future PLN in patients with SLE.

Clin J Am Soc Nephrol 8: 1702–1708, 2013. doi: 10.2215/CJN.01910213

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that can involve multiple organ systems with varying severity (1,2). More than half of patients with SLE develop lupus nephritis (LN), often after the original diagnosis of SLE (3). LN can present with a wide range of clinical and histopathologic findings. The heterogenicity of disease presentation and progression suggests a complex mechanism of disease. Although significant progress has been made in the past decades, the pathophysiology of LN has yet to be fully elucidated (4–9).

Anti–double-stranded DNA (anti-dsDNA) antibody is one of multiple autoantibodies implicated in the pathogenesis of LN. *In vitro* human and *in vivo* animal studies suggest that anti-dsDNA antibodies are directly pathogenic. Elution of kidney tissue from patients with LN reveals anti-dsDNA antibodies. Injection of anti-dsDNA antibodies into mice models induces histopathologic findings similar to LN (4–9). Recent animal data support the theory that LN is triggered by the direct binding of anti-dsDNA antibody to the glomerular basement membrane (GBM), although previous studies purport that the antibody needs to complex with nucleosomes in circulation or with chromatin *in situ* (10). There is a strong association between anti-dsDNA antibodies and LN, and, more specifically, proliferative LN (PLN), at the time of diagnosis (3,11–14). Absolute quantitative level and rate of increase in anti-dsDNA antibodies in LN have not been previously evaluated. In addition, to our knowledge no previous studies have assessed the temporal relationship between the elevation of anti-dsDNA antibody and the elevation of C-reactive protein (CRP), with CRP representing a nonspecific asymptomatic inflammation surrogate for subclinical disease.

We hypothesized that a much larger percentage of patients with SLE and PLN have an elevated and increasing anti-dsDNA antibody level approaching diagnosis compared with controls with SLE but without LN matched for patient age, sex, race, and age of serum sample. We also hypothesized that anti-dsDNA antibody levels would increase before CRP did.

Materials and Methods

Participants

We performed a retrospective case-control serum bank study comparing anti-dsDNA antibody and CRP levels years before PLN diagnosis to matched controls who have SLE without LN. This study was approved by the Human Use Committee at Walter Reed National Military Medical Center, and the need for informed consent was waived.

We identified 23 patients with biopsy-proven PLN (World Health Organization class III or IV) from the Walter Reed Army Medical Center renal biopsy database from 1993 to 2009. A comprehensive electronic database review was performed for each patient with PLN to populate a clinical background data collection sheet. The SLE Disease Activity Index (SLEDAI) as well as National Institutes of Health (NIH) Activity and Chronicity Indices were tabulated (15–17). The Department of Defense Serum Repository (DoDSR), described in previous publications, identified 21 controls with SLE without LN who were matched for patient age, sex, race, and age of serum samples (18). To maximize specificity, each control had at least one hospitalization or three outpatient International Classification of Diseases, Ninth Revision (ICD-9), codes for SLE (711.0) without an ICD-9 code for LN (583.81) or any other urinary abnormalities to suggest undiagnosed LN. The DoDSR also provided a list of all other ICD-9 codes for each matching control to document comorbid conditions. Controls from Walter Reed who had SLE without LN would not have been matched effectively for age, sex, race, and age of serum sample. The DoDSR then pulled the oldest, the second to last, and the most recent 0.5-ml serum samples before PLN or SLE without LN diagnosis and sent them to Quest Diagnostics Nichols Institute (Chantilly, VA).

