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The effects of sampling from a peripheral venous catheter compared to repeated venepunctures on markers of coagulation, inflammation, and endothelial function

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Abstract

Peripheral venous (PV) catheters are often used for serial blood sampling, but studies suggest that PV catheters increase markers of coagulation activation and inflammation. Whether the increase is caused by irritation of the vessel wall or diurnal variation is unknown. We therefore compared the effects of a PV catheter and repeated venepunctures on markers of coagulation, inflammation, and endothelial function.

A PV catheter was inserted at 07:45 in a hand vein in 10 healthy subjects, and blood samples were collected at 8:00, 10:00, 12:00, and 14:00. In the contralateral arm, blood was simultaneously obtained by venepunctures. Measures of coagulation, i.e. activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen, prothrombin fragment 1+2 (F1+2) and thrombin-antithrombin (TAT), inflammation, i.e. interleukin 6 (IL-6) and C-reactive protein (CRP), and endothelial function, i.e. plasminogen activator inhibitor 1 (PAI-1), tissue plasminogen activator (tPA), von Willebrand factor (vWF), and tissue factor (TF) were measured in plasma.

The concentrations of TAT and F1+2 were significantly increased (10:00; $p < 0.01$, 12:00; $p < 0.05$, and 14:00; $p < 0.01$) in PV catheter samples compared with venepuncture samples. There was a minor increase in PT and INR and no increase in APTT, fibrinogen, CRP, PAI-1, tPA, vWF, and TF, with no differences between sampling methods. IL-6 concentrations increased in many PV catheter samples and venepuncture samples, but the response varied between the subjects.

Blood collection through a PV catheter induces coagulation activation, whereas endothelial function is not affected. More studies are needed to disclose the effect of blood sampling on IL-6.

Key words: Serial blood sampling, peripheral venous catheter, coagulation activation, interleukin-6, plasminogen activator inhibitor 1, prothrombin fragment 1+2, thrombin-antithrombin, fibrinogen, C-reactive protein, tissue plasminogen activator, von Willebrand factor

Introduction

Thrombosis can be caused by disturbances of the haemostatic balance which may be reflected as changes in the levels of plasma markers involved in the pathways of coagulation and fibrinolysis. The intrinsic and extrinsic pathways of coagulation are routinely assessed by the activated partial thromboplastin time (APTT) and prothrombin time (PT), respectively, whereas plasma biomarkers, such as prothrombin fragment 1+2 (F1+2), thrombin-antithrombin (TAT), fibrinogen and D-Dimer, are used as measures of coagulation activation and fibrin metabolism [1-4]. The haemostatic system is interrelated with inflammatory pathways as well as the vascular endothelium. Activation of coagulation is thereby linked to the plasma levels of inflammatory markers such as interleukin-6 (IL-6) and C-reactive protein (CRP), but also endothelial derived proteins such as tissue plasminogen activator (tPA), plasminogen activator inhibitor 1 (PAI-1), von Willebrand factor (vWF), and tissue factor (TF) [5-8]. These biomarkers are often analysed in clinical studies on cardiovascular risk.

During blood sampling, caution must be taken not to trigger the activation of these pathways leading to falsely elevated levels of biomarkers. Standard venepuncture is the recommended blood sampling technique for coagulation testing studies, and the optimal collection procedure is to use a straight, clean, and visible vein from an area free from skin lesions and scars. However, repeated venepuncture is inconvenient and frequently associated with pain, bruising, and skin damage, increasing the risk of hematoma, nerve injury and infection [9]. A peripheral venous (PV) catheter with only a single venepuncture may be the most suitable option in clinical trials requiring serial blood samples obtained at hourly intervals for measuring markers of coagulation, inflammation, and endothelial function. However, a PV catheter may irritate the vessel wall and falsely affect the markers compared with repeated venepunctures.

Several studies have investigated the use of a PV catheter with respect to markers of coagulation and inflammation, but the results are inconsistent due to different study designs and methodologies [10-16]. Some of the studies suggest that the use of medical devices (such as a catheter) interferes with measured levels of the coagulation activation markers TAT and F1+2 [10,15] and the inflammation marker IL-6 [11,13,14], but these studies did not compare repeated venepuncture samples collected simultaneously to repeated catheter samples. In the present study, serial blood samples were obtained simultaneously by a PV catheter in one arm and repeated venepunctures in the contralateral arm. The study aim was to compare the effects of a PV catheter

and repeated venepunctures in healthy volunteers on selected markers of coagulation, inflammation, and endothelial function in serial blood samples.

