

Letters to the Editor

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Letter to the Editor regarding ‘effect of rivaroxaban, in contrast to heparin, is similar in neonatal and adult plasma’

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We are writing in relation to the paper by Novak *et al.* [1] entitled ‘Effect of rivaroxaban, in contrast to heparin, is similar in neonatal and adult plasma’, which was recently published in *Blood Coagulation and Fibrinolysis*.

We read this publication with interest as it represents an important contribution to the knowledge of how anti-coagulants interact with the plasma coagulation factors *in vitro*.

However, we are extremely concerned with the fact that the title of this study refers to ‘neonatal’ plasma, when in fact the samples that were used were cord blood samples. Blood samples obtained from neonates and blood samples obtained from cord blood are significantly different biological samples [2]. The cord plasma does not reflect the impact of vasculature (endothelium) and circulating cells, which plasma obtained from neonates does, and further, the changes in the neonatal coagulation system are so rapid that significant differences exist over the first few days of life [3]. Comparing results of previously published studies using neonatal plasma samples to results obtained using cord blood samples is not appropriate.

Hence, we believe that the more appropriate and importantly more accurate title for this study would replace the word ‘neonatal’ with ‘cord blood’.

In fact, we believe that there should be a general principle that studies of the cord blood should always use the terminology ‘cord blood’ rather than claim to represent neonatal studies.

Although the results of this study are interesting, studies on blood samples collected from neonates are more relevant studies when investigating how novel anti-coagulants such as rivaroxaban function in neonates, as it is neonates who will potentially receive the drug.

A second concern with this study is the concentrations of anticoagulants used during the experiments, which are far greater than clinical therapeutic doses.

Adequately designed studies are required to advance our understanding of the haemostatic system in neonates

and children and to allow clinicians to provide the best standard of care to these patient populations. Given the significance of such research and its translation into practice, it is essential that appropriate biological samples and clinically relevant drug concentrations are used.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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Prophylactic administration of fibrinogen concentrate in a pregnant woman with congenital hypofibrinogenemia and a positive obstetric history of severe bleeding in previous cesarean section

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Introduction

Congenital disorders of fibrinogen are uncommon and affect either the quantity (afibrinogenemia and hypofibrinogenemia) or/and the quality (dysfibrinogenemia/hypodysfibrinogenemia) of circulating fibrinogen. They consist hereditary diseases, associated mainly with bleeding episodes [1,2].

In a significant number of patients clinical manifestations, however, include thrombosis [3]; in the case of dysfibrinogenemia 10–20% of patients are reported with thrombotic complications [4].

Congenital hypofibrinogenemia is inherited either as an autosomal recessive trait of afibrinogenemia or in an autosomal dominant way with variable penetrance. It is associated with severe complications of pregnancy, such as recurrent spontaneous abortions, abruption placentae and bleeding during delivery or post partum [4,5].

Case

A 34-year-old woman presented at our hospital during her second pregnancy, at 39 weeks of gestation. She was admitted for programmed delivery by cesarean section. She had a positive family history of hypofibrinogenemia; her grandmother, her father and her sister had low fibrinogen plasma levels, but they had not suffered any episodes of hemorrhage or thrombosis. General medical history of the patient presented here was unremarkable, but her obstetric history included one abruption placentae at week 10 of gestation with subsequent abortion of the fetus and severe bleeding during her first cesarean section which required transfusion of 3 units of packed red blood cells.

On admission platelet (PLT) count was 161 K/ μ l. Routine clotting studies revealed low fibrinogen levels (78 mg/dl) and prolongation of thrombin time (TT = 42.3 s), whereas prothrombin time (PT) and partial thromboplastin time (PTT) were normal (10.9 and 26 s, respectively). A complete workup for her coagulation profile had been carried out but no abnormality had been detected.

Because of the positive obstetric history, fibrinogen replacement therapy was initiated, in order to increase fibrinogen levels up to 200 mg/dl before the cesarean section. Since cryoprecipitate was not available at that time, fibrinogen concentrate was the only therapeutic option. The administered dose of fibrinogen concentrate (mg/kg) was calculated according to the formula provided by the manufacturer: $\text{dose (mg/kg)} = [\text{target level (mg/dl)} - \text{measured level}] / 1.7$.