Laboratory Assays

Quest Diagnostics used the BioPlex 2200 flow immunoassay for the quantitative measurement of anti-dsDNA antibodies (19). Dyed beads uniquely coated with dsDNA were incubated with an aliquot of approximately 300 μl of patient serum. After a wash cycle, a fluorescent (phycoerythrin) conjugated IgG antibody–bound residual anti-dsDNA antibody. After another wash, the samples were run through a detector for quantification using relative fluorescence intensity. Titer levels for anti-dsDNA antibodies were reported as negative ($\leq$4 IU/ml), indeterminate (5–9 IU/ml), or positive ($\geq$10 IU/ml). Average intra-assay reproducibility rates for a high positive panel and a low positive panel were 2.2% and 3.1%, respectively. Average interassay reproducibility rates for a high positive panel and a low positive panel were 5.2% and 5.4%, respectively. The test demonstrated linearity throughout the assay range (R^2=0.9994). A latex assay was used to quantify CRP levels using a 100-μl serum sample. Serum CRP agglutinates with latex particles coated with monoclonal anti-CRP mouse antibodies in a glycine buffer stabilized with 0.09% sodium azide. The precipitate is determined turbidimetrically at 552 nm by a Roche Cobra Integra Analyzer. The analyzer automatically makes a 1:1, 1:1.5, 1:2, 1:4, and 1:16 dilution in addition to using a 0-mg/L standard. The reference range for normal CRP is <0.8 mg/dl.

Statistical Analyses

The percentage of patients with PLN who had anti-dsDNA antibodies above selected threshold values before diagnosis was compared with the percentage of controls with SLE without LN using the Fisher exact probability test. Odds ratios and 95% confidence intervals were also calculated using the VassarStats software. The same statistical analysis was used for all secondary outcomes and subgroup analysis.

Results

Demographics

The study population consisted of predominantly African American women younger than 40 years of age. The patients with PLN had a higher SLEDAI score than the controls (median [25%, 75%], 16 [12, 22] versus 10 [6, 14]) but had the same median SLEDAI as the control group when limited to only extrarenal SLE manifestations (10 [4, 14] versus 10 [6, 14]). NIH Activity and Chronicity Index scores (median [25%, 75%]) were 8 (5, 10) and 3 (1, 3), respectively (Table 1).

Anti-dsDNA Antibody

More patients with PLN had a single elevated dsDNA level (>/10 U/ml) than did controls at any time before diagnosis (78% versus 5%; P<0.001), <1 year before diagnosis (82% versus 8%; P<0.001), 1–4 years before diagnosis (53% versus 0%; P<0.001), and >4 years before diagnosis (36% versus 0%; P=0.04) (Table 2).

A greater percentage of patients with PLN had an elevated anti-dsDNA antibody level >40 IU/ml compared with matched controls at any time before diagnosis (70% versus 0%; P<0.001), <1 year before diagnosis (82% versus 0%; P<0.001), and 1–4 years before diagnosis (27% versus 0%; P=0.04) (Table 2). More patients with PLN had an anti-dsDNA antibody level in the indeterminate range (>4 IU/ml) than did matched controls at any time before diagnosis (78% versus 14%; P<0.001), <1 year before diagnosis (82% versus 15%; P<0.001), and 1–4 years before diagnosis (60% versus 0%; P=0.009)) (Table 2).

Elevated anti-dsDNA antibody levels >50 IU/ml (100% in patients versus 41% in controls; P=0.02) were associated with future crescentic LN.

Time Course of Antibody Development. The anti-dsDNA antibodies became elevated (>/10 IU/ml) an average of 2.7 years before diagnosis and in <5 years before 83% of diagnoses. The 2.7 years is an indeterminate underestimation because all patients with PLN probably experienced an increase in anti-dsDNA antibody levels before the banked serum sample with the first elevation was obtained. An institutional serum sample limit of three per patient can leave large temporal gaps between data points. In the most extreme cases, there was a 10-year window in which the first elevation in anti-dsDNA antibodies could have occurred unrecorded.

Increases in anti-dsDNA antibodies >1 IU/ml per year (70% versus 0%; P<0.001) and >50% per year (70% versus 0%; P<0.001) were highly specific for future PLN (Table 3).