Materials and Methods

Study participants

The study included 10 healthy non-fasting volunteers employed at local departments at the University Hospital of Southern Denmark, Esbjerg, Denmark. The study was conducted according to the Danish Law and the Declaration of Helsinki, and informed consent was obtained from all participants. According to the Danish regulations, no ethical approval was required since all blood samples and data were stored and handled anonymously in a methodological quality assurance study. There were no exclusion criteria.

Blood sampling

Blood sampling was performed according to the guidelines recommended by the Danish Society for Clinical Biochemistry [9,17,18], and the procedure was carried out by highly experienced technicians. A BD Venflon™ Pro Safety Shielded IV Catheter (Becton Dickinson, Franklin Lakes, USA) was inserted into the cephalic hand vein at 07:45. Blood sampling from both arms of each subject was performed simultaneously after 10 minutes of standardized rest in a chair. While drawing blood samples from the catheter, a tourniquet was applied to the contralateral arm (minimal stasis), and a 21G needle with a winged collection set (butterfly) was used to draw blood from the antecubital vein. The blood samples were collected using the BD vacutainer system (Becton Dickinson, Plymouth, UK) at serial time points (08:00, 10:00, 12:00 and 14:00), and from the same position in the antecubital vein. The first 5 ml of blood was discarded, and the next 4 x 2.7 ml blood were collected into 0.3 ml (0.109 M) sodium citrate tubes (BD Ref:363048) and 3.0 ml blood were collected into EDTA tubes (BD Ref: 368856). Before and after each sampling, the catheter was flushed with 5 ml saline. Platelet poor plasma was prepared by centrifugation at 2000xg for 20 min. at 20°C.

A visual check for haemolysis was performed after each blood sampling, and samples with haemolysis were excluded prior to analysis. Plasma was aliquoted and stored at –80°C until analysed. All sample analyses were completed within 12 months of storage.

Laboratory methods

Commercial coagulation assays from Diagnostica Stago (Asnières-sur-Seine, France) were used to measure APTT (sec, STA[®]-PTT), PT (sec, STA[®]SPA+), fibrinogen ($\mu\text{mol/L}$, STA[®]-Liquid Fib), and D-dimer (STA[®] -LIATEST D-DI PLUS) on an automatic Stago STA-R[®] Evolution Coagulation Analyser. The International Normalized Ratio (INR) was calculated as a normalized PT raised to the power of the international sensitivity index (ISI) corresponding to the thromboplastin being used.

Commercial ELISA methods were used to measure the plasma concentrations of F1+2 (pmol/L, Enzygnost[®] F1+2 (monoclonal), Siemens Healthcare Diagnostics Products, Marburg, Germany), TAT ($\mu\text{g/L}$, Enzygnost TAT-micro, Siemens Healthcare Diagnostics Products), IL-6 (pg/mL, Human IL-6 Quantikine HS, R&D Systems, Minneapolis, USA), and TF (TF:Ag, pg/mL, Human TF Quantikine, R&D Systems). Concentrations of CRP (mg/L, CardioPhase[®] hsCRP) were determined on a nephelometer (Siemens Healthcare Diagnostics Products). The plasma concentration of vWF (vWF:Ag, %) was determined by an in-house ELISA using rabbit anti-human vWF polyclonal IgG as capture and detection antibodies (DAKO, Glostrup, Denmark, Ref. Nr. A0082). Results were expressed relative to a reference plasma (HemosIL calibration plasma 0020003700) calibrated (in percentage) against WHO's International Standard for vWF. Concentrations of PAI-1 (PAI-1:Ag, ng/mL) were measured using an in-house ELISA, as previously described [19].

Concentrations of tPA (tPA:Ag, ng/mL) were determined by an in-house ELISA using mouse anti-human tPA monoclonal IgG as capture (clone 15-4-21) and detection (clone 15-4-6) antibodies. In brief, MaxiSorp ELISA plates (NUNC, Roskilde, Denmark) were coated with 2 $\mu\text{g/mL}$ monoclonal anti-tPA antibody 15-4-21 in PBS overnight at 4°C. The plates were washed three times in PBS + 0.05% Tween-20, and the calibrator and samples were diluted in PBS + 20 mmol/L EDTA + 0.05% Tween-20 and applied to the plates in duplicates and incubated for 2 hours at room temperature. The detection antibody (biotinylated anti-tPA monoclonal antibody 15-4-6) was applied (2 $\mu\text{g/mL}$) and incubated for one hour at room temperature. Secondary detection was performed with HRP-conjugated streptavidin (GE Healthcare; catalog no. RPN1051) diluted 1:3000, and the reaction was initiated with o-phenylene-diamine (Kem-En-Tec, Taastrup, Denmark)/H₂O₂. The reaction was stopped by adding 1 mol/L sulphuric acid, and the plates were read at an optical density of 490 nm. Serial dilutions of a plasma pool spiked with 100 ng/mL single chain tPA from Technoclone, Vienna, Austria were included to establish the calibration curve.

