Letter to the Editor

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Influence of vitamin K antagonist treatment on activated partial thromboplastin time

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To the Editor,

Vitamin K antagonists (VKA) perform their anticoagulant effect by inhibiting vitamin K epoxide reductase, an enzyme that recycles oxidized vitamin K to its reduced form. Vitamin K is a cofactor for the post-translational carboxylation of several vitamin K-dependent proteins. Consequently, VKA result in defective synthesis of the vitamin K-dependent clotting factors: factor (F) II, VII, IX, and X. The recommended method used for monitoring VKA is determination of the international normalized ratio (INR) which is derived from the prothrombin time (PT) [1]. This test is sensitive to the levels of FII, FVII and FX, but is not responsive to reduction of FIX, which can be detected by activated partial thromboplastin time (aPTT). APTT measures the efficacy of both the intrinsic and the common coagulation pathway and is also influenced by vitamin K-dependent clotting factors (FIX, FX and FII). Therefore, among patients treated with VKA, a prolonged aPTT is observed. The relationship between an elevated INR in a patient on VKA treatment with the aPTT is not known and probably depends on the FIX level. Very low FIX in patients on VKA treatment, due to mutation in the FIX gene, with prolonged aPTT have been described, but is very rare [2]. This relationship likely depends also on the combination of aPTT reagent and instrument utilized and aPTT reagents differ largely in sensitivity to coagulation factor deficiency [3]. Although aPTT measurement is not indicated in VKA monitoring, the aPTT is widely used as part of a coagulation screening panel. In case of VKA-treated patients a prolonged aPTT is often due to the VKA, although the measure of prolongation of aPTT in VKA-treated patients is unknown. Misinterpretation of a prolonged aPTT should be avoided and therefore the sensitivity of the aPTT reagent towards INR in AVK-treated patients can be informative.

In this study, we have evaluated the relationship between INR and aPTT in patients on VKA anticoagulant treatment and the sensitivity of three different aPTT reagents, PTT-A (Diagnostica Stago, Asnières-sur-Seine, France), Cephascreen® (Diagnostica Stago) and C.K. Prest® (Diagnostica Stago).

Patients from the Ghent University Hospital treated with VKA and monitored by PT/INR were collected. The study was approved by the Belgian regulatory agency (B670201419819). The trial was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Ghent University Hospital (registration no. 2014/101). Samples with an INR >1.5 were prospectively and consecutively collected from January to April 2014 and frozen at –20 °C in different aliquots within 4 h after sampling. The aPTT with three different reagents (PTT-A, C.K. Prest® and Cephascreen®) and PT/INR (Neoplastine® CI plus, Diagnostica Stago) were measured. A simple linear regression analysis with 95% and 90% prediction limits between aPTT and INR was made using SPPS® Statistics 20 (IBM Business Analytics, Armonk, NY, USA). Samples with disproportionate aPTT ('outliers') were identified as those with an aPTT outside 90% prediction limits and were further analyzed. To measure clotting factor activity the one-stage clotting assay was used. STA®-Thrombin 2 (Diagnostica Stago) and Biophen Heparin LRT (Hyphen BioMed, Andresy, France) were used to measure, respectively, thrombin time (TT) and anti-Xa activity.

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Lupus anticoagulant (LA) was detected according to the SSC guidelines using the dilute Russell's Viper Venom Test (dRVVT) (LA screen and LA confirm, Life Diagnostics, DSRV, Clarkstons, MI, USA) and a sensitive aPTT assay (PTT-LA and Staclot® LA, Diagnostica Stago) [4]. C-reactive protein (CRP) was measured on Cobas®8000 (Roche Diagnostics, Belgium).

Seven hundred samples were collected. In a first step the aPTT with the three different reagents and PT/INR were measured (Figure 1). A Grubb's outlier test (α=0.05) on bias between INR measured during the study and the routinely measured INR was performed to exclude samples (n=45) influenced by the freezing/thawing cycle. Linear regression analysis between aPTT and INR (n=655) was performed. Samples of patients with disproportionate aPTT were identified by using the 95% and 90% prediction limits (Figure 2). In total 55 outliers were identified, five samples with a disproportionate short aPTT and 50 with a disproportionate prolonged aPTT for corresponding INR. In Table 1 the mean aPTT of all samples within the prediction limits and the upper limit of aPTT was calculated for the three aPTT reagents for INR of 2, 3 and 4.

