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ORIGINAL ARTICLE

Determinants of the APTT- and ETP-based APC sensitivity tests

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Summary. *Background:* A reduced sensitivity for activated protein C (APC) is associated with an increased risk of venous thrombosis even in the absence of the factor (F)V Leiden mutation. This risk has been demonstrated with two APC sensitivity tests, which quantify the effects of APC on the activated partial thromboplastin time (APTT) and the endogenous thrombin potential (ETP), respectively. *Objectives:* We examined determinants of both APC sensitivity tests in the control group of the Leiden Thrombophilia Study (LETS). *Methods:* Multiple linear regression analysis was performed with normalized APC-SR_{APTT} or APC-SR_{ETP} as dependent variable and putative determinants [levels of FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII A subunit, FXIII B subunit, protein S total, protein S free, protein C, tissue factor pathway inhibitor (TFPI) total, TFPI free, antithrombin and fibrinogen] as independent variables. *Results and conclusions:* The major determinant of the APTT-based test was FVIII level, followed by FII level. The ETP-based test was influenced most by free protein S and free TFPI levels. In both tests FXa formation plays a major role, as the effect of FVIII and TFPI on the tests seems to be executed via FXa. The ETP-based test was also strongly influenced by oral contraceptive use, even when we adjusted for all the clotting factors listed above. This means that the effect of oral contraceptives on the ETP-based test is not fully explained by the changes of coagulation factor levels investigated in this study, and that the molecular basis of acquired APC resistance during use of oral contraceptives remains to be established.

Keywords: activated protein C resistance, factor V Leiden.

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Introduction

Activated protein C (APC), in combination with its cofactor protein S, acts as an anticoagulant by inactivating the activated cofactors factor (F)Va and FVIIIa by specific peptide bond cleavages. Factor V acts together with protein S as a cofactor of APC in the inactivation of activated FVIII. In 1993, Dahlbäck *et al.* reported a new mechanism for thrombophilia, characterized by a poor anticoagulant response of plasma to APC [1]. Most cases of this so-called APC resistance are caused by a mutation in one of the APC cleavage sites (Arg506) of FV (FV Leiden) [2]. This mutation is a common risk factor for venous thrombosis.

Several methods for the detection of APC resistance have been developed. The original test of Dahlbäck *et al.* evaluates the increase of the activated partial thromboplastin time (APTT) after addition of a fixed amount of APC to plasma [1]. Reduced prolongation of the APTT indicates resistance to the anticoagulant action of APC. The sensitivity and specificity for the FV Leiden mutation is dependent on the APTT reagent used [3]. A disadvantage of the APTT-based test is that it cannot be used in patients using oral anticoagulants or with lupus anticoagulants, because of their prolonged baseline APTT.

Rosing and co-workers developed an APC sensitivity test which is based on quantification of the effect of APC on the endogenous thrombin potential (ETP), i.e. on the time integral of thrombin generated in plasma in which coagulation is initiated via the extrinsic pathway [4,5].

In addition to the above-mentioned APTT- and ETP-based tests several other APC sensitivity tests were developed based on the prolongation by APC of the prothrombin time, the FXa clotting time, the Russell Viper venom time, or the textarin time. Also modified tests, in which patient plasma is diluted in FV-deficient plasma, have been developed which have a high sensitivity and specificity for the FV Leiden mutation also in patients using oral anticoagulants and in patients with lupus anticoagulants. A disadvantage of these latter tests is that they cannot detect a reduced sensitivity for APC in the absence of FV Leiden.

