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Case Report

Lupus anticoagulant: a clinical and laboratory diagnostic dilemma

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ABSTRACT

Lupus anticoagulants are a group of diverse autoantibodies that interfere in vitro in phospholipid-dependent clotting tests, and inhibit both the common and intrinsic pathways of coagulation. Paradoxically, they are implicated to cause hypercoagulability, thrombotic events in vivo in varied clinical settings like obstetrics and oncology.

A 56-year female was referred to the laboratory with complaint of repeated de novo clotting of drawn plasma samples. She was a post-operative case of surgery for superior mesenteric venous thrombosis, and a previously diagnosed case of squamous cell carcinoma of the buccal mucosa, not on treatment. The patient was evaluated clinically for new onset hypercoagulability and the history of exposure to heparin was negative, which ruled out heparin induced thrombocytopenia. The differential diagnosis were lupus anticoagulant, staphylococcal septicemia, and thrombotic thrombocytopenic purpura. On advanced work up, inhibitor screen was negative, dilute Russell's viper venom time was positive.

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INTRODUCTION

Antiphospholipid antibodies (aPL) are implicated as acquired risk factors for thrombophilia and adverse pregnancy outcomes, this presentation is called antiphospholipid syndrome (APS).[1] aPL are group of heterogenous antibodies directed against various proteins with affinity for several clotting factors, plasma proteins that are generally negatively charged phospholipids.[1]

The latest classification for APS recognizes three different antibodies with some overlapping specificity. Out of three, two antibodies are named after their respective plasma

protein/ lipid, i.e., β2-glycoprotein I (β2GPI), phospholipid cardiolipin (CL), or combination of β2GPI and CL. The anticardiolipin antibodies (aCL) and anti-β2-glycoprotein I antibodies (aβ2GPI) are detected by solid phase assays, whereas lupus anticoagulants (LA) are detected by the functional behaviour of LA in the medley of coagulation-based assays.[2] LA can be due to presence of anti-β2GPI or antiprothrombin antibodies, or may be even in absence of these antibodies. LA causes in vitro prolongation of clotting assays, but paradoxically it is associated with increased risk for thrombosis and adverse pregnancy outcome.[1]

 # CASE REPORT

A 56-year female was referred to the laboratory with complaint of repeated de novo clotting of drawn plasma samples. She was a post-operative case of surgery for superior mesenteric venous thrombosis, and a previously diagnosed case of squamous cell carcinoma of the buccal mucosa, not on treatment. The patient was evaluated clinically for new onset hypercoagulability and the history of exposure to heparin was negative, which ruled out heparin induced thrombocytopenia. The differential diagnosis included lupus anticoagulant (LA), Staphylococcal septicemia, thrombotic thrombocytopenic purpura (less likely).

The basic hematological investigations consisted of hemoglobin 9.1 gm/dL, total leucocyte count 16,710/cumm, total platelet count 1.93 lacs/dL, base line prothrombin time and INR were within normal limits. Peripheral smear examination showed normocytic normochromic picture with absence of schistocytes. D-dimer value was within normal limits. On aerobic culture & sensitivity, enterococcus species was isolated.

Advanced investigations for LA were undertaken:

Inhibitor Screen:

Patient's value: 30.1 sec, Control value: 26.3 sec

Mixing studies with normal pooled plasma (NPP), 50:50 APTT, patient's value: 27.4 sec

Mixing studies with NPP, 50:50 APTT at 60 minutes: patient's value:33.0 sec

Mixing studies with NPP, 50:50 APTT at 120 minutes: patient's value: 34.8 sec

Inhibitor Screen: Negative.

LA test: Dilute Russell's viper venom time (dRVVT) method:

LA1 screening:

Patient's value:73.8 sec (reference range:31-44.0 sec)

Control value: 42.4 sec

Mixing study: patient plasma: NPP: 50:50, patient's value: 59.0 sec

LA 2 confirmatory:

Patient's value: 56.1 sec (reference range:30-38.0 sec)

Control value: 35.1 sec

Mixing study: patient plasma: NPP: 50:50, patient's value: 43.0 sec

Interpretation: Positive for LA

On the basis of the laboratory and clinical spectrum, the patient was diagnosed with lupus anticoagulant and treatment with Rivoraxaban was instituted. As the patient had very advanced stage of carcinoma, the patient was started on palliative treatment. The patient finally succumbed to the complications of metastatic carcinoma.

