Complement Activation Predicts Adverse Pregnancy Outcome in Patients with Systemic Lupus Erythematosus and/or Anti-Phospholipid Antibodies

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Abstract

Objective: Studies in mouse models implicate complement activation as a causative factor in adverse pregnancy outcomes (APOs). We investigated whether activation of complement early in pregnancy predicts APOs in women with systemic lupus erythematosus (SLE) and/or antiphospholipid antibodies (aPL).

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Methods: The PROMISSE Study enrolled pregnant women with SLE and/or aPL antibodies (n=487) and pregnant healthy controls (n=204) at <12 weeks gestation and evaluated them monthly. APOs were: fetal/neonatal death, preterm delivery <36 weeks because of placental insufficiency or preeclampsia, and/or growth restriction <5th %ile. Complement activation products were measured on serial blood samples obtained at each monthly visit.

Results: APO occurred in 20.5% of SLE and/or aPL pregnancies. As early as 12–15 weeks, levels of Bb and sC5b-9 were significantly higher in patients with APOs and remained elevated through 31 weeks compared to those with normal outcomes. Moreover, Bb and sC5b-9 were significantly higher in SLE and/or aPL patients without APOs compared to healthy controls. In logistic regression analyses, Bb and sC5b-9 at 12–15 weeks remained significantly associated with APO (OR_{adj}=1.41 per SD increase; 95% CI: 1.06–1.89; p=0.019 and OR_{adj}=1.37 per SD increase; 95% CI: 1.05–1.80; p=0.022, respectively) after controlling for demographic and clinical risk factors for APOs in PROMISSE. When analyses were restricted to patients with aPL (n=161), associations between Bb at 12–15 weeks and APOs became stronger (OR_{adj}=2.01 per SD increase; 95% CI: 1.16–3.49; p=0.013).

Conclusion: In pregnant SLE and/or aPL patients, increased Bb and sC5b-9 detectable early in pregnancy is strongly predictive of APOs and supports activation of complement, particularly the alternative pathway, as a contributor to APOs.

INTRODUCTION

Women with systemic lupus erythematosus (SLE) and/or antiphospholipid (aPL) antibodies are at increased risk for adverse pregnancy outcomes (APOs), including preeclampsia, fetal and neonatal death, and fetal growth restriction. Although we have made progress in identifying clinical risk factors and dysregulated circulating anti-angiogenic factors as predictors of APOs, identification of patients destined for complications remains challenging.[1,2]

Studies in mice implicate inflammation, particularly complement activation and recruitment of neutrophils, as an essential and causative factor in placental insufficiency, fetal loss, and growth restriction.[3–5] Activation of complement stimulates infiltrating leukocytes to release TNF-α and soluble fms-like tyrosine kinase-1 (sFlt1), a potent antiangiogenic factor, which are both associated with impaired development of the placenta and preeclampsia.[5–7] Complement activation is initiated by classical, alternative and lectin pathways. The convergence of the three pathways on C3 results in generation of common effectors: anaphylatoxins, opsonins, and the membrane attack complex. Mice deficient in alternative and classical pathway complement components (factor B, C4, C3 and C5) and mice treated with inhibitors of complement activation (anti-C5 mAb, anti-factor B mAb, C5a receptor antagonist peptide) are resistant to fetal injury induced by aPL,[3,8] indicating that both pathways contribute to damage.

Studies in humans support the role of complement in aPL-associated pregnancy complications,[9–11] and in preeclampsia and growth restriction in non-autoimmune women. [12,13] Complement fragment C4d, a marker of classical pathway activation, is present in placentae from women with SLE and/or antiphospholipid syndrome (APS) and from women with preeclampsia.[12,14–16] Mild hypocomplementemia has also been reported in primary APS in two studies.[17,18] The presence of risk variants in complement regulatory proteins in patients with SLE and/or aPL antibodies who develop preeclampsia, and in preeclampsia patients without an associated autoimmune disease, links complement activation to disease pathogenesis.[9] Furthermore, in a prospective study of non-autoimmune patients, elevated levels of the alternative pathway complement activation product Bb, was strongly associated with preeclampsia.[19] There have been no longitudinal studies examining complement activation in high risk pregnancies.

Accordingly, we investigated whether alterations in plasma levels of complement activation fragments before mid-second trimester predict APOs in women with SLE and/or aPL using data from the **PROMISSE** Study (**P**redictors of p**R**egnancy **O**utcome: bio**M**arkers **I**n antiphospholipid antibody **S**yndrome and **S**ystemic Lupus **E**rythematosus). PROMISSE is the largest multi-center, multi-ethnic, and multi-racial study to prospectively assess clinical and laboratory predictors of APO in women with aPL and/or SLE with inactive or mild/moderate activity at conception. We hypothesized that complement activation products are elevated early in pregnancy in patients destined for APOs.