Next, we further analyzed the samples beyond the 90% prediction limit with at least one of the aPTT reagents. Samples with a disproportional short aPTT (n=5) were examined for FVIII and FIX activity. In four out of the five samples the elevated FVIII level >150% (normal range, 60%–150%) could explain the disproportional short aPTT. One sample showed a normal FVIII activity of 103% and the FIX activity was low (23.3%) and thus could not explain the disproportion. FII, FX and FVII were, respectively, 46%, 28%, and 2%. FVII influences strongly the PT-INR [5]. The discrepancy between the relatively high FII and FX level and very low FVII level could explain the disproportional low aPTT (respectively, 67.4 s, 48.8 s, and 63.4 s with PTT-A, C.K. Prest® and Cephascreen®) for high INR of 14.1.

Samples with disproportional prolonged aPTT (n=50) were further analyzed for TT and anti-Xa activity to rule out the presence of heparin (UFH) or low molecular weight heparin (LMWH). In 37 out of 50 samples (74%) presence of UFH or LMWH was confirmed. We explored the remaining 13 out of 50 samples by consulting the medical and laboratory history of these patients and extra analyses. Five out of 13 samples showed the presence of LA. LA interferes with clotting in phospholipid dependent clotting tests, such as aPTT and explains the prolonged aPTT [4]. In four out of the 13 samples CRP was >180 mg/dL