The inter-assay coefficients of variation (CV) were 2.1 % for APTT, 4.6 % for PT, 4.3 % for fibrinogen, 3.3 % for D-dimer, 7.8 % for F1+2, 5.0 % for TAT, 6.5 % for IL-6, 6.3 % for TF:Ag, 3.1 % for CRP, 4.5 % for vWF, 8.2 % for PAI-1:Ag, and 5.1% for tPA:Ag. The inter-assay CVs for in-house developed assays were calculated as the average value of two inter-assay CVs based on 30 high and 30 low internal quality controls, respectively. For commercial assays, the inter-assay CVs were provided by the manufacturers and confirmed in our laboratory using the quality control included in the assay.

Statistics

The results were analysed using non-parametric tests. Variation over time in each group (PV catheter and venepuncture) was analysed using a Friedman test. When significant time effects were observed, baseline levels (at 08:00) were compared with levels at 10:00, 12:00, and 14:00 using a Wilcoxon Signed Ranks Test. The difference between blood sample techniques at 08:00, 10:00, 12:00, and 14:00 was analysed using Wilcoxon Signed Ranks Test.

Results are presented as median values and interquartile ranges. P-values <0.05 were considered statistically significant. The SPSS program (version 24; IBM SPSS Inc., Chicago, IL, USA) was used for the statistical analyses.

Results

There was a marked increase in the coagulation markers F1+2 ($p=0.001$) and TAT ($p=0.0001$) over time when blood samples were obtained with a PV catheter, but no increase over time when using repeated venepunctures (Figure 1). The plasma concentrations of F1+2 and TAT were significantly higher in samples obtained by the PV catheter at 10:00 (F1+2 and TAT: $p=0.005$), 12:00 (F1+2: $p=0.025$, TAT: $p=0.017$), and 14:00 (F1+2 and TAT: $p=0.005$) compared to samples obtained by venepuncture (Figure 1).

There was no increase in APTT, CRP and fibrinogen in blood sampled from a PV catheter. There was a minor increase in PT and INR over time in both PV catheter samples ($p=0.02$) and repeated venepunctures ($p=0.0001$), but no differences were observed between the sampling methods (Table 1).

There was no increase in markers of endothelial function when blood was drawn with a PV catheter. There was a marked decrease in PAI-1:Ag ($p=0.0001$) over time both when blood was obtained with a PV catheter and repeated venepunctures (Table 1). Also, a modest decrease in

tPA:Ag and vWF:Ag was observed over time although only significant in PV catheter samples for tPA:Ag ($p=0.039$; $p=0.16$ for vWF:Ag) and in venepuncture samples for vWF:Ag ($p=0.033$; $p=0.17$ for tPA:Ag). Concentrations of TF did not change over time (Table 1). When comparing the sampling methods, there were no significant differences at 08:00, 10:00, 12:00, and 14:00 for concentrations of PAI-1, tPA, vWF, and TF, respectively (Table 1).

The concentrations of IL-6 increased over time for both sampling methods although only significant when blood samples were obtained with a PV catheter ($p=0.001$) and not when using repeated venepunctures ($p=0.39$). There were no significant differences between concentrations of IL-6 at 10:00, 12:00, and 14:00 when comparing the PV catheter and venepunctures, but concentrations were significantly higher in venepuncture samples than in catheter samples obtained at 08:00 (Table 1). However, the IL-6 response varied between subjects indicating a large inter-individual variation. For instance, an increase in IL-6 concentrations over time using both sampling techniques was observed in six subjects, but with highly different concentration ranges. In the other four subjects, there was either no change in IL-6 over time ($n=2$) or increases in IL-6 with catheter only ($n=1$) or venepunctures only ($n=1$) (data not shown).

D-dimer could not be detected since most plasma concentrations of D-dimer were below the lower detection limit of the assay working range of $0.27 \mu\text{g/ml}$ (results not shown).

