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# Laboratory Identification of Lupus Anticoagulants in Thai Patients

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***Background:*** *Lupus anticoagulants (LA) are acquired heterogeneous groups of antibodies that cause prolongation of the phospholipid-dependent coagulation tests. Due to the heterogeneity of LA and the differences in the reagents and tests, the diagnosis of LA varies from laboratory to laboratory.* ***Objective:*** *To study the pattern of LA positivity by different coagulation tests and the prevalence of LA among blood samples sent for LA screening to our laboratory.* ***Methods:*** *Blood samples from a group of 278 Thai patients sent for LA screening were analyzed. Criteria for the diagnosis of LA were based on prolongation of at least one of the screening coagulation tests, evidence of inhibitor by mixing study and evidence of phospholipid dependency. The screening tests included activated partial thromboplastin time (APTT), kaolin clotting time (KCT), dilute activated partial thromboplastin time (dAPTT), and dilute Russell's viper venom time (dRVVT). Mixing study was performed if an individual screening coagulation test was abnormal. If the initial mixing study showed correction of the abnormal clotting time, then an assay for time-dependent inhibition was performed. There must be at least one abnormal result in the mixing studies of the abnormal screening tests to warrant the presence of a circulating inhibitor. The confirmatory studies consisted of platelet neutralization procedure (PNP) and tissue thromboplastin inhibition time (TTIT).* ***Results:*** *Of the 278 blood samples, 12 (4.3%) were LA positive. The prevalence would have been higher (9.5% or 12/126) if the tests had been done only in patients clinically more likely to have LA. The results of screening dAPTT, APTT, KCT and dRVVT were abnormal in 12/12 (100%), 11/12 (91.7%), 10/12 (83.3%) and 9/12 patients (75.0%), respectively. However, only 8/12 (66.7%), 9/11 (81.8%), 6/10 (60%) and 6/9 (66.7%) of the afore-mentioned abnormal screening tests gave positive results with the immediate 1:1 mixing studies. One sample each showed time-dependent inhibition in the dAPTT and dRVVT. For confirmatory step, the PNP-APTT, PNP-dRVVT, and TTIT were positive in 8/11 (72.7%), 8/9 (88.9%), and 11/11 patients (100%), respectively.* ***Conclusion:*** *The prevalence of the LA positivity was quite low in this group of Thai patients. The dAPTT was the most sensitive screening test while the TTIT was the most sensitive confirmatory test. About 10% of our LA showed time-dependent inhibition. This study also confirmed the need of various tests to detect LA.*

***Key Words :*** ● *Lupus anticoagulants* ● *Laboratory identification*

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Lupus anticoagulants (LA) are acquired heterogeneous groups of circulating antibodies belonging to the antiphospholipid antibody family. It has been believed that these antibodies were directed against anionic phospholipids (PL). Recent findings suggest that they are directed against modified phospholipids, lipid - protein products and phospholipid binding proteins with affinity for anionic (phospholipid) surface. Some of the antigenic targets of these antibodies are β2- glycoprotein I[1-3], prothrombin[4], annexin V[5], (activated) protein C and protein S[6]. These antibodies cause prolongation of in vitro clotting tests by inhibiting the activity of prothrombinase complex (FXa - FVa - $Ca^{++}$ - phospholipid complex) which are required for the conversion of protrombin to thrombin.[7-9] LA were first recognized in patients with systemic lupus erythrematosus (SLE).[10] Subsequent studies have shown that LA are associated with arterial and venous thrombosis[11-14], thrombocytopenia[15-17], pregnancy loss[18-20] and a wide variety clinical situations of many branches of medicine such as neurology[21-23], cardiology[24-25], dermatology[26] and infectious disease.[27] Due to the heterogeneity of LA and the differences in the reagents and tests, the diagnosis of LA varies from laboratory to laboratory. The purposes of this study are to analyze the pattern of LA positivity in Thai patients using a combination of coagulation tests recommended by the Scientific and Standardization Committee for standardization of LA[28] and to determine its prevalence in blood samples sending to our laboratory for LA screening.

