

## ORIGINAL ARTICLE

### Practice and performance of lupus anticoagulant tests: A single centre experience

Kyaw TAY ZA, S. JAYARANEE, Hemalatha SHANMUGAM

Division of Laboratory Medicine, Department of Pathology, University Malaya Medical Centre

#### Abstract

**Introduction:** Lupus anticoagulant (LA) is a well-known risk factor for thrombosis. Correct diagnosis of LA is essential in patient management with anticoagulation. The objectives of this study were to document the clinical and laboratory characteristics of patients tested for LA and to evaluate existing LA testing methods in our laboratory with the aim of improving the performance of LA test interpretation and reporting. **Methods:** Tests for LA include dilute Russell's viper venom time (dRVVT) and Kaolin clotting time (KCT). Patients with LA ratio (dRVVT screen ratio/dRVVT confirm ratio) of  $\geq 1.2$  were considered as LA positive irrespective of KCT results. KCT was considered positive if there was a prolongation in KCT screening test which was not corrected on mixing with normal plasma. **Results:** Of 577 patients' results, 295 were normal, 178 were KCT positive with negative dRVVT and 104 were LA positive. Incidences of thrombosis, connective tissue disease (CTD) and bad obstetric events were noted in 13%, 16% and 44% of normal patients, 9%, 22% and 49% of KCT+ patients and 23%, 37% and 17% of LA+ patients respectively. On further evaluation of dRVVT screen ratios, 431 had a ratio of  $< 1.1$ , 59 had a ratio between 1.1 and 1.2 and 87 had a ratio of  $> 1.2$ . Positive LA results were found in 3%, 29% and 87% of patients with dRVVT screen ratios of  $< 1.1$ , 1.1 - 1.2 and  $> 1.2$  respectively. **Conclusion:** LA+ patients had higher incidences of thrombosis and CTD as compared to normal and KCT only positive patients. There was no significant difference in clinical characteristics between normal and KCT+ patients which suggests the presence of a high rate of false-positive KCT results. Since confirmatory testing for KCT is not widely used, the option of using another LA screening test method should be considered. In regard to dRVVT testing, confirmatory test should only be performed in patients with prolonged dRVVT screening result which was not corrected upon mixing with normal plasma as required by the International Society of Thrombosis and Haemostasis guidelines on LA testing. This practice will not only result in significant cost reduction but also avoid diagnostic confusion.

**Keywords:** lupus anticoagulant, antiphospholipid, Kaolin clotting time, dilute Russell's viper venom time, thrombosis

#### INTRODUCTION

Antiphospholipid antibodies (APL) are a heterogeneous class of immunoglobulins that may develop spontaneously or as a result of autoimmune disease. They are mainly directed towards phospholipid-binding proteins such as  $\beta 2$ -glycoprotein I and prothrombin. Some APL can prolong phospholipid-dependent coagulation tests and are known as lupus anticoagulant (LA).<sup>1</sup> LA is a risk factor for venous or arterial thrombosis and recurrent pregnancy loss.<sup>2</sup> Since there is considerable thrombotic potential in patients with LA, correct laboratory diagnosis

of LA is essential for risk assessment and long-term patient management with anticoagulant therapy.<sup>3,4</sup>

The diagnostic criteria of LA have essentially not changed since their introduction by Brandt *et al*<sup>5</sup> in 1995. It requires (1) screening test: demonstration of the prolongation of a phospholipid-dependent clotting time; (2) mixing test: confirmation of the presence of an inhibitor and the exclusion of a coagulation factor deficiency; and (3) confirmatory test: confirmation that inhibitor is phospholipid-dependent and not directed against specific

clotting factor. The laboratory diagnosis of LA is still a major challenge for any haematology laboratory. This in large is contributed by the heterogeneity of lupus anticoagulant in reacting with phospholipid-associated proteins.<sup>6</sup> Moreover, current available laboratory assays and methods show substantial differences in detecting LA and no single LA test is capable of detecting all LAs.<sup>7-11</sup>

This study documents the clinical and laboratory characteristics of patients tested for LA and evaluates our existing LA testing strategies which include Kaolin clotting time (KCT) and dilute Russell's viper venom time (dRVVT) test systems with the aim of improving the performance of LA test interpretation and reporting.