METHODS

Patient Population

The PROMISSE Study enrolled pregnant women between September 2003 and August 2013 from 8 U.S. sites and 1 Canadian site. Institutional review boards at each site approved protocols and consent forms; written informed consent was obtained from all subjects. Consecutive pregnant women referred to the study with diagnoses of SLE,[20] aPL (defined in Table 1) or both were recruited at <12 weeks' gestation as previously described.[21] Healthy control patients matched for ethnicity/race were also included.

Inclusion criteria were: live singleton intrauterine pregnancy confirmed by ultrasound; age 18–45 years; hematocrit >26%. Exclusion criteria, to minimize confounding by known causes of APOs not specifically associated with SLE and/or aPL included: prednisone >20 mg/day; urine protein (mg)/creatinine (gram) ratio 1000 on random sampling or 24 hour collection; erythrocyte casts on urinalysis; serum creatinine >1.2 mg/dL; type I or II diabetes mellitus; blood pressure >140/90 mmHg.

Detailed medical and obstetrical information and serial blood samples were obtained at screening and monthly from 12 weeks' gestation until the end of pregnancy and rheumatology assessments at each trimester.[1,2]

Adverse Pregnancy Outcomes

APOs defined for PROMISSE included one or more of the following: 1) fetal death after 12 weeks' gestation unexplained by chromosomal abnormalities, anatomical malformation, or

congenital infection; 2) neonatal death before hospital discharge due to complications of prematurity and/or placental insufficiency; 3) indicated preterm delivery at less than 36 weeks due to gestational hypertension, preeclampsia, or placental insufficiency; and 4) small-for gestational-age (SGA) neonate (<5th percentile).

Laboratory assays

Complement levels were measured in a blinded manner by MicroVue™ EIA kits for Bb Plus Fragment, sC5b-9 Plus Fragment, Ba Fragment, C3a Plus Fragment, C4d Fragment, iC3b Fragment and C5a. Duplicate samples were run in two plates and averaged to obtain final results. Angiogenic factor (sFlt1 and PlGF) levels were measured as described.[2]

Statistical methods

Categorical and continuous variables were compared between groups using Fisher's exact and T-tests, respectively. Correlations were estimated using Pearson's correlation. Odds ratios for the associations between APO status and complement levels were estimated using logistic regression models including the following covariates selected *a priori* based on clinical considerations and prior studies: race/ethnicity, SLE status, lupus anti-coagulant (LAC) status, history of thrombosis, screening diastolic pressure, aspirin use, anti-hypertensive use, and BMI. Model performance was evaluated using the Hosmer-Lemeshow (HL) goodness-of-fit test and area under the ROC curve (AUC). To address the potential for over-fitting, leave-one-out cross-validation was performed. Complement levels measured at the time of or after an APO were excluded. Missing data were handled with both listwise deletion and multiple imputation (MI) using the Markov chain Monte Carlo approach. Missing data rates were 0%-6% for baseline variables and 29.5%-31.1% for 12–15 and 16–19 week complement variables. Given similarity in parameter estimates across missing data methods, MI results are provided in supplementary Table S1. Change in complement levels over time were analyzed by linear mixed effects models. All analyses were conducted using SAS version 9.4 (SAS Institute, Inc.).

RESULTS

Study Population and Pregnancy Outcomes

Of 770 pregnant women with SLE and/or aPL screened for PROMISSE, 487 who met study inclusion/exclusion criteria, had documented outcomes, and had at least one blood sample evaluated for complement levels during pregnancy were included: 326 (66.9%) with SLE and without aPL, 60 (12.3%) with SLE and aPL, and 101 (20.7%) with only aPL. APOs occurred in 100 cases (20.5%): fetal death in 27 (5.5%), neonatal death in 5 (1.0%), indicated preterm delivery for placental insufficiency or gestational hypertensive disease in 49 (10.1%), and SGA in 47 (9.7%). Also included were 204 healthy controls, 7 of whom had APOs (3.4%).

Baseline Demographic and Clinical Risk Factors

Women with SLE and/or aPL who had subsequent APOs were more likely to be positive for aPL and LAC, have higher systolic and diastolic blood pressures, higher BMI, history of thrombosis, and exposure to heparin and anti-hypertensive medications, compared to women

without APOs (Table 1). Race/ethnicity, age, parity, history of lupus nephritis, proteinuria, aspirin use, and smoking status at enrollment were not significantly associated with pregnancy outcome in bivariate analyses.