Time Course of Increase in Antibody versus CRP. The anti-dsDNA antibody level increased before CRP did in most patients with an antecedent elevation (92% versus 8%; P<0.001) (Table 4). The one patient with an antecedent

Table 1. Background information on 23 patients with systemic lupus erythematosus and proliferative lupus nephritis and 21 matched controls without lupus nephritis

Characteristic	Patients with SLE and PLN	Controls with SLE without LN
Median age (yr)	25 (23, 41)	25 (23, 41)
Race (%)		
White	15	15
African American	60	60
Other	25	25
Women (%)	65	65
History of hypertension (%)	83 (19/23)	36
History of diabetes mellitus (%)	0 (0/23)	9
Arthralgia (%)	70 (16/23)	0
Dermatologic (%)	48 (11/23)	73
Hematologic (%)	53 (12/23)	46
Cardiac involvement (%)	35 (8/23)	41
CNS involvement (%)	9 (2/23)	23
Lung involvement (%)	35 (8/23)	9
Liver involvement (%)	9 (2/23)	5
Hematuria (>3 RBCs per high-power field)	100 (23/23)	
Proteinuria (>300 mg)	100 (23/23)	
Nephrotic-range proteinuria (>3.5 g)	35 (8/23)	
Median proteinuria (g)	1.60 (0.63, 3.40)	
Median serum creatinine (mg/dl)	1.0 (0.8, 1.2)	
Antinuclear antibody (% positive)	96 (22/23)	
dsDNA antibody (% positive)	78 (18/23)	
Median titer (n=18)	1:320 (1:160, 1:640)	
Median level (U/ml; n=6)	200 (147, 228)	
dsDNA antibody or antinuclear antibody (% positive)	100 (23/23)	
Antiphospholipid antibody (% positive)	38 (8/23)	
Anti-SM antibody (% positive)	38 (6/16)	
Anti-RNP antibody (% positive)	50 (8/16)	
Median CRP level (mg/dl)	1.37 (0.83,3.88)	
CRP level >0.8 mg/dl (%)	79 (11/14)	
Biopsy (%)		
WHO class III	22 (5/23)	
WHO class IV	78 (18/23)	
WHO class IV and V	35 (8/23)	
Crescent formation	23 (6/23)	
Median NIH Activity Index	8 (5, 10)	
Median NIH Chronicity Index	3 (1, 3)	
Median SLE Disease Activity Index	16 (12, 22)	10 (4, 14)
Immunosuppression before biopsy (%)		
Prednisone	22 (5/23)	
Hydroxychloroquine	39 (9/23)	
Other (cyclophosphamide, mycophenolate mofetil, rituximab)	9 (2/23)	

Data on patients with lupus nephritis were based on review of electronic medical record charts. Data on controls without lupus nephritis are based on International Classification of Diseases, Ninth Revision, codes provided by the Department of Defense Serum Repository. Medians are presented with 25% and 75% values in parentheses for continuous data because they were not normally distributed. Prednisone dose was ≤10 mg/d. Some percentages are accompanied by n/n in parentheses. Other immunosuppression was discontinued at least 6 months before confirmatory kidney biopsy. Not all patients had information available for each laboratory measurement. Laboratory data were not available for the control patients. SLE, systemic lupus erythematosus; PLN, progressive lupus nephritis; LN, lupus nephritis; CNS, central nervous system; RBC, red blood cell; dsDNA, double-stranded DNA; SM, smooth muscle; RNP, ribonucleoprotein; CRP, C-reactive protein; NIH, National Institutes of Health.

CRP elevation had a normal CRP level (0.05 mg/dl) with an elevated anti-dsDNA antibody level (58 IU/ml) 5 years later, suggesting an initial infectious cause. The most significant individual example was a patient with a 477-IU/ml anti-dsDNA antibody level and a 0.05-mg/dl CRP level 212 days before diagnosis.

Discussion

Our novel results describe the natural history of anti-dsDNA antibodies before PLN diagnosis. The study cohort had an average SLEDAI score, average NIH Activity and Chronicity indices, and a prevalence of elevated anti-dsDNA antibody at diagnosis that were similar to those noted in

Table 2. Percentage of patients with systemic lupus erythematosus and progressive lupus nephritis versus percentage of matched controls without lupus nephritis with anti–double-stranded DNA antibody levels above specific thresholds (all time periods, <1 year, 1–4 years, and >4 years)

