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Evaluation of different ways to identify persistent positivity of lupus anticoagulant in systemic lupus erythematosus

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ABSTRACT

Objective Persistent positivity for lupus anticoagulant has been associated with an increased risk of thrombosis among patients with SLE. Persistent positivity is often defined as having two positive assessments separated by more than 90 days. Our objective was to determine whether frequent repeated lupus anticoagulant testing would identify more patients with persistent positivity, and whether the additional patients identified were still at increased risk of thrombosis.

Methods Using a large longitudinal cohort with frequent lupus anticoagulant testing, we compared three different hypothetical clinical strategies for identifying persistent positivity: (1) assessment of lupus anticoagulant twice more than 90 days apart; (2) assessment of lupus anticoagulant annually, with repeat testing if an annual assessment was positive; and (3) assessment of lupus anticoagulant 16 times (approximately quarterly for 4 years). The prevalence of persistent positivity was compared between the approaches and by demographic subgroups. Subgroups based on these definitions were compared with respect to the risk of thrombosis in subsequent follow-up using discrete survival analysis.

Results Among the 785 patients included in our analysis, the prevalence of persistent lupus anticoagulant as defined by the first two patient assessments was 4.3%. Annual assessment resulted in a prevalence of 6.6%, and using all 16 assessments resulted in a prevalence of 10.5%. The prevalence was substantially higher in men than in women, and in Caucasians than in African-Americans ($p<0.01$ for all comparisons). The rate of thrombosis was significantly elevated among those with persistently positive lupus anticoagulant by *any* definition (HR ranging from 2.75 to 3.42) relative to those without persistently positive lupus anticoagulant.

Conclusion While there are other risk factors for thrombosis (including other antiphospholipid subtypes), more frequent testing (not limited to twice over 3 months) for lupus anticoagulant would be useful for identifying more patients with SLE at elevated risk for thrombosis.

Significance

Already known
- Lupus anticoagulant is the antiphospholipid antibody most strongly associated with thrombosis.
- The association is much stronger if the patient is persistently positive (two or more positive findings separated by at least 12 weeks).

New findings
- Persistent positivity (two positive tests separated by more than 90 days) was defined in three different ways, based on (1) the first two assessments, (2) annual assessments or (3) 16 assessments.
- The prevalence of persistent lupus anticoagulant was 4.3%, 6.6% and 10.5%, respectively.
- The rate of thrombosis was significantly elevated among those with persistently positive lupus anticoagulant by *any* of the three definitions (HR 2.75–3.42).

Change in clinical practice
- Frequent testing (not limited to twice over 3 months) for lupus anticoagulant would identify more patients with SLE at increased risk for thrombosis.

INTRODUCTION

Patients with antiphospholipid antibodies (lupus anticoagulant, anticardiolipin and anti-beta 2 glycoprotein) are at increased risk of thrombosis or adverse pregnancy outcomes.[1] The association between the different antiphospholipid antibodies and the outcomes of antiphospholipid syndrome differs widely. Lupus anticoagulant has a much stronger correlation with thrombosis[2 3] and pregnancy morbidities[4 5] in SLE. Even in the absence of antiphospholipid antibodies, patients with SLE have an increased risk of thromboembolic events.[3]

Although there is an association with transient antiphospholipid positivity, the association is thought to be much stronger with persistent positivity.[3 5] The definition of 'persistent positivity' has varied in different iterations of classification criteria for antiphospholipid syndrome and has never been based on evidence. The Sapporo criteria (1999) defined persistent positivity as two or

more positive findings separated by at least 6 weeks.[6] The definition in the Sydney criteria (2006) was changed to 12 weeks.[1] Although Kaul *et al*[7] suggested that the 2006 criteria might be superior with regard to limiting inclusion to a homogeneous group of patients and providing a risk-stratified approach, another position paper concluded that the Sapporo criteria were also satisfactory for classification of 'true' antiphospholipid syndrome.[8] The positive predictive value of the Sapporo criteria for classification of 'true' antiphospholipid syndrome may be higher than the Sydney criteria.[9]

