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LETTER TO THE EDITOR

False positive lupus anticoagulant tests in patients with high C-reactive protein: A comparison of two hexagonal phase reagents

Dear Editors,

Lupus anticoagulants (LA) are a heterogeneous group of autoantibodies that bind to negatively charged phospholipids or protein/phospholipid complexes. The design of a laboratory testing algorithm is complicated by the biological heterogeneity of LA and by the variable sensitivity, specificity, and patterns of interference for different reagents. Three expert groups have published recommendations on LA testing: the International Society on Thrombosis and Haemostasis (ISTH),[1] the Clinical Laboratory Standards Institute (CLSI),[2] and the British Committee for Standards in Haematology (BCSH).[3,4] The laboratory criteria for the diagnosis of antiphospholipid antibody syndrome (APLAS) are the presence of one or more of the following: LA, anticardiolipin antibody (ACL) (IgG/IgM), or anti-ß2 glycoprotein I antibody (anti-ß2GPI) (IgG/IgM) in two specimens collected more than 12 weeks apart.[5,6]

The acute phase reactant C-reactive protein (CRP) is known to bind negatively charged phospholipids, causing false positive results in the PTT-LA screen (PTT-LA, Diagnostica Stago, Inc.) and hexagonal phase (HexP) confirmatory assays.[6–10] The ISTH recommends that LA screening not be performed during the acute phase.[1]

We compared susceptibility to CRP interference in two hexagonal (HexP) reagents: CRYOcheck HexLA (Precision BioLogic, Inc., Dartmouth, Canada) (hereafter, PB) and Staclot LA 20 (Diagnostica Stago S.A.S, Asnières-sur-Seine, France) (hereafter, STAclot). We tested 30 STAclot positive or borderline specimens with high CRP using the PB and dilute Russell's viper venom time (dRVVT) and, when sufficient volume remained, ACL and anti-ß2GPI (IgG/IgM) antibody tests. The 30 STAclot positive/borderline have high CRP (>8 mg/L) drawn within 24 h of the LA test.

We also compared the overall percent positive of STAclot and PB results on 83 consecutive specimens submitted to the lab for LA testing over 4 consecutive weeks beginning on October 4, 2022. For this comparison, specimens with elevated CRP levels and/or borderline STAclot results were excluded.

For all patient specimens, venous blood was collected into sodium citrate tubes, centrifuged to achieve a platelet count of <10 000/µL, and either tested immediately or frozen at −70°C and later thawed in a 37°C water bath. All samples were collected as part of routine clinical activity at Massachusetts General Hospital (MGH) and an institutional review board approved this study.

PTT-LA, STAclot LA, and CRYOcheck HexLA were performed using a STA-R Max analyzer (Diagnostica Stago, Inc.) per manufacturer instructions. Positive cutoffs for STAclot (8 s) and PB (6 s) were derived from the package inserts and verified by us. Our laboratory has a "borderline" category for STAclot (5–7.9 s), adopted because normal donors in our initial validation studies rarely had results exceeding 5 s.

Anti-Xa tests were performed using anti-Xa screen (Stachrom Heparin or Stago STA-Liquid Anti-Xa 8, Diagnostica Stago, Inc.). When indicated, heparin up to 2 international units in 1 mL of plasma was removed from the specimen by digestion with Hepzyme (Siemens, Munich, Germany) for 15 min at room temperature before LA testing. Patients on apixaban could be included if apixaban was <450 ng/mL or an anti-Xa screen <2.0 IU/mL, based on our internal reflex protocol that was established during the assay validations. Patients on rivaroxaban or direct thrombin inhibitors (DTI) were excluded.

dRVVT testing was performed using a Werfen ACL TOP 750 LAS and the HemosIL dRVVT (Werfen, Barcelona, Spain) screen/confirm reagents. The cutoffs for dRVVT screen (>44.0 s) and total ratio (TR), calculated as the normalized screen ratio over the normalized confirm ratios (>1.21) were determined by our validation study. The use of the dRVVT screen value in seconds (rather than a normalized ratio) was consistent with the practice of the reference laboratory we used at the time. ACL and anti-ß2GPI IgG/IgM tests were performed using the QUANTA Lite ACA III (Werfen), QUANTA Lite anti-ß2GPI (Werfen) on the DSX automated ELISA platform (Dynex Technologies, Inc., Chantilly, VA). Values >15 GPL/MPL units are considered positive.