Although the patient received two doses of 3 g fibrinogen (hemocomplettan 1 g) within 6 h intervals, the fibrinogen plasma levels were still low (112 mg/dl). Therefore, it was decided to administer an additional dose of 3 g fibrinogen, and cesarean section was carried out immediately afterwards. At that time, the fibrinogen levels had been finally raised up to 144 mg/dl, TT had been reduced to 23.4 s, whereas PT and PTT values were normal (11 and 27.8 s, respectively). Unfortunately rotational thromboelastometry is not available in our hospital, so we could not further evaluate the effectiveness of the fibrinogen concentrate. The procedure was successfully completed and a healthy 3050-g male baby was born.

The postpartum course had no hemorrhagic complications. For that reason and to avoid any thrombotic

complications, we decided to administer prophylactic dose of low-molecular-weight heparin (Tinzaparin 3,500 anti-Xa IU, subcutaneously, once daily) 12 h post cesarean section and during the hospitalization period (overall 3 days). The patient was discharged 4 days later and by that time the fibrinogen plasma levels had been decreased to 91 mg/dl. PLT count was 160 K/ μ l on discharge and during patient's hospital stay remained within normal range (155–168 K/ μ l).

Discussion

Fibrinogen, a glycoprotein circulating in plasma, has a pivotal role in hemostasis [3,6] and it is also important for implantation and placentation during pregnancy; fibrinogen levels start elevating early in pregnancy in order to avoid placental abruption [4,7–9].

It is also known that fibrinogen disorders, quantitative or qualitative, are associated with pregnancy complications [4,7].

Fibrinogen replacement treatment is usually recommended for pregnant women with congenital hypofibrinogenemia [4]. According to the literature, fibrinogen levels should be maintained above 60 mg/dl during pregnancy to prevent placental abruption and at least 200 mg/dl at labor to prevent bleeding complications [4,10]. Substitution therapy with fibrinogen concentrate, though, should be weighed up carefully in each case, because of the risk of severe thrombotic events in patients with fibrinogen disorders [5]. It has been reported that severe thrombotic events occur in 25% of patients with dysfibrinogenemia [11] and in 4% of patients with afibrinogenemia [12]. Furthermore, treatment with fibrinogen substitute in the latter group of patients significantly raised the frequency of thrombotic complications, even though it prevented or treated hemorrhage [12,13]. On the contrary, it seems that the complication of congenital hypofibrinogenemia with thrombosis, including during pregnancy, has been attributed to the co-existence of a structural defect of the fibrinogen molecule [4]. Thus, identification of that specific population among pregnancies with hypofibrinogenemia before prophylactic replacement treatment is considered crucial. In those patients with dys-hypofibrinogenemia, the fibrinogen immunologic/fibrinogen clottable ratio would be greater than 1 since in patients with only quantitative disorder of fibrinogen the above ratio would be 1 or less. Unfortunately, co-existence of fibrinogen functional disorders could not be excluded via immunological or molecular assays in our patient. However, the final decision of initiating prophylactic therapy with fibrinogen was based on the fact that the woman had neither a hereditary nor an individual history of thrombotic events and, on the contrary, she had a severe bleeding episode during her previous cesarean section. In addition, as prophylactic administration of heparin at the time of therapy with fibrinogen concentrate is recommended, especially during postpartum period [3,5], we

initiated prophylactic co-administration of low-molecular-weight heparin 12 h post surgery and during the hospitalization period.

Fibrinogen levels in our patient failed to rise up to the target level of 200 mg/dl during delivery, yet postpartum course went uneventfully. It is well known that fibrinogen requirements increase significantly during the last weeks of gestation and that for several cases continuous infusion of fibrinogen is required during labor to prevent placental abruption. A target level of at least 150 mg/dl of fibrinogen is generally acceptable as well tolerated for surgery and cesarean section, although higher concentrations are considered preferable [14,15]. We believe that due to the patient's condition higher doses of fibrinogen were probably needed. Congenital hypofibrinogenemia is a rare entity; therefore, more experience is needed in order to establish a risk stratification of patients and an optimal fibrinogen concentration at which neither bleeding nor thrombotic complications occur.