The likelihood of identifying a patient with persistent positivity based on this definition clearly depends on the frequency with which it is assessed. An open clinical question is how frequently patients should be assessed for lupus anticoagulant. Since 2003, the patients in the Hopkins Lupus Cohort have been assessed for lupus anticoagulant every 3 months. Using the resulting information, we examined the impact of various testing strategies for the identification of patients with persistent positivity for lupus anticoagulant. In addition, we examined the degree to which these different approaches identified patients with SLE at increased risk for future thrombosis. We also examined other approaches to identifying patients with SLE at highest risk for thrombosis.

MATERIALS AND METHODS

The Hopkins Lupus Cohort is a longitudinal cohort study established in 1987 of over 2000 patients with SLE who have been seen quarterly. Written consent to participate and publish was obtained from all patients who participated in the study. SLE was diagnosed and classified based on either the revised American College of Rheumatology[10] or the Systemic Lupus International Collaborating Clinics classification criteria.[11] This analysis was based on all cohort patients who had at least 16 lupus anticoagulant assessments after 2003 (excluding those on anticoagulants).

Assessment of lupus anticoagulant

Since 2003, lupus anticoagulant by dilute Russell viper venom time (dRVVT) has been assessed at every cohort visit. For those with dRVVT of 45 s or more, mixing test and confirmatory test were performed.[12] For 20% of the patients with elevated dRVVT, the confirmatory test was missing as it was not done in patients whose insurance mandated testing outside of the Johns Hopkins University Hematology Laboratory. These values were imputed using multiple imputation, based largely on the degree of elevation of dRVVT. Other variables in the imputation model included age, race, sex, calendar year, hydroxychloroquine use and proportion of other observations for the same person that were confirmed positive.

Definitions of persistent positivity

Only cohort participants who had at least 16 lupus anticoagulant assessments were included in the analysis. Patient visits when patients were on anticoagulation therapy were not included. Each patient was defined as 'persistently positive' using various definitions based on the first 16 lupus anticoagulant assessments during cohort participation. We chose 16 in order to have enough assessments to compare different definitions of persistence, yet leave enough cohort follow-up time after the 16 assessments to determine the association between various definitions and future risk of thrombosis. Results of analyses based on 12 and 20 assessments are provided in the online supplemental file. Because cohort clinic visits were performed quarterly, the period spanned by these 16 visits was generally around 4 years. However, the span was shorter for those who required more frequent clinic visits and longer for those who missed some visits.

The following approaches were used to classify patients as having 'persistently positive' lupus anticoagulant based on the first 16 lupus anticoagulant assessments. These correspond to different possible hypothetical clinical strategies for identifying those with persistently positive lupus anticoagulant.

- Persistent positivity was defined based on the first two assessments that were separated in time by more than 90 days. If the patient was confirmed positive for lupus anticoagulant on both of those assessments, the patient was classified as persistently positive.
- Persistent positivity was defined based on an annual assessment of lupus anticoagulant. If the patient was positive for lupus anticoagulant at an annual assessment, we examined the reassessment 90 days later. If the patient was positive at an annual assessment and at the reassessment, they were defined as persistently positive.
- Persistent positivity was defined as having any two or more of the 16 assessments confirmed positive for lupus anticoagulant, separated by at least 90 days.

We also categorised patients based on the proportion of the 16 lupus anticoagulant assessments that were positive and by the mean value of dRVVT.

Definition of thrombosis

The occurrence of thrombotic events was determined during follow-up after the first 16 lupus anticoagulant assessments. Arterial thrombosis included cerebrovascular accident, myocardial infarction and other arterial thrombosis, including digital gangrene. Venous thrombosis was defined as the occurrence of deep venous thrombosis, pulmonary embolus or other venous thrombosis. Deep venous thrombosis was defined by ultrasound or venogram and pulmonary embolus by ventilation/perfusion scan or spiral CT. Arterial thrombosis, in case of stroke, was defined by brain MRI or CT and, in case of myocardial infarction, by appropriate electrocardiographic changes, creatine kinase or troponin change or cardiac imaging. Other arterial thrombosis was defined as appropriate for the site involved.