Complement activation products: Bb and sC5b-9

We initially focused on Bb and sC5b-9, the complement measures which varied the least among the healthy controls (i.e., smallest coefficients of variation at all visits). As early as 12–15 weeks, patients with APOs demonstrated significant elevations in Bb and sC5b-9 compared to patients without APO (Figure 1A, 1B). These differences tended to increase through week 31. SLE and/or aPL patients without APO had consistently higher levels of Bb and sC5b-9 compared to the reference group, the healthy controls without APO (N=197). Bb levels were elevated at 12–15 weeks even in the subset of 138 SLE patients with SLEPDAI 2 at baseline, compared to the reference group (p<0.001). In all groups, Bb decreased as pregnancy progressed, but the rate of decrease was smaller in patients with APOs compared to patients without APOs (p=0.06) and compared to the reference group (p=0.002), suggesting increased complement activation and cleavage of Bb. Patients with APO also showed less decline in sC5b-9 compared to patients without APO (p=0.02) and the reference group (p=0.002). Correlations between Bb and sC5b-9 in diseased patients ranged from 0.49 (p<0.001) at 12–15 weeks to 0.68 (p<0.001) at 36–39 weeks.

Early elevations in complement tended to persist throughout pregnancy: correlations between 12–15 and 36–39 week levels were 0.62 for Bb (p < 0.001) and 0.44 for sC5b-9 (p< 0.001). In ROC analysis of the 12–15 week measures, the optimal cut-points for discriminating APO from non-APO in diseased patients when sensitivity (SE) and specificity (SP) are equally weighted were >1.04 μg/ml for Bb (SE=46%, SP=75%, PPV=33%, NPV=84%) and >347 ng/ml for sC5b-9 (SE=52%, SP=68%, PPV=29%, NPV=85%).

Multivariable Analyses of Complement Levels

Bb at 12–15 weeks remained significantly associated with APOs after controlling for potential confounders (Table 2; OR_{adj}=1.41 per SD increase; 95% CI: 1.06–1.89; p=0.019). SC5b-9 at 12–15 weeks was also significantly associated with APO in logistic regression analyses (OR_{adj}=1.37 per SD increase; 95% CI: 1.05–1.80; p=0.022). At 16–19 weeks, odds ratios were OR_{adj}=1.59 per SD increase for Bb (95% CI: 1.16–2.17; p=0.004) and OR_{adj}=1.26 per SD increase of sC5b-9 (95% CI: 0.94–1.68; p=0.12). Between 20–31 weeks, Bb was significantly associated with APO at all visits; sC5b-9 levels at 28–31 weeks and not earlier were significant [results not shown].

Results above were based on diseased patients. Among all healthy subjects, Bb, but not sC5b-9, was significantly elevated at 12–15 weeks in the 7 who had APO compared to non-APO (Bb: 0.91 vs 0.71 μg/ml, p = 0.03; sC5b-9: 241.5 vs 226.5 ng/ml; p=0.66). To assess the independent effects of disease status and complement on APO, a separate logistic regression was fit with main effects for Bb at 12–15 weeks and disease (yes/no), based on all diseased and healthy subjects. The adjusted odds ratio for APO was 4.82 (95% CI: 2.01–11.55; p<0.001) comparing diseased and healthy subjects, and for Bb was 1.61 (95% CI:

1.26–2.06; p<0.001) per SD increase. The odds ratio for sC5b-9 at 12–15 weeks after adjusting for disease status was 1.34 per SD increase (95 CI: 1.06–1.70; p=0.01). Adjustment for additional covariates was not feasible due limited APOs in the healthy group.

Relationship with angiogenic factors

Given prior evidence that complement activation stimulated release of sFlt1 by leukocytes[22] and that angiogenic dysregulation early in pregnancy is associated with APOs,[2] we examined the relationship between levels of complement activation products and angiogenic factors. Correlations between Bb and sC5b-9 measured at or before 12–15 weeks with sFlt1, PlGF, and sFlt1/PlGF measured at the same and subsequent visits were modest and did not exceed 0.25. Adjustment for sFlt1/PlGF in multivariable analyses did not affect the association between APOs and Bb measured at 12–15 weeks (OR_{adj}=1.39; 95% CI: 1.03–1.88; p=0.034) or at 16–19 weeks (OR_{adj}=1.60; 95% CI: 1.14–2.2; p=0.006). SC5b-9 results were also minimally affected after adjusting for sFlt1/PlGF at 12–15 weeks (OR_{adj}=1.34; 95% CI: 1.00–1.78; p=0.047) and 16–19 weeks (OR_{adj} =1.30; 95% CI: 0.95–1.76; p=0.10).