It has been demonstrated with both the APTT- and the ETP-based APC sensitivity test that a reduced sensitivity for APC not because of FV Leiden also increases the risk of venous thrombosis [6–8]. Causes of such a reduced response to APC are for instance pregnancy, oral contraceptive use, hormone replacement therapy, lupus anticoagulants, and high FVIII levels. The detection of these acquired APC-resistant phenotypes depends on the test that is used, e.g. in the ETP-based test plasma of women using oral contraceptives appeared to be considerably APC-resistant [9], while in the APTT-based test FVIII levels seemed to be a major determinant [6,10]. So, the APTT- and the ETP-based APC sensitivity tests are both global tests that can predict thrombosis risk [6–8], but they seem to have different determinants. The aim of this study was to examine extensively the determinants of the APTT- and ETP-based APC sensitivity tests. Knowledge of the main determinants may help us understand the mechanisms leading to APC resistance. For our investigation we used a large group of individuals without a history of venous thrombosis (the control group of the Leiden Thrombophilia Study, LETS).

Patients and methods

Subjects

The investigated subjects were 474 individuals (202 men and 272 women) without a personal history of thrombosis from a population-based case–control study on venous thrombosis (LETS) [11]. For the present investigation all subjects with the FV Leiden mutation were excluded ($n = 14$). Furthermore, analysis was restricted to subjects in which all investigated parameters [FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII A and B subunit, protein S total, protein S free, protein C, tissue factor pathway inhibitor (TFPI) free, TFPI total, antithrombin, fibrinogen, normalized APC-SR_{APTT} and normalized APC-SR_{ETP}] were measured. Therefore, we excluded 47 subjects without free protein S data, one subject with missing TFPI data, four subjects with missing normalized APC-SR_{APTT}, and one subject who used oral anticoagulants and had missing free protein S and normalized APC-SR_{APTT} data. This selection resulted in 407 subjects available for analysis (166 men and 241 women with a mean age of 46 years; range: 16–73). For analyses concerning oral contraceptive use 391 subjects (166 men, 53 women using oral contraceptives and 172 women not using oral contraceptives) were used, because oral contraceptive use was unknown for 16 women.

Blood collection of LETS samples

Blood was collected into tubes containing 0.1 volume of 0.106 M trisodium citrate. Plasma was prepared by centrifugation for 10 min at 2000 *g* at room temperature and stored at −70 °C.

APTT-based APC sensitivity test

The sensitivity of the plasma APTT to APC was measured in Leiden as described before with Cephotest® (Nycomed Pharma, Oslo, Norway) as activator [11,12]. Because this APTT reagent contains a high concentration of phospholipids, the test is not influenced by the presence of residual platelets in plasma. The APC sensitivity ratio (APC-SR_{APTT}) is defined as the APTT in the presence of APC divided by the APTT in the absence of APC. To reduce between-assay variation results are expressed as normalized APC sensitivity ratios (n-APC-SR_{APTT}). The normalized APC-SR_{APTT} is calculated by dividing the APC-SR_{APTT} of the sample by the APC-SR_{APTT} of pooled normal plasma, which is measured in the same run. The pooled normal plasma was prepared in the same way as plasma of the LETS samples.

ETP-based APC sensitivity test

The sensitivity of the plasma ETP to APC was measured in Maastricht as described before [8,9]. Under these conditions the test is insensitive for small amounts of phospholipid present in plasma [13]. The normalized APC-SR_{ETP} is defined as the ratio of α2-macroglobulin-thrombin (α2M-IIa) determined in the presence and absence of APC divided by the same ratio determined in pooled normal plasma. The pooled normal plasma used for the ETP-based test was obtained using 0.1 volume of 0.13 M trisodium citrate as anticoagulant [9].

Determination of coagulation parameters

Measurements of FII activity [14], FV ag [15], FVII activity [16], FVIII activity [17], FIX ag [18], FX ag [19], FXI ag [20], FXII activity [21], FXIII A and B subunit ag [22], protein C activity [23], total protein S ag [23], free protein S ag [23], free TFPI ag [24], total TFPI ag [24], antithrombin activity [23], and fibrinogen levels [16] have been described before. The above-mentioned parameters were all expressed in U dL^{-1}, except fibrinogen levels that were expressed in g L^{-1} and free and total TFPI levels that were expressed in ng mL^{-1}. By definition 1 mL pooled normal plasma contains 1 unit.