Current therapeutic options for supplementation in patients with fibrinogen deficiencies are plasma, cryoprecipitate and fibrinogen concentrate [4,15,16]. From the one hand plasma and cryoprecipitate are less expensive, but usually require administration of larger volumes and lack virus inactivation/elimination processes. Especially for pregnant women this safety profile of fibrinogen concentrate against viral transmission is of great importance. Moreover, administration of fluids in large volume is also of concern in the peripartum period, since women in labor often receive other treatments (e.g. oxytocin) that predispose to fluid overload and edema. Even more importantly, plasma and cryoprecipitate contain unnecessary proteins, such as factor VIII, von Willebrand factor, that could be harmful [15]. In the case of pregnant women, these factors are already elevated, so administration of even more of these factors could trigger a thrombotic complication. Finally anaphylatoxins in plasma and cryoprecipitate could lead in severe allergic reactions [15].

In general, congenital disorders of fibrinogen in pregnant women are rare and there are not enough data in the literature about either pathogenesis or about proper management of related situations. In the case that was presented, administration of fibrinogen concentrate was judged absolutely necessary and successful in the prevention of severe bleeding during cesarean section and postpartum period, without any complications.

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Conflicts of interest

There are no conflicts of interest.

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Anaphylaxis with tissue plasminogen activator: case report and literature review

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Cases of anaphylaxis have been reported in patients receiving thrombolytic agents including urokinase, streptokinase, and rarely alteplase. Estimates of its occurrence range from 0.02% during alteplase treatment of acute myocardial infarction (MI), to approximately 2% during acute stroke [1]. Given that alteplase is structurally identical to endogenous plasminogen activator, the

incidence of anaphylaxis is much less than other fibrinolytics such as streptokinase [1]. Commercially available alteplase is produced by recombinant DNA technology from a human melanoma cell line (Chinese Hamster Ovary cells). Although fermentation is carried out in a medium using gentamicin, the antibiotic is not detectable in the final product. The product is a preservative-free lyophilized powder, which is reconstituted with Sterile Water for Injection, USP [2].

We present a case of an anaphylaxis event during alteplase administration and compare this presentation to other reports in the literature to identify commonalities.

A 23-year-old woman with past medical history of anemia and recent childbirth was diagnosed with a deep vein thrombosis (DVT) located in her right leg after presenting to the emergency department with diffuse pain in the region. The patient was started on warfarin until transitioned to enoxaparin subcutaneous injections twice daily.

Three weeks later, the patient presented for a planned inferior vena cava filter placement and catheter-guided thrombolysis using alteplase given as 16 mg intravenously (i.v.). During the procedure the patient noted numbness in her tongue and felt lip swelling. The patient developed urticarial rash in the upper thigh area without respiratory distress or wheezing, and received methylprednisolone 100 mg i.v. and diphenhydramine 50 mg i.v. Rigors

developed and the patient was given meperidine 25 mg i.v. All symptoms resolved within 30–60 min and the medical intensive care unit was consulted.

The patient was seen to be in no acute distress, breathing comfortably on room air without wheezes, and with mild bilateral redness on the upper thigh area. The patient was cleared for transfer from the postanesthesia care unit to the general medicine service for 24 h monitoring on telemetry.

Purposed mechanisms include initiation of the complement cascade by cleavage of C1 and C3 via plasmin leading to histamine release, as well as indirectly stimulating the breakdown of kininogen into bradykinin [1]. Early studies discovered that streptokinase, urokinase, and alteplase activate the complement cascade causing initial speculation that increased plasmin is associated with this phenomena [3].

