

**Case report:**

**Lupus Cofactor Effect of Mixing Study: Example of a Case Report and Revisit on Theoretical Aspects**

Wan Suriana Wan Ab Rahman<sup>1</sup>, Zefarina Zulkafli<sup>2</sup>, Mohd Nazri Hassan<sup>3</sup>, Wan Zaidah Abdullah<sup>4</sup>

**Abstract**

**Background:** Lupus anticoagulants (LA) are in vitro inhibitors paradoxically associated with thrombosis and performing coagulation test on a mixture of test plasma and pooled normal plasma to evidence this property is a mainstay in LA detection. **Case report:** We report a 20-year-old man who presented with one week history of fever, associated with productive cough, jaundice, and lethargy. He was a known case of systemic lupus erythematosus for 10 years. Isolated activated partial thromboplastin time (APTT) were prolonged. Interestingly, the APTT mixing study was not corrected and showed more prolonged results than baseline APTT. LA study showed presence of a strong LA, which is related to the underlying autoimmune disease. **Discussion:** This case focuses on the 'rebound phenomenon' in APTT mixing test, from a strong LA that gives a cofactor inhibitory effect to the entire test system after adding the normal plasma.

**Keywords:** lupus anticoagulant; mixing study; inhibitor

Bangladesh Journal of Medical Science Vol. 18 No. 02 April'19. Page : 430-433  
DOI: <https://doi.org/10.3329/bjms.v18i2.40721>

**Introduction**

Antiphospholipid syndrome (APS) is an acquired autoimmune disorder characterized by a wide range of clinical manifestation, mainly thrombotic and/or pregnancy morbidity<sup>1</sup>. Three criteria antibodies employed in diagnosis of APS includes lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti-beta-2-Glycoprotein-I antibodies (a $\beta$ 2GPI)<sup>2</sup>. Lupus anticoagulants are in vitro inhibitors associated with thrombosis, and performing coagulation test on a mixture of test plasma and normal pooled plasma to evidence non-corrected property is a mainstay in LA investigation. As an in-house procedure, mixing test is advocated for cases with prolonged APTT. In this article, a rebound phenomenon during a mixing test study of APTT is described in a patient with LA associated with systemic lupus erythematosus (SLE)

complicated by septicaemia. We will highlight about this phenomenon that could occur during a mixing test previously described as 'lupus cofactor' effect.

**Method**

Venous blood was collected into silicone treated vacutainer tubes which contain 0.5 ml of anticoagulant trisodium citrate which introduces a 9:1 ratio of blood to anticoagulant. Prothrombin time (PT), activated partial thromboplastin time (APTT), Clauss fibrinogen and D-dimers were performed with Neoplastine® CI Plus, STA-PTT® A, (Liquid Fib) and (STA Liatest D-Di) respectively (Diagnostica Stago, Asnieres, France) on a STA-R Evolution® automated coagulation analyser (Diagnostica Stago). Pooled normal plasma (PNP) was prepared in-house from routine normal samples from 20 healthy donors. To interpret the mixing test, the 10% rule was used

1. Wan Suriana Wan Ab Rahman, Mpath (Haemato), 1 School of Dental Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian Kelantan.
2. Zefarina Zulkafli, MPath (Haemato), 3 Department of Hematology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian Kelantan.
3. Mohd Nazri Hassan, MPath (Haemato), 2 Department of Hematology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian Kelantan.
4. Wan Zaidah Abdullah, Mpath (Haemato), 2 Department of Hematology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian Kelantan.

**Correspondence to:** Dr Wan Suriana Wan Ab Rahman, Address: School of Dental Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian Kelantan, E-mail: [suriana@usm.my](mailto:suriana@usm.my);

: a) 90/100 x patient APTT(or patient PT) and b) 110/100 xPNPAPTT (or PNP PT). The result is considered partially corrected if patient's result is between 'a' and 'b'. The result is considered corrected if patient's result is more than 'b' and it is considered not corrected if patient's result is more than 'a'. Correction of more than +10% is considered factor deficiency while correction more than -10% is considered strong inhibitor. However, correction between -10% and +10% is borderline and possible of weak inhibitor, but factor deficiency may be shown up in this range.

Lupus anticoagulant assays was done by using APTT-LA and diluted Russell's viper venom time (dRVVT), by using cephalin and a particulate activator (silica) for APTT LA, and STA®- Staclot® dRVV Screen and STA®- Staclot® dRVV confirm containing Russell's viper venom, phospholipids, calcium and phospholipid inhibitor for dRVVT test on a STA-R Evolution® automated coagulation analyser (Diagnostica STAGO, France). A mixing test was performed in a 1:1 proportion with PNP and the reference values were locally derived. Index of circulating anticoagulant (ICA) also known as Rosner index (RI) was used. Value of less than 15% is considered full correction which is probably related to factor deficiency while no correction is considered when the percentage is more than 15.

