Usefulness of a Diluted Prothrombin Time for Accurately Diagnosing Antiphospholipid Syndrome

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Abstract: The usefulness of lupus anticoagulant (LA) and several antibodies for predicting thrombosis was assessed in patients with idiopathic thrombocytopenic purpura (ITP), systemic lupus erythematosus (SLE), lupus like disease (LLD), recurrent abortion and primary antiphospholipid syndrome (APS). LA was measured using the diluted Russell Viper Venom test (DRVVT) and the diluted prothrombin time (dPT).

In healthy volunteers, the median (range) of the DRVVT and dPT ratio were 0.97 (0.88 - 1.08) and 1.05 (0.85 - 1.29), respectively. The positive percent of dPT ratio was high in patients with ITP, SLE, recurrent abortion and primary APS. The sensitivity for thrombosis was highest for the dPT ratio and the specificity for thrombosis was highest for the DRVVT ratio. The sensitivity and the Odd's ratio for both the dPT and DRVVT ratio were high. A positive predictive value in DRVVT and a negative predictive value in dPT were high. The receiver operating characteristic (ROC) curve analysis indicates that the dPT ratio might be more useful for predicting thrombosis than the DRVVT ratio. The DRVVT and dPT ratios are useful for both the diagnosis of APS as well as predicting thrombosis.

Keywords: Diluted prothrombin time, antiphospholipid syndrome, diluted russell viper venom test, lupus anticoagulant.

INTRODUCTION

Patients with Antiphospholipid syndrome (APS) are subject to thrombosis in arteries and/or veins, pregnancy loss, and thrombocytopenia, associated with antiphospholipid antibodies [1, 2]. Anti-phospholipid antibodies (APA) include lupus anticoagulant (LA), anti-cardiolipin (aCL), anti-prothrombin (aPT) and anti-β2-glycoproteinI (anti-β2-GPI) antibodies [3-6]. Lupus anticoagulants (LA) are considered to be immunoglobulins which are directed to either prothrombin or β2-GPI bound to phospholipids and which are highly sensitive for APS [7]. Almost all patients with APS have either β2-GPI- or prothrombin-dependent antibodies. Therefore, accurate detection of LA is important in patients suspected of having APS. The combination of the dilute activated partial thromboplastin time (dAPTT) and the dilute Russell viper venom time (DRVVT)-based LA tests are a simple and reproducible way of detecting the presence of LAs with a high sensitivity and specificity [8]. The dilute prothrombin time (dPT) is a well documented screening test for LA [9]. However, when using a crude mixture of tissue factor and PL, it tends to have a poor reproducibility and specificity [10]. Arnout *et al.* [9] showed that the dPT performed with human tissue thromboplastin obtained by recombinant DNA technology considerably improved the test results.

In this study, we investigated the usefulness of dPT for the detection of LA in addition to DRVVT.

MATERIALS AND METHODS

Several APA were examined in 100 patients with suspected of having LA and consisting of 28 men and 72 women ranging in age from 35 to 59.5 years, with a mean age of 47.0. The underlying diseases of the patients were as follows; 40 idiopathic thrombocytopenic purpura (ITP), 34 lupus like disease (LLD), 12 systemic lupus erythematosus (SLE), 6 habitual pregnancy loss, 5 primary antiphospholipid syndrome (primary APS) and 3 hemophilia. The presence of recurrent arterial and venous thrombosis, pregnancy complications of 100 patients were as follows; 10 had brain thrombosis, 7 had deep vein thrombosis (DVT)/ pulmonary embolism (PE), 2 had central retinal artery occlusion (CRVO), 6 had habitual miscarriage and 2 had other diseases.

DRVVT was measured using a DVV-test (American Diagnostica Inc., ADI, Stamford, CT, USA) and DVV-confirm (ADI). The patients who have more than 1.2 of DVV-test/

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1567-2700/09 2009 Bentham Open

DVV ratio are considered as LA positive. dPT was measured using a ACTICLOT dPT (ADI). A low lipid concentrated PT/ high lipid concentrated PT ratio of higher than 1.3 was considered as LA positive. The cutoff and the mean value for the DRVVT and dPT ratios were examined in 60 healthy volunteers consisting of 43 men and 17 women ranging in age from 21 to 54 years old, and the mean age was 31.5 years old.

Anti-phospholipid (aPL) antibody, aPT antibody or anti-β2GP1 antibody was measured using an enzyme-linked immunosorbent assay (ELISA) using the IMUCLONE aPL IgG ELISA Kit (ADI), the IMUCLONE Anti-Prothrombin IgG ELISA Kit (ADI), or the IMUCLONE Anti-β2GP1 IgG, ELISA Kit (ADI).