## Materials and Methods

### Blood samples

Blood samples were taken from a group of 278 Thai patients, aged 1-81 years, by their physicians. They were sent to our laboratory during October 1996 to October 1998 for LA assay. None of the patients were on anticoagulant therapy at the time of blood drawing.

### Blood collection

Blood samples of patients and normal controls were collected in plastic tubes containing 0.109 M sodium citrate (9:1 ratio) using two syringe technique. Platelet poor plasma (PPP) was obtained by double centrifugation at 2,000 x g for 20 min. at 4°C. Plasma with residual platelet count less than $10x10^9$/L was either assayed immediately or frozen at -80°C for later testing.

### Coagulation tests

#### Step 1 : Screening tests

Activated thromboplastin time (APTT)[29] using kaolin-inosithin suspension (1:100, v:v, of 3.8% inosithin in 0.15 M sodium chloride and 2.5 mg% kaolin suspension in veronal buffer), 1:5 dilute APTT in veronal buffer and dilute Russell viper venom time (dRVVT)[30] using viper venom (Murex Diagnostic Ltd, Temple Hill, Dartford, England) 5 μg/mL in tris buffered saline were performed in MLA 65°C coagulometer (Medical Laboratory Automation, Pleasantville, NY). Kaolin clotting time (KCT)[31] was performed manually. Kaolin (Sigma, St. Louis, MO) was suspended in 0.85% NSS at a concentration of 20 mg/mL.

#### Step 2 : Mixing studies (to demonstrate evi-

**dence of inhibition)**

1:1 mixing studies of patients and pooled normal plasma were performed immediately if any of the screening tests was abnormal to verify the presence of an inhibitor. If the immediate mixing study showed correction of the abnormal clotting time, an assay for time-dependent inhibition (2 hours incubation at 37°C) was performed. No correction of prolonged clotting time of the mixing studies indicates an inhibitor in the sample. Correction means clotting time of the mixture is equal to or less than the upper limit of normal value of that screening test.

**Step 3 : Confirmatory tests (to demonstrate evidence of phospholipid-dependence of inhibitors)**

The confirmatory tests were done if the mixing studies of the test giving an abnormal screening assay indicated the presence of a circulating inhibitor. Platelet neutralization procedure (PNP)[32] in both APTT and dRVVT systems were done on MLA 65°C coagulometer. Suspension of platelet, as the source of phospholipid, in tris buffered saline was added in APTT and dRVVT tests. The antiphospholipid activity of LA demonstrated by shortening of the clotting time in test sample was equal to or less than 5 seconds of NSS control. Tissue thromboplastin time inhibition test ('TTIT')[33] was done by manual technique. Simplastin (Organon Tenika Corporation, Durham, North Carolina) was diluted 1:500 with 0.85% NSS. The ratio of clotting time of patient's plasma to pooled normal plasma equal to or more than 1.3 was considered positive.

Criteria for the diagnosis of LA followed those recommended by the Scientific and Standardization Committee for standardization of LA 199528, i.e. prolongation of at least one of the screening tests, presence of inhibitor shown by no correction of the prolonged screening tests by mixing studies and evidence of phospholipid dependency in the afore-mentioned inhibitory activity by the shortening of increased clotting time after adding excess phospholipid in the confirmatory tests.

## Results

During the period of two years, 278 samples were analyzed for the presence of LA. Twelve were found to have LA (4.3 %). However the prevalence of LA increased to 12/126 (9.5%) if only patients clinically more likely to have APA were considered. LA was positive in 5/49 (10.6%) of patients with a history of venous thrombosis, 2/4 (50%) with recurrent abortion and 5/58 (8.6%) with SLE; three of them had thrombocytopenia. None of patients with arterial thrombosis (0/5) or stroke (0/6) was positive for LA.