Subgroup analyses

Because studies in mouse models of aPL-associated APO strongly implicate complement activation and reveal complement deposition on trophoblasts,[8] we analyzed complement analytes in the subgroup of aPL-positive patients with or without SLE (n=161). Associations between Bb and APO increased for both the 12–15 week measures (OR_{adj}=2.01 per SD increase; 95% CI: 1.16–3.49; p=0.013) and 16–19 week measures (OR_{adj}=2.46 per SD increase; 95% CI: 1.27–4.76; p=0.008) after adjusting for the same variables in Table 2; Bb results remained highly significant after further adjustment for heparin (p=0.01 and p=0.009, respectively). Odds ratios for sC5b-9 in aPL positive subjects were similar to those based on the overall study population using the 12–15 week levels (OR_{adj}=1.35 per SD increase; 95% CI: 0.86–2.12; p=0.19) and 16–19 week levels (OR_{adj}=1.16 per SD increase; 95% CI: 0.69–1.94; p=0.57).

Among SLE patients without aPL antibodies (n=326), associations between Bb with APO were weaker (12–15 weeks: OR_{adj} =1.28 per SD increase; 95% CI: 0.87–1.86; p=0.21; 16–19 weeks: OR_{adj} =1.45 per SD increase; 95% CI: 0.99–2.14; p=0.06) than corresponding results in the aPL positive group. In contrast, associations of sC5b-9 with APOs in the SLE only patients were comparable to those in the aPL positive group (12–15 weeks: OR_{adj}=1.39 per SD increase; 95% CI: 0.98–2.01; p=0.07; 16–19 weeks: OR_{adj}=1.33 per SD increase; 95% CI: 0.93–1.90; p=0.12). Baseline SLEPDAI scores were significantly correlated with Bb and sC5b-9 at both 12–15 and 16–19 weeks (ρ=0.22–0.30; p<0.001 for all correlations). In addition, among all SLE patients, mean Bb was significantly elevated at both visits in those who were LAC-positive versus negative (12–15 weeks: 1.21 μg/ml vs. 0.87 μg/ml; p<0.001; 16–19 weeks: 1.19 μg/ml vs. 0.85 μg/ml; p<0.001); mean sC5b-9 was non-significantly elevated (12–15 weeks: 399.5 ng/ml vs 356.3 ng/ml, respectively; p=0.28; 16–19 weeks: 433.3 ng/ml vs 358.5 ng/ml; p=0.19).

Other complement factors

C5a, C4d, and iC3b levels at 12–15 weeks and 16–19 weeks were not significantly associated with APOs (Supplementary Figures). C3a was significantly associated with APO in bivariate analyses, but not when added to the multivariable models that included Bb or sC5b-9, whereas Bb remained significant (12–15 weeks: p=0.057; 16–19 weeks: p=0.004). Limited data were available for Ba (>40% had missing values), precluding rigorous evaluation of this analyte. However, consistent with the contribution of alternative pathway activation, Ba appeared to be a stronger predictor of APOs than sC5b-9 at both 12–15 weeks (OR_{adj}=1.74 per SD increase; 95% CI: 1.24–2.43; p=0.001) and 16–19 weeks (OR_{adj}=1.47 per SD increase; 95% CI: 1.06–2.05; p=0.02), when added to the models in Table 2. It was also a stronger predictor of APOs than Bb at 12–15 weeks (OR_{adj}=1.59 per SD increase; 95% CI: 1.10–2.30; p=0.01) but not at 16–19 weeks (OR_{adj}=1.25 per SD increase; 95% CI: 0.89–1.75; p=0.20).

