COMPLEMENT ACTIVATION IN PERIPHERAL BLOOD IN RELATION TO LUPUS, ANTIPHOSPHOLIPID AND RHEUMATOID ARTHRITIS AUTOANTIBODIES: INSIGHTS FROM CLINICAL LABORATORY EVALUATIONS

Thierry Dervieux, John Conklin, Joanne Ligayon, Rowena Lafon, Armida Sace, Tyler O'Malley, Roberta Alexander, and Claudia Ibarra
Exagen Diagnostics, Vista, CA

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ABSTRACT

PURPOSE

Abnormal activation of the complement system is emerging as useful biomarker in the laboratory evaluation of patients presenting with symptoms of autoimmune rheumatic diseases. We sought to evaluate the relationships between complement activation and autoantibodies associated with systemic lupus erythematosus (SLE), antiphospholipid Syndrome (APS) and rheumatoid arthritis (RA).

METHODS

From May 2014 to November 2016, a cohort of 32,404 patients within the United States (mean 51±15 [SD] years, 86% females) was tested. EDTA whole blood and serum were collected within 48 hours of patient examination. and processed centrally in a CLIA certified/CAP accredited clinical laboratory. Abnormal complement activation was detected using peripheral blood and C4d bound to erythrocyte or B lymphocyte levels above the 99th percentile of normal healthy group. The panel of 15 autoantibodies (all determined by immunoassays) consisted of 5 SLE associated autoantibodies (anti-dsDNA confirmed using Crithidia, anti-U1 RNP, anti-C1q, anti-ribosomal P, anti-Smith, all IgGs), 6 APS associated autoantibodies (anti-cardiolipin, anti-beta2 glycoprotein 1, anti-phosphatidylserine/prothrombin complex antibodies, IgM and IgG) and 4 RA associated autoantibodies (anti-CCP, anti-MCV, IgM RF, IgA RF). Patient data were de-identified prior to analysis. The relationships between abnormal complement activation and the presence of the autoantibodies were analyzed using multivariate logistic regression with abnormal complement activation as the dependent variable and the presence of autoantibodies as independent predictors. Adjusted Odds ratio were calculated for each of the autoantibodies.

RESULTS

Of the 32,404 patients tested 12% of them presented with abnormal complement activation. The overall incidence of autoantibodies ranged from 1% (anti-Sm) to 13.1% (IgM RF). The presence of SLE and APS antibodies were all associated with abnormal complement activation with adjusted OR ranging from 1.40 for anti-C1q (CI95%: 1.24-1.58) to 4.52 for anti-dsDNA (CI95%: 3.95-5.17), and from 1.47 for anti-cardiolipin IgG (CI95%: 1.22-1.77) to 3.2 for anti-PS/PT IgM (CI95%: 3.20-2.92), respectively ($p<0.02$). Of the 4 RA associated antibodies, only anti-CCP (adjusted OR=1.25, CI95%: 1.02-1.54) and IgM RF (adjusted OR=1.17, CI95%: 1.04-1.32) were significantly associated with complement activation ($p<0.05$). Figure 1 illustrates the relationship between the cumulative presence of lupus, APS and RA autoantibodies and abnormal complement activation. Across the cumulative range of SLE and APS associated antibodies, a 319-fold (CI95%: 240-424), and 120-fold (CI95%: 94-153) increase in the likelihood of abnormal complement was detected. In contrast, the cumulative presence of RA autoantibodies yielded minimum impact of the likelihood of abnormal complement activation (adjusted OR=2.3; CI95%: 2.0-2.7)

CONCLUSION

These diagnostic immunology data suggest that complement activation in peripheral blood is intimately related to SLE and APS antibodies.

OBJECTIVE

Evaluate the relationships between complement activation and autoantibodies associated with SLE, APS and RA from a large diagnostic immunology database.