Discussion

The study aimed to determine whether a PV catheter can be used as an alternative to repeated venepunctures when measuring markers of coagulation, inflammation, and endothelial function in serial blood samples collected at hourly intervals in healthy individuals. Our study demonstrated increased coagulation activation measured as significantly increased plasma levels of F1+2 and TAT when a PV catheter was inserted for 2-6 hours and compared with repeated venepunctures (Figure 1). Also, PT and INR increased over time, but with no difference between sampling methods. There was no increase in APTT, fibrinogen, and CRP or in the endothelial derived proteins tPA, PAI-1, vWF and TF (Table 1). The marker of local vessel wall inflammation, IL-6, increased over time for both sampling methods although only significant in the PV catheter samples, and with a large inter-individual variation in the IL-6 response.

In our study, blood samples were obtained simultaneously from a PV catheter in one arm and by repeated venepunctures in the contralateral arm in order to use the venepuncture samples as controls for the catheter samples. The significant increase in F1+2 and TAT confirms previous

studies reporting increases in these markers following exposure to prolonged catheter and needle insertion [10,15,20]. Our study therefore demonstrates that insertion of a PV catheter is not suited for the determination of F1+2 and TAT.

Irritation of the endothelial vessel wall is a potential mechanism for increased coagulation activation due to release of TF, an initiator of the TF-pathway. However, the PV catheter did not increase the concentrations of TF, or the endothelial proteins tPA, PAI-1 and vWF, suggesting that the increase in F1+2 and TAT is not TF-dependent. Instead, we observe a significant decrease in tPA and PAI-1 which may be due to a known circadian variation [21].

Contact activation induced by artificial surfaces (e.g. a catheter) may result in coagulation activation [22], and cause the time dependent increase in F1+2 and TAT observed in the samples collected by PV catheter. Here, blood collected in presence of corn trypsin inhibitor (CTI), which specifically inhibits FXIIa [23], could elucidate whether catheter-induced contact activation is responsible for the recorded increase in F1+2 and TAT. This issue, however, was not addressed in the present study.

Several studies have observed that IL-6 increases in serial blood samples collected during the day, and these studies suggest that the use of a PV catheter is a possible confounder [11,13,14]. On the other hand, two studies have measured IL-6 in serial blood samples drawn by a catheter without reporting any effects on IL-6 [16,24]. There seems to be a diurnal variation in IL-6 with a drop in the morning followed by an increase in the afternoon, which is also observed in the present study [25]. However, the large variation in IL-6 could indicate that IL-6 is a sensitive marker that is prone to local effects induced by serial sampling, irrespective of the sampling method. Thus, the release of IL-6 might depend on activated endothelial cells, immune cells and nerves located at the sampling site [14,26]. More studies are needed to disclose the effect of blood sampling on IL-6.

The commonly used routine variables APTT, PT, INR, fibrinogen, and CRP did not differ between sampling methods. The PT was subjected to a minor, but significant prolongation over time, most likely due to diurnal variation [27]. These routine variables do not reflect local effects at the sampling site, but are either synthesized by the liver (fibrinogen and CRP) or reflect combined effects of liver-synthesized coagulation proteins (APTT, PT, and INR), and the results are therefore as expected and can be used as negative controls in the study.

The strengths of the present study are the simultaneous collection of serial blood samples by PV catheter and venepuncture, but also that blood samples were collected and handled by highly experienced technicians and nurses using standardized procedures. To our knowledge, this is the

first study to investigate the effect of a PV catheter on haemostatic endothelial proteins (PAI-1, tPA, vWF, and TF). Our results, however, are limited to blood samples obtained 2-6 hours after insertion of a PV catheter in healthy subjects. Some markers may show a different pattern in atherosclerotic or hypercoagulable patients or in blood samples collected over a longer period of time. Also, we mainly used immunological assays, and therefore we cannot make conclusions about activity levels. The study only included 10 subjects, and the non-significant increase in IL-6 in venepuncture samples might be due to a type 2 error. Ideally, the catheter should be inserted into the antecubital vein, the same anatomical site as the venepuncture sampling, but mobilization of the arm throughout the day could disturb the position of the catheter and by that cause false positive results. Further, the size and type of catheter may influence the results, but our study does not allow conclusions about e.g. central catheters compared with PV catheters.