We performed a spiking study with purified CRP in normal control plasma. CRP spiking studies were performed using a CRP 3 mg/L stock solution (EMD Millipore) and normal pooled plasma (Precision BioLogic).[11] CRP was added to plasma to make 2 high concentrations (250 and 200 mg/L based on manufacturer's product insert; measured as 289 and 262 mg/L), and each was serially diluted 1:1 in plasma to make 5 concentrations each. The CRP level was measured using CRP Ultra Wide Range Reagent kit (Sekisui Diagnostics, Inc., Burlington, MA) on a Cobas c502 (Roche Diagnostics Corp., Basel, Switzerland).

Table 1 lists the test results of 30 patients with positive/borderline STAclot results and high CRP. None had DOAC detectable

TABLE 1 Thirty patient specimens with high CRP values (8 mg/L or higher), ordered by CRP concentration.

Patient	CRP (mg/L)	STAclot Δ (sec)	STAclot (qual)	PB Δ (sec)	PB (qual)	dRVVT (TR)	dRVVT (qual)	ACL IgM	ACL IgG	anti-GPI IgM	Anti-2GPI IgG
1	8	9.8	POS	3.2	NEG	1.01	NEG	NEG	NEG	ND	ND
2(h)	43	7.4	BOR	2.3	NEG	1.34	POS	NEG	NEG	NEG	NEG
3	48	6.1	BOR	6.8	POS	1.31	POS	POS	NEG	NEG	NEG
4	51	5.4	BOR	3.0	NEG	1.17	NEG	NEG	NEG	NEG	NEG
5	53	7.4	BOR	3.6	NEG	0.99	NEG	NEG	NEG	NEG	NEG
6	71	8.3	POS	4.5	NEG	1.02	NEG	POS	NEG	NEG	NEG
7(c)	73	12.3	POS	-2.1	NEG	1.27	POS	NEG	NEG	NEG	NEG
8(h)	81	10.4	POS	4.7	NEG	1.14	NEG	NEG	NEG	NEG	NEG
9	86	20.3	POS	25.4	POS	1.86	POS	NEG	NEG	NEG	NEG
10	87	9.5	POS	3.1	NEG	NC	NEG	NEG	NEG	NEG	NEG
11	89	9.8	POS	4.8	NEG	1.06	NEG	NEG	NEG	NEG	NEG
12(h)	110	8.6	POS	4.7	NEG	1.11	NEG	ND	ND	ND	ND
13	130	15.7	POS	9.6	POS	1.21	NEG	NEG	NEG	ND	NEG
14(c)	140	12.8	POS	17.6	POS	1.57	POS	POS	NEG	POS	NEG
15	146	14.8	POS	0.8	NEG	1.00	NEG	NEG	NEG	NEG	NEG
16	147	17.7	POS	5.5	NEG	1.01	NEG	NEG	NEG	NEG	NEG
17	147	6	BOR	1.9	NEG	0.95	NEG	ND	ND	ND	ND
18	147	12.5	POS	4.4	NEG	1.16	NEG	ND	ND	ND	ND
19	153	10.1	POS	2.8	NEG	1.08	NEG	NEG	NEG	NEG	NEG
20	153	10.2	POS	7.0	POS	1.12	NEG	NEG	NEG	NEG	NEG
21	159	13.1	POS	4.7	NEG	NC	NEG	NEG	NEG	NEG	NEG
22	159	10.3	POS	6.9	POS	1.03	NEG	POS	NEG	NEG	NEG
23	184	14	POS	5.3	NEG	1.12	NEG	NEG	NEG	NEG	NEG
24	186	6.1	BOR	0.7	NEG	0.95	NEG	ND	ND	ND	ND
25	241	17.5	POS	6.6	POS	1.09	NEG	NEG	NEG	NEG	NEG
26	246	17	POS	3.4	NEG	1.16	NEG	NEG	NEG	NEG	NEG
27	266	6.6	BOR	5.1	NEG	NC	NEG	NEG	NEG	NEG	NEG
28	271	15.6	POS	2.7	NEG	1.25	POS	NEG	NEG	ND	NEG
29	319	10.7	POS	3.2	NEG	1.05	NEG	ND	ND	ND	ND
30	401	15.7	POS	5.7	NEG	1.27	POS	NEG	NEG	NEG	NEG