Statistical analysis

To assess putative determinants of the normalized APC-SR multiple linear regression analysis was performed with normalized APC-SR_{APTT} or APC-SR_{ETP} as dependent variable and the determinants (levels of FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII A subunit, FXIII B subunit, protein S total, protein S free, protein C, TFPI total, TFPI free, antithrombin and fibrinogen) as independent variables (model 1, $n = 407$). The range and distribution of coagulation factor levels and normalized APC-SR_{APTT} and APC-SR_{ETP} differs. To facilitate comparison of the two tests all variables (normalized APC-SRs and coagulation factors) were expressed as standardized values (Z scores). Multiple linear regression analysis was performed

with these standardized values. The resulting standardized regression coefficient β for a coagulation factor indicates the increase of the normalized APC-SR (expressed in SD) when that particular coagulation factor increases with 1 SD and all other variables in the model are unchanged. To investigate the influence of sex, age, and oral contraceptive use, multiple linear regression analysis was performed with age (standardized), sex (0 = male, 1 = female), and oral contraceptive use at venepuncture (0 = no, 1 = yes) as additional independent variables (model 2, $n = 391$). In this model, the regression coefficient for a dichotomized variable (e.g. sex) indicates the average increase of the normalized APC-SR (in SD) for a particular subgroup (e.g. women) compared with another subgroup (e.g. men).

Results

In Fig. 1 the normalized APC-SR_{ETP} is plotted against the normalized APC-SR_{APTT}. Because APC inhibits thrombin formation and prolongs the APTT, plasmas with a reduced sensitivity for APC will give a decrease of the normalized APC-SR in the APTT-based test and an increase in the ETP-based test. The two normalized ratios were associated, but there was considerable variation (regression coefficient $\beta = -4.9$; 95% CI: −6.1 to −3.6). In Table 1, the mean normalized APC ratios for the two tests are shown for the whole population and stratified for sex and oral contraceptive use.

Results of the two APC sensitivity tests in the complete population-based case–control study have been described elsewhere [6,8]. The main purpose of the present study was to assess determinants of both tests by a multiple linear regression model. In this model (model 1, Table 2) the main determinant of the APTT-based test appeared to be FVIII level (standardized regression coefficient $\beta = -0.55$) followed by FII (prothrombin) level ($\beta = -0.25$). The ETP-based test was influenced most by free TFPI ($\beta = -0.39$) and free protein S levels ($\beta = -0.33$). Addition of sex, age, and oral contraceptive use to the model (model 2, Table 3), only resulted in marginal changes in the effect of the coagulation factors on the APTT-based test. The regression coefficients for the two most important determinants, FVIII ($\beta = -0.52$) and FII level ($\beta = -0.23$), hardly changed with this adjustment. However, adjustment for sex, age, and oral contraceptive use reduced the effects of the major determinants of the ETP-based test, namely free TFPI level ($\beta = -0.25$) and free protein S level ($\beta = -0.26$). Oral contraceptive use appeared to be a strong determinant of the ETP-based test. Even after adjustment for many coagulation factors (model 2), oral contraceptive use led to an average increase of the normalized APC-SR_{ETP} with 0.78 SD. The influence of oral contraceptive use on the APTT-based test was less ($\beta = -0.21$). In both tests women had a reduced sensitivity for APC compared with men. Sex had a larger effect on the ETP-based test ($\beta = 0.44$) than on the APTT-based test ($\beta = -0.24$). There was no influence of age (SD: 13.3) on the ETP-based test and only a small effect on the APTT-based test ($\beta = -0.13$).

