Journal of Thrombosis and Haemostasis, **4**: 372–376

ORIGINAL ARTICLE

Thrombin generation time is a novel parameter for monitoring enoxaparin therapy in patients with end-stage renal disease

D. F. BROPHY,*†§ E. J. MARTIN,§¶ T. W. B. GEHR,† A. M. BEST,‡ K. PAUL† and M. E. CARR Jr§¶
*Department of Pharmacy Practice, Virginia Commonwealth University/Medical College of Virginia, Richmond, VA; †Department of Internal Medicine, Virginia Commonwealth University/Medical College of Virginia, Richmond, VA; ‡Department of Biostatistics, Virginia Commonwealth University/Medical College of Virginia, Richmond, VA; §The Coagulation Special Studies Laboratory, Virginia Commonwealth University/Medical College of Virginia, Richmond, VA; and ¶Hemodyne, Inc., Richmond, VA, USA

To cite this article: Brophy DF, Martin EJ, Gehr TWB, Best AM, Paul K, Carr ME Jr. Thrombin generation time is a novel parameter for monitoring enoxaparin therapy in patients with end-stage renal disease. *J Thromb Haemost* 2006; **4**: 372–6.

Summary. *Background:* Patients with end-stage renal disease (ESRD) who receive enoxaparin are at increased risk for adverse bleeding episodes. This phenomenon appears to occur despite judicious monitoring of antifactor Xa (aFXa) activity. Better monitoring parameters are needed to quantify the anticoagulant effects of enoxaparin in the ESRD population. *Objectives:* The objective of this study was to determine the utility of using thrombin generation time (TGT), platelet contractile force (PCF) and clot elastic modulus (CEM) to monitor the degree of anticoagulation in ESRD subjects, and to compare these results to aFXa activity, the current gold-standard monitoring parameter. *Methods:* Eight healthy volunteers without renal dysfunction and eight ESRD subjects were enrolled into this study. Subjects received a single dose of enoxaparin 1 mg kg^{-1} subcutaneously, and blood samples were obtained for the determination of aFXa activity, TGT, PCF and CEM at baseline, 4, 8, and 12 h postdose. *Results:* Baseline, 4, 8, and 12-h aFXa activity concentrations were not different between groups. However, the corresponding TGT at 8 and 12 h was significantly prolonged in the ESRD group ($P = 0.04$, and $P = 0.008$, respectively). The 4-h peak TGT trended toward significance ($P = 0.06$). There were no differences in PCF or CEM across time. *Conclusions:* These data suggest that the parameter aFXa activity is a poor predictor of the anticoagulant effect of enoxaparin in patients with ESRD. Thrombin generation time appears to be more sensitive to the antithrombotic effects of enoxaparin in this population. Further large-scale trials are needed to corroborate these data.

Keywords: antifactor Xa activity, enoxaparin, thrombin generation time.

Correspondence: D. F. Brophy, Virginia Commonwealth University/Medical College of Virginia, School of Pharmacy, PO Box 980533, Richmond, VA 23298-0533, USA.
Tel.: +804 827 1455; fax: +804 828 8359; e-mail: dbrophy@vcu.edu

Received 15 July 2005, accepted 10 October 2005

Introduction

End-stage renal disease (ESRD) patients who are anticoagulated with low molecular weight heparin (LMWH) drugs such as enoxaparin are at high-risk for adverse bleeding events [1,2]. There is a significant body of literature documenting this phenomenon.

The prevailing explanation for the increased bleeding risk associated with enoxaparin in this population is that its antifactor Xa (aFXa) activity is eliminated via renal mechanisms. Pharmacokinetic investigations have documented a significant linear correlation between creatinine clearance and enoxaparin clearance [3,4]. Therefore, as the glomerular filtration rate (GFR) declines, there is a corresponding reduction in antifactor Xa elimination in this population. To prevent overdosing and to potentially reduce adverse bleeding events, the Seventh American College of Chest Physicians Conference on Antithrombotic and Thrombolytic Therapy [1] and the manufacturer of enoxaparin [5] recommend judicious monitoring of aFXa activity in patients with renal dysfunction.

Although aFXa activity is the current gold-standard monitoring marker for the plasma concentration of enoxaparin, it is poorly correlated to efficacy or toxicity [6–8]. Indeed, the relationship between the target range for aFXa activity and clinical efficacy and toxicity remains unclear. Debate still exists as to its clinical utility as a meaningful laboratory monitor [9,10]. This may be explained, in part, because factor Xa is merely an intermediate step in a highly complex biochemical pathway.

Our new understanding of coagulation revolves around the evolving mechanisms of thrombin generation as demonstrated by the working groups of Dr Mann and Dr Roberts [11–19]. These proposed models of coagulation have recently extended our understanding of the critical roles of thrombin and platelets in the coagulation process. Thrombin not only converts fibrinogen to fibrin, but it is also one of the most potent stimuli for platelet activation. Our laboratory has developed a whole blood assay (Hemodyne Hemostasis Analyzer™;

© 2006 International Society on Thrombosis and Haemostasis

Hemodyne®, Inc., Richmond, VA, USA) that provides a global evaluation of the integrity of the coagulation system by reporting the parameters thrombin generation time (TGT), platelet contractile force (PCF), and clot elastic modulus (CEM). TGT is the speed at which thrombin is generated in whole blood. PCF is the force produced by platelets during clot retraction and therefore a measure of platelet function during clotting. CEM is measured simultaneously with PCF and it reflects the structural integrity of the clot. The normal ranges for TGT, PCF and CEM are 4–8 min, 6.6–9.1 kdyne, and 13.2–24.4 kdyne cm^{-2}, respectively [20]. We have successfully used these parameters to monitor the pharmacological effects of various antiplatelet, anticoagulant and hemostatic drugs [20–25]. Moreover, we have used these parameters to preliminarily describe the *in vitro* effects of enoxaparin in patients with renal dysfunction [26–28].