### Case report

A 20-year-old Malay man presented with fever for one week duration. The symptom was associated with lethargy, jaundice, reduce effort tolerance, and productive cough for the past one month prior to admission. He was a known case of SLE since

10 years of age and received hydroquine therapy, however poorly compliant to the medication. He has no past history of thrombosis. Physical examination revealed presence of jaundice and pallor. There was no organomegaly and no sign of thrombosis or renal failure. Laboratory investigation showed features of autoimmune hemolytic anaemia (AIHA) by evidence of anaemia with Hb of 5.6 g/dL, increase total bilirubin (96 µmol/L) with predominantly indirect bilirubin (86 µmol/L), and positive direct Coomb's test. Peripheral blood film showed severe anaemia with presence of polychromatic cells and spherocytes. There was no fragmented red cells to suggest microangiopathic hemolytic anaemia and the platelet count was normal. Erythrocyte sedimentation rate (ESR) was raised (117 mm/h). His serum electrolyte, urea and creatinine were normal. Coagulation screening test showed normal prothrombin time (PT) and prolonged activated partial thromboplastin time (APTT). Plasma fibrinogen (3.89 g/L) and D-dimer (<0.45µg/mL) were within normal range. Mixing test for APTT was done (Table 1). LA study for Dilute Russell Viper Venom Time (DRVVT) test and APTT-LA was done twice, during admission and 3 months after admission. Both LA studies showed presence of strong LA. The anticardiolipin antibody levels were normal (IgG<10 GPL and IgM<7 MPL). However anti-beta 2-glycoprotein I was not done in this patient due to some logistic reasons. This result has confirmed the persistent nature of this pathological antibody. Table 1 and table 2 demonstrates the coagulation test results of this patient during admission.

**Table 1 Result of PT and mixing test of APTT**

No	Test Sample	<sup>a</sup> PT (sec)	<sup>b</sup> APTT (sec)	Interpretation (Based on 10% rule calculation)
<b>Mixing Test</b>				
1	Control Normal (Pool), 1 ml	11.8	35.8	
2	Patient, 1 ml	13.6	58.9	
3	Control normal + patient Mix 1:1		85.9	Immediate mixing test for <sup>b</sup> APTT wasnot corrected

<sup>a</sup>PT=prothrombin time; <sup>b</sup>APTT=activated partial thromboplastin time

Table 2 Result of lupus anticoagulant study

A. LA Screening test					
	Patient (neat)	Patient (mix)	Reference range (sec)	Calculation of Rosner index (RI):	
P T T - L A (sec)	102.9	106.5	Cut off point 53.8	$\frac{CT_{mix} - CT_{PNP}}{CT_{PP}} \times 100 = 65.89$	
B. Dilute Russell's viper venom time (DRVVT)					
	Screening (cut off point: 45.8 sec)		Confirm (cut off point: 37.6 sec)		
	Neat (sec)	Mix 1:1 (sec)	Neat (sec)	Mix 1:1 (sec)	
Patient	84.3	73.6	37.6	37.2	
Screen Ratio = Patient Screen/Mean of Screen Normal Range					
Confirm Ratio = Patient Confirm/Mean Confirm Normal Range					
Lupus ratio (LR): Normalized LA ratio=Screen Ratio/Confirm Ratio = 2.05					

<sup>a</sup>PT=prothrombin time; <sup>b</sup>APTT=activated partial thromboplastin time; <sup>c</sup>LA= Lupus anticoagulant; <sup>d</sup>CNP= controlled normal plasma

Blood culture and sensitivity was done due to high grade fever and was positive for *Salmonella sp.* This patient has LA which is related to the autoimmune disease (SLE) and developed *Salmonella sp.* bacteremia few days after admission. He was treated with intravenous antibiotic sensitive to the isolated organism (Ciprofloxacin) and also manage for his AIHA condition with intravenous immunoglobulin (IVIg) and immunosuppression therapy.

#### **Consent:**

Verbal informed consent was obtained from the patient for publication of this case report.

We warrant that the manuscript submitted is our own original work; All authors participated in the work in a substantive way and are prepared to take public responsibility for the work.

#### **Ethical Clearance:**

This case report was submitted for publication after getting Ethical approval from the Ethics Committee of the Universiti Sains Malaysia, 16150 Kubang Kerian Kelantan,

#### **Discussion**

Lupus anticoagulant was first described in SLE patients, however it also occurs in other conditions such as Antiphospholipid syndrome, rheumatoid arthritis, lymphoma, acquired immune deficiency syndrome (AIDS), and in individual with no identified medical problems. Lupus anticoagulant is an in vitro anticoagulant, usually immunoglobulins G (IgG) that interfere with in vitro phospholipid-dependent coagulation tests, such as PT, APTT, and DRVVT<sup>3</sup>. These antibodies are directed to phospholipid-binding

proteins, and more specifically, they are often directed against cryptic epitopes exposed upon binding to phospholipid<sup>4</sup>. It is identified using clot-based assays in the laboratory. It shows the strongest association with adverse thrombotic and obstetric complication related to APS. Presence of this antibody can cause a wide variety of pre-analytical and analytical issues that can compromise inter-laboratory portability and clinical utility<sup>5</sup>.