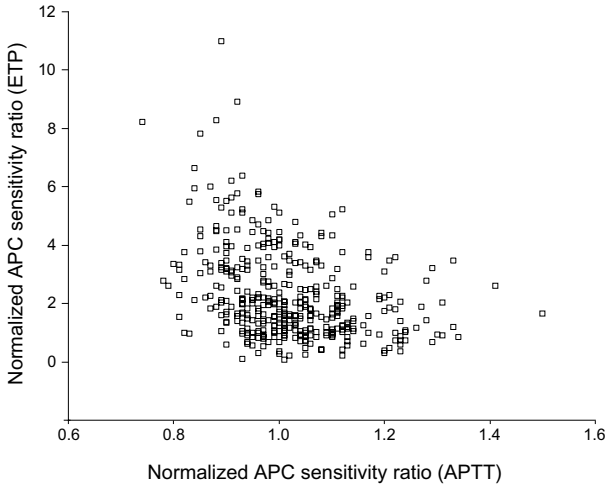

Fig. 1. Normalized activated protein C (APC)-SR_{ETP} vs. normalized APC-SR_{APTT}. Regression line: n-APC-$SR_{ETP} = 7.3 - 4.9 \times$ n-APC-SR_{APTT}.

Table 1 Mean APC sensitivity in healthy individuals

	n	n-APC-SR_{APTT} (95% CI)	n-APC-SR_{ETP} (95% CI)
All	407	1.02 (1.01–1.03)	2.33 (2.18–2.48)
Sex			
Men	166	1.05 (1.04–1.07)	1.38 (1.27–1.49)
Women	241	0.99 (0.98–1.00)	2.99 (2.78–3.19)
Use of oral contraceptives*			
Yes	53	0.94 (0.92–0.96)	4.88 (4.44–5.32)
No	172	1.00 (0.99–1.02)	2.43 (2.26–2.61)

*Oral contraceptive use at venepuncture was unknown for 16 women. APC, activated protein C; APTT, activated partial thromboplastin time; CI, confidence interval.

Because an APC sensitivity test is a global test, it is not surprising that the test is influenced by many of the examined variables. We examined more closely the coagulation parameters with the strongest effect on the two tests, i.e. levels of FII, FVIII, free protein S, and free TFPI. These four variables were stratified into quartiles and for each quartile the mean normalized APC-SR_{APTT} and the mean normalized APC-SR_{ETP} were calculated (Table 4). When FVIII levels increase, the mean normalized APC-SR_{APTT} decreases, while such a clear trend was not observed for the normalized APC-SR_{ETP}. A similar trend, but weaker, was seen for FII levels. In contrast, free protein S levels and free TFPI levels show an association with the normalized APC-SR_{ETP} and not with the normalized APC-SR_{APTT}.

Discussion

A reduced sensitivity for APC, as measured with the APTT- or the ETP-based APC sensitivity test, is associated with an increased risk of venous thrombosis both in the absence and presence of FV Leiden [6,8,11]. In this study we found that many coagulation factors have an effect on these global tests, with the APTT-based APC sensitivity test mainly influenced by

Table 2 Mean change in normalized APC-SR (SD*) with 1 SD increase in coagulation factor (model 1)

		β (95% CI)	
	SD	APTT-based test	ETP-based test
Factor II	15.0	−0.25 (−0.33 to −0.18)	0.15 (0.07–0.23)
Factor V	31.8	0.15 (0.07–0.22)	−0.14 (−0.21 to −0.06)
Factor VII	21.7	−0.09 (−0.17 to −0.02)	0.10 (0.03–0.18)
Factor VIII	32.5	−0.55 (−0.62 to −0.48)	0.05 (−0.02 to 0.12)
Factor IX	19.8	−0.12 (−0.21 to −0.04)	0.20 (0.11–0.28)
Factor X	16.8	−0.12 (0.21 to −0.04)	0.11 (0.02–0.19)
Factor XI	19.5	−0.07 (−0.14 to −0.001)	0.06 (−0.01 to 0.13)
Factor XII	28.4	0.08 (0.01–0.15)	0.02 (−0.06 to 0.09)
Factor XIII A	23.7	0.03 (−0.07 to 0.13)	0 (−0.10 to 0.10)
Factor XIII B	18.5	0.01 (−0.10 to 0.12)	0.02 (−0.09 to 0.13)
Protein S total	19.7	0.01 (−0.08 to 0.11)	−0.18 (−0.27 to −0.09)
Protein S free	20.5	0.10 (0.01–0.19)	−0.33 (−0.42 to −0.23)
Protein C	18.1	−0.15 (−0.22 to −0.07)	0.02 (−0.06 to 0.10)
TFPI total	15.1	−0.02 (−0.11 to 0.07)	−0.03 (−0.12 to 0.06)
TFPI free	4.81	0.13 (0.03–0.22)	−0.39 (−0.49 to −0.30)
Antithrombin	10.5	0.02 (−0.05 to 0.09)	−0.07 (−0.15 to −0.01)
Fibrinogen	0.65	0.07 (−0.01 to 0.16)	−0.03 (−0.11 to 0.06)