## MATERIALS AND METHODS

In our coagulation unit, LA tests are carried out mainly to investigate patients with bad obstetric history (such as recurrent miscarriages and intrauterine death), with arterial or venous thrombosis (particularly in young patients) and with connective tissue disease (as a part of screening for antiphospholipid antibodies). The activated partial thromboplastin time (APTT) test is performed for all samples before proceeding to LA testing which includes KCT and dRVVT. dRVVT and APTT are performed by using automated coagulation analyser, ACL TOP® 500 CTS (Beckman Coulter, USA). KCT is carried out manually. During the study period between September 2008 and November 2011, 577 patients in our centre were tested for LA. The test results of KCT, dRVVT and APTT were obtained from the laboratory information system. Demographic and clinical data of the patients were obtained from the request forms and case notes.

LA testing is performed on venous blood collected in plastic tubes (Becton-Dickinson, Franklin Lakes, NJ, USA) containing 1/10 volume of 3.2% trisodium citrate (0.109M) as an anticoagulant. Platelet-poor plasma (PPP) for APTT testing is obtained by centrifugation of the samples at 3500g for 10 minutes at room temperature. The samples for LA testing are further centrifuged at 3500g for another 10 minutes at room temperature and then filtered using 0.2 µm filter papers (Sartorius Stedim, Goettingen, Germany). The plasma is stored in small aliquots at -50° C and tested within a week of sample collection. Samples are thawed at 37°C for 5 minutes before LA testing is done.

### *Normal pooled plasma (NPP)*

Blood is obtained from 10 apparently healthy volunteers and platelet poor plasma is prepared in the same manner as the patient's plasma and then pooled. Volunteers who show abnormal coagulation screening results with APTT and/or prothrombin time are excluded. KCT and dRVVT (screen and confirm) are performed for the NPP. The use of commercial NPP was initiated in August 2009.

### *Kaolin Clotting Time Test*

200 µl of patient's plasma is incubated with 100 µl of 2% kaolin (Sigma, Chemical Co., St Louis, MO, USA) for three minutes at 37°C. 200 µl of calcium chloride is then added and the clotting time recorded. KCT is performed on NPP and patient's plasma. KCT mixing study is carried on 1:1 mixture of patient's plasma and NPP for all samples irrespective of patient KCT result.

Rosner index is calculated as follows:  $(b - c) / a \times 100$ , where a = the clotting time of the patient plasma, b = the clotting time of a 1:1 mix of patient and normal plasma and c = the clotting time of normal pooled plasma. Rosner index value of > 15 is considered as a non-corrected mixing test for KCT.<sup>12</sup> KCT test was considered positive if there was a prolonged screening time by KCT which was not corrected upon mixing with normal plasma.

### *Dilute Russell's Viper Venom Time Test*

dRVVT tests are performed according to the manufacturer's instruction. dRVVT screen (LAC Screen; HemosIL) and dRVVT confirm (LAC Confirm; HemosIL) tests are carried out simultaneously for all cases. Mixing studies for dRVVT screen and confirm are performed if either test result of the patient is higher than that of normal pooled plasma. dRVVT screen ratio (patient dRVVT screen/NPP dRVVT screen) and dRVVT confirm ratio (patient dRVVT confirm/NPP dRVVT confirm) were determined. LA ratio (dRVVT screen ratio/dRVVT confirm ratio) is then calculated. dRVVT test is considered positive when LA ratio is  $\geq 1.2$  following the manufacturer's cut-off value.

### *Activated Partial Thromboplastin Time Test*

APTT test is performed within two hours of receipt of the sample as per manufacturer's instructions. APTT reagent (PTT-SP; HemosIL) is not mentioned as LA sensitive by the manufacturer. APTT is considered prolonged if the result is higher than the normal reference range.