DISCUSSION

In our study that included nearly 500 pregnant patients with SLE and/or aPL, increased levels of Bb and sC5b-9 early in pregnancy were significantly associated with APOs. This supports the concept that complement activation, particularly the alternative pathway, contributes to abnormal placental development that leads to pregnancy complications. That the association of alternative complement activation with APO was greatest in the patients with aPL is consistent with our experiments showing that mice deficient in factor B or treated with an inhibitor of factor B activation were protected from aPL-induced fetal resorptions and growth restriction.[3,23]

Bb levels fell as pregnancy progressed in uncomplicated pregnancies in both diseased and healthy subjects. However, in those destined for APOs, Bb levels were significantly higher throughout pregnancy, and the rate of decrease during pregnancy was lower. Lynch et al. similarly showed that Bb levels decreased with gestational age in normotensive women, while dysregulation of Bb activation occurred between 10–20 weeks gestation in those who developed preeclampsia.[24] Others have shown that elevated Bb is present in amniotic fluid of women with severe preeclampsia, supporting the concept that complement is activated at the maternal-fetal interface.[25,26]

We also found a significant association between elevated sC5b-9 levels and APOs, but this was not the case in a study of non-autoimmune women who developed preeclampsia.[27] Nonetheless, a role for terminal pathway complement activation, as a cause or consequence of preeclampsia, is supported by reports of markedly increased urinary sC5b-9 excretion and correlations between urinary sC5b-9 and angiogenic dysregulation.[28,29]

Although antibodies are essential to the pathogenesis of SLE and aPL, the alternative pathway fragment Bb, rather than classical pathway components, was the strongest predictor of APO. The correlation between Bb and sC5b-9 further supports that the alternative pathway is a key driver of complement activation in pregnancy complications. Whether the complement cascade is triggered by the classical, lectin, or alternative pathway, activation is amplified by the alternative pathway. Classical pathway activation depends on affinity and

subclass of multiplexed antibodies, and aPL are skewed towards IgG2 subclass, relatively ineffective classical pathway activators.[30] Excess deposition of non-classical pathway activating antibodies can inhibit membrane-bound complement regulators and allow unabated activation of the alternative pathway. Furthermore, cleavage of complement components by neutrophil- and platelet-derived proteases can by-pass the classical pathway. [31]

In normal pregnancy, the human placenta is subjected to complement-mediated immune attack at the maternal-fetal interface.[32,33] Complement activation is controlled in successful pregnancies by inhibitory proteins on trophoblast cells.[34–36] In patients with APL and/or SLE, the excessive complement activation, evident in the circulation and in the placenta, [12,14–16] overwhelms regulatory pathways and places the fetus at risk.

Infiltrating leukocytes recruited and activated by complement are a source of the excess anti-angiogenic factor sFlt1 that impairs early placental development. Subsequent hypoperfusion of the intervillous space stimulates trophoblasts to synthesize large amounts of sFlt-1 as pregnancy progresses. Because the latter is not directly driven by complement, the modest relationship between sFlt1 levels and complement activation fragments was not surprising.

Although PROMISSE patients had quiescent, stable, or mildly-active disease, even those without APO had significantly higher levels of Bb and sC5b-9 at baseline (6–11 weeks) compared to healthy controls. Prolonged activation of complement, also described in non-pregnant patients with inactive SLE and those with primary APS, may lower the threshold for exaggerated, uncontrolled activation that recruits leukocytes and unleashes potent inflammatory and anti-angiogenic mediators associated with placental insufficiency.[17,37,38]

Interestingly, baseline SLEPDAI scores correlated significantly with early measures of Bb and sC5b-9. A small study also reported that elevations of Bb and sC5b-9 accompanied disease activity in pregnant SLE patients.[39] Interpretation of complement is confounded in pregnancy because circulating complement reflects both synthesis (enhanced by estrogen) and consumption.[40] Our findings of early increased complement activation products in women destined for APOs, and our previous report that less increase in C3 levels from baseline to second trimester was predictive of APO, argue that excess complement activation is the driver rather than consequence of APOs. The presence of classical pathway complement fragment C4d deposition on placentae from women with SLE and/or APS and women with preeclampsia, and in kidneys of women with preeclampsia,[12–16] provides further evidence for local activation of classical or lectin pathways by aPL or necrotic fetoplacental debris. Such activation, regardless of the source, is then augmented through the alternative pathway. Importantly, circulating levels of complement fragments may not reflect the extent of activation in the placenta.

Our study had limitations. We did not measure pre-pregnancy levels of complement activation products so could not differentiate whether increased complement consumption was due to SLE or placental inflammation. Sample sizes did not allow for analyses stratified by APO type. Whether patient aPL antibodies were complement-fixing was not determined, and not possible for LAC, the aPL associated with greatest risk.