Statistical Analysis

The data are expressed as the mean ± SD or median; range. Differences between the groups were examined for significance using the Mann-Whitney U test while any correlations between the 2 variables were tested by Pearson's correlation analysis. A P value < 0.05 was considered significant. The usefulness of D-dimer and soluble fibrin (SF) for the diagnosis of thrombosis, DVT and disseminated intravascular coagulation (DIC) was examined by a receiver operating characteristic (ROC) analysis. The cutoff values were determined by an ROC analysis. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

RESULTS

Fig. (**1**) shows the DRVVT and dPT ratio in healthy volunteers and those who do not demonstrate a normal distribution. The median (range) of the DRVVT and dPT ratios were 0.97 (0.88 - 1.08) and 1.05 (0.85 - 1.29), respectively. Next, the cutoff value was determined to be 1.2 for the DRVVT ratio and 1.3 for the dPT ratio.

The positive percentage of the dPT ratio (more than 1.3) and DRVVT ratio (more than 1.2) was 45.0 % and 17.5 % in patients with ITP, 23.5 % and 23.5 % in those with LLD, 66.7 % and 66.7 % in those with SLE, 83.3 % and 16.7 % in those with recurrent abortion, 80.0 % and 20.0 % in those with primary APS, and 0.0 % and 0.0 % in those with hemophilia, respectively (Table **1**). The positive percentage of aPT and anti-β2GP1 antibody were high in the patients with SLE but low in the other groups. The positive percentage of aPL was low in all groups.

The dPT ratio (median; range) was significantly higher in the patients with thrombosis (1.37; 1.27 – 1.56) than in those without thrombosis (1.12; 1.01 – 1.30) but not the DRVVT ratio (Fig. **2**). The sensitivity for thrombosis was the highest for the dPT ratio (92.6 %) while the specificity for thrombosis was highest for the DRVVT ratio (82.2 %) (Table **2**).

An ROC analysis shows the dPT ratio to be more useful for the diagnosis of thrombosis than the DRVVT ratio (Fig. **3**). These data indicated the adequate cutoff value for thrombosis to be 1.09 for the DRVVT ratio and 1.26 for the dPT ratio. At this cutoff value, the sensitivity and Odd's ratio for both the dPT and DRVVT ratios were high, while the positive predictive value (PPV) for the DRVVT ratio and the negative predictive value (NPV) for the dPT ratio were high (Table **3**).

DISCUSSIONS

From the data obtained in healthy volunteers, the cutoff values for DRVVT and dPT ratios were 1.2 and 1.3, respectively. The value of DRVVT was similar to that previously described [7, 11]. The positive percentage of the dPT ratio was high in ITP, SLE, recurrent abortion and primary APS. The frequency of LA was the highest in collagen diseases. Previously, APS has been frequently reported in collagen diseases [8, 10]. As LA was observed in both patients with ITP and SLE without thrombosis and healthy volunteers, LA

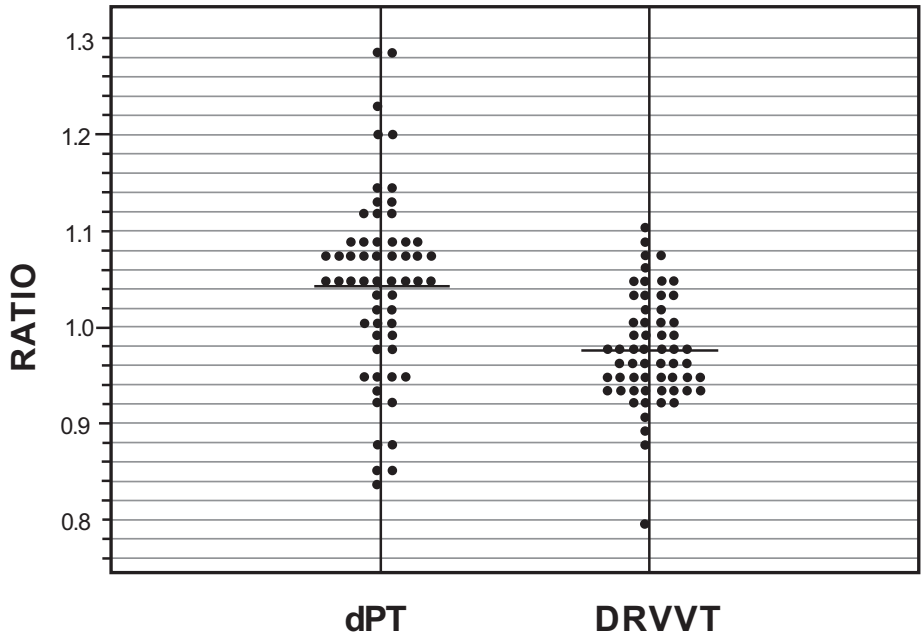

Fig. (1). The dilute prothrombin time (dPT) and diluted Russell Viper Venom test (DRVVT) ratios in healthy volunteers.