Compared with C4 and C5, C3a is the most potent activator of mast cell degranulation and histamine release [1]. After alteplase administration to patients with acute MI, striking increases in C4a, C3a, and C5a have been observed [4]. Peak levels of C4a and C3a have been identified 15–30 min after alteplase initiation [5]. Another complement, C2-kinin, involved in this process is a potent vasodilator attributable to the hypotension observed in some reports [1]. Aside

Previous case reports

	Age (years), sex	Alteplase indication	Symptoms	Treatment (all via injection unless otherwise indicated)	Outcome
Case 1 [1]	74, Female	Stroke	Diffuse urticarial rash, facial angioedema, hypotension, oropharyngeal swelling	Intubation, hydrocortisone, diphenhydramine, ranitidine, crystalloid fluids	Eventual expiration 2 weeks later due to arrhythmia
Case 2 [1]	76, Female	Stroke	Facial angioedema	Methylprednisolone 80 mg, diphenhydramine 50 mg, ranitidine 25 mg	Symptom resolution within hours
Case 3 [6]	69, Male	MI	Facial angioedema, neck swelling, urticaria	Hydrocortisone 200 mg, chlorpheniramine 10 mg	Symptom resolution within hour
Case 4 [7]	70, Female	Stroke	Tachycardia, hypotension	Catecholamines, antihistamines	Symptom resolution within hour
Case 5 [8]	73, Female	Stroke	Facial angioedema	Intubation, hydrocortisone 200 mg, prochlorperazine 25 mg, ranitidine 50 mg, epinephrine 1 mg	Resolution of anaphylaxis and eventual discharge
Case 6 [9]	25, Male	DVT	Urticarial rash, severe epigastric pain	Change to urokinase with further epigastric pain; prednisolone 50 mg, antihistamines	Symptom resolution within hours; initiation of oral anticoagulants
Case 7 [10]	47, Female	Stroke	Facial angioedema	Clemastine, methylprednisolone	Symptom resolution within hours
Case 8 [10]	68, Female	Stroke	Facial angioedema	Clemastine, methylprednisolone	Symptom resolution within hours
Case 9 [11]	n/a	Stroke	Oropharyngeal swelling	Steroids, antihistamines	Symptom resolution within hours
Case 10 [11]	n/a	Stroke	Oropharyngeal swelling	Intubation, steroids, antihistamines	n/a
Case 11 [12]	75, Female	Stroke	Oropharyngeal swelling, tachycardia, urticarial rash	Intubation, methylprednisolone, diphenhydramine, epinephrine	Resolution of anaphylaxis and eventual discharge
Case 12 [13]	65, Female	Pulmonary embolism	Oropharyngeal swelling	Intubation, methylprednisolone, antihistamines	Resolution of anaphylaxis and eventual discharge
Case 13 [14]	51, Female	Stroke	Oropharyngeal swelling	Nasal trumpet, methylprednisolone 125 mg, diphenhydramine 50 mg	Symptom resolution within hours
Case 14 [15]	61, Female	Stroke	Hypotension, loss of consciousness, cold sweat, persistent gastrointestinal symptoms occurring minutes after exposure	Intubation, crystalline fluids, dopamine 10 mcg/kg per min	Anaphylaxis resolution within 1 h

from cleaving C3 and C1 to initiate the complement cascade, plasmin also activates factor XIIa converting prekallikrein to kallikrein causing breakdown of kininogen into bradykinin [1]. Bradykinin is also a potent vasodilator and may exacerbate hypotensive events.

Our patient experienced a similar course of anaphylaxis from alteplase consistent with previous case reports, characterized by urticarial rash and angioedema that was resolved within hours after treatment with corticosteroids and antihistamines. No previous patient experienced rigors, as this was a unique presentation during the anaphylaxis event.

Although our patient was not on an angiotensin-converting enzyme inhibitor (ACE inhibitor), it is noteworthy to mention that concomitant use of ACE inhibitors has been implicated in causing an exacerbated anaphylaxis event. ACE inhibitors also inhibit plasma kininases that would otherwise break down bradykinin to prevent prolonged vasodilation. Given the proposed mechanism of anaphylaxis development with alteplase wherein plasmin increases levels of bradykinin, the inhibition of its breakdown may potentially exacerbate hypotensive events. Case 1 had a previous history of angioedema after initiation of ACE inhibitors and beta-blockers in the past, suggesting predisposition to the severe anaphylactic reaction seen when given alteplase [1]. Case 10, 11, 13, and 14 were concurrently on an ACE inhibitor during the severe anaphylactic reactions to alteplase requiring mechanical intervention to maintain respiration [11,12,14,15].