DsDNA	Patients (%)	Controls (%)	Odds Ratio (95% Confidence Interval)	*P* Value (Fisher Exact Test)
(>2 IU/ml): All	83 (19/23)	24 (5/21)	15 (3.5 to 66)	<0.001
<1 yr	82 (14/17)	15 (2/13)	26 (4 to 181)	<0.001
1–4 yr	73 (11/15)	25 (4/16)	8.3 (1.7 to 41)	0.01
>4 yr	50 (7/14)	7 (1/14)	13 (1.3 to 128)	0.03
(>4 IU/ml): All	78 (18/23)	14 (3/21)	22 (4.5 to 104)	<0.001
<1 yr	82 (14/17)	15 (2/13)	26 (4 to 181)	<0.001
1–4 yr	60 (9/15)	13 (2/16)	11 (2 to 64)	0.009
>4 yr	36 (5/14)	7 (1/14)	7.2 (0.7 to 73)	0.17
(>10 IU/ml): All	78 (18/23)	5 (1/21)	72 (7.7 to 676)	<0.001
<1 yr	82 (14/17)	8 (1/13)	56 (5 to 612)	<0.001
1–4 yr	53 (8/15)	0 (0/16)	23 (2 to 218)[a]	<0.001
>4 yr	36 (5/14)	0 (0/14)	13 (1.3 to 128)[a]	0.04
(>40 IU/ml) All	70 (16/23)	0 (0/21)	65 (7.1 to 593)[a]	<0.001
<1 yr	82 (14/17)	0 (0/13)	90 (7 to 1116)[a]	<0.001
1–4 yr	27 (4/15)	0 (0/16)	10 (1.03 to 97)[a]	0.04
>4 yr	7 (1/14)	0 (0/14)	2.2 (0.2 to 27)[a]	1.0

The Department of Defense Serum Repository could not assign a matching control for one patient. The samples for a second control were lost in processing, leaving 21. Not all patients had samples available for each subgroup time period. If multiple serum samples were present for a patient in a specific subgroup analysis time period, the highest antibody level dictated group assignment. All serum samples available for the one control with an anti–double-stranded DNA antibody level >/10 IU/ml were obtained <1 year before diagnosis. Percentages are accompanied by *n/n* in parentheses. dsDNA, double-stranded DNA.
[a]Estimated because of actual infinite value.

Table 3. Percent of patients with systemic lupus erythematosus and progressive lupus nephritis versus percentage of matched controls without lupus nephritis who had both an absolute and a percentage rate of increase in anti–double-stranded DNA antibody above specific thresholds

DsDNA	Patients (%)	Controls (%)	Odds Ratio (95% Confidence Interval)	*P* Value (Fisher Exact Test)
Change in IU/ml per yr				
>0 IU/ml	75 (15/20)	22 (4/18)	11 (2.3 to 47)	0.003
>0.5 IU/ml	75 (15/20)	0 (0/18)	76 (8 to 751)[a]	<0.001
>1 IU/ml	70 (14/20)	0 (0/18)	57 (6 to 542)[a]	<0.001
Change in % per yr				
>0%	80 (16/20)	22 (4/18)	14 (2.9 to 67)	<0.001
>10%	75 (15/20)	11 (2/18)	24 (4 to 143)	<0.001
>50%	70 (14/20)	0 (0/18)	60 (6.3 to 569)[a]	<0.001

Only 20 patients and 18 controls had multiple serum samples for evaluation of change in anti–double-stranded DNA antibody levels over time. Percentages are accompanied by *n/n* in parentheses. dsDNA, double-stranded DNA.
[a]Estimated because of actual infinite value.

previous reports (11,17,20,21). Anti-dsDNA antibody was elevated months to years before 78% of patients with PLN but in only one control with SLE without LN. A similar percentage of known patients with SLE manifest elevated anti-dsDNA antibody before LN flare (21–24). In addition, the one control who had anti-dsDNA antibody–positive SLE without LN developed a positive titer less than a year before diagnosis and could conceivably develop PLN in the future.

Using the same serum bank, Arbuckle *et al.* reported only 55% of patients with SLE and a positive anti-dsDNA antibody titer before diagnosis (25). However, this study did not distinguish between patients with and those without LN or quantify anti-dsDNA antibodies. It is possible that most of the 55% of patients with positive anti-dsDNA antibody titers had or went on to develop LN, consistent with the known prevalence of LN in patients with SLE. Perhaps more important, to our knowledge our study shows for the first time that the rate of increase in anti-dsDNA antibody level may help to distinguish between future PLN and SLE without LN. A rate of increase of 1 IU/ml per year was highly specific for disease. There are no previous studies of the rate of change in anti-dsDNA antibody over time for comparison.