In conclusion, we demonstrate that complement pathway activation is associated with APOs in patients with SLE and/or APL. Aberrant complement activation, whether initiated by immune complexes in SLE or by aPL, may trigger or amplify inflammation at the maternal fetal-interface and thereby contribute to the pathogenesis of APOs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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A

Figure showing Mean Bb (μg/ml) versus Gestational Age (weeks)
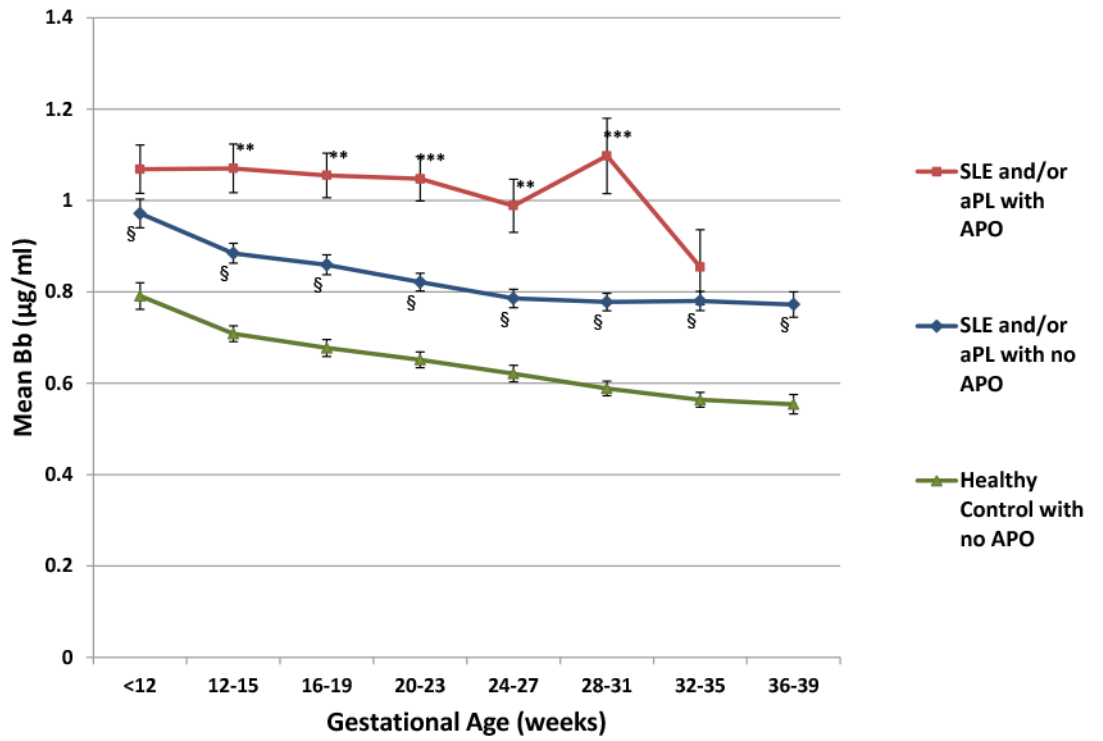
Sample size	<12	12-15	16-19	20-23	24-27	28-31	32-35	36-39
SLE and/or aPL with APO	66	71	62	62	51	43	27	
SLE and/or aPL with no APO	260	269	270	285	260	266	270	149
Healthy Control with no APO	88	149	155	149	145	152	145	122
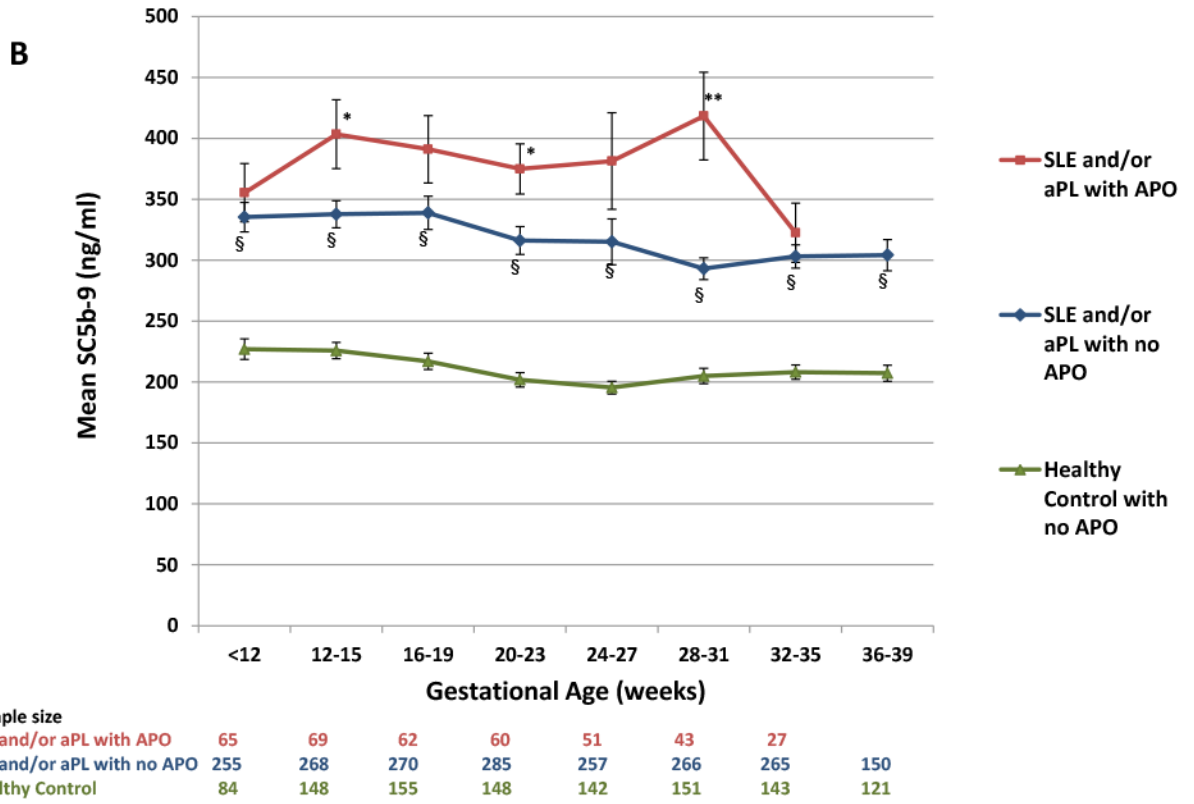
Figure 1.
(A) Levels of Bb and (B) levels of sC5b-9 during pregnancy for patients with SLE and/or aPL with and without APO and healthy control women (no SLE, no APL) without APO. Values represent means and standard errors. Sample size is the number of specimens available from individual patients from each group at each time point.
P-values by two sample t-tests:
SLE and/or aPL with APO vs. SLE and/or aPL with no APO: * = <0.05, **= <0.01, *** = <0.0001;
SLE and/or aPL with no APO vs Healthy Control: § = <0.0001