*Interpretation of results*

Samples were defined as LA positive if they had a positive dRVVT test with LA ratio of  $\geq 1.2$ . According to the manufacturer's instructions, the normal LA ratio for dRVVT test ranges from 0.91 to 1.14. Samples with LA ratio between 1.15 and 1.19 and positive KCT test were interpreted as borderline. Samples with LA positive and borderline results as well as KCT alone positive results were requested to have a repeat test after 12 weeks.<sup>13</sup> Samples with normal KCT and negative dRVVT with LA ratio of less than 1.2 were reported as normal.

*Statistics*

Fisher's Exact Test (using the approximation of Woolf) was performed for comparisons of categorical variables. Rosner index and LA ratio values were compared by the unpaired student *t*-test.

**RESULTS**

The clinical and laboratory characteristics of patients are shown in Table 1. Of 577 samples tested for LA in the study period, three groups of patients were identified. Group 1 includes 295 (51%) patients with normal LA test results, group 2 consists of 178 (31%) patients with positive KCT alone with prolonged screening time by KCT and non-corrected KCT mixing test and group 3 is composed of 104 (18%) patients with positive dRVVT tests (LA ratio  $\geq 1.2$ ) with or without KCT positivity. APTT prolongation was observed in 7% (21/295) of group 1, 10% (18/178) of group 2 and 67%

(70/104) of group 3. The mean age of patients in the three groups was  $33\pm 10$  SD,  $33\pm 9$  SD and  $38\pm 16$  SD years respectively. Patients in group 3 were significantly older than groups 1 and 2 [ $P < 0.0001$  (group 3 Vs group 1),  $P = 0.0004$  (group 3 Vs group 2)]. In all three groups, the majority of the patients were female (273/295, 166/178 and 56/80 respectively). However, the proportion of male patients was significantly higher in group 3 compared to other two groups [ $P < 0.0001$  (group 3 Vs group 1),  $P = 0.0001$  (group 3 Vs group 2)].

The underlying reasons for performing LA testing were broadly divided into five categories: bad obstetric history (BOH) (235/577), thrombosis (79/577), established CTD (125/577), suspected CTD (53/577) and miscellaneous (85/577). BOH was the predominant reason of screening for LA in groups 1 and 2 whereas CTD and thrombosis were found to be the commonest causes of screening for LA in group 3 (Fig. 1). Incidence of thrombosis was significantly higher in group 3 compared to other two groups [ $P = 0.0276$  (group 3 Vs group 1),  $P = 0.0014$  (group 3 Vs group 2)]. The diagnosis of CTD was also significantly higher in group 3 [ $P < 0.0001$  (group 3 Vs group 1),  $P = 0.0088$  (group 3 Vs group 2)]. In contrast, the number of patients with bad obstetric history were significantly lower in group 3 compared to the other two groups [ $P < 0.0001$  (group 3 Vs group 1),  $P = 0.0004$  (group 3 Vs group 2)] (Table 1, Fig. 1). In patients with CTD, the diagnosis of systemic lupus erythematosus (SLE) was noted in 32 out of 48 in group 1, 24 out of 39 in group 2 and 28 out of 38 in group 3 (Table 1).

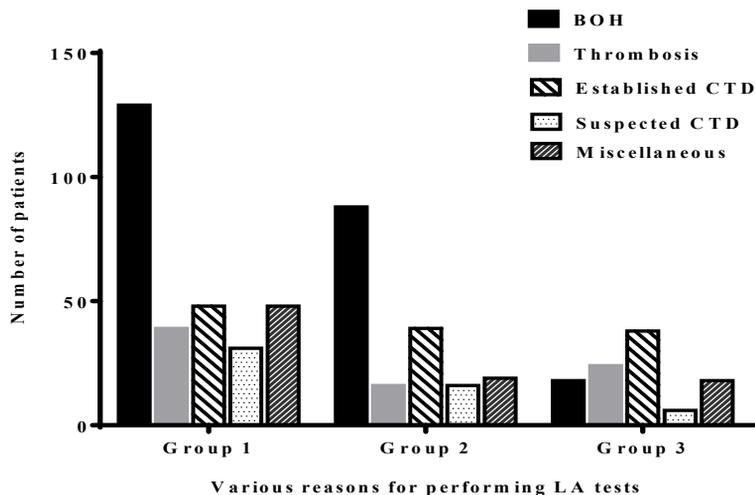


FIG. 1: Reasons for performing LA tests in patients in group 1 (normal results), group 2 (KCT positive and dRVVT negative) and group 3 (dRVVT positive with or without positive KCT).