Statistical methods

We determined the prevalence of persistent positivity for lupus anticoagulant using the definitions described

Table 1 Characteristics of patients with SLE included in the analysis

Characteristics	n (%)
Sex	
Female	728 (93)
Male	57 (7)
Ethnicity	
Caucasian	406 (52)
African-American	316 (40)
Other	63 (8)
Age (years) at first included visit	
<30	99 (13)
30–44	291 (37)
45–59	292 (37)
60+	103 (13)
Duration of time for lupus anticoagulant assessment (years)	
<3	82 (10)
3–5	474 (60)
>5	229 (29)
Years of follow-up after lupus anticoagulant assessment	
<2	122 (16)
2–5	165 (21)
5–8	151 (19)
8+	347 (44)

above. The association between persistent positivity for lupus anticoagulant and future risk of thrombosis was assessed using discrete survival analysis. Statistical inference was based on generalised estimating equations to account for the fact that some patients experienced more than one thrombosis. All analyses were performed using SAS V.9.4.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting or dissemination plans of our research.

RESULTS

Definitions of persistent positivity

Table 1 shows the characteristics of the 785 patients used in the analysis. Our cohort is predominantly Caucasian and African-American. Most are female, with a wide age distribution. For 89% of the patients, the first 16 assessments took place over a time period of 3 years or more.

Table 2 shows the frequency distribution of the number of confirmed lupus anticoagulant positives in the first 16 assessments. Of the patients, 80.4% were negative at all 16 assessments. Of the remaining 19.6%, most were positive at less than 6 of the 16 assessments. There were a small number of patients (2.9%) positive at more than 75% of their assessments.

Table 2 Distribution of the number of confirmed lupus anticoagulant positive assessments out of the first 16 assessments

Confirmed positives (n)	Percentage of patients	95% CI
None	80.4	77.4 to 83.4
1–2	11.2	8.8 to 13.6
3–5	3.5	2.2 to 4.8
6–8	0.7	0.1 to 1.3
9–12	1.3	0.5 to 2.1
13–16	2.9	1.7 to 4.1

Table 3 shows the estimated prevalence of persistent positivity based on the different hypothetical strategies for identifying those with persistent positivity. It can be seen that if only the first two lupus anticoagulant assessments are used, 4.3% of the patients were identified as being persistently positive. Additional patients were identified as being persistently positive if an assessment was made annually (6.6%). Finally, more than double the number (10.5%) of patients were defined as being persistently positive if all 16 lupus anticoagulant assessments were considered. Similar gradients of increased percentages defined as persistently positive were seen if we based the classification on 12 or 20 assessments (online supplemental table S1).

Table 3 also shows the relationship between demographic characteristics and persistent positivity. There was a strikingly higher prevalence of persistent positivity for men (vs women) and for Caucasians (vs African-Americans).

Assessment of thrombotic risk

The patients in this analysis were followed for a total of 5411 person-years after their first 16 lupus anticoagulant assessments. During that follow-up, 91 thrombotic events were experienced by 80 patients. These consisted of 52 arterial thromboses, 36 venous thromboses and 3 classified as both. Arterial events consisted of 17 strokes, 13 myocardial infarctions, 7 cases of digital gangrene, and 18 classified as other types of arterial thromboses or mixed events. Of the venous thromboses, 35 were classified as deep vein thrombosis/pulmonary embolus and 4 as another venous thrombosis distribution or mixed events.