Standardized regression coefficients β were obtained with multivariate linear regression analysis.
*Standard deviation n-APC-SR_{APTT} = 0.11 and standard deviation n-APC-SR_{ETP} = 1.54.
APC, activated protein C; CI, confidence interval; APTT, activated partial thromboplastin time; ETP, endogenous thrombin potential; TFPI, tissue factor pathway inhibitor.

Table 3 Multiple linear regression including age, sex, and oral contraceptive use (model 2)

	β (95% CI)	
	APTT-based test	ETP-based test
Factor II	−0.23 (−0.31 to −0.15)	0.07 (−0.001 to 0.15)
Factor V	0.13 (0.06–0.21)	−0.11 (−0.17 to −0.04)
Factor VII	−0.07 (−0.15 to 0.01)	0.07 (0.001–0.14)
Factor VIII	−0.52 (−0.60 to −0.45)	0.04 (−0.03 to 0.11)
Factor IX	−0.11 (−0.20 to −0.03)	0.16 (0.08–0.24)
Factor X	−0.15 (−0.24 to −0.05)	0.03 (−0.05 to 0.12)
Factor XI	−0.04 (−0.12 to 0.03)	0.03 (−0.03 to 0.10)
Factor XII	0.09 (0.02–0.17)	−0.03 (−0.10 to 0.04)
Factor XIII A	0.05 (−0.05 to 0.16)	−0.02 (−0.12 to 0.07)
Factor XIII B	−0.01 (−0.12 to 0.11)	0.06 (−0.05 to 0.16)
Protein S total	0.01 (−0.09 to 0.11)	−0.09 (−0.18 to 0.01)
Protein S free	0.09 (−0.01 to 0.18)	−0.26 (−0.35 to −0.17)
Protein C	−0.13 (−0.21 to −0.05)	−0.01 (−0.08 to 0.07)
TFPI total	−0.02 (−0.11 to 0.07)	0.02 (−0.06 to 0.11)
TFPI free	0.09 (−0.01 to 0.19)	−0.25 (−0.34 to −0.15)
Antithrombin	−0.01 (−0.09 to 0.06)	−0.05 (−0.12 to 0.02)
Fibrinogen	0.10 (0.01–0.18)	−0.05 (−0.12 to 0.03)
Age	−0.13 (−0.21 to −0.04)	−0.04 (−0.12 to 0.04)
Sex*	−0.24 (−0.39 to −0.08)	0.44 (0.29–0.59)
Oral contraceptive use†	−0.21 (−0.51 to 0.10)	0.78 (0.50–1.07)

Standardized regression coefficients β were obtained with multivariate linear regression analysis.
*0 = male and 1 = female.
†0 = no and 1 = yes.
CI, confidence interval; APTT, activated partial thromboplastin time; TFPI, tissue factor pathway inhibitor; ETP, endogenous thrombin potential.

FVIII levels and the ETP-based test by free TFPI and free protein S levels. The latter test was also highly dependent on oral contraceptive use.