**TABLE 1: Clinical and laboratory characteristics of patients tested for LA**

	Normal results (Group 1)	*KCT+ & dRVVT- (Group 2)	**dRVVT+ & KCT± (Group 3)
Total No. = 577			
No [%]	295 (51%)	178(31%)	104(18%)
Age (years) (mean ± SD)	33±10	33±9	38±16
Gender [No. (%)]:			
Male	22 (7%)	12 (7%)	24 (23%)
Female	273 (93%)	166 (93%)	80 (77%)
Thrombosis [No. (%)]	39 (13%)	16 (9%)	24 (23%)
BOH [No. (%)]	129 (44%)	88 (49%)	18 (17%)
JCTD [No. (%)]	48 (16%)	39 (22%)	38 (37%)
APTT	21 (7%)	18 (10%)	70 (67%)

\*Group 2; KCTonly+ and dRVVT- with LA ratio < 1.2

\*\*Group 3; dRVVT+ with LA ratio ≥ 1.2 with or without KCT positivity

‡ Total of 84 SLE cases in CTD patients in 3 groups: 32/48 in group 1, 24/39 in group 2 and 28/38 in group 3. Number of CTD patients mentioned do not include suspected CTD cases.

LA positive patients of group 3 were further divided into 2 sub-groups: patients who had positive dRVVT as well as positive KCT results (group 3a) and patients with dRVVT only positive (group 3b). 75 out of 104 (72%) LA positive patients were found to be in group 3a and the remaining 29 patients (28%) were in group 3b. On evaluation, LA ratios were significantly higher in group 3a than in group 3b [P < 0.0001]. Mean LA ratios were 1.67±0.49 in group 3a as compared to 1.29±0.17 in group 3b (Fig. 2).

According to the ISTH guidelines<sup>5</sup>, confirmatory test for dRVVT needs to be performed only when the dRVVT screen is prolonged and dRVVT mixing study is non-corrected. However, in our laboratory,

dRVVT screen and confirm tests are performed simultaneously for all cases. For the purpose of this study, the results of the dRVVT screen ratio were divided into three cut-off values: screen ratios of less than 1.1, 1.1 to 1.2 and greater than 1.2. We correlated these three dRVVT screen ratio cut-off values with their corresponding LA ratios. Of 577 patients, 431 had dRVVT screen ratio of less than 1.1, 59 had dRVVT screen ratio between 1.1 and 1.2 and 87 had dRVVT screen ratio of >1.2. Of 431 patients, 420 (97%) had negative LA ratio of < 1.2 and 11 (3%) had positive LA ratio of ≥ 1.2. Of the 59 patients with dRVVT screen ratio between 1.1 and 1.2, 17 (29%) had positive LA ratio and 42 (71%) has a negative LA ratio. Of the remaining 87

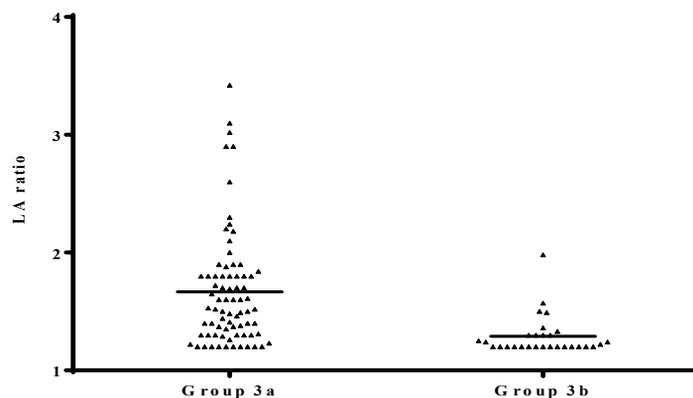


FIG. 2: LA ratios in patients positive for both KCT and dRVVT (group 3a) are compared with LA ratios in patients with positive for dRVVT only.