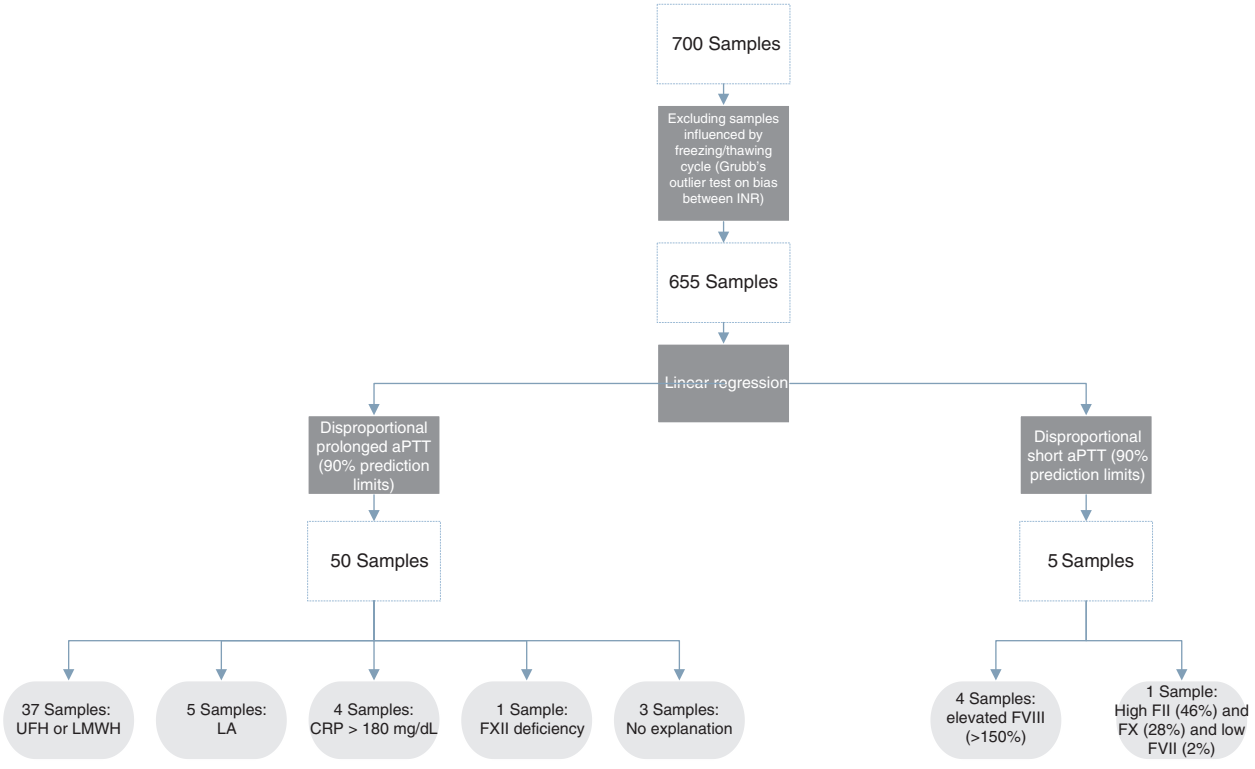

Figure 1 Flowchart of the selection of the samples and the results.
aPTT, activated partial thromboplastin time; CRP, C reactive protein; F, factor; INR, international normalized ratio; LA, lupus anticoagulant; LMWH, low molecular weight heparin; PT, prothrombin time; UFH, unfractioned heparin.