METHODS

- A total of 32,404 patients within the United States (mean 51±15 [SD] years, 86% females) was tested.
- Abnormal complement activation was measured in peripheral blood and C4d bound to erythrocyte or B lymphocyte levels above the 99th percentile of normal healthy group: EC4d>14 net MFI or BC4d>60 net MFI.
- Autoantibodies were measured using immunoassays

	Auto-antibody Measured	Cumulative score
SLE	Anti-dsDNA confirmed using Crithidia [INOVA] Anti-U1 RNP; Anti-Smith [EIA , Thermofisher] Anti-C1q [INOVA]; Anti-ribosomal P [INOVA]	Range 0-5
APS	Anti-cardiolipin IgM ang IgG [INOVA] Anti-beta2 glycoprotein 1 [INOVA] Anti-PS/PT antibodies IgM and IgG) [INOVA]	Range 0-6
RA	Anti-CCP2 [EIA , Thermofisher] Anti-MCV [Orgentec] IgM and IgA RF [EIA , Thermofisher]	Range 0-4

- Statistical analysis: multivariate logistic regression with abnormal CB-CAPS as the dependent variable and the presence of antibodies as the independent predictors.

CB-CAPs AND AUTOANTIBODIES

The table highlights the incidence of abnormal CB-CAPs and autoantibodies observed in the diagnostic immunology database.

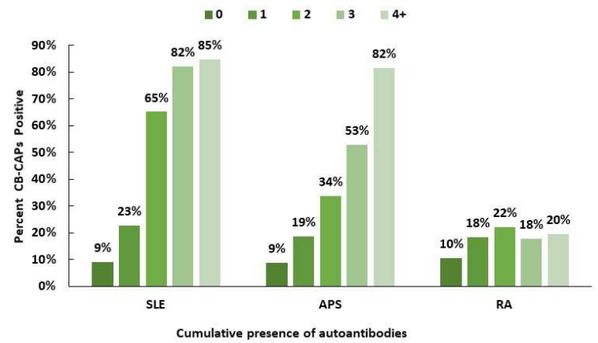
	Percent Positive
Abnormal CB-CAPs	12.3%
Anti-C1q (>20 Units)	8.3%
Anti-dsDNA confirmed (>301 Units)	7.5%
Anti-ribosomal P (>20 units)	0.8%
Anti-U1 RNP (>10 units)	3.8%
Anti-cardiolipin IgM (>20 units)	4.9%
Anti-cardiolipin IgG (>20 units)	3.8%
Anti-beta2 glycoprotein 1 IgM (>20 units)	2.0%
Anti-beta2 glycoprotein 1 IgG (>20 units)	3.5%
Anti-CCP2 (>10 units)	5.4%
Anti-MCV (>70 units)	4.1%
IgM RF (>5 units)	13.1%
IgA RF (>10 units)	7.2%
Anti-PS/PT IgM (>30 units)	11.6%
Anti-PS/PT IgG (>30 units)	4.6%

The incidence of the SLE, APS and RA antibodies (cumulative) is presented below

Score	0	1	2	3	4+
SLE	86%	11%	2%	1%	0%
APS	79%	16%	3%	1%	1%
RA	78%	13%	4%	2%	2%

BIOMARKERS AND COMPLEMENT ACTIVATION

Positive marker	Familly	Odds ratio	-95%CI	+95%CI	p-value
igM RF	RA	1.04	0.90	1.21	0.56
Anti-MCV	RA	1.07	0.86	1.33	0.56
IgM RF	RA	1.17	1.04	1.32	0.01
Anti-CCP	RA	1.25	1.02	1.54	0.03
Anti-C1q	SLE	1.40	1.24	1.58	0.02
Anti-RiboP	SLE	1.47	1.07	2.00	<0.001
Anti-cardiolopin IgG	APL	1.47	1.22	1.77	<0.001
Anti-PS/PT IgG	APL	1.49	1.28	1.73	<0.001
Anti-cardiolopin IgM	APL	1.49	1.26	1.78	<0.001
Beta2 GP IgM	APL	1.78	1.39	2.27	<0.001
Anti-Smith	SLE	2.32	1.67	3.21	<0.001
Anti- U1 RNP	SLE	2.86	2.46	3.33	<0.001
Beta2 GP IgG	APL	3.10	2.60	3.71	<0.001
Anti-PS/PT IgM	APL	3.20	2.92	3.51	<0.001
Anti-dsDNA	SLE	4.52	3.95	5.17	<0.001



Multivariate Analysis	Cumulative Score APS	Cumulative Score SLE	Cumulative Score RA
Odds ratio (range)	120.0	319.2	2.3
CI 95%	[94.1-153.0]	[240.4-424.0]	[2.0-2.7]

CONCLUSION

Complement activation is intimately related to SLE and APS antibodies

Contact: tdervieux@exagen.com