Evaluation of a Novel Lupus Anticoagulant Assay Designed to Reduce False Positive Results Due to Iatrogenic Anticoagulation: A Retrospective Study at a Single Health System

Jessica D. Anderson; Tamara Sabih; Yuying Xing; Lili Zhao; Marc D. Smith

Beaumont Hospital, Royal Oak, MI

Introduction

Lupus anticoagulants (LA) are antibodies directed against phospholipids/phospholipid-protein complexes involved in coagulation and are associated with thrombotic events and recurrent fetal loss. There is no gold standard test for LA, therefore at least two LA-specific tests are recommended before excluding LA. Potential testing methods involve dilute Russell viper venom time (dRVVT) and hexagonal phase phospholipid assays (HPPA); however, many of these tests are susceptible to interference with anticoagulant therapy which can lead to false positive results. In this study, we compared the performance of our current HPPA, Staclot LA (SCLA), with a novel assay reported to have relatively minimal interference by anticoagulation therapy, CRYOcheck Hex LA (CCLA).

Methods

We performed a retrospective study using 74 samples submitted for lupus anticoagulant testing in 3.2% sodium citrate vacutainer tubes. Samples were spun for 15 minutes at 3400 rpm obtain platelet poor plasma. SCLA samples were selected for further testing and chart review for diagnosis of LA. These samples were frozen and retested by CCLA. We calculated the sensitivity, specificity and positive predictive value of both tests, assessed agreement between test methods (HPPA vs. dRVVT/anticardiolipin antibodies/beta-2 glycoprotein I antibodies) using kappa statistics, and estimated the extent to which positive results were affected by anticoagulant therapy using Fisher's exact test. Statistical analysis was performed on R (version 4.2.1). Statistical significance was defined as $p < 0.05$.

Results

Of the 74 samples, 48 were from women and 21 were from men, with an average age of 54 years. Using ISTH criteria, the presence or absence of LA was able to be determined in 69 out of the 74 samples. 15 samples were positive for LA, and 54 samples were negative (Table 1). Of the 15 positive samples, all were detected by both SCLA and CCLA. Of the 54 negative samples, 27 tested positive by SCLA, and one was positive by CCLA (Table 2). Thus, the positive predictive value for SCLA was 35.7% and 93.8% for CCLA (Table 3). Overall agreement between CCLA and true LA patients and CCLA and dRVVT, were good (Table 4; kappa=0.96, $p<0.0001$ for true LA; kappa=0.67, $P<0.0001$ for dRVVT), which was better than SCLA. Additionally, 27 patients were taking anticoagulants at the time their sample was drawn. The rate of anticoagulation therapy was significantly higher in SCLA samples with false positive results (Table 5; 61.5% in false positive vs 14.3% in true positive; P=0.007).

Conclusion

Diagnosis of LA can be challenging, particularly when the patient is being treated with anticoagulation. In our study, hexagonal phase phospholipid assays (SCLA and CCLA) showed high sensitivity (100%); however, SCLA showed a significant false positive rate, largely due to patients on anticoagulation therapy. CCLA on the other hand, shows promising resistance to anticoagulant therapy, and therefore, should be a strong consideration for labs offering LA testing in patient populations that are frequently anticoagulated.