Table 4 Mean normalized APC-SR_{APTT} and mean normalized APC-SR_{ETP} for quartiles of factor II, factor VIII, protein S free, and TFPI free

	Number of subjects	Mean n-APC-SR_{APTT} (95% CI)	Mean n-APC-SR_{ETP} (95% CI)
Factor II (U dL^{-1})			
< 94	99	1.07 (1.05–1.09)	2.21 (1.94–2.47)
94–103	106	1.03 (1.01–1.06)	2.21 (1.93–2.49)
103–113	97	1.01 (0.99–1.04)	2.57 (2.17–2.97)
> 113	105	0.96 (0.94–0.97)	2.36 (2.10–2.62)
Factor VIII (U dL^{-1})			
< 99	101	1.11 (1.09–1.14)	2.11 (1.89–2.34)
99–119	105	1.03 (1.02–1.05)	2.39 (2.06–2.73)
119–143	99	0.99 (0.98–1.01)	2.32 (2.01–2.63)
> 143	102	0.93 (0.92–0.94)	2.50 (2.18–2.83)
Protein S free (U dL^{-1})			
< 83	99	1.02 (1.00–1.05)	3.34 (2.98–3.71)
83–96	104	1.03 (1.01–1.06)	2.54 (2.25–2.83)
96–111	99	1.02 (1.00–1.04)	1.82 (1.61–2.03)
> 111	105	1.00 (0.98–1.02)	1.66 (1.45–1.87)
TFPI free (ng mL^{-1})			
< 9.5	101	0.99 (0.97–1.01)	3.96 (3.63–4.30)
9.5–12.5	102	1.05 (1.02–1.07)	2.24 (2.02–2.46)
12.5–15.3	102	1.02 (1.00–1.03)	1.84 (1.67–2.02)
> 15.3	102	1.02 (1.00–1.04)	1.31 (1.15–1.46)

APC, activated protein C; APTT, activated partial thromboplastin time; ETP, endogenous thrombin potential; TFPI, tissue factor pathway inhibitor; CI, confidence interval.

We found that increased FII (prothrombin) levels reduced the sensitivity for APC in the APTT-based test. In a model system containing purified proteins it has been demonstrated before that prothrombin inhibits the ability of APC to inactivate FVa [25]. Furthermore, it has been shown in a group of 285 normal subjects that hyperprothrombinemia may

lead to APC resistance [26]. By diluting normal plasma in FII-deficient plasma de Ronde and Bertina previously demonstrated that a reduction in prothrombin level leads to an increased sensitivity for APC in the APTT-based test [12]. Castaman *et al.* obtained the same results by adding purified prothrombin to normal plasma [27]. In the same study, the response to APC was assessed in families with the prothrombin 20210A variation, which is associated with increased prothrombin levels [14]. A reduced sensitivity for APC was found in 20210A heterozygotes compared with wild-type carriers [27]. All these studies suggest that FII levels influence the APTT-based APC sensitivity test. Our results show, however, that this effect is moderate and weaker than the observed effect of FVIII levels on the APTT-based test.

A reduction in sensitivity for APC at increased FVIII levels was demonstrated earlier both with our local APTT-based APC sensitivity test (with Cephotest® as activator) [6] and with a commercial APTT-based APC sensitivity test (Coatest® APC™ Resistance, Chromogenix, Milan, Italy) [10,28]. No significant effect of FVIII was observed in the ETP-based test, although the data in Table 4 suggest that FVIII levels may have a minor effect on the normalized APC-SR_{ETP}. The insensitivity of the ETP-based test for FVIII levels is probably due to the high tissue factor concentration present in the ETP test used in this study. Lowering the tissue factor concentration probably will make thrombin generation more dependent on the concentration of the components of the tenase complex (FIX, FVIII) [29]. It will be of interest to see whether reduction of the tissue factor concentration will result in a higher sensitivity of the ETP-based test for FVIII levels.