Note: The difference between before and after hexagonal phase phospholipid addition in seconds (columns 3 and 5) and the qualitative interpretation (columns 4 and 6) for STAclot HexP (STAclot) and Precision BioLogic (PB) HexP reagents. Cutoffs for STAclot are <5 s negative, 5–7.9 s borderline, and 8 s or above positive. PB HexP has no borderline range, but 6 s or above is positive. Column 7 and 8: normalized screen and confirm ratios (TR, normalized screen ratio/normalized confirm ratio). TR >1.21 are interpreted as positive. "NC" indicates that the TR was not calculated because the initial dRVVT screen was negative (<44.0 s). "ND" indicates that the test was not performed on the specimen at all. Columns 9–12: Qualitative results for anticardiolipin antibody (IgG/IgM) (ACL) and anti-β-2 glycoprotein I antibody tests (IgG/IgM) (anti-β2GPI). >15 GPL/MPL units is considered positive. Patients with "c" were prescribed coumadin and had INRs of 2.2. "h" indicates the heparin screen was positive, and the specimen was treated with hepzyme before testing. For CRP, the color saturation increases as the CRP increases. Otherwise, green is a normal/negative value, red is a positive/abnormal value, yellow is a borderline value, and gray is not completed.

by anti-Xa assay. Five were prescribed coumadin but only 2 of them (#7 and #14) had therapeutic INRs (both 2.2). Three specimens (#2, #8, #12) had detectable anti-Xa activity (UFH) and were treated with hepzyme before testing. Nineteen of the 30 specimens were positive or borderline by the STAclot but negative by PB and dRVVT. Chart review of these 19 cases reveals either no history of

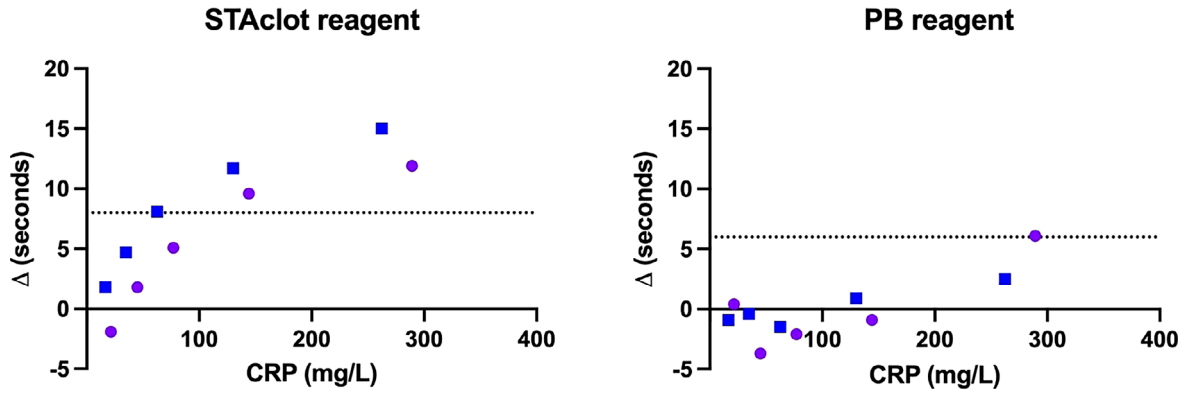

FIGURE 1 Results of a spiking study of CRP into pooled normal plasma. CRP (mg/dL) is graphed on the *X*-axis and the hexagonal phase Δ (in s) is graphed on the *Y*-axis. A dotted line on the *Y*-axis shows the manufacturer's suggested cutoff value for positive, 8 s for STAclot and 6 s for PB. Blue squares and purple circles are used to distinguish the two series of 1:1 dilutions from each other. Numerical values are in Table 2.

TABLE 2 Results of C-reactive protein (CRP) spiking study.

Calculated CRP(mg/L)	Measured CRP(mg/L)	STAclot Δ (s)	STAclot (qual)	PB Δ (s)	PB (qual)	dRVVT S (s)	dRVVT TR	dRVVT (qual)
250	289	11.9	POS	6.1	POS	ND	ND	N/A
200	262	15.0	POS	2.5	NEG	46.4	0.96	NEG
125	144	9.6	POS	−0.9	NEG	ND	ND	N/A
100	130	11.7	POS	0.9	NEG	45.7	0.98	NEG
62	77	5.1	NEG	−2.1	NEG	46.3	0.99	NEG
50	62	8.1	POS	−1.5	NEG	45.2	0.96	NEG
31	45	1.8	NEG	−3.7	NEG	46.5	0.99	NEG
25	35	4.7	NEG	−0.4	NEG	ND	ND	N/A
16	21	−1.9	NEG	0.4	NEG	ND	ND	N/A
12.5	17	1.8	NEG	−0.9	NEG	42.3	0.95	NEG