Table 4. Temporal relationship between C-reactive protein and anti–double-stranded DNA antibody

DsDNA	Before CRP >0.8 mg/dl (%)	After CRP >0.8 mg/dl (%)	Odds Ratio (95% Confidence Interval)	*P* Value (Fisher exact test)
>10 IU/ml	92 (12/13)	8 (1/13)	144 (8.0 to 2578)	<0.001
>4 IU/ml	55 (12/13)	5 (1/13)	144 (8.0 to 2578)	<0.001
>2 IU/ml	100 (14/14)	0 (0/14)	225 (12.9 to 3940)[a]	<0.001
>0 IU/ml	100 (17/17)	0 (0/17)	324 (18.8 to 5589)[a]	<0.001

Antecedent elevation of C-reactive protein or anti–double-stranded DNA antibodies above the specified threshold could not be established for all patients. If both became elevated in the same sample or if neither was elevated in any sample, no antecedent elevation was determined. Total patients vary in some groups because more patients demonstrated a clear antecedent elevation of anti–double-stranded DNA antibody when thresholds were set lower. Percentages are accompanied by *n/n* in parentheses. dsDNA, double-stranded DNA; CRP, C-reactive protein.
[a]Estimated because of actual infinite value.

Our data also further clarify the pathogenic role of anti-dsDNA antibody in SLE. Anti-dsDNA antibody is present before even subclinical systemic inflammation. Ninety-two percent of study patients with a clear antecedent elevation first developed anti-dsDNA antibodies before CRP elevation or PLN diagnosis. In addition, although 79% of our study cohort had an elevated CRP level at diagnosis, only 26% of patients had an elevated CRP before diagnosis. CRP only became elevated in this subgroup <9 months before diagnosis (3–258 days). If anti-dsDNA antibody were simply a passive marker of disease, it would be expected to increase after an elevation in CRP from another culprit. However, even in this subset of patients with PLN, passive elevation of anti-dsDNA antibodies after another inciting event and before CRP elevation cannot be completely ruled out. CRP measurement has both general and SLE-specific limitations. Although concurrent malignancies, chronic infections, and other inflammatory diseases were excluded, elevations due to undocumented infection cannot be ruled out. In addition, although CRP levels are above normal in most patients with SLE at diagnosis, these elevations are blunted compared with other inflammatory disease processes, such as vasculitis, and may be more associated with arthritis, pleuritis, and pericarditis than nephritis (26–28). It is hypothesized that serum CRP is consumed in SLE by anti-CRP antibodies, renal deposition, and binding to apoptotic debris or complement (26,29). Despite these limitations, our findings are consistent with previous reports that CRP does not directly correlate with anti-dsDNA antibody, disease severity, or impending LN flare (26,29).

Not only do more patients with PLN have an elevated anti-dsDNA antibody level before diagnosis, a greater percentage also have anti-dsDNA antibody levels above thresholds within the normal range in comparison with controls who have SLE without LN. This statistically significant difference may exist decades before diagnosis, reflecting a baseline "first hit" genetic risk factor, but our study had insufficient power to evaluate specific time periods more distant than >4 years from diagnosis. The anti-dsDNA antibody threshold of >/10 IU/ml was established on the basis of the evaluation of patients with active clinical disease. It is possible that abnormal subclinical anti-dsDNA antibody levels occur in the currently accepted normal range. We previously demonstrated that more patients with anti-GBM have detectable anti-GBM antibody within the accepted normal range before diagnosis compared with matched controls (18).