Whereas all the cases mentioned involved anaphylaxis to a first time administration of alteplase, Case 6 had been receiving alteplase for 7 continuous days for treatment of a persistent thrombus before onset of symptoms. Continuing thrombolysis therapy was deemed necessary in this patient and urokinase was initiated with immediate epigastric pain [9].

Anaphylaxis to alteplase described in the cases has a consistent symptomology and course. The proposed mechanisms have been supported with laboratory data showing increased complement levels both *in vitro* and *in vivo*. The overproduction of plasmin both activates the complement cascade leading to histamine release and indirectly causes increased bradykinin production; together these mechanisms may cause angioedema, urticarial rash, and hypotension. Although a majority of the case reports detail occurrence of angioedema and urticarial hives with swift resolution after corticosteroid and antihistamine administration, intubation was required in two cases, one of which resulted in fatality. Alteplase is often given in emergency situations and although anaphylaxis does appear in the product labeling for alteplase, clinicians need to remain cognizant and prepared for anaphylaxis in patients.

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Conflicts of interest

There are no conflicts of interest.

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Glycosylation influences the stability of human plasminogen activator inhibitor-1

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Plasminogen activator inhibitor-1 (PAI-1) inhibits the activity of both tissue-type plasminogen activator

(t-PA) and urokinase-type plasminogen activator (u-PA) and, therefore, is an important regulator of the plasminogen activator system [1]. Under physiological conditions, active PAI-1 is rapidly converted to the latent conformation with an apparent half-life of 1–2 h at 37°C. Glycosylated PAI-1 can be produced in eukaryotic cells, cultured at 37°C. Due to the thermal instability of wild-type PAI-1 (PAI-1-wt) at 37°C, virtually all PAI-1-wt is converted to the latent conformation prior to purification. Glycosylated PAI-1 can be reactivated *in vitro* using denaturing agents, but the efficiency of these reactivation procedures remains rather low. In order to increase the PAI-1 stability, various mutants have been constructed, ultimately resulting in a PAI-1 variant with a half-life of about 150 h [2,3] when expressed as a nonglycosylated form in *Escherichia coli*. This stable variant has also been overexpressed in transgenic mice [4]. Next to an age-dependent coronary arterial thrombosis, these mice also revealed a number of phenotypic abnormalities including systemic amyloidosis, Alzheimer disease, extramedullary hematopoiesis and alopecia areata [4,5].

In the current study, we investigated whether these stabilizing mutations previously reported for nonglycosylated human PAI-1 (i.e. N150H, K154T, Q301P, Q319L and M354I) [3] result in a similar stabilization of glycosylated human PAI-1.

The pcDNA3.1(+)-human-PAI-1-N150H-K154T-Q301P-Q319L-M354I plasmid was successfully constructed using site-directed mutagenesis. The corresponding mutated protein (PAI-1-stab) was expressed in HEK293T cells and PAI-1 antigen and activity levels were verified 24, 48, 72 and 96 h posttransfection revealing an optimum activity/antigen ratio after 48 h. Purification on heparin sepharose yielded an average of $21 \pm 11 \mu\text{g}$ glycosylated PAI-1-stab protein per millilitre-conditioned medium.

PAI-1 activity toward t-PA was determined using a plasminogen-coupled chromogenic assay as described previously [6]. The specific activity of purified glycosylated PAI-1-stab was $168\,000 \pm 9\,800 \text{ U/mg}$ (mean $\pm$ SD;

$n=4$) corresponding to $23 \pm 1.3\%$ of the theoretical maximum value (i.e. $\pm 745\,000 \text{ U/mg}$). In contrast, for purified glycosylated PAI-1-wt, virtually no activity could be detected after purification (i.e. $4\,927 \pm 177 \text{ U/mg}$ or $0.66 \pm 0.024\%$ of the theoretical maximum value). In comparison, the t-PA inhibitory activity of purified nonglycosylated PAI-1-stab and nonglycosylated PAI-1-wt, expressed in *E. coli*, was $277\,000 \pm 64\,000$ and $270\,000 \pm 57\,000 \text{ U/mg}$, corresponding to 37 ± 8.6 and $36 \pm 7.7\%$ of the theoretical maximum value, respectively.