Note: Two sets of 1:1 serial dilutions were performed. Column 1 is calculated CRP (mg/L), calculated based on the manufacturer's estimate of stock concentration. Column 2 is measured CRP. Columns 3–6 are the hexagonal phase delta results (in s) and qualitative interpretation (qual) for the STAclot HexP and Precision BioLogic (PB) HExP reagents. Columns 7–9 lists the dRVVT screen in seconds and the total ratio (TR, normalized screen ratio/normalized confirm ratio). A qualitative positive for dRVVT TR is >1.21.
Abbreviations: N/A, not applicable, ND, note done.

thrombosis/miscarriage/preeclampsia or a hematology consult stating that the result was a likely to be a false positive. The hematology consults cite clinical presentation, inconsistent (negative) repeat testing, and/or acute inflammation. The six subjects that had repeated testing >12 weeks later were negative. Four of the 30 specimens were positive or borderline by both STAclot and PB but negative by dRVVT (#13, #20, #22, and #25). Three of those had no thrombosis at all (inappropriately sent), and the one that had a hematology consult (#25) was deemed a false positive. None were repeated. Four of the 30 specimens were positive or borderline by STAclot and dRVVT but not by PB (#2, #7, #28, and #30). Patient #2 had line-associated thrombosis at the time of the positive result but was negative on repeat. Patient #28 had thrombosis in the context of sepsis and was negative on repeat testing. Patient #30 had no history of thrombosis at all. Only patient #7 met the laboratory criteria for APLAS. Finally, 3 of the 30 specimens were positive or borderline for STAclot, PB, and dRVVT (#3, #9, and #14). #9 had no history of thrombosis/pregnancy complications. #3 and #14 had multiple clots in the context of acute illness and tested positive for ACL, anti-ß2GPI antibodies, or both. #3 died soon after testing. #14, though negative on repeat testing, remains on anticoagulation.

In summary, 25 of 30 specimens are from patients who either had no history of thrombosis or were deemed probable false positives in a hematology consult. These are clinically false positives and probable analytical false positives. One patient (#7) is likely a true positive. The remaining four (#2, #28, #3, and #14) have some provoked thrombosis but are so medically complex that no reasonable conclusion can be drawn regarding the role of CRP interference.

Figure 1 and Table 2 depict the spiking study of purified CRP serially diluted in PB normal pooled plasma and analyzed with STAclot and PB HexP. STAclot reaches the positive threshold with a CRP of 62.2 mg/L, consistent with previously published results.[9] Although the dRVVT screen was positive for values as low as 45 mg/L, the TR (normalized screen/control ratio) was negative for all CRP values

tested. The PB assay does not result as positive at CRP concentrations as high as 262 mg/L. This is consistent with the PB manufacturer claim that CRP does not interfere with result interpretation but that it may increase the delta correction of LA-positive samples.

The lack of a gold standard for a LA assay and positive reference plasma limits the ability to objectively compare reagents. It is not possible to prove that CRP interference is responsible for the specimens in Table 1 that are positive for STAclot, even if they are negative for the PB and dRVVT assays, negative 12 weeks later, or have no thrombosis. It is possible for a genuine LA to be transient.

Although the spiking study suggests a dose-dependent increase in the STAclot delta, we know that there are patients high CRP levels who have negative STAclot results. Unlike reagent phospholipids, the structural diversity and relative abundance of endogenous phospholipids vary both between individuals and at varying timepoints within the same individual. The level of endogenous phospholipid in a patient's plasma and the relative affinity of those endogenous phospholipids for CRP could account for differences in the threshold value of CRP required to cause a false-positive result in a patient without a LA.

Because the 30 samples in Table 1 were selected based on STAclot positivity, our study has an inherent bias against false negatives on STAclot. Had we selected for positives on the PB HexP assay, some might not have reproduced on the STAclot. To compare general performance, we ran all specimens submitted during a 4 week period on STAclot and PB (data not shown). Eighty-three were submitted for testing. Fourteen were excluded due to high CRP and 5 were excluded due to borderline STAclot results. The PB HexP positive rate (36%) and the STAclot positive rate (33%) were broadly comparable in this cohort, suggesting that the insensitivity to CRP does not reflect a general lack of sensitivity for the PB reagent.

AUTHOR CONTRIBUTIONS

RM, SU: experiment design, data analysis, manuscript drafting and editing. BB, GM: experiment design, data analysis. SM and MR: data analysis, manuscript drafting and editing.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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