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SHORT COMMUNICATION

Assessment of Normative Range and Deriving Cut-Off Values for Lupus Anticoagulant Testing: An Experience from a Tertiary Care Center in Southern India

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Abstract Lupus anticoagulant (LA) testing is a demanding laboratory test. The most common among recommended tests for LA are dilute Russell viper venom time (dRVVT) and LA sensitive activated partial thromboplastin time (LA-aPTT). Although integrated test systems come with manufacturer assigned cut-off, but according to guidelines cut-off should be locally-derived. This study was carried out to find normative range of LA using LA-aPTT and dRV VT tests in south Indian population (n = 100) and using locally derived cut-off values to interpret patient samples (n = 152) in a one-year study period. Cut-off (mean+2.3 SD) for aPTT, LA-aPTT, dRVVT screen and dRVVT confirm assays were 37.5, 45.7, 45.5 and 41.3 s and for normalized ratio and percentage correction of ratio were 1.16 and 15.8% respectively. Among patients, 25 (16.4%) were positive of whom 14 were positive by normalized ratio, seven by LA-aPTT and four were positive by both. Four additional patients could be identified using local cut-off. While the specificity of all screening tests was high, sensitivity of only dRVVT screen was high. All testing laboratories should attempt to derive normative range and locally-derived cut-off. While dRVVT is a good screening test with high sensitivity, however combining LA-aPTT offers advantage of picking up cases negative by dRVVT.

Keywords Lupus anticoagulant · Cut-off · Locally-derived · Normative range · dRVVT · LA-aPTT · Normalized ratio

Introduction

Various laboratory tests are available to detect the presence of lupus anticoagulant (LA); among them dilute Russell viper venom time (dRVVT) and LA sensitive activated partial thromboplastin time (LA-aPTT) tests are most commonly used worldwide [1] and recommended in International Society on Haemostasis and Thrombosis (ISTH) 2009 [2], the British Committee for Standards in Haematology (BCSH) 2012 [3] and the Clinical and Laboratory Standards Institute (CLSI) 2014 [4] guidelines. The order of tests to be done varies in the different recommendations as screen-mix-confirm [2, 3] or screen-confirm-mix [4]. Final interpretation for the presence of LA depends on the normalized ratio cut-off or by percentage correction method. The cut-off values also vary as per recommendation as 99th percentile or 97.5th percentile [1]. Most of the integrated test systems have combined screen-confirm reagent and a manufacturer assigned cut-off. According to ISTH 2009 guidelines local cut-off should be derived by using data from a minimum of 40 healthy volunteers and 99th percentile which is equal to mean+2.3 SD [2]. Hence there is a necessity for each laboratory to establish their own normative range in the local population. This study was carried out to derive a normative range of LA using LA-aPTT and dRVVT tests in south Indian population and using locally-derived cut-off values to interpret patient samples.

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Materials and Methods

The study was carried out in a large tertiary care hospital in southern India over a period of one year. Initially, a total of 100 healthy controls (54 females and 46 males; mean age 29 years) all from local population were tested for LA-aPTT, aPTT and dRVVT screen and confirm (PTT LA, CK PREST, STA CLOT ® dRVV screen, STA CLOT ® dRVV confirm respectively) in automated coagulometer (STA compact, Diagnostic Stago). Platelet poor plasma (PPP) was prepared by double centrifugation at 2000 g for 15 min. Aliquot of normal pooled plasma (NPP) from 20 healthy donors was used with every batch. The normalized ratio and percentage correction of ratio were calculated using the formulae given below:

(1) Normalized ratio = dRVVT screen ratio/dRVVT confirm ratio
(2) Percentage correction of ratio= {(dRVVT screen ratio - dRVVT confirm ratio)/dRVVT screen ratio} $\times 100$

A cut-off of 2.3SD above mean corresponding to 99th percentile was taken as per ISTH guideline[2]. Cut-offs for normalized ratio were calculated in two ways; first was by using NPP control values as denominator and the second was by taking reference interval mean (RI mean) as denominator. RI mean represents the statistical average of control sample values.

A total of 152 patient samples (143 females and 9 males; mean age 28.8 years) sent for LA testing in this period were processed in the same way. Patients already on anticoagulants were excluded from analyses. Majority of the patients had SLE (70, 46%) or pregnancy complications (50, 32.9%). Others were sent for venous thrombosis (8, 5%) or arterial thrombosis (5, 3%) or other miscellaneous indications (20, 13.2%). Manufacturer-assigned cut-off as well as locally derived cut-off values were applied to interpret patients' samples.

Using our locally-derived cut-offs, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of a prolonged screening test taking first NPP-derived normalized ratio as gold standard and second as NPP-derived normalized ratio or LA-aPTT or both positive (LA positive) as gold standard were calculated.