In conclusion, the use of a PV catheter has a significant effect on coagulation activation reflected as increased concentrations of F1+2 and TAT, but no effects on APTT, PT, INR, fibrinogen, CRP, or the endothelial derived proteins PAI-1, tPA, vWF, and TF. The effects of blood sampling techniques on IL-6 are complex and need further investigation. The present study results indicate that future studies should use venepuncture as blood drawing technique for investigation of coagulation activation markers in serial blood samples.

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Disclosure statement

No potential conflict of interest was reported by the authors

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Table 1. Values of PAI-1, tPA, vWF, TF, IL-6, APTT, PT, INR, fibrinogen, and CRP in blood samples obtained by venepuncture and PV catheter over time

Variable	Venepuncture (n=10)	PV catheter (n=10)	P-value [#]
PAI-1:Ag (ng/mL)			
08:00	20.9 (17.7-29.0)	23.4 (17.5-29.1)	0.594
10:00	15.9 (13.0-19.6)**	17.5 (15.3-24.5)**	0.285
12:00	13.0 (8.7-16.3)**	14.0 (9.9-20.8)**	0.241
14:00	10.7 (7.8-18.5)**	12.2 (7.6-19.2)**	0.241
tPA:Ag (ng/mL)			
08:00	4.10 (2.37-7.12)	4.35 (2.45-7.85)	0.139
10:00	3.70 (2.20-6.87)	3.75 (2.27-7.25)*	0.437
12:00	3.75 (2.75-7.40)	3.45 (2.17-7.10)**	0.080
14:00	2.80 (2.20-7.17)	2.85 (2.47-7.05)	0.538
vWF:Ag (%)			
08:00	91 (67-111)	88 (68-109)	0.593
10:00	87 (69-126)	85 (72-117)	0.547
12:00	84 (69-119)	83 (69-104)	0.944
14:00	83 (67-109)	83 (70-111)	0.172
TF:Ag (pg/mL)			
08:00	49.2 (40.0-52.8)	49.7 (39.6-52.3)	0.417
10:00	45.2 (34.8-50.3)	45.7 (38.3-52.6)	0.308
12:00	44.2 (35.4-51.8)	45.8 (34.2-53.2)	0.508
14:00	46.9 (39.5-54.7)	46.7 (36.9-52.9)	0.059
IL-6 (pg/mL)			
08:00	1.03 (0.72-1.43)	0.91 (0.61-1.36)	0.005
10:00	1.02 (0.87-1.50)	0.99 (0.84-1.25)	0.185
12:00	1.69 (1.02-3.10)	1.31 (1.04-1.71)*	0.169
14:00	1.39 (1.10-2.89)	1.53 (1.16-2.44)*	0.959
APTT (sec)			
08:00	33.6 (30.8-35.6)	33.2 (31.3-35.6)	0.646
10:00	33.2 (30.9-35.9)	33.6 (30.4-36.0)	0.959
12:00	33.6 (30.5-35.3)	33.3 (30.7-35.3)	0.202
14:00	34.0 (30.6-35.8)	33.5 (30.4-35.5)	0.123
PT (sec)			
08:00	23.3 (21.6-26.1)	23.2 (21.7-26.7)	0.395
10:00	23.5 (21.4-26.8)	23.5 (21.8-26.7)	0.066
12:00	23.6 (21.9-27.0)**	24.0 (22.0-26.8)*	0.073
14:00	23.8 (22.0-27.5)**	23.8 (21.9-27.3)**	0.399

INR

08:00	0.99 (0.92-1.10)	0.99 (0.93-1.13)	1.000
10:00	0.99 (0.91-1.13)	1.00 (0.92-1.13)	0.084
12:00	1.01 (0.93-1.14)*	1.02 (0.94-1.13)*	0.230
14:00	1.01 (0.93-1.16)*	1.01 (0.93-1.15)*	0.739

Fibrinogen ($\mu\text{mol/L}$)

08:00	9.00 (7.37-9.72)	8.95 (7.45-9.77)	0.858
10:00	8.80 (7.30-9.60)	8.45 (7.55-9.82)	0.906
12:00	8.90 (7.40-9.42)	8.75 (7.50-9.12)	0.877
14:00	8.65 (7.42-9.15)	8.70 (7.37-9.30)	0.856

CRP (mg/L)

08:00	0.39 (0.32-1.21)	0.38 (0.32-1.21)	0.495
10:00	0.39 (0.31-1.24)	0.36 (0.30-1.29)	0.718
12:00	0.39 (0.29-1.26)	0.38 (0.29-1.26)	0.497
14:00	0.39 (0.29-1.24)	0.39 (0.29-1.20)	0.526