Table 4 shows the rate of thromboses in subgroups defined by the various definitions of persistent lupus anticoagulant. It can be seen that for all three definitions, those with persistent positivity had a higher rate of thrombosis. The elevated rate ratios for those with persistent positivity were even higher after adjustment for age, race and sex. The rate of thrombosis was similar irrespective of the way persistent positivity was identified. Similar findings were observed when we based the analysis on 12 or

Table 3 Per cent prevalence of persistently positive lupus anticoagulant based on different strategies for identifying patients

	Persistent positivity identified by the first two clinical assessments	Persistent positivity identified by annual assessments with reassessment of positives	Persistent positivity identified by two or more positives among 16 assessments
All patients	4.3 (2.9 to 5.7)	6.6 (4.8 to 8.3)	10.5 (8.3 to 12.7)
Sex			
Female	3.7 (2.3 to 5.1)	5.7 (4.0 to 7.4)	9.7 (7.5 to 11.9)
Male	12.3 (3.5 to 21.1)*	17.5 (7.4 to 27.7)‡	20.7 (9.8 to 31.6)¶
Race			
Caucasian-American	6.5 (4.1 to 8.9)	8.1 (5.4 to 10.8)	12.2 (9.0 to 15.4)
African-American	1.7 (0.3 to 3.2)†	4.3 (2.0 to 6.7)§	8.6 (5.4 to 11.8)**
Other	3.2 (0.0 to 7.6)	7.9 (1.0 to 14.8)	9.5 (2.1 to 17.0)
Age group			
<30	8.1 (2.6 to 13.5)	9.1 (3.3 to 14.9)	11.0 (4.7 to 17.4)
30–44	3.4 (1.3 to 5.5)	7.1 (4.1 to 10.1)	10.6 (7.0 to 14.3)
45–59	4.4 (2.0 to 6.8)	5.8 (3.0 to 8.6)	11.2 (7.5 to 14.9)
60+	2.9 (0.0 to 6.2)	4.9 (0.6 to 9.1)	7.8 (2.5 to 13.0)

*P=0.051 comparing men with women.
†P=0.0011 comparing African-Americans with Caucasians.
‡P=0.021 comparing men with women.
§P=0.038 comparing African-Americans with Caucasians.
¶P=0.053 comparing men with women.
**P=0.12 comparing African-Americans with Caucasians.

20 lupus anticoagulant assessments (online supplemental table S2).

Another approach to identifying patients at higher risk of thrombosis would be to simply look at the proportion of visits that a patient was confirmed positive for lupus anticoagulant. As seen in table 4, there is a trend such that the more frequently one is positive, the greater the risk of thrombosis. Yet another approach would be to calculate

Table 4 Rates of thromboses in subgroups defined by different assessments of persistently positive lupus anticoagulant

Patient group	Events	Person-years	Rate per 100 person-years	Rate ratio	P value	Adjusted rate ratio*	P value
Persistent positivity based on the first two lupus anticoagulant assessments							
No	81	5178	1.5	1.00 (ref)		1.00 (ref)	
Yes	10	232	4.3	2.75 (1.40 to 5.41)	0.0034	3.42 (1.76 to 6.65)	0.0003
Persistent positivity based on annual assessments							
No	76	5059	1.5	1.00 (ref)		1.00 (ref)	
Yes	15	352	4.2	2.75 (1.60 to 4.73)	0.0003	3.08 (1.83 to 5.19)	<0.0001
Persistent positivity based on the first 16 lupus anticoagulant assessments							
No	70	4851	1.4	1.00 (ref)		1.00 (ref)	
Yes	21	560	3.8	2.62 (1.60 to 4.28)	0.0001	2.75 (1.71 to 4.42)	<0.0001
Proportion of the first 16 assessments positive for lupus anticoagulant							
None	61	4338	1.4	1.00 (ref)		1.00 (ref)	
1%–49%	18	823	2.2	1.60 (0.90 to 2.86)	0.11	1.51 (0.84 to 2.72)	0.17
50%+	12	250	4.8	3.42 (1.88 to 6.23)	<0.0001	3.86 (2.17 to 6.87)	<0.0001
Mean value of dRVVT in the first 16 assessments							
<35 s	31	2555	1.2	1.00 (ref)		1.00 (ref)	
35–45 s	43	2503	1.7	1.40 (0.86 to 2.27)	0.18	1.33 (0.82 to 2.16)	0.25
45+ s	17	352	4.8	3.96 (2.25 to 6.99)	<0.0001	4.14 (2.36 to 7.27)	<0.0001

*Adjusted for age, race and sex.
dRVVT, dilute Russell viper venom time; ref, reference group.

the mean dRVVT for each patient without considering the confirmatory test. As seen in table 4, those with the highest mean dRVVT had the highest risk of thrombosis.