Our patient is a known case of SLE, therefore coagulation screening test (PT and APTT) was performed during admission and also indicated for his septic condition. APTT showed prolongation and therefore mixing test for APTT was done. Mixing tests are a simple procedure, used as a first line investigation in the case of abnormal screening coagulation test to guide for further investigations. Factor deficiencies are usually corrected in mixing tests while inhibitors is expected to exhibit partial or non-correction, although weak antibodies may cause false-negative mixing tests in LA detection due to the dilution effect<sup>5</sup>. In this case, mixing test for APTT resulted more prolongation on the mixture than on the patient plasma alone. This phenomenon has been termed as "lupus cofactor effect". Inhibitors of clotting factors or anticoagulants are circulating endogenously produced substances that interfere with various in vitro tests of coagulation<sup>6</sup>.

Lupus cofactor refers to a plasma component that was required for the expression of lupus anticoagulant. The lupus cofactor effect is a paradoxical effect that cause further prolongation of a patient's clotting

time upon mixing with PNP<sup>7</sup>. Although the effect is suggestive of LA, it is not a pathognomonic for LA. The phenomenon is due to prozone effect. It is commonly occur in APTT test and commonly seen when a 4:1 patient to normal mix is used<sup>8</sup>. This case however did not proceed to 4:1 dilution since the LA was strongly detected by 1:1 ration. However, the 1995 ISTH guideline described limitations of the 4:1 dilution and indicated that 1:1 mixing tests were the most commonly employed at the time and are now standard practice. Ratios variation of test and normal plasma is expected to be rarely seen in current practice. The LA cofactor effect in this case is more convincing with routine reagent and it is not exhibited in LA sensitive test such as APTT LA and DRVVT. This is probably the nature of this inhibitor that incapable to overcome the 'correcting effect' in this APTT reagent or the inconsistent sensitivity of APTT reagent as compare to LA sensitive test<sup>9</sup>.

### **Conclusion**

Circulating LA can cause 'rebound phenomenon' in APTT mixing test by showing higher results than the neat plasma APTT. This interesting phenomenon known as lupus cofactor effect, as a result of additional inhibitory effect of LA to the entire coagulation test system after adding the normal plasma. Therefore,

correct evaluation when dealing with unexpected mixing test results should be undertaken and understanding this effect could rule out any suspicion of preanalytical or technical errors that could be suspected during mixing test procedure. However a proper LA evaluation by specific tests should be done in order to confirm the presence of LA in samples having this effect on mixing test.

### **Conflict of interest**

We declare that we have no conflict of interest.

Source of support: nil

**Acknowledgement:** We thank Dr Salfarina Ibrahim and the staff of Haematology Laboratory Unit, Hospital Universiti Sains Malaysia for their help.

### **Authors' Contributions:**

Data gathering and idea owner of this study: Wan Suriana Wan Ab Rahman, Zefarina Zulkafli

Study design: Wan Suriana Wan Ab Rahman, Mohd Nazri Hassan

Data gathering: Wan Suriana Wan Ab Rahman, Wan Zaidah Abdullah

Writing and submitting manuscript: Wan Suriana Wan Ab Rahman, Zefarina Zulkafli,

Editing and approval of final draft: Wan Suriana Wan Ab Rahman, Mohd Nazri Hassan

### **References:**

1. Eby C. Antiphospholipid review. *Clin Lab Med Ju*. 2009;29(2): 305-19.
2. Favaloro EJ, Wong RC. Antiphospholipid antibody testing for the antiphospholipid syndrome: a comprehensive practical review including a synopsis of challenges and recent guidelines. *Pathology*. 2014; 46(6): 481-495.
3. Francine R. Dembitzer, Marlies R. Ledford Kraemer, Piet Meijer, and Ellinor I.B. Peer. Lupus Anticoagulant Testing: Performance and Practices by North American Clinical Laboratories. *Am J Clin Pathol* 2010;134:764-773.
4. Martinuzzo ME, Forastiero RR and Carreras LO. Binding properties of antibodies to prothrombin and  $\beta$ -glycoprotein I ( $\beta$ 2-GPI) assayed by ELISA and dot blot. *Clin Exp Immunol*. 1999 ; 118(3): 480-486.
5. CLSI. Laboratory Testing for the Lupus Anticoagulant; Approved Guideline. CLSI document H60-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
6. Della Valle P, Crippa L, Garlando AM, et al. Interference of lupus anticoagulants in prothrombin time assays: implications for selection of adequate methods to optimize the management of thrombosis in the antiphospholipid-antibody syndrome. *Haematologica*. 1999;84(12):1065-1074.
7. Thomas L. Ortel. Antiphospholipid Syndrome Laboratory Testing and Diagnostic Strategies. *Am J Hematol*. 2012 ; 87(Suppl 1): S75-S8.
8. Triplett DA. Laboratory diagnosis of lupus anticoagulants. *Semin Thromb Hemost*. 1990;16:182-192.
9. Brandt J.T., Triplett D.A., Alving B. & Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. *Thrombosis and Haemostasis*. 1995; 74, 1185-1190.