The fact that the APTT-based test is greatly influenced by FVIII levels suggests that FXa formation plays an important role in this test. An increase in FVIII level will lead to increased activation of FX. Increased FXa formation can result in a reduced sensitivity for APC, as it was shown that FXa protects FVa from inactivation by APC [30–34] by selectively blocking cleavage at Arg506 [35], which results in slow inactivation of FVa via APC-mediated cleavage at Arg306 [36–38]. FXa formation also seems to be important in the ETP-based test. Namely, in agreement with previous findings [39], we found that the latter test is influenced by free TFPI levels, with a reduction in free TFPI level leading to a reduced sensitivity for APC. TFPI is an inhibitor that regulates the extrinsic (tissue factor-induced) coagulation pathway. TFPI inhibits FXa and the FXa/TFPI complex is a potent inhibitor of the tissue factor/FVIIa complex (for a review see Ref. [40]). When free TFPI levels are reduced this will lead to an initial increase in FXa formation, which, by the above-mentioned mechanism (i.e. protection at Arg506) [35], might result in a reduced sensitivity for APC. Curvers *et al.* [13] demonstrated that the efficacy by which APC inhibits thrombin formation in the ETP-based test is decreased at high tissue factor concentrations [13]. In view of this observation it is not surprising that the ETP-based test is influenced by free TFPI levels.

We confirmed that a reduction in free protein S level leads to a reduced sensitivity in the ETP-based APC sensitivity test [39,41,42]. In contrast, de Ronde and Bertina showed before by diluting normal plasma in protein S-deficient plasma that the APTT-based test is only affected by very low protein S levels (< 20 U dL^{-1}) [12]. Protein S is a cofactor of APC, which specifically accelerates APC-mediated cleavage at Arg306 of FVa [35]. A reduced protein S level will lead to a reduced cleavage of FVa at Arg306 by APC, resulting in a delayed FVa inactivation and in increased thrombin formation. The fact that the ETP-based test is affected by protein S level whereas the APTT-based test is not, might suggest that the Arg306 cleavage is more important in the ETP-based test than in the APTT-based test.

The APTT-based APC sensitivity test was only moderately influenced by sex and oral contraceptive use, while the ETP-based test was strongly affected by these variables. In the latter test women have a reduced response to APC compared with men and oral contraceptive users have the lowest response. This effect cannot be explained by a reduction of protein S levels in oral contraceptive users, because in our study population free protein S levels were not materially different in women using oral contraceptives compared with non-users [43,44]. Furthermore, addition of oral contraceptive use to the multiple regression model (model 2) only slightly reduced the effect of free protein S on the test. Part of the effect of oral contraceptives on the APC response may be executed via TFPI. It has been reported that women taking oral contraceptives have reduced TFPI levels [45] and we showed here that adjustment for oral contraceptive use reduced the influence of free TFPI level on the test. Initiation of coagulation occurs in the case of the APTT via the intrinsic pathway and in the case of the ETP via the extrinsic pathway (tissue factor/FVIIa). This indicates that the basis of the impaired APC response in women using oral contraceptives may be sought in the activity or regulation of the extrinsic pathway. Because oral contraceptive use affects the concentrations of many proteins involved in blood coagulation [46] a combination of these effects will most likely lead to a reduced sensitivity for APC. However, in our analysis a strong effect of oral contraceptive use on the ETP-based test persisted ($\beta = 0.78$) when several coagulation factors were taken into account (model 2). This means that the effect of oral contraceptives on the ETP-based test is mediated by variables (e.g. coagulation factors or other plasma components) that were not included in our analysis. Also the differences in APC sensitivity between men and women in both the ETP-based and the APTT-based test are not fully explained by the other variables in the model, because an effect of sex ($\beta = -0.24$ for the APTT-based test and $\beta = 0.44$ for the ETP-based test) on the APC sensitivity still exists (model 2).

We observed a moderate correlation between the APC sensitivity measured with the APTT-based test and the sensitivity measured with the ETP-based test (see Fig. 1). It is therefore good to realize that a person who is typed 'APC-resistant' in the ETP-based test will not by definition be APC-resistant in the APTT-based test. The two global tests evaluate APC resistance via different pathways. It will be of interest to

investigate whether it is possible to design a test, which is sensitive for FVIII and prothrombin as well as free protein S, and free TFPI.

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