Table 1. Positive Percent of Anti-Phospholipid Antibody in Various Diseases

	ITP	LLD	SLE	Abortion	PAPS	Hemophilia
aPT	12.5 %	6.7 %	41.7 %	16.7 %	0 %	0 %
Anti-β2GPI	12.5 %	8.8 %	50.0 %	33.3 %	20 %	0 %
aPL	0 %	3.3 %	27.3 %	16.7 %	20 %	0 %
dPT ratio	45.0 %	23.5 %	66.7 %	83.3 %	80 %	0 %
DRVVT ratio	17.5 %	23.5 %	66.7 %	16.7 %	40 %	0 %

aPT: anti-prothrombin, anti-β2-GPI: anti-β2-glycoprotein I, aPL: Anti-phospholipid, dPT: dilute prothrombin time, DRVVT: diluted Russell Viper Venom test, ITP: idiopathic thrombocytopenic purpura, LLD: lupus like disease, SLE: systemic lupus erythematosus, PAPS: primary anti-phospholipid syndrome.

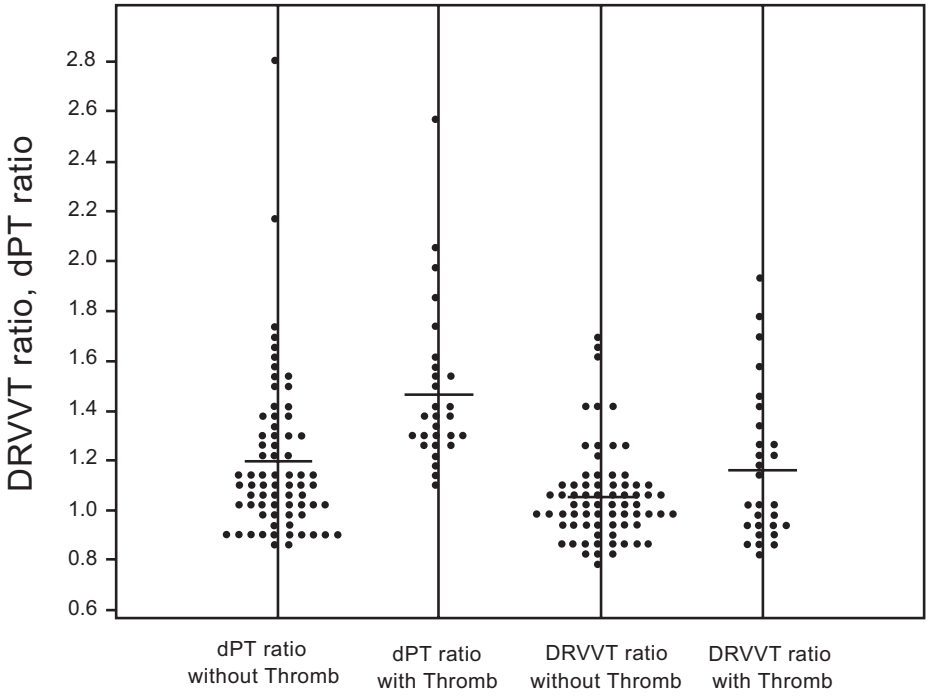

Fig. (2). The dilute prothrombin time (dPT) and diluted Russell Viper Venom test (DRVVT) ratios in patients with or without thrombosis
**: $p < 0.01$
Thromb: thrombosis

is not always a risk factor for thrombosis. LA was also reported in other patients without thrombosis [12, 13]. Since a positive frequency was higher for the dPT ratio than for the DRVVT ratio, the dPT ratio was thus suggested to possibly be a sensitive assay for LA.

Table 2 Sensitivity and Specificity of Anti-Phospholipid Antibody for Thrombosis Based on the Cutoff Values from Healthy Volunteers

	Sensitivity	Specificity
dPT ratio	92.6 %	75.3 %
DRVVT ratio	48.1 %	82.2 %

dPT: dilute prothrombin time, DRVVT: diluted Russell Viper Venom test, Cutoff value: dPT ratio; 1.30, DRVVT ratio; 1.20.