***Table 1.* SCLA and CCLA sample data.**

Demographic Data		Pertinent Clinical History	Diagnosis Based on All Laboratory Data	Hexagonal Phase Phospholipid Assay Comparison				Additonal LA Testing		Iatrogenic Anticoagulation	
Age	Gender			Staclot LA Result	Delta	Cryocheck Hex LA Result	Delta	dRVVT	Inhibitor Screen	Anticoagulation	Drug Type
Staclot positive/Hex LA positive											
52	F	History of SLE	Positive for lupus anticoagulant	pos	23.2	pos	35.7	pos	pos	N	N/A
68	F	No thrombosis	Positive for lupus anticoagulant	pos	26.5	pos	17.9	pos	pos	N	N/A
69	F	Unknown	Positive for lupus anticoagulant	pos	26.6	pos	17.7	pos	pos	N	N/A
37	F	History of antiphospholidpid antibody	Positive for lupus anticoagulant	pos	22.1	pos	63.5	pos	N/A	N	N/A
65	F	Deceased	Positive for lupus anticoagulant	pos	22.9	pos	45.2	pos	pos	N	N/A
71	M	Deceased	Positive for lupus anticoagulant	pos	14.2	pos	13.3	neg	borderline	N	N/A
79	F	Positive ANA	Positive for lupus anticoagulant	pos	24	pos	19.9	neg	N/A	N	N/A
54	F	DVT, PE	Positive for lupus anticoagulant	pos	21.1	pos	47.3	pos	N/A	Y	apixaban
74	M	Deceased	Positive for lupus anticoagulant	pos	63.7	pos	63.2	pos	N/A	Unk	N/A
43	M	CVA	Positive for lupus anticoagulant	pos	20	pos	24.6	pos	N/A	N	N/A
90	F	CVA	Positive for lupus anticoagulant	pos	>90	pos	200.1	pos	pos	N	N/A
68	F	Unknown	Positive for lupus anticoagulant	pos	26.5	pos	19.7	pos	pos	N	N/A
38	F	SLE	Positive for lupus anticoagulant	pos	57.8	pos	45	pos	pos	N	N/A
68	F	PE	Equivocal (excluded from statistical analysis)	pos	25.8	pos	26.9	pos	N/A	Y	UFH
33	M	DVT	Positive for lupus anticoagulant	pos	17.8	pos	23.7	neg	N/A	Y	unknown
61	F	Unknown	Positive for lupus anticoagulant	pos	34.2	pos	42.7	pos	pos	N	N/A
60	F	Hx positive ANA	Positive for lupus anticoagulant	pos (low)	9.2	pos	15.3	neg	pos	N	N/A
Staclot positive/Hex LA negative											
53	F	Provoked DVT	Negative for lupus anticoagulant	pos	15.5	neg	-0.8	neg	N/A	Y	UFH
38	F	Sepsis	Negative for lupus anticoagulant	pos	19.7	neg	5.3	neg	N/A	Y	UFH
56	F	DVT, PE, previous Hx APS	Equivocal (excluded from statistical analysis)	pos	13.4	neg	-2	pos	N/A	Y	UFH
72	F	no thrombus or bleeding	Equivocal (excluded from statistical analysis)	pos	14.4	neg	12.6	neg	N/A	N	N/A
70	F	SLE	Equivocal (excluded from statistical analysis)	pos	12.2	neg	8.1	neg	N/A	N	N/A
62	M	DVT, PE, Prothrombin 20210A HOMOZYGOUS POSITIVE	Negative for lupus anticoagulant	pos	15.5	neg	8.8	pos	N/A	Y	apixaban
80	F	Unknown	Negative for lupus anticoagulant	pos	20	neg	-3.9	neg	neg (delayed)	N	N/A
19	F	Acquired factor VIII deficiency	Negative for lupus anticoagulant	pos	16.3	neg	3.7	neg	pos	N	N/A
61	F	DVT/PE	Negative for lupus anticoagulant	pos	15.6	neg	3.7	neg	N/A	Y	apixaban
60	M	DVT/PE	Negative for lupus anticoagulant	pos	21.6	neg	4.5	neg	N/A	Y	dabigatran
74	M	DVT/PE	Negative for lupus anticoagulant	pos	16.3	neg	3.3	neg	N/A	Y	UFH, argatroban
51	M	DVT	Negative for lupus anticoagulant	pos	18.4	neg	-3.2	neg	N/A	Y	LMWH
40	M	PE	Negative for lupus anticoagulant	pos	15.5	neg	-0.6	neg	N/A	Y	unknown
53	F	Ischemic infarcts in brain	Negative for lupus anticoagulant	pos (low)	10.5	neg	-0.8	neg	N/A	Y	UFH
54	F	Unknown	Negative for lupus anticoagulant	pos (low)	11.9	neg	11.1	neg	N/A	N	N/A
41	F	DVT, PE	Negative for lupus anticoagulant	pos (low)	9.2	neg	-1.2	neg	N/A	Y	apixaban
53	F	Unknown	Negative for lupus anticoagulant	pos (low)	9.6	neg	-2.1	neg	N/A	Y	apixaban
18	F	Ehlers Danlos, SLE	Negative for lupus anticoagulant	pos (low)	8.1	neg	1.1	neg	N/A	N	N/A
43	M	PE	Negative for lupus anticoagulant	pos (low)	9.5	neg	-1.6	neg	N/A	Y	rivaroxaban
54	F	PE	Negative for lupus anticoagulant	pos (low)	11.2	neg	9.1	N/A	N/A	Y	UFH
36	F	PE	Negative for lupus anticoagulant	pos (low)	10.3	neg	0	pos	N/A	Y	rivaroxaban
47	F	PE	Negative for lupus anticoagulant	pos (low)	7.6	neg	6.9	neg	N/A	Y	UFH
39	M	positive ANA	Negative for lupus anticoagulant	pos (low)	11.9	neg	1.9	neg	N/A	Y	apixaban
79	M	partial FXII deficiency, CVA	Negative for lupus anticoagulant	pos (low)	9.6	neg	7.8	neg	borderline	N	N/A
62	M	positive ANA	Negative for lupus anticoagulant	pos (low)	11.7	neg	-0.4	neg	N/A	N	N/A
55	F	unknown	Negative for lupus anticoagulant	pos (low)	9.6	neg	9	neg	N/A	N	N/A
91	M	DVT	Negative for lupus anticoagulant	pos (low)	9.5	neg	-0.4	N/A	N/A	Y	UFH
34	F	TIA	Equivocal (excluded from statistical analysis)	pos (low)	11.1	neg	1.4	neg	N/A	Unk	N/A
52	F	CVA	Negative for lupus anticoagulant	pos (low)	9.8	neg	2.8	pos	N/A	N	N/A
72	F	arterial thrombosis	Negative for lupus anticoagulant	pos (low)	7.9	neg	-0.4	neg	N/A	N	N/A