DISCUSSION

LA are included in Sydney criteria for diagnosis of APS. The antibodies excluded in the laboratory criteria for diagnosis of APS are termed as Non-Criteria Antiphospholipid Antibodies. These antibodies are namely, IgA aPL isotypes (IgA aCL and IgA aβ2-GPI), antibodies against plasma proteins (namely prothrombin [aPT], vimentin, annexin V), anionic phospholipids (namely phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol), and to protein/ phospholipid complexes (aPS/PT), and antibodies against the domain 1 of β2-GPI. IgA isotypes are often present in APS patients but are not included in the laboratory criteria for diagnosis of APS, as their presence coincides with presence of IgG, IgM isotypes. Antibodies like anti-prothrombin antibodies, autoantibodies against epitopes in the domain 1 of β2- GPI have recently been found to be associated with thrombotic events in APS, especially in triple-positive patients. Based on recent evidence, it is inferred that non-criteria antibody play role in APS pathogenesis, seronegative APS (SNAPS).[2,3]

The guidelines that pertain to preanalytical, analytical, and post analytical phases of LA testing, have been published by: 1) the International Society of Thrombosis and Haemostasis Scientific Standardization Committee (ISTH SSC), revised in 2009; 2) the British Committee for Standards in Haematology (BCSH), in 2012; and 3) the Clinical and Laboratory Standards Institute (CLSI), in 2014.[4] The data generated over time has established the heterogeneity of the LA antibody and the testing reagents

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Table 1: List of coagulation-based assays for diagnosis of LA with the principle5

Assay types based on pathway	List of Assays
Intrinsic pathway-based assays	LA-responsive routine APTT(LAR), Dilute APTT, Kaolin Clotting Time (KCT), Silica Clotting Time (SCT)
Extrinsic pathway-based assays	dilute Prothrombin Time (dPT), Activated Seven Lupus Anticoagulant assay (ASLA)
Common pathway-based assays FX activation	dRVVT, Vipera lebetina venom time (VLVT)
Common pathway-based assays FII activation	Taipan snake venom time (TSVT), Textarin time

Table 2: Comparison between ISTH-SSC guidelines, BCSH guidelines, and CLSI guidelines

Variables	ISTH-SSC guidelines2,5,7,8	BCSH guidelines2,5,7,8	CLSI guidelines2,5,7,8
Lymphovascular invasion	1. Double Centrifugation	1. Double Centrifugation	1. Double Centrifugation/ Single centrifugation if target is achieved.
	2. Target final platelet count of <10 X 109/L is advocated.	2. Target final platelet count of <10 X 109/L is advocated.	2. Target final platelet count of <10 X 109/L is advocated.
	3. Defer usage of plasma filtration through 0.22-lm	3. Ultracentrifugation is discouraged, due to possible micro particle formation.	3. Ultracentrifugation is discouraged, due to possible micro particle formation.
		4. Reject use of plasma filtration through 0.22-lm	4. Defer usage of plasma filtration through 0.22-lm
First line test of screening test	1. dRVVT followed by LAC sensitive APTT. 2. Tests based on different assay principle. 3. Dilute phospholipid in APTT	1. dRVVT and aPTT with proven LA sensitivity and/or others (modified APTT or dilute prothrombin time)	1. dRVVT and LAR APTT and/or others. 2.Tests based on different assay principle + representing different arm of coagulation pathway. 3. Described alternative to dRVVT, (VLVT), which employs the FX activator from Blunt-nosed viper venom.
Activator	Silica	Silica, ellagic acid, Kaolin	Silica, ellagic acid, Kaolin
Number of specific assays to use in a LAC panel	dRVVT and a LAC sensitive APTT only	No limit on the number of assays	No limit on the number of assays
Cut-off value for a positive LAC assay	99th percentile	97.5th percentile (if Gaussian)	97.5th percentile (if Gaussian)
Testing order	Screen-mix-confirm. Confirm only when mixing study is positive.	Screen-mix-confirm. States that in the absence of other causes of prolonged clotting times, samples with negative mixing tests but clear positive screen and confirm tests on undiluted plasma can be considered LA positive.	Screen-confirm- mix. Considers the mixing step as the last one and unnecessary in specific circumstances.
Ratio derivation	Normal Pooled Plasma (NPP) denominator	NPP denominator	Reference interval (RI) mean denominator
Mixing Tests	Should be performed on a 1:1 mixture of index plasma and NPP.	Should be performed on a 1:1 mixture of index plasma and NPP.	Should be performed on a 1:1 mixture of index plasma and NPP.
Interpretation of Mixing studies	Interpret with ICA (index of circulating anticoagulant) or mixing test-specific cut-off		Interpret with ICA, or mixing test-specific cut-off
Type of Confirmatory test	Confirmatory tests be performed by increasing the phospholipid concentration, with bilayer or hexagonal (II) phase phospholipid.	Suggests employing high phospholipid concentration, platelet neutralization procedure (PNP), or LA-insensitive reagent.	1. Should be based on same assay principle as initial, abnormal screening assay. 2. Solid phase immune assays for antibodies against phospholipid (e.g. ACL, B2GP1) are not regarded as confirmatory tests for LA detection. 3. Details about available confirmatory tests for each screening test, including considerations (about limitations)