Functional distribution of PAI-1 was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis followed by quantitative densitometric scanning of the reaction products formed during the incubation of PAI-1 with a two-fold molar excess of t-PA. Under these conditions, glycosylated PAI-1-stab resulted in the formation of a covalent complex ($48 \pm 4.1\%$) corresponding to the fraction of active PAI-1, small amounts of cleaved PAI-1 ($17 \pm 4.2\%$), originating from the substrate pathway of PAI-1 and residual nonreactive PAI-1 corresponding to latent PAI-1 ($35 \pm 1.4\%$) (Table 1a). Nonglycosylated PAI-1-stab displayed a significantly increased substrate behavior in comparison to nonglycosylated PAI-1-wt and glycosylated PAI-1-stab. In line with its full latency, no complex formation was detected when glycosylated PAI-1-wt was incubated with t-PA. When PAI-1 was incubated at 37°C, the decrease in activity was in all cases associated with a concomitant increase in latent conformation (data not shown).

Two different methods were used to determine the functional stability of the active PAI-1 variants (Table 1b). In both methods, PAI-1 samples were incubated at 37°C and aliquots were collected at various time points. The percentage of active PAI-1 after different time intervals at 37°C was calculated either by the functional distribution assay or by the plasminogen-coupled chromogenic assay. The residual PAI-1 activity for each aliquot was expressed as percentage of the initial amount of active PAI-1 and the decay in PAI-1 activity was used to calculate the half-life

Table 1 Functional distribution versus t-PA (a) and functional stability (b) of the PAI-1 variants

(a)	Active	Nonreactive	Substrate
Glycosylated PAI-1-stab	48 ± 4.1	35 ± 1.4	17 ± 4.2
Glycosylated PAI-1-wt	<2	79 ± 4.9	21 ± 5.0
Nonglycosylated PAI-1-stab	57 ± 1.4	10 ± 3.5	$33 \pm 2.4^*$
Nonglycosylated PAI-1-wt	46 ± 3.8	41 ± 1.3	13 ± 2.5
(b)	Half-life (based on the functional distribution assay)	Half-life (based on the plasminogen coupled chromogenic assay)	
Glycosylated PAI-1-stab	$28 \pm 6.8^{**}$	$21 \pm 5.3^{**}$	
Glycosylated PAI-1-wt	NA	NA	
Nonglycosylated PAI-1-stab	185 ± 22	195 ± 62	
Nonglycosylated PAI-1-wt	1.8 ± 0.48	1.2 ± 0.20	

PAI-1, plasminogen activator inhibitor-1; PAI-1-wt, wild-type PAI-1; t-PA, tissue-type plasminogen activator. (a) Results are expressed as percentage of PAI-1 protein (mean $\pm$ SD; $n \geq 3$). (b) Results are expressed in hours (mean $\pm$ SD; $n \geq 3$). * $P < 0.05$ in comparison to nonglycosylated PAI-1-wt, glycosylated PAI-1-wt and glycosylated PAI-1-stab (unpaired Student's *t*-test). ** $P < 0.01$ in comparison to the half-life of nonglycosylated PAI-1-wt and nonglycosylated PAI-1-stab (unpaired Student's *t*-test).

(one-phase decay, GraphPad Prism software; GraphPad Inc. La Jolla, California, USA).

Glycosylated PAI-1-stab exhibited a half-life of 21–28 h (Table 1b). This half-life is significantly shorter than the half-life of nonglycosylated PAI-1-stab (i.e. 185–195 h) but significantly longer than the half-life observed for nonglycosylated PAI-1-wt (i.e. 1–2 h). The half-lives observed for nonglycosylated PAI-1-stab and nonglycosylated PAI-1-wt are in the same range as reported previously [2,3].