Table 1 shows the different pattern of LA positive in 12 patients. One sample each showed time-dependent inhibition in the dAPTT and dRVVT. The sensitivity of different LA tests is shown in table 2 dAPTT, APTT, KCT and dRVVT were abnormal in 12/12 (100%), 11/12 (91.7%), 10/12 (83.3%) and 9/12 patients (75.0%), respectively. However, only 8/12 (66.7%), 9/11 (81.8%), 6/10 (60%), and 6/9 (66.7%)

**Table 1** Pattern of LA positive in 12 patients. All values are in seconds.

| | Screening tests | | | | Mixing studies | | | | | | Confirmatory tests | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Immediate | | | | 2-hour incubation | | | | |
| **No** | **dAPTT** | **APTT** | **KCT** | **dRVVT** | **dAPTT** | **APTT** | **KCT** | **dRVVT** | **dAPTT** | **dRVVT** | **PNP-A** | **PNP-dR** | **TTIT** |
| 1 | >200 | >200 | >200 | >200 | NC | NC | NC | NC | ND | ND | P | P | P |
| 2 | 110.9 | 53.5 | 194.4 | 35.8 | NC | NC | NC | NC | ND | ND | P | P | P |
| 3 | 154 | 59.7 | 135.2 | 54.4 | NC | NC | NC | NC | ND | ND | P | P | ND |
| 4 | 73.8 | 60.2 | 127.0 | 35.2 | NC | NC | NC | NC | ND | ND | N | P | P |
| 5 | 76.2 | 34.5 | 102.2 | 33.2 | C | ND | C | NC | NC | ND | ND | P | P |
| 6 | 72.2 | 52.3 | 93.0 | 28.4 | NC | NC | ND | C | ND | NC | N | P | P |
| 7 | >200 | >200 | >200 | 20.7 | C | NC | C | ND | C | ND | P | ND | P |
| 8 | 87.8 | 55.6 | 107.9 | 85.5 | NC | NC | C | C | ND | C | N | P | P |
| 9 | 90 | 82.3 | 263.3 | 23.3 | NC | NC | NC | ND | ND | ND | P | ND | P |
| 10 | 73.2 | 49.8 | 141.0 | 23.5 | C | C | NC | ND | C | ND | P | ND | P |
| 11 | 62.6 | 48.3 | 106.1 | 36.6 | NC | C | C | NC | ND | ND | P | N | P |
| 12 | 72.4 | 44.6 | 88.5 | 31.8 | C | NC | ND | C | C | C | P | P | P |
| Normal range | 40.5-61.8 | 28.5-42.1 | 44.3-93.4 | 20.1-28.0 | | | | | | | | | |

*APTT: activated partial thromboplastin time; KCT: kaolin clotting time; dAPTT: diluteactivated partial thromboplasin time; dRVVT: dilute Russell's viper venom time; PNP-A, PNP-dR: platelet neutralization procedure APTT(A) or dRVVT(dR) system.*

*C: correction (clotting time of the 1:1 mixture of patients' and pooled normal plasma is ≤ the upper limit of mormal of that test).*

*NC: no correction (clotting time of the 1:1 mixture of patients' and pooled normal plasma is higher than the upper limit of normal of that test)*

*P: positive; N: negative; ND: not done*

**Table 2** Sensitivity of different LA tests

| | | |
|---|---|---|
| dAPTT | 12/12 | 100.0% |
| APTT | 11/12 | 91.7% |
| KCT | 10/12 | 83.3% |
| dRVVT | 9/12 | 75.0% |
| mixing dAPTT | 8/12 | 66.7% |
| mixing APPT | 9/11 | 81.8% |
| mixing KCT | 6/10 | 60.0% |
| mixing dRVVT | 6/9 | 66.7% |
| PNP-APTT | 8/11 | 72.7% |
| PNP-dRVVT | 8/9 | 88.9% |
| TTIT | 11/11 | 100% |

of the afore-mentioned abnormal screening tests gave positive results with the immediate 1:1 mixing studies. For confirmatory step, PNP-APTT, PNP-dRVVT and TTIT were positive in 8/11 (72.7%), 8/9 (88.9%) and 11/11 patients (100%), respectively.