Table 1

Demographic and Clinical Variables by Outcome Status at Baseline among Patients with aPL and/or SLE

Variable	APO (N = 100)	No APO (N = 387)	P-value[*]
Demographic characteristics			
Race/ethnicity, N (%)			0.135
Non-Hispanic white	52 (52)	223 (58)	
Hispanic white	5 (5)	30 (8)	
African American	21 (21)	57 (15)	
Asian	10 (10)	42 (11)	
Other	2 (2)	17 (4)	
Do not know	10 (10)	18 (5)	
BMI, N (%)			0.0009
<25	43 (45)	228 (63)	
25-30	25 (26)	83(23)	
>30	28 (29)	52(14)	
Missing	4	24	
Maternal age, Mean (SD)	30.49 (5.17)	31.55 (4.79)	0.054
Clinical history			
Parity, N (%)			0.20
Nulliparous	53 (53)	231 (60)	
Parous without history of PE	36 (36)	132 (34)	
Parous with history of PE	11 (10)	24 (6)	
APL[**]/SLE status, N (%)			0.0004
APL+/SLE−	28 (28)	73 (19)	
APL+/SLE+	21 (21)	39 (10)	
APL−/SLE+	51 (51)	275 (71)	
Lupus nephritis ever, N (%)			0.28
No	71 (71)	295 (76)	
Yes	29 (29)	92 (24)	
Thrombosis, N (%)			<0.0001
No	74 (74)	352 (91)	
Yes	26 (26)	35 (9)	
Systolic, Mean (SD)	116.63 (13.05)	110.32 (11.42)	<0.0001
Diastolic, Mean (SD)	71.72 (10.01)	66.42 (8.77)	<0.0001
Smoking, N (%)			0.48
Never	71 (71)	290 (75)	
Ever	24 (24)	84 (22)	
Current	5 (5)	11 (3)	
Laboratory values			