In conclusion, we expressed successfully a stabilized form of glycosylated human PAI-1 that displays measurable activity after purification, that is 34-fold higher activity than purified glycosylated PAI-1-wt. Strikingly, the half-life of glycosylated human PAI-1-stab (21–28 h at 37°C) is about eight-fold lower than that of the nonglycosylated stabilized variant, but significantly increased (i.e. 16 fold) in comparison to the half-life of nonglycosylated human PAI-1-wt. It is not clear at present why introduction of the same mutations does not result in a similar stabilizing effect in glycosylated versus nonglycosylated human PAI-1. The presence of glycan chains probably results in a slightly different structural conformation, thereby interfering with the stabilizing effect of one or more of the mutations. In addition, our data also illustrate that for PAI-1 mutants with altered properties that have been studied extensively *in vitro* using nonglycosylated variants [2,3], extrapolation of their properties *in vivo* (thus in the glycosylated form) [4,7] should be done with caution.

Nonetheless, the increased stability will facilitate further investigation of the glycosylated human PAI-1 structure and function and provides the additional benefit that active glycosylated PAI-1 is readily available for evaluation of PAI-1 inhibitory compounds that should react with glycosylated PAI-1 *in vivo*.

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Conflicts of interest

There are no conflicts of interest.

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Discard tube for coagulation testing: the debate continues

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In a recent article, Tekkeşin *et al.* [1] concluded that discard tubes are still required for prothrombin time (PT)/international normalized ratio (INR) and activated partial thromboplastin time (APTT) testing in order to avoid tissue factor contamination and sample activation that may be present in the first tube, but not in subsequent tubes. With this conclusion, derived by evidence, the authors asserted a statistically significant difference in coagulation test results between the first collection tube and the second collection tube. We refute their conclusion regarding the need for a discard tube based on their findings for several reasons.

First and foremost, although the authors reported a statistically significant difference between test times obtained by the first and second tubes, as based on a paired *t*-test, there was no evidence provided of any clinically significant difference. The magnitude of absolute differences was very small, being 0.18 s for PT, 0.03 for INR units, and 0.27 s for APTT. The Bland–Altman difference plots clearly showed nearly equal variation above and below the 0% point, consistent with random error, rather than systematic bias, and no clinically significant bias was evident in these data. That the differences observed were both positive (i.e. longer coagulation time in the second tube and, hence, potentially attributable to release of thromboplastin or other similar procoagulant substances in the former) and negative (i.e. shorter coagulation time in the second tube) is simply incompatible with the systematic presence of contaminating prothrombotic substances.

Accordingly, although a statistically significant variation of PT/INR and APTT were reported in this study, it seems implausible that this was due to systematic tissue factor contamination and sample activation. The authors' reported finding that clinically significant differences in INR were present in approximately 60% of total samples is simply inconsistent with the data presented in their report. Finally, the choice of a paired *t*-test for statistical evaluation of their dataset is also potentially questionable, as the authors provided no evidence that the data they obtained showed a normal distribution.

The recommendation provided by Tekkeşin *et al.* to continue to collect a discard tube for routine coagulation testing is in marked contradiction with the current Clinical and Laboratory Standards Institute (CLSI) guidelines [2], an expert group consensus guideline endorsed on the basis of results of a number of previous studies [3–6], demonstrating that that a discard tube is an unnecessary and unjustifiably expensive practice, at least for routine coagulation tests such as PT/INR and APTT. This conclusion has also been recently reinforced by studies by Raijmakers *et al.* [7] and Smock *et al.* [8] for both routine coagulation tests as well as more specialized tests of haemostasis.

We, thereby, conclude that the results reported by Tekkeşin *et al.* should be interpreted with caution. We continue to support the current CLSI recommendations in this regard, and assert that a discard tube is unnecessary when collecting samples for haemostasis tests, except in selected circumstances [9]. This would include when using butterfly devices, because the air space in the tube of this collection system may cause under-filling of the first-drawn tube [9,10], as well as perhaps when collecting

for testing of platelet function such as by the Platelet Function Analyzer-100 [11].

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Conflicts of interest

There are no conflicts of interest.

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