## Discussion

Because LA were reported in a wide variety of clinical conditions, hemostatic laboratories are faced with increasing requests for LA tests. They have been described as heterogeneous in both titer and specificity. The prevalence of LA in a group of Thai patients in our study was quite low (4.3%, 12/278). However the prevalence of LA increased to 12/126 (9.5%) if only patients likely to have APA were included. Associated diseases or conditions in those LA positive patients were venous thrombosis 5/12 (41.6%), recurrent abortion 2/12 (16.1%) and SLE 5/12 (41.6%). The prevalence of LA from the multicenter study in consecutive samples referred for LA testing by the Socie'te' Francaise de Biologie Clinique was 20.7% (147/535)[34]. In other studies, the prevalence of LA varied from 6 to 73% in SLE patients, from 0 to 87% in non-SLE patients[35-38], and from 1 to 2% in healthy population[39]. The difference in the prevalence of LA could be explained by the heterogeneity of the study populations.

The result of our study showed the different patterns of abnormal results. For extreme cases (1, 2, 3, 4) all of screening, mixing and almost all of confirmatory tests were abnormal. The extreme abnormal results of these cases might indicate a higher titer of the antibodies. Among the remainders showed abnormal results in some of screening tests and also only some mixing studies and confirmatory tests were abnormal. Cases 5 and 6 showed time-dependent inhibition with a two-hour incubation. Therefore, in suspicious clinical setting all of screening tests should be performed before excluding of LA. Otherwise some patients will be missed.

These different patterns of positive LA results confirmed the heterogeneity of these antibodies. The dAPTT had the highest sensitivity (100%) for screening tests followed by APTT (91.7%) while KCT (83.3%) and dRVVT (75%) had lower sensitivity. The positive results of mixing studies were quite low, even though we have reduced the amount of platelets to less than $10x10^9$/L by double centrifugation at 2,000 g for 20 min. However, the shortening effect of clotting time by residual platelets might

still occur. For the mixing study, immediate APTT mixing gave the highest sensitivity (81.8%). For the confirmatory tests, TTIT showed the highest positive results (100%), followed by PNP-dRVVT (88.9%) and PNP-APTT (72.7%). Similar results were reported by Triplett et al[40]. They reported sensitivity of 100% for APTT, 70% for immediate APTT, 90% for PNP-APTT and 70% for TTIT. However, the sensitivity of coagulation tests reported by Lazarchick and Kizer[41] was relatively high. The sensitivity of the tests varied: APTT was 94.2%, dRVVT 95.2%, immediate APTT 75%, 2 hours APTT 75%, PNP-APTT 85.7% and TTIT 95.2%. Subsequent studies by Forastiero et al[42] and Brandt et al[43] also showed high positive results of these tests. On the other hand, Lui HW et al[44] studied in Chinese LA patients and reported a rather low sensitivity of APTT (64.3%), immediate APTT (57.1%) and PNP-APTT (57.1%), but high sensitivity of TTIT index, KCT and mixing and dRVVT (100%). Adcock et al[45] reported the sensitivity of 100% for APTT, 83% for DRVVT, 26% for immediate mixing APTT, 69% for 2 hours mixing APTT and 78% for PNP in LA patients. Lo et al[46] reported 100% sensitivity of KCT in SLE and non SLE patients while the result of KCT from Feng et al[38] was 43.7%.

The heterogeneity of LA leads to the variation of coagulation tests sensitivity. The different results have been suggested to be due to the type of binding surfaces of antibodies to plasma proteins and concentrations of antigens and antibodies. APTT is the most common screening test but the sensitivity to LA of the APTT reagents varies. KCT and dRVVT are also sensitive screening tests. However, all screening tests are non-specific to LA, because they are affected by the concentration of clotting factors and by the presence of inhibitors other than LA. Prolonged clotting time due to factor deficiency is corrected by normal plasma in the mixing study. Since LA are antiphospholipid antibodies, the presence of excess phospholipid in the test mixture will neutralize the antibodies activity resulting in the shortening of prolonged clotting time. Therefore, laboratory diagnosis of LA required the combination of different screening tests, mixing studies and confirmatory assays.