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Testing patients on vitamin K antagonists	Undiluted plasma if INR < 1.5, 1:1 dilution of patient plasma: NPP, if INR between 1.5 and < 3.0	Screening and confirm with 1:1 mixture of patient plasma: NPP. Taipan snake venom time (TSVT) with PNP or ET	Screening and confirm with 1:1 mixture of patient plasma: NPP. TSVT with PNP or ET
Testing patients on unfractionated heparin	Interpretation with caution	Not recommended	Can detect LA in some cases where heparin neutralizer is effective
Retesting	After 12 weeks after initial LA positive result.	After 12 weeks after initial LA positive result.	After 12 weeks after initial LA positive result.

available, which mandates the usage of multiple assays with different principles for the screening purposes.[4] The ISTH SSC guidelines recommends for screening purposes to dilute Russell's viper venom time (dRVVT) and activated partial thromboplastin time (APTT) with low phospholipid concentration, followed by mixing studies on abnormal test results, and repeat testing with phospholipids to confirm the phospholipid dependence of the antibody.[4] ISTH SSC recommends, dRVVT for its specificity to clinically relevant antibodies & APTT with low phospholipid concentration for its sensitivity.[5] However, BCSH mandates to combine dRVVT with second test which can be either an APTT with proven LA sensitivity, or a modified APTT, or a dilute prothrombin time (PT). CLSI 2014 guidelines recommend combination of dRVVT and LA responsive APTT as the first line screening tests, advises additional tests with varying analytical principles to increase the sensitivity.[6]

Most of the laboratories employ dRVVT with APTT pairing to detect LA, yet other laboratories are continuing use of other assays also, as in certain situations other tests have genuine clinical relevance.

The traditional screen-mix-confirm algorithm can increase false negative results, as mixing studies introduce dilution factor. It has been established that test and reagent with higher specificity can clearly detect the presence of LA without dilution, hence CLSI 2014 recommends against performing mixing studies on all samples, but leaves room for mixing studies in relevant cases.[7]

Hence, many diagnostic laboratories have either retained/discontinued mixing studies for LA testing. This has led to introduction and wider acceptance of integrated testing.[2] Integrated testing means when screen and confirm assays (i.e., low and high phospholipid) are performed simultaneously on every sample and directly percent correction or screen/confirm ratio is calculated. This is irrespective of screen being elevated or not. The advantage of integrated testing is that weaker LA can be diagnosed in patients where basal clotting time is prolonged but not to that extent that it exceeds the cut off, but screen-confirm discrepancy reveals the presence of LA. As per CLSI 2014 guidelines, "true" integrated tests are assays that include dilution in NPP.[7]

New anticoagulants:

CLSI 2014 describes interferences associated with newer anticoagulants. Direct thrombin inhibitors (DTIs) can cause false positive results in all LA assays, also mixing assays cannot correct the effects of DTI or direct factor Xa (FXa) inhibitors because there is no associated factor deficiency.[7] Rivaroxaban interferes with both dRVVT, APTT, has variable effect on PT based LA assays. According to recent study, in the setting of rivaroxaban therapy TSVT with ET combination is very sensitive for detection of LA, with no interference by rivaroxaban therapy. Because both venoms used are prothrombin activators and are not affected by FXa inhibition.[6]

CONCLUSIONS

The diagnosis of LA anticoagulant should always be entertained in the patients with de novo hypercoagulability in the setting of untreated or advanced stage malignancies. The uniformity between the three panels on many relevant variables like sample preparation, choice of tests, usage of dRVVT, use of ratios, calculations for phospholipid dependence, and report interpretation is a step towards harmonization of practices. BCSH, CLSI guidelines have paved a way for use of other tests like TSVT with ET, TSVT with PNP in relevant clinical settings. Presently there is no single robust assay that can cover the heterogenous nature of LAC, hence coagulation based LA assays continue to be part of diagnostic medley for LA detection.

Conflict of interest: None

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