There are multiple theoretical explanations for these findings. We propose that baseline anti-dsDNA antibody represents one hit in a multiple-hit mechanism of disease previously described by Dr. Tsokos. Baseline genetic abnormalities manifesting as altered C4, C1Q, and cytokine production, as well as abnormal apoptotic and T cell function, could preserve self-reactive B cells and nuclear debris. This inflammatory milieu would support low-level anti-dsDNA antibody production (1,30–32). Intrinsic or external stimuli, such as epigenetic alterations or antigen mimicry, then may provide the secondary hit to augment preexisting antibody production. The resulting large anti-dsDNA antibody burden could then instigate clinical disease.

Our data set could also provide clinical relevance. Up to 35% of patients with SLE develop LN after diagnosis, most frequently in the African American population (24). A predictive anti-dsDNA antibody model may identify the SLE subpopulation at high risk for development of LN. This population could warrant closer follow-up and a lower threshold for confirmatory renal biopsy. A more prompt diagnosis would ensure more renal function to preserve at the time of therapeutic intervention. There is some precedence for proactive therapeutic intervention. A randomized, placebo-controlled trial found that early steroid intervention for serologically active but clinically quiescent SLE prevented severe flares (33). On the basis of our findings, any patient with SLE who has an anti-dsDNA antibody level >2 IU/ml would be monitored with serial anti-dsDNA antibody serologic testing. A consistent rate of increase >1 IU/ml per year or an absolute level of 10 IU/ml per year, especially in the setting of even mildly abnormal urine sediments, would necessitate a renal biopsy to rule out early proliferative disease. However, rate of change in IU/ml per year assumes a linear rate of increase in anti-dsDNA antibody over time before diagnosis. This has not been proven. Before implementation, our proposed diagnostic model would require prospective validation on a cohort of patients with newly diagnosed SLE without LN.

Before any prospective validation, our model would benefit from further investigation to address current

limitations. We plan to compare the anti-dsDNA antibody trajectory in patients with PLN with that in controls who have mesangial and membranous LN. Comparison with mesangial LN is particularly important because it can have an early clinical presentation similar to that of PLN. In addition, we plan to describe the prediagnostic natural history of Smith, ribonucleoprotein, ANCA, C1q, nucleosome, and histone antibodies, which are also associated with LN at diagnosis (3,34). This is essential because anti-dsDNA antibody cannot independently account for the pathogenesis of PLN. More than 15% of patients with PLN had a normal anti-dsDNA antibody level 1 year before and at diagnosis. Defining the prediagnostic trajectory and temporal relationship of all LN-associated autoantibodies may establish a more comprehensive predictive autoantibody profile for PLN risk. Data already suggest that dual-positive anti-dsDNA antibody and anti-C1q antibody are more sensitive and specific for LN flare and portend a worse renal prognosis than either autoantibody individually (20,21). Even a comprehensive autoantibody profile will probably not predict development of PLN in all cases. Antibody avidity, cross-reactivity, subclass, tissue target specificity, and cell penetration with nuclear localization probably contribute. T cell activity, cytokine production, complement activity, and presence of Fc receptor glomerular tissue expression targets are also implicated (4–9,35–37).

The DoDSR has unique limitations described previously (18). In this specific study, we cannot rule out that some of the controls with SLE without LN went on to develop LN. The erroneous placement of a study patient into the control group would also only narrow any difference between groups and marginalize the statistical significance of our findings. Inability to directly link results to specific patient background clinical data due to DoDSR required de-identification precludes robust secondary analysis, such as correlation of anti-dsDNA antibody level to NIH Activity Index. Also, exclusion of other classes of LN prevents purposeful evaluation of positive and negative predictive values. In addition, controls were not universally treated at Walter Reed, introducing the potential for practice pattern and documentation variability. Study design also prevented description of baseline CRP and antibody levels for the controls at diagnosis. Finally, our study cohort skewed toward young African American women. Our results may not translate to patients with other demographic characteristics.

The diagnostic synergy between the DoDSR and Walter Reed National Military Medical Center renal biopsy databases allowed us to establish an association between elevated and rising anti-dsDNA antibody and future PLN.

Acknowledgments

The views expressed in this manuscript are those of the authors and do not reflect the official policy of the Department of the Army, Department of Defense, or the U.S. government.

Disclosures

None.