In other antibodies, the positive percent of aPT and anti-β2GP1 antibody were high in patients with SLE but low in the other groups. The positive percent of aPL was low in all groups. The frequency of anti-β2GP1 IgG was markedly high in patients with cerebral thrombosis but only slightly high in patients with DVT, thus suggesting this frequency to be related to thrombosis, especially arterial thrombosis [7]. Cerebral ischemia associated with anti-phospholipid antibody is the most common arterial thrombotic manifestation in APS [14, 15]; however, the importance of anti-phospholipids as a cardiovascular risk factor remains controversial. The frequency of aPT IgG antibody was moderately high in patients with DVT and low in those with cerebral thrombosis and SLE, thus suggesting that it is related to thrombosis, especially venous thrombosis [7]. Prothrombin, another phospholipid binding protein, was first proposed as a possible cofactor for LA by Loeliger in 1959 [16]. A positive correlation between the presence of aPT antibody and DVT

Table 3. Sensitivity and Specificity of the DRVVT and dPT Ratios for Thrombosis Based on a Cutoff Value from an ROC Analysis

	dPT Ratio	DRVVT Ratio
Cutoff value	1.26	1.09
Sensitivity	75.0 %	73.6 %
Specificity	70.8 %	67.9 %
PPV	50.0 %	85.5 %
NPV	87.9 %	50.0 %
Odd's ratio	7.29	5.89
AUS	0.794	0.702

dPT: dilute prothrombin time, DRVVT: diluted Russell Viper Venom test,
PPV: positive predictive value, NPV: negative predictive value
AUC: area under the curve
Cutoff value: dPT ratio; 1.26, DRVVT ratio; 1.09.

was reported in a SLE population [17]. In a study of 265 cases of DVT or PE, the risk of thrombotic events was shown to significantly increase in carriers of aPT antibody [18].

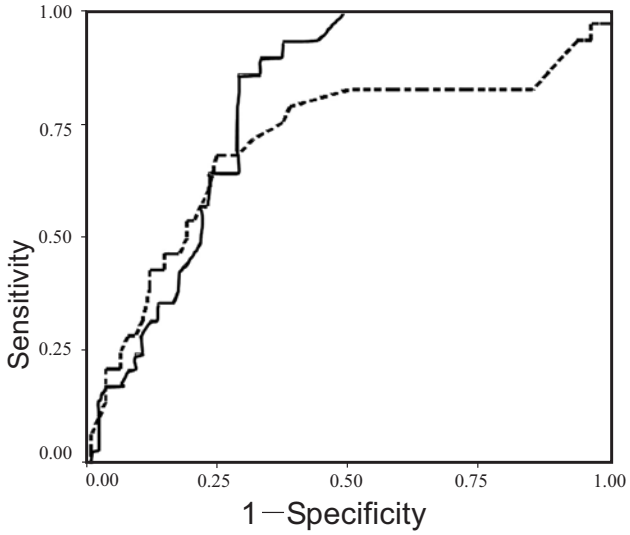

Fig. (3). Receiver operating characteristic (ROC) analysis of dilute prothrombin time (dPT) and diluted Russell Viper Venom test (DRVVT) ratio for thrombosis
—dPT ratio, ----- DRVVT ratio

The sensitivity for thrombosis was the highest for the dPT ratio and the specificity for thrombosis was the highest for the DRVVT ratio, thus suggesting that a lightly lower cutoff value of the DRVVT ratio might thus be better for diagnosing of thrombosis. Indeed, an adequate cutoff value of the DRVVT ratio for thrombosis from an ROC analysis was slightly low; the sensitivity and Odd's ratio for both the dPT and DRVVT ratios were high, the PPV for the DRVVT ratio and the NPV for the dPT ratio were high. An ROC analysis and Odd's ratio show that the dPT might therefore be more useful for predicting thrombosis than DRVVT.

APA is reported to interfere with tissue factor pathway inhibitor (TFPI), which is an important inhibitor for the extrinsic pathway of blood coagulation [19]. Especially, TFPI regulates the inhibitory effect of blood coagulation on the phospholipid surface [20]. Therefore, a dPT assay may be a sensitive tool for diagnosing thrombosis.

ACKNOWLEDGEMENTS

This work was supported in part by research grants from the Japanese Ministry of Health and Welfare and Japanese Ministry of Education Culture and Science.

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Received: July 12, 2008 Revised: July 18, 2008 Accepted: July 19, 2008