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# Laboratory Identification of Lupus Anticoagulants in Thai Patients

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***บทคัดย่อ:*** *Lupus anticoagulants (LA) เป็นกลุ่มของแอนติบอดี้ที่มีความหลากหลายและเกิดขึ้นมาในภายหลัง เป็นสาเหตุหนึ่งที่ทำให้ clotting time จาก phospholipid-dependent coagulation tests ยาวผิดปกติ เนื่องจากความหลากหลายของ LA รวมทั้งการใช้น้ำยาและการทดสอบที่แตกต่างกัน ทำให้การวินิจฉัย LA มีความผันแปรจากห้องปฏิบัติการหนึ่งสู่อีกห้องห้องปฏิบัติการหนึ่ง เพื่อศึกษารูปแบบของ LA positivity ใน coagulation tests ที่แตกต่างกันและอุบัติการณ์ของแอนติบอดี้ในตัวอย่างเลือดที่ต้องการตรวจหา LA ที่ส่งมายังห้องปฏิบัติการของหน่วยโลหิตวิทยาภาควิชาอายุรศาสตร์ โรงพยาบาลรามาธิบดี จึงทำการศึกษาตัวอย่างเลือดจากผู้ป่วยจำนวน 278 รายที่ส่งมาตรวจหา LA หลักเกณฑ์ในการวินิจฉัยคือมีความผิดปกติของ screening tests อย่างน้อยหนึ่งอย่าง มีข้อบ่งชี้ว่ามี inhibitor จาก mixing studies และมีข้อบ่งชี้ว่า inhibitor นั้นเป็น phospholipid-dependent โดย screening tests จะประกอบด้วย activated partial thromboplastin time (APTT), kaolin clotting time (KCT), dilute activated thromboplastin time (dAPTT) และ dilute Russell's viper venom time (dRVVT) ถ้า screening tests ผิดปกติจึงจะทำ mixing study ถ้าค่าที่ผิดปกติของ screening tests กลับมาปกติใน immediate mixing study จะทำ time-dependent mixing study ต่อ จะต้องมี mixing study ของ abnormal screening test อย่างน้อยหนึ่งอย่างผิดปกติเพื่อยืนยันว่ามี inhibitor ส่วน confirmatory tests ประกอบ platelet neutralization procedure (PNP) และ tissue thromboplastin time (TTIT) จากผลการทดลองในผู้ป่วย 278 คนพบว่ามี LA 12 คน (ร้อยละ 4.3) อุบัติการณ์เพิ่มขึ้นเป็น ร้อยละ 9.5 (12/126) ในผู้ป่วยที่มีอาการทางคลินิกน่าจะมี LA ใน screening dAPTT, APTT, KCT และ dRVVT พบความผิดปกติ 12/12 (ร้อยละ 100), 11/12 (ร้อยละ 91.7), 10/12 (ร้อยละ 83.3) และ 9/12 ราย (ร้อยละ 75.0) ตามลำดับ อย่างไรก็ตาม immediate 1:1 mixing study ของ screening tests ที่กล่าวมาแล้วผิดปกติเพียง 8/12 (ร้อยละ 66.7), 9/11 (ร้อยละ 81.8), 6/10 (ร้อยละ 60) และ 6/9 ราย (ร้อยละ 66.7) ตามลำดับ ส่วน time- dependent mixing study แสดงผลผิดปกติอย่างละ 1 รายใน dAPTT และ dRVVT สำหรับ confirmatory tests พบว่า PNP-APTT, PNP-dRVVT และ TTIT ให้ผลผิดปกติ 8/11 (ร้อยละ 72.7), 8/9 (ร้อยละ 88.9), 11/11 ราย (ร้อยละ 100) ตามลำดับ อุบัติการณ์ของ LA ในผู้ป่วยไทยที่ศึกษากลุ่มนี้ค่อนข้างต่ำ จากการศึกษาในครั้งนี้การทดสอบที่มีความไวมากที่สุดของ screening tests คือ dAPTT ขณะที่ TTIT เป็นการทดสอบที่ไวที่สุดของ confirmatory tests ประมาณร้อยละ 10 ของ LA ที่พบในคนไข้กลุ่มนี้จะแสดงความผิดปกติออกมาต่อเมื่อใช้เวลาในการทำปฏิกิริยานานขึ้น (time-dependent) การศึกษาในครั้งนี้ยืนยันถึงความจำเป็นที่ต้องการการทดสอบที่หลากหลายสำหรับการตรวจหา LA*

***Key Words :*** ● *Lupus anticoagulants* ● *Laboratory identification*

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