DISCUSSION

The Hopkins Lupus Cohort protocol of quarterly visits with lupus anticoagulant and anticardiolipin antibodies at each visit allowed us to analyse both the behaviour of antiphospholipid antibodies and the associated risk of thrombosis. There were multiple surprises in the study.

First, we found that, among those who were ever positive for lupus anticoagulant, the majority of patients with SLE were positive on 25% or less of their visits. This contrasts with the results of Erkan *et al*,[13] who found that more than three-quarters of subsequent tests of lupus anticoagulant were consistent with baseline test results, regardless of the laboratory. This fact—that most patients with SLE make lupus anticoagulant only a small proportion of time—has profound implications, not just for the definition of 'persistence', but also in determining treatment. For example, just because a patient has been negative in the past does not rule out the presence at the current, or future, visits. Due to the sporadic nature of lupus anticoagulant, different strategies for defining and testing for persistent positivity result in different determinations of prevalence. We acknowledge, though, that in clinical practice the detection of a positive lupus anticoagulant should not rely on a single method and that confirmatory tests should be used.[4 14 15]

The second surprise was that the prevalence of persistent positivity among patients with SLE differed strikingly by patient demographics. Depending on the definition, the prevalence of persistent lupus anticoagulant was 2–3.3 times higher in men than in women. This finding is consistent with the findings from the **Lupus** in Minorities: Nature versus Nurture (LUMINA) cohort.[16] Depending on the definition, the prevalence of persistent lupus anticoagulant was 1.4–3.8 times higher in Caucasians than in African-American patients with SLE. We are not aware of previous reports that looked at the relationship between ethnicity and prevalence of persistent lupus anticoagulant.

A third surprise was that the association between persistently positive lupus anticoagulant and thrombosis was similar, *irrespective* of how persistent positivity was defined. Thus, for example, the rate of thrombosis during follow-up among those with *any* two positives in the first *16* assessments was similar to the rate found among those who were positive on their *first two* lupus anticoagulant assessments. Given the fact that more than twice as many high-risk patients were identified using the former strategy, this suggests that clinicians should test for lupus anticoagulant more frequently and allow for a wider definition of persistent positivity. Clinicians might consider prophylactic therapy in those found to be persistently positive using our broader definition.

Our findings are relevant to the question of the necessity for mixing and confirmatory lupus anticoagulant testing for identifying patients with SLE at high risk. We found that the average value (in seconds) of dRVVT time served as an equally strong predictor of future risk of thrombosis as did persistent positivity based on mixing and confirmatory tests. Thus, gradations in the classic three-part definition of lupus anticoagulant confer risk. The antiphospholipid score also found that the initial screen was a risk factor (not just the confirmatory test).[17] This does not negate, though, the role of mixing and confirmatory steps in the official definition.

With our large cohort, frequent clinic visits and repeated assessments of antiphospholipid antibodies, the Hopkins Lupus Cohort provided a rich opportunity to explore the behaviour of lupus anticoagulant over time and the relationship with thrombosis. However, one limitation of this study is that it was carried out at a single clinical centre. Thus, the findings may mainly apply to patients similar to the Caucasian and African-American patients with SLE seen in Baltimore, Maryland. Due to the fact that the study was performed in a real-world clinical cohort, there was some variation in the span of time needed to accrue 16 lupus anticoagulant assessments. Our findings need to be interpreted in this light.

Contributors MAP contributed to the conception, study design, collection of all data, interpretation of the results and preparation of the manuscript. MA contributed to the conception, study design, interpretation of the results and preparation of the manuscript. LSM contributed to the conception, study design, analysis of data, interpretation of the results and preparation of the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request from MAP (mpetri@jhmi.edu).

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