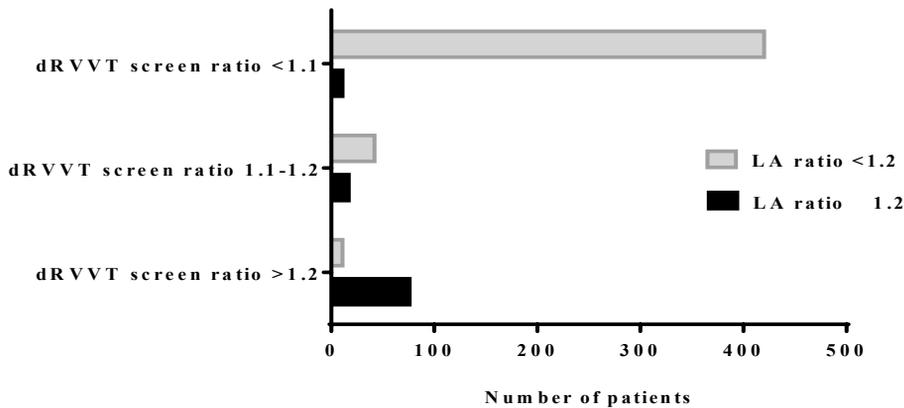


FIG. 3: Correlation of three dRVVT screen cut-off values and their respective LA ratios

with a dRVVT screen ratio of  $>1.2$ , 76 (87%) showed positive LA ratio and 11 (13%) revealed a negative LA ratio (Fig. 3).

## DISCUSSION

The term lupus anticoagulant was first used by Feinstein and Rappaport<sup>14</sup> in 1972 to describe antibodies that inhibited phospholipid-dependent coagulation *in vitro*. LA is a double misnomer as most patients do not have systemic lupus erythematosus and *in vivo* LA reacts as procoagulant.<sup>15</sup> LA is a well-known risk factor for thrombosis.<sup>16, 17</sup> Compared to the anticardiolipin antibodies (ACA), LA was found to be a stronger risk factor for thrombosis.<sup>17</sup> Our study revealed that thrombosis and CTD were more prevalent in LA positive patients (group 3) compared to patients with normal results (group 1) and those with KCT only positive (group 2) (Table 1). There was a total of 84 SLE cases out of 125 patients with CTD screened for LA (Table 1). According to the review by Love and Santoro<sup>18</sup>, the prevalence of LA in SLE patients was 34%. Similarly, nearly one-third (28/84) of our SLE patients were LA positive. There was also a significant difference in the clinical characteristics between patients in group 3 and patients in other two groups. LA positive patients were significantly older and nearly one-fourth of them were found to be males. In contrast, patients in group 1 and 2 were significantly younger with only 7% of males in each group (Table 1). This is because the majority of the patients in group 1 and 2 were investigated for BOH (Fig 1).

Some LA can be assessed better in some testing systems than in others. An adapted dRVVT was more sensitive to  $\beta$ 2-glycoprotein

I dependent LAC, whereas KCT and aPTT appeared to be more sensitive to LA positive anti-prothrombin antibodies.<sup>19</sup> There is no single test which can identify all LA. Hence it is recommended to perform at least two tests with different assay principles to exclude the presence of LA.<sup>13</sup> Diagnosis of LA in our coagulation unit involved using two test systems: KCT and dRVVT. dRVVT is commonly used in diagnostic haemostasis laboratories for detection of LA and believed to be highly specific in identifying patients with high risk of thromboembolic events.<sup>20</sup> As for KCT, it was once considered as a highly sensitive test for screening of LA.<sup>8</sup> In the absence of exogenous phospholipid, the sensitivity of KCT test which mainly depends on the residual cell membrane fragments and plasma lipid could be compromised significantly by platelet contamination.<sup>21</sup>