Table legend:
ANA=anti-nuclear antibody, CVA=cerebral vascular accident, DVT=deep vein thrombosis, F=female, LMWH=low molecular weight heparin, M= male, PE= pulmonary embolism, SLE= systemic lupus erythematosis, UFH=unfractionated heparin

***Table 2.* Statistical Summary**

		Diagnosis of Lupus anticoagulant	
Variable*	All	Negative	Positive
Age			
Mean (SD)	54.17 (16.88)	52.72 (16.84)	59.40 (16.51)
Gender			
Female	48 (69.6%)	36 (66.7%)	12 (80.0%)
Male	21 (30.4%)	18 (33.3%)	3 (20.0%)
Delta Staclot LA			
Negative	27 (39.1%)	27 (50.0%)	0 (0.0%)
Positive	42 (60.9%)	27 (50.0%)	15 (100.0%)
Delta Hex LA			
Negative	53 (76.8%)	53 (98.1%)	0 (0.0%)
Positive	16 (23.2%)	1 (1.9%)	15 (100.0%)
Iatrogenic Anticoagulation			
No	40 (59.7%)	28 (52.8%)	12 (85.7%)
Yes	27 (40.3%)	25 (47.2%)	2 (14.3%)
dRVVT			
Negative	51 (76.1%)	48 (92.3%)	3 (20.0%)
Positive	16 (23.9%)	4 (7.7%)	12 (80.0%)
IgA ACA			
Negative	62 (91.2%)	50 (94.3%)	12 (80.0%)
Positive	6 (8.8%)	3 (5.7%)	3 (20.0%)
IgM ACA			
Negative	49 (80.3%)	42 (85.7%)	7 (58.3%)
Positive	12 (19.7%)	7 (14.3%)	5 (41.7%)
IgG ACA			
Negative	58 (87.9%)	50 (96.2%)	8 (57.1%)
Positive	8 (12.1%)	2 (3.8%)	6 (42.9%)
IgG beta-2 glycoprotein I antibody			
Negative	55 (83.3%)	49 (96.1%)	6 (40.0%)
Positive	11 (16.7%)	2 (3.9%)	9 (60.0%)
IgM beta-2 glycoprotein I antibody			
Negative	60 (90.9%)	49 (96.1%)	11 (73.3%)
Positive	6 (9.1%)	2 (3.9%)	4 (26.7%)
Sample Size	69	54	15