References

1. Tsokos GC: Systemic lupus erythematosus. N Engl J Med 365: 2110–2121, 2011
2. Rahman A, Isenberg DA: Systemic lupus erythematosus. *N Engl J Med* 358: 929–939, 2008
3. Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I, Khamashta MA, D'Cruz D, Hughes GRV: Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. *Ann Rheum Dis* 62: 556–560, 2003
4. Deshmukh US, Bagavant H, Fu SM: Role of anti-DNA antibodies in the pathogenesis of lupus nephritis. *Autoimmun Rev* 5: 414–418, 2006
5. van Bavel CC, van der Vlag J, Berden JH: Glomerular binding of anti-dsDNA autoantibodies: The dispute resolved? *Kidney Int* 71: 600–601, 2007
6. Yung S, Chan TM: Anti-DNA antibodies in the pathogenesis of lupus nephritis—the emerging mechanisms. *Autoimmun Rev* 7: 317–321, 2008
7. Waldman M, Madaio MP: Pathogenic autoantibodies in lupus nephritis. *Lupus* 14: 19–24, 2005
8. Putterman C: New approaches to the renal pathogenicity of anti-DNA antibodies in systemic lupus erythematosus. *Autoimmun Rev* 3: 7–11, 2004
9. Wellmann U, Letz M, Herrmann M, Angermüller S, Kalden JR, Winkler TH: The evolution of human anti-double-stranded DNA autoantibodies. *Proc Natl Acad Sci U S A* 102: 9258–9263, 2005
10. Krishnan MR, Wang C, Marion TN: Anti-DNA autoantibodies initiate experimental lupus nephritis by binding directly to the glomerular basement membrane in mice. *Kidney Int* 82: 184–192, 2012
11. Yu F, Tan Y, Liu G, Wang SX, Zou WZ, Zhao MH: Clinicopathological characteristics and outcomes of patients with crescentic lupus nephritis. *Kidney Int* 76: 307–317, 2009
12. Munoz LE, Gaipl US, Herrmann M: Predictive value of anti-dsDNA autoantibodies: Importance of the assay. *Autoimmun Rev* 7: 594–597, 2008
13. Davis P, Percy JS, Russell AS: Correlation between levels of DNA antibodies and clinical disease activity in SLE. *Ann Rheum Dis* 36: 157–159, 1977
14. Cortés-Hernández J, Ordi-Ros J, Labrador M, Buján S, Balada E, Segarra A, Vilardell-Tarrés M: Antihistone and anti-double-stranded deoxyribonucleic acid antibodies are associated with renal disease in systemic lupus erythematosus. *Am J Med* 116: 165–173, 2004
15. Austin HA 3rd, Muenz LR, Joyce KM, Antonovych TT, Balow JE: Diffuse proliferative lupus nephritis: Identification of specific pathologic features affecting renal outcome. *Kidney Int* 25: 689–695, 1984
16. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH; The Committee on Prognosis Studies in SLE: Derivation of the SLEDAI. A disease activity index for lupus patients. *Arthritis Rheum* 35: 630–640, 1992
17. Austin HA 3rd, Boumpas DT, Vaughan EM, Balow JE: Predicting renal outcomes in severe lupus nephritis: Contributions of clinical and histologic data. *Kidney Int* 45: 544–550, 1994
18. Olson SW, Arbogast CB, Baker TP, Owshalimpur D, Oliver DK, Abbott KC, Yuan CM: Asymptomatic autoantibodies associate with future anti-glomerular basement membrane disease. *J Am Soc Nephrol* 22: 1946–1952, 2011
19. *BioPlex 2200 System Operation Instruction for Use Manual: Anti-Double Stranded DNA Antibody*. Hercules, California, BioRad
20. Yang XW, Tan Y, Yu F, Zhao MH: Combination of anti-C1q and anti-dsDNA antibodies is associated with higher renal disease activity and predicts renal prognosis of patients with lupus nephritis. *Nephrol Dial Transplant* 27: 3552–3559, 2012
21. Matrat A, Veysseyre-Balter C, Trolliet P, Villar E, Dijoud F, Bienvenu J, Fabien N: Simultaneous detection of anti-C1q and anti-double stranded DNA autoantibodies in lupus nephritis: Predictive value for renal flares. *Lupus* 20: 28–34, 2011
22. Linnik MD, Hu JZ, Heilbrunn KR, Strand V, Hurley FL, Joh T; LJP 394 Investigator Consortium: Relationship between anti-double-stranded DNA antibodies and exacerbation of renal disease in patients with systemic lupus erythematosus. *Arthritis Rheum* 52: 1129–1137, 2005
23. ter Borg EJ, Horst G, Hummel EJ, Limburg PC, Kallenberg CGM: Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. A long-term, prospective study. *Arthritis Rheum* 33: 634–643, 1990