Approximately one-third of patients investigated for LA (178/577) were positive for KCT only. There was no significant difference in clinical characteristics between KCT only positive patients and those with normal LA screen in group 1. This likely suggests a high rate of false-positive KCT results. High false-positive rate of KCT results was also evidenced by finding of normal KCT results in majority of repeated samples. Out of 178 patients in group 2, 33 of them had repeated the LA tests 12 weeks later. Majority of these patients (23/33) showed negative KCT on repeated samples. The highly sensitive nature of the KCT test and lack of implementation of relevant confirmatory test probably could have resulted in an increase of false-positive results. KCT was found to have many drawbacks. It was not suitable for use in coagulation analysers that use optical detection systems due to the particulate nature of the

kaolin reagent. Kaolin caused sedimentation within the coagulometer dispensing systems and cuvettes.<sup>8</sup> Although it was a highly sensitive test, confirmatory procedure was not commercially available or widely used.<sup>22</sup> Furthermore, it was associated with a poor reproducibility.<sup>8</sup> Hence, KCT was no longer recommended to be used for identification of LA by British Committee for Standards in Haematology (BCSH) guidelines.<sup>23</sup>

In the study conducted by Pengo *et al*<sup>4</sup>, they found that the majority of LA positive patients were positive for both dRVVT and KCT. Similar findings were observed in our study where 72% of LA positive patients had dRVVT and KCT positivity (group 3a) and the remaining 28% had dRVVT positivity alone (group 3b) (Fig. 2). Although our APTT reagent is not mentioned as LA sensitive by the manufacturer, most of the LA positive patients (70%) had prolonged APTT whereas only 7% and 10% of patients in group 1 and 2 showed APTT prolongation (Table 1). On further evaluation of LA positive samples, the LA ratio was significantly higher in patients with both KCT and dRVVT positive results than in those with dRVVT alone (Fig. 2). Pengo *et al*<sup>4</sup> also reported that samples with potent LA were those that were positive in more than one test system. However, whether potent LAs are stronger risk factors for thrombosis and pregnancy morbidity is not precisely known.

The correlation between the dRVVT screen ratio and LA ratio was further evaluated by using three cut-off values of dRVVT screen ratio. Of the patients with dRVVT screen ratio of less than 1.1, almost all cases had negative LA ratio of less than 1.2. In contrast, nearly one-third of patients with dRVVT screen ratio of 1.1 to 1.2 and the majority of patients with dRVVT screen ratio of greater than 1.2 were found to have positive LA ratio (Fig. 3). According to this data, the majority of patients tested for LA had dRVVT screen cut-off ratio of < 1.1 which is likely consistent with negative screening test and running of confirmatory tests in these cases is not cost-effective. The laboratory needs to establish its own reference range for dRVVT screening test using healthy individuals and a confirmatory dRVVT test should be performed only in cases with positive dRVVT screening results.

Another problem of doing dRVVT screen and dRVVT confirm together is the difficulty in the interpretation of cases with negative dRVVT screen results and positive LA ratios. In our

analysis, we had 11 such cases with dRVVT screen ratio of < 1.1 and positive LA ratio. We had reported these cases as LA positive as only LA ratio was taken into consideration in the interpretation of the dRVVT test. Retrospectively, it raises a question of whether these 11 cases were true LA positive. Jun Teruya also addressed this issue in his publication on LA assays. According to him, answers from experts in this matter were equivocal.<sup>2</sup> The first step of ISTH recommendation for diagnosis of LA requires prolongation of a phospholipid dependent screening clotting assay before proceeding to confirmatory test.<sup>5</sup> Strict adherence to ISTH criteria for LA testing is essential to avoid the above-mentioned dilemma.

In summary, lupus anticoagulant patients in this study had significantly higher incidences of thrombosis and connective tissue disease but not bad obstetric events. In regards to KCT performance, there was a high rate of false-positive results which was evidenced by findings such as similar clinical characteristics between KCT only positive patients and those with normal LA screen as well as majority of repeated KCT tests showing normal results. In addition, the confirmatory test for KCT procedure is not commercially available or widely used. Hence, the option of using another LA screening test system needs to be considered. As for dRVVT testing, it is important to establish a local reference range for dRVVT tests in each laboratory and the dRVVT confirmatory test should only be performed in patients with positive dRVVT screening result and non-corrected dRVVT mixing study in order to be more cost-effective and to avoid diagnostic confusion in the interpretation of dRVVT test results.

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