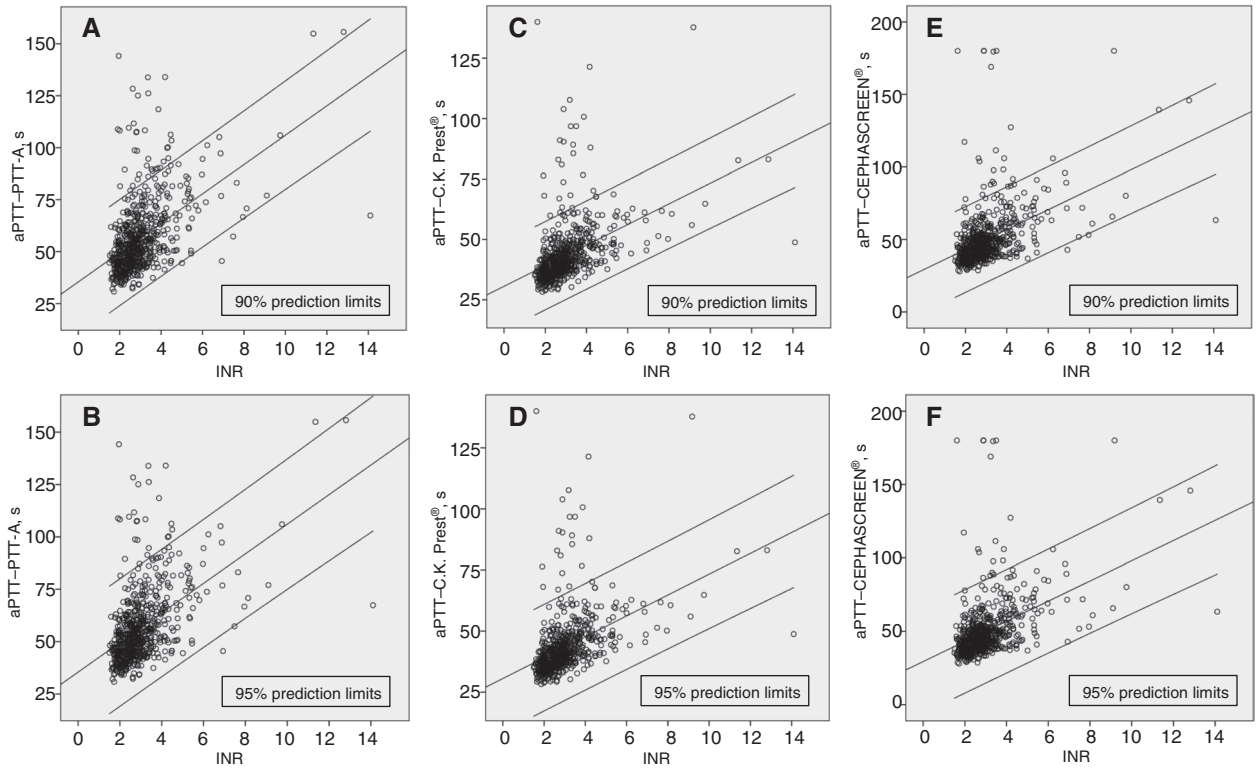

Figure 2 APTT values in relation to INR for 655 samples.
APTT measured with three different reagents, respectively, PTT-A (A, B), C.K. Prest®(C, D) and CEPHASCREEN® (E, F). The linear regression analysis revealed the following relation between INR and aPTT: aPTT (PTT-A)=35.4+7.1 INR, aPTT (C.K. Prest®)=30.6+4.3 INR and aPTT (CEPHASCREEN®)=29.4+6.9 INR. The lines indicate the linear regression line and the upper and lower 90% and 95%, respectively, in A, B, C and D, E and F, prediction limits of the linear regression between the aPTT and INR.

(Table 2). CRP interacts with phospholipids and results in an in vitro prolongation of phospholipid-dependent clotting tests, similar to the in vitro prolongation of coagulation tests by LA due to interference with accumulation of coagulation factors on negatively charged phospholipids [6, 7]. The influence is reagent dependent as observed in the present data and explains the disproportional prolonged aPTT with Cephascreen® and PTT-A, reagents sensitive to high CRP concentration and not with C.K. Prest®, a reagent insensitive for CRP interference [7]. Furthermore, we found one sample with a factor FXII deficiency.

For three out of the 13 samples we could not identify a cause for the disproportional prolonged aPTT (CRP

Table 1 Mean aPTT for three different aPTT reagents (PTT-A, C.K. Prest® and Cephascreen®) excluding 5% and 10% of the data, with disproportionate prolonged and shortened aPTT and the upper limit of the expected aPTT with the 95% and 90% prediction limits of the linear regression analysis for INR of 2, 3 and 4.

aPTT reagent	INR	Mean aPTT, s – 5% data	Mean aPTT, s – 10% data	aPTT, s 95% PL	aPTT, s 90% PL
PTT-A	2	46.9	46.8	<79.1	<74.6
	3	54.8	54.2	<86.1	<81.7
	4	62.7	61.6	<93.2	<88.7
C.K. Prest®	2	37.6	37.5	<60.3	<56.6
	3	41.9	41.7	<64.6	<60.8
	4	46.2	45.9	<68.9	<65.1
Cephascreen®	2	40.8	40.8	<76.4	<71.7
	3	47.8	47.6	<83.3	<78.6
	4	54.8	54.5	<90.2	<85.5

aPTT, activated partial thromboplastin time; PL, prediction limit; PT, prothrombin time.

Table 2 INR and aPTT with three different reagents (PTT-A, C.K. Prest® and Cephascreen®) of four samples with CRP >180 mg/L.

INR	aPTT PTT-A, s	aPTT C.K. Prest®, s	aPTT Cephascreen®, s	CRP, mg/L
4.3	99.9	56.3	91.8	184.4
6.2	101.2	62.8	105.8	332.4
11.4	154.9	82.7	139.4	405.9
12.8	155.7	83.1	145.8	>400

aPTT, activated partial thromboplastin time; CRP, C reactive protein; INR, international normalized ration.

level, respectively, 5, 8 and 42 mg/L). One of these samples showed a FIX activity of only 7.6% for an INR of 3.8. As described by Legnani et al. vitamin K antagonist can greatly influence the factor IX activity but the sensitivity of factor IX activity to VKA varies greatly between individuals with a similar anticoagulation intensity level as measured by INR [2]. See Supplemental Data, Table 1, for an overview of the aPTT values and INR of the 55 samples with disproportional short and prolonged aPTT and indication of the cause.

In conclusion, the rate of aPTT prolongation in VKA-treated patients is reagent dependent. We calculated the expected prolongation of aPTT with three different reagents (PTT-A, C.K. Prest® and Cephascreen®) for INR of 2, 3 and 4. An extensively prolonged aPTT in VKA-treated patients may not be due to VKA alone. Disproportional prolonged aPTT in patients on VKA treatment merit further attention and should be investigated to avoid misinterpretation. In 47 of the 50 samples (94%) we found another cause resulting in a prolonged aPTT: presence of UFH/LMWH (n=37), LA (n=5) high CRP (n=4) and FXII deficiency (n=1).

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