Table legend:
DRVVT= dilute russel viper venom time, IgA ACA= IgA anticardiolipin antibody, IgM ACA= IgM anticardiolipin antibody, IgG ACA= IgG anticardiolipin antibody

***Table 3.* Sensitivity, specificity, and positive predictive value of Staclot LA and Hex LA**

	Sensitivity	Specificity	Positive Predictive Value
Staclot LA	1.00 (95% CI 1.00 - 1.00)	.50 (95% CI 0.37 - 0.63)	35.7%
Hex LA	1.00 (95% CI 1.00 - 1.00)	0.98 (95% CI 0.95 - 1.00)	93.8%

***Table 4.* Agreement analysis of true value (cases diagnosed positive for LA), Staclot LA and Hex LA with dRVVT, anticadiolipin antibodies (IgA, IgM, IgG) and beta-2 glycoprotein I antibodies (IgM, IgG)**

Positive for Lupus Anticoagulant				
	kappa*	95% CI		P value
		Lower	Upper	
SCLA	0.30	0.16	0.45	0.00
CCLA	0.96	0.88	1.00	<.0001
dRVVT	0.71	0.50	0.91	<.0001
IgA ACA	0.18	-0.08	0.44	0.08
IgM ACA	0.27	-0.02	0.56	0.03
IgG ACA	0.46	0.19	0.74	<.0001
IgM-B2	0.29	0.02	0.56	0.01
IgG-B2	0.62	0.38	0.86	<.0001

Staclot LA				
	kappa*	95% CI		P value
		Lower	Upper	
dRVVT	0.19	0.02	0.36	0.04
IgA ACA	0.12	0.02	0.22	0.04
IgM ACA	0.02	-0.17	0.20	0.84
IgG ACA	0.12	0.00	0.25	0.08
IgM-B2	0.12	0.02	0.21	0.04
IgG-B2	0.22	0.09	0.35	0.00

Hex LA				
	kappa*	95% CI		P value
		Lower	Upper	
dRVVT	0.67	0.46	0.88	<.0001
IgA ACA	0.17	-0.08	0.41	0.11
IgM ACA	0.25	-0.04	0.53	0.05
IgG ACA	0.43	0.16	0.70	<.0001
IgM-B2	0.27	0.01	0.53	0.01
IgG-B2	0.59	0.35	0.82	<.0001

Table legend:
DRVVT= dilute russel viper venom time, IgA ACA= IgA anticardiolipin antibody, IgM ACA= IgM anticardiolipin antibody, IgG ACA= IgG anticardiolipin antibody, IgM-B2= IgM Beta-2 glycoprotein, IgG-B2= IgG Beta-2 glycoprotein

***Table 5.* Staclot LA and Hex LA true positives and false positives with and without anticoagulant use.**

Staclot LA				Hex LA			
	Iatrogenic Anticoagulation				Iatrogenic Anticoagulation		
	No	Yes	Total		No	Yes	Total
False Positive	10	16	26	False Positive	1	0	1
	38.5	61.5			100	0	
True Positive	12	2	14	True Positive	12	3	15
	85.7	14.3			80	20	
Total	22	18	40	Total	13	3	16

P25821m_050423