24. Bastian HM, Roseman JM, McGwin G Jr, Alarcón GS, Friedman AW, Fessler BJ, Baethge BA, Reveille JD; LUMINA Study Group. LUpus in MInority populations: NAture vs nurture: Systemic lupus erythematosus in three ethnic groups. XII. Risk factors for lupus nephritis after diagnosis. *Lupus* 11: 152–160, 2002
25. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, Harley JB: Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 349: 1526–1533, 2003
26. Amezcua-Guerra LM, Springall R, Arrieta-Alvarado AA, Rodríguez V, Rivera-Martinez E, Castillo-Martinez D, Bojalil R: C-reactive protein and complement components but not other acute-phase reactants discriminate between clinical subsets and organ damage in systemic lupus erythematosus. *Clin Lab* 57: 607–613, 2011
27. Lee SS, Singh S, Link K, Petri M: High-sensitivity C-reactive protein as an associate of clinical subsets and organ damage in systemic lupus erythematosus. *Semin Arthritis Rheum* 38: 41–54, 2008
28. Williams RC Jr, Harmon ME, Burlingame R, Du Clos TW: Studies of serum C-reactive protein in systemic lupus erythematosus. *J Rheumatol* 32: 454–461, 2005
29. Birmingham DJ, Irshaid F, Nagaraja HN, Zou X, Tsao BP, Wu H, Yu CY, Hebert LA, Rovin BH: The complex nature of serum C3 and C4 as biomarkers of lupus renal flare. *Lupus* 19: 1272–1280, 2010
30. Munoz LE, van Bavel C, Franz S, Berden J, Herrmann M, van der Vlag J: Apoptosis in the pathogenesis of systemic lupus erythematosus. *Lupus* 17: 371–375, 2008
31. Mevorach D: Clearance of dying cells and systemic lupus erythematosus: The role of C1q and the complement system. *Apoptosis* 15: 1114–1123, 2010
32. Stuart L, Hughes J: Apoptosis and autoimmunity. *Nephrol Dial Transplant* 17: 697–700, 2002
33. TsengCE, Buyon JP, Kim M, Belmont HM, Mackay M, Diamond B, Marder G, Rosenthal P, Haines K, Ilie V, Abramson SB. The effect of moderate-dose corticosteroids in preventing severe flares in patients with serologically active, but clinically stable, systemic lupus erythematosus: findings of a prospective, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 54: 3623–3632, 2006
34. Pradhan VD, Badakere SS, Bichile LS, Almeida AF: Anti-neutrophil cytoplasmic antibodies (ANCA) in systemic lupus erythematosus: Prevalence, clinical associations and correlation with other autoantibodies. *J Assoc Physicians India* 52: 533–537, 2004
35. van Bavel CC, Fenton KA, Rekvig OP, van der Vlag J, Berden JH: Glomerular targets of nephritogenic autoantibodies in systemic lupus erythematosus. *Arthritis Rheum* 58: 1892–1899, 2008
36. D'Andrea DM, Coupaye-Gerard B, Kleyman TR, Foster MH, Madaio MP: Lupus autoantibodies interact directly with distinct glomerular and vascular cell surface antigens. *Kidney Int* 49: 1214–1221, 1996
37. Yung S, Cheung KF, Zhang Q, Chan TM: Anti-dsDNA antibodies bind to mesangial annexin II in lupus nephritis. *J Am Soc Nephrol* 21: 1912–1927, 2010

Received: February 15, 2013 **Accepted:** May 28, 2013

Published online ahead of print. Publication date available at www.cjasn.org.