Results

The locally-derived cut-off values are tabulated in Table 1. Local cut-off (mean + 2.3 SD) for aPTT, LA-aPTT, dRVVT screen and dRVVT confirm assays were 37.5, 45.7, 45.5 and 41.3 s respectively. The cut-off for

Table 1 Locally derived normative range and cut-off values for lupus anticoagulant testing

Parameters	aPTT (s)	LA-aPTT (s)	dRVVT screen (s)	dRVVT confirm (s)	dRVVT screen ratio	dRVVT confirm ratio	Normalised ratio	dRVVT screen ratio	dRVVT confirm ratio	Normalised ratio	Percentage correction of ratio using RI mean	Percentage correction of ratio using NPP
					Ratio using RI Mean as denominator			Ratio using NPP Value as denominator				
Mean	30.36	37.06	37.51	35.45	1	1	0.99	0.93	0.91	1.01	− 0.35	1.65
SD	3.12	3.8	3.49	2.54	0.09	0.07	0.05	0.13	0.11	0.06	5.91	6.17
Maximum	40	50.6	44.2	47.6	1.18	1.34	1.18	1.26	1.37	1.19	14.91	16.3
Minimum	21	28.2	30.2	31.2	0.81	0.88	0.84	0.66	0.71	0.87	− 18.48	− 15.2
Cut off (Mean+2.3 SD)	**37.53**	**45.79**	**45.52**	**41.3**	1.21	1.16	**1.13**	1.23	1.16	**1.16**	**13.25**	**15.86**

Table 2 Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of positive screening test with locally derived cut-off taking normalized ratio or LA positive as gold standard

	Taking NPP Normalised ratio/percentage correction of ratio as gold standard			
	Sensitivity	Specificity	PPV	NPV
aPTT	11.1	98.5	50	89.2
LA-aPTT	22.2	94.8	36.4	90
dRVVT screen	94	87.3	50	99.1
	Taking either NPP Normalised ratio or LA-aPTT or both positive (LA+) as gold standard			
	Sensitivity	Specificity	PPV	NPV
aPTT	16	100	100	85.8
LA-aPTT	44	100	100	90
dRVVT screen	76	88	55.8	94.9

normalized ratio was 1.16 taking NPP as denominator and 1.13 taking RI mean as denominator. There were 2 false positive control samples using both cut-offs. The cut-off for percentage correction of ratio was 15.8% using NPP and 13.2% using RI mean.

The RI mean cut-off was much lower than manufacturer cut-off of 1.2 for normalized ratio. Hence the NPP derived cut-off was used to avoid over-diagnosis. Interpretation of patient samples (n = 152) in the same period using locally derived cut-off yielded 25 (16.4%) positive patients of whom 14 were positive by normalized ratio, seven were positive by LA-aPTT and four were positive by both. Among the 18 patients positive by normalized ratio, 14 had more than manufacturer cut-off of 1.2 whereas 4 were more than locally derived cut-off of 1.16. Hence four additional patients could be identified. All the 18 cases that were positive by normalized ratio were also positive by percentage correction of ratio taking a cut-off of 15.8%. The clinical profile of LA positive patients was SLE in 12 (48%), pregnancy complication in 6 (24%), venous thrombosis in 3 (12%) and others in 4 (16%). All the three patients with venous thrombosis were LA-aPTT positive, one of whom was positive by both LA-aPTT and normalized ratio.

The sensitivity, specificity, PPV and NPV of positive screening test is given in Table 2. The specificities and NPV of all the screening tests were high. Sensitivity of aPTT, LA-aPTT and dRVVT taking normalized ratio as gold standard was 11.1% and 22.2% and 94% respectively and taking LA positive as gold standard was 16%, 44% and 76% respectively.

Discussion and Conclusion

In this study, locally-derived cut off (mean+2.3 SD) for LA testing was established using 100 healthy controls for aPTT, LA-aPTT, dRVVT screen and dRVVT confirm assays, and for derived parameters like normalized ratio and percentage correction of ratio. In a similar study done by Saldarriaga et al. [5] LA aPTT, dRVVT screen, dRVVT confirm tests were performed in 36 healthy volunteers to establish local reference interval.

Normalization of LA results gives uniformity to interpretation [6] and it is the most widely used method. ISTH 2009 recommends minimum 40 donors [2] whereas BCSH 2012 cites validating previously established cut-off from reagent manufacturer, using smaller number that is 20 to 60 normal donors [3]. While 99th percentile cut-off reduces sensitivity, which leads to false negative, use of 97.5th percentile may produce false positive results [1]. In this study, four additional patients could be identified using local cut-off. Hence it is suggested that every laboratory should establish its own normalized ratio cut-off for detecting the presence of LA.

LA testing should be done carefully as per guidelines and result should be interpreted by correlating with clinical history. Majority of the LA positive patients had SLE followed by pregnancy complications. The problem of LA testing on patients without clinical features is the false positivity as was seen in 2% of our healthy control samples.

Although, the specificity of all screening tests was high, sensitivity of only dRVVT screen was high making it an ideal screening test. However, combining an additional test as recommended like LA-aPTT done in this study helped in picking up cases negative by dRVVT.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical Approval Informed consent was taken from all the study participants and the study was approved by the Institute Ethics Committee.

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