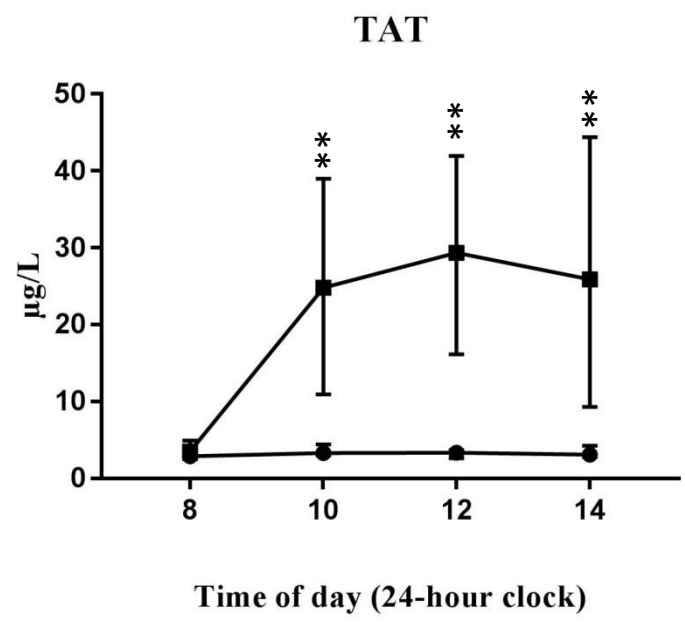
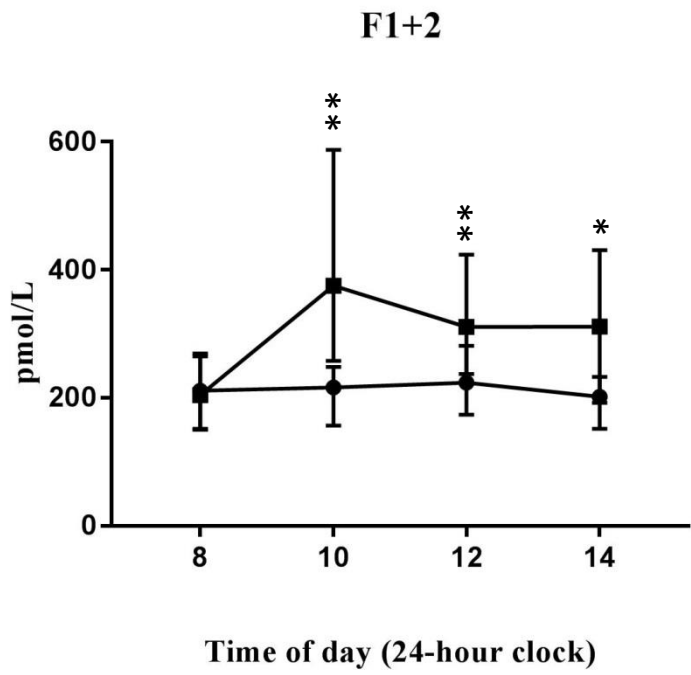


Figure 1

Legends to figures and tables

Figure 1. Concentrations of F1+2 and TAT in blood samples obtained by venepuncture (●) and PV catheter (■). Values are medians (error bars: interquartile range). Variation over time was analysed using a Friedman Test. When significant time effects were observed, pairwise comparisons with baseline (08.00) were performed using a Wilcoxon Signed Ranks Test; * $p < 0.05$, ** $p < 0.01$. F1+2: prothrombin fragment 1+2, TAT: thrombin-antithrombin, PV: peripheral venous.

Table 1. Values are medians (interquartile ranges). Variation over time was analysed using a Friedman test. When significant time effects were observed, pairwise comparisons with baseline (08:00) were performed using a Wilcoxon Signed Ranks Test (* $p < 0.05$, ** $p < 0.01$). # The difference between venepuncture and PV catheter was analysed using a Wilcoxon Signed Ranks test. PAI-1:Ag: plasminogen activator inhibitor 1 antigen, tPA:Ag: tissue plasminogen activator antigen, vWF:Ag: von Willebrand factor antigen, TF:Ag: tissue factor antigen, IL-6: interleukin 6, APTT: activated partial thromboplastin time, PT: prothrombin time, INR: international normalized ratio, CRP: C-reactive protein, PV: peripheral venous.

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