Variable	APO (N = 100)	No APO (N = 387)	P-value[*]
LAC, N (%)			<.0001
No	59 (59)	334 (86)	
Yes	41 (41)	53 (14)	
Proteinuria (500-1000 mg/day), N (%)			0.122
No	89 (89)	362 (94)	
Yes	11 (11)	25 (6)	
Disease activity (SLE only, n=386)			
SLEPDAI, Mean (SD)	3.81 (3.31)	2.52(2.76)	0.003
SLEPDAI >4			0.0002
No	47 (67%)	264 (86%)	
Yes	23 (33%)	44 (14%)	
PGA, Mean (SD)	0.57 (0.63)	0.34 (0.49)	0.006
PGA >1			0.002
No	51 (80%)	278 (92%)	
Yes	13 (20%)	23 (8%)	
Current Medications			
Aspirin N (%)			0.37
No	50 (50)	174 (45)	
Yes	50 (50)	213 (55)	
Antihypertensive, N (%)			<0.0001
No	82 (82)	366 (95)	
Yes	18 (18)	21 (5)	
Heparin, N (%)			0.0002
No	46 (46)	256 (66)	
Yes	54 (54)	131 (34)	
Glucocorticoids, N (%)			0.33
Current	35 (35%)	119 (31%)	
Prior	23 (23%)	118 (30%)	
Never	42 (42%)	150 (39%)	
Daily dose (mg, current users), Mean (SD)	9.13 (7.48)	7.66 (5.68)	0.29

[*] By Pearson's chi-square tests for the categorical variables or two-sample t-tests for the continuous variables.

[**] APL positive is defined as anticardiolipin antibody [aCL: IgG 40 GPL units; IgM 40 MPL units] and/or positive lupus anticoagulant [RVVT, kaolin, dilute TTI or PTT LA] and/or anti-β2 glycoprotein I [antiβ2GPI: IgG 40 GPL units; IgM 40 MPL units] at least twice between 6 weeks and 5 years apart of which one must be during the PROMISSE pregnancy at a core laboratory, as previously described)[21].

Table 2

Adjusted odds ratios for complement factors measured at 12-15 and 16-19 weeks

Predictor variable	Bb (12-15 weeks) N=323 subjects, 70 events		Bb (16-19 weeks) N= 317 subjects, 60 events		sC5b-9 (12-15 weeks) N= 320 subjects, 68 events		sC5b-9 (16-19 weeks) N= 317 subjects, 60 events	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Non-Hispanic White (yes vs no)	0.74 (0.39-1.40)	0.35	0.64(0.32-1.27)	0.20	0.70 (0.37-1.33)	0.27	0.55 (0.28-1.09)	0.086
SLE status (positive vs negative)	1.27 (0.51-3.22)	0.61	0.82 (0.31-2.18)	0.69	1.07 (0.43-2.71)	0.88	0.72 (0.27-1.95)	0.52
LAC status (positive vs negative)	5.81 (2.55-13.25)	<0.0001	2.41 (0.94-6.15)	0.067	6.58 (2.89-14.98)	<.0001	2.85 (1.12-7.26)	0.028
History of thrombosis (yes vs no)	2.51 (1.11-5.65)	0.027	2.85 (1.26-6.46)	0.012	2.35 (1.04-5.29)	0.040	2.73 (1.23-6.08)	0.014
Diastolic pressure > 80 mm Hg (yes vs no)	3.13 (1.42-6.91)	0.005	4.46 (2.01-9.88)	0.0002	2.82 (1.29-6.20)	0.010	4.27 (1.97-9.26)	0.0002
Aspirin Use (yes vs no)	0.57 (0.29-1.11)	0.095	0.58 (0.28-1.17)	0.13	0.57 (0.29-1.12)	0.104	0.65 (0.32-1.31)	0.22
Current anti-hypertensive (yes vs no)	3.82(1.44-10.10)	0.007	4.37(1.60-11.95)	0.004	4.61 (1.75-12.14)	0.002	4.37 (1.64-11.63)	0.003
BMI		0.43		0.48		0.27		0.18
<25	1		1		1		1	
25-30	0.78 (0.38-1.59)		0.82 (0.36-1.82)		0.70(0.34-1.45)		0.68(0.31-1.51)	
>30	1.28 (0.54-3.02)		1.33 (0.53-3.36)		1.29(0.55-3.05)		1.42 (0.57-3.51)	
Bb per SD increase *	1.41 (1.06-1.89)	0.019	1.59 (1.16-2.17)	0.004	-----		----	
sC5b-9 per SD increase *	-----		-----		1.37 (1.05-1.80)	0.022	1.26 (0.94-1.68)	0.12
Hosmer-Lemeshow Goodness-of-fit test	0.85		0.32		0.30		0.22	
AUC	0.80		0.80		0.80		0.80	
Cross-validated AUC	0.76		0.75		0.76		0.74	

*
SD for Bb = 0.38 µg/ml at 12-15 weeks and 0.37 µg/ml at 16-19 weeks; SD for sC5b9 = 196 ng/ml at 12-15 weeks and 223ng/ml at 16-19 weeks.