The primary objective of this study was to determine the *in vivo* utility of measuring these parameters to describe the degree of anticoagulation following enoxaparin exposure in ESRD patients, compared with the gold-standard monitoring parameter aFXa activity.

Methods

Study design and population

This was a prospective, open-label, *in vivo*, study of the effects of enoxaparin in patients with and without renal dysfunction. The Virginia Commonwealth University (VCU) Institutional Review Board and VCU General Clinical Research Center approved this study prior to subject enrollment, and this study was conducted in compliance with the Declaration of Helsinki. A total of 16 subjects were enrolled in this study: eight healthy normal volunteers and eight ESRD subjects who received maintenance hemodialysis. All study interventions and measurements were obtained during an off-dialysis day. All subjects were anticoagulant naïve, non-thrombosed, and otherwise healthy. Subjects were admitted into this study if they were > 18 years of age and provided written informed consent. In addition, subjects with ESRD must have received maintenance hemodialysis for at least 3 months. Subjects were excluded from this investigation if they had any of the following: active bleeding or thrombotic disorder; pregnancy; recent trauma or surgery (< 7 days); cirrhosis or other liver abnormality; hematocrit $< 30\%$; active cancer; had received a blood transfusion within 1 week of study enrollment; thrombocytopenia (platelets $< 100\,000$ mL^{-1}); documented history of antithrombin III, protein C or protein S deficiency; concurrent use of anticoagulant or antiplatelet drug therapy. All subjects who provided written informed consent and who met the above criteria underwent screening evaluation, which consisted of a routine physical examination and laboratory evaluation [basic metabolic panel, complete blood count (CBC), international normalized ratio (INR), activated partial thromboplastin time (aPTT), prothrombin time (PT)].

Study procedures

Each eligible subject was admitted to the VCU General Clinical Research Center for a 12-h dosing and blood sampling period. Pre-dose blood samples (3.2% sodium citrate blue top tubes) were collected via aseptic venipuncture to determine baseline aFXa activity, TGT, PCF and CEM. Subjects then received a single-dose of enoxaparin 1 mg kg^{-1} subcutaneously (s.c.) in their abdomen. Blood sampling was conducted at 4, 8, and 12 h postdose to determine the pharmacokinetics of aFXa as well as the pharmacodynamics of TGT, PCF and CEM over time.

Specimen processing and analysis

Baseline chemistries (basic metabolic profile, CBC, PT, aPTT, INR) and aFXa concentrations were processed and analyzed at the VCU Health System Department of Pathology. Plasma enoxaparin aFXa activity was measured using a validated, commercially available chromogenic method (STA® heparin colorimetric analyzer; Diagnostica Stago, Parsippany, NJ, USA), and the results expressed as IU mL^{-1}. The lower limit of aFXa detection for this assay was 0.05 IU mL^{-1}; the coefficient of variation (CV) was $\pm 3\%$. Whole blood samples were analyzed for TGT, PCF and CEM at the VCU Coagulation Special Studies Laboratory. These parameters were simultaneously measured using the Hemodyne Hemostasis Analysis System™ (Hemodyne®). Batroxobin was purchased as Atroxin®, *Bothrops atrox* venom protein (Sigma Diagnostics, St Louis, MO, USA), dissolved in deionized water and used without further modification. It was maintained on ice and used within 3 h of being reconstituted. Nanopure water was used in the preparation of all solutions. All clots were formed using 700 μL of citrated whole blood. Clotting was initiated at time zero by adding calcium chloride ($CaCl_2$) and batroxobin. Final clotting conditions included: $CaCl_2$ 10 mM, batroxobin 0.21 μg mL^{-1}, pH 7.4, ionic strength 0.15 M and a final volume of 0.750 mL. PCF development was measured for 1200 s (20 min) and recorded in kdynes. CEM was measured concurrently and reported in kdynes cm^{-2}, and the time between calcium addition and initial platelet contractile force development was designated as the TGT. The coefficient of variation (CV) for this instrument was $\pm 7\%$.

Statistical analysis

All statistical procedures were performed using JMP statistical software version 5.1 (SAS Institute, Cary, NC, USA). Descriptive statistics (mean ± SD) characterized each group's baseline subject characteristics and laboratory parameters. Descriptive statistics (least squares mean ± SD, maximum and minimum range) for baseline and 4, 8, and 12-h aFXa activity, TGT, PCF and CEM were performed for each group. Repeated measures analysis of variance was used to detect intergroup differences in each parameter (aFXa, TGT, PCF

© 2006 International Society on Thrombosis and Haemostasis

and CEM) at each time interval. All statistical tests were performed at the significance level $\alpha = 0.05$.

Results

Sixteen subjects completed this study. Their demographic data are listed in Table 1. Intergroup baseline demographics were similar with the exception of age, renal function, platelet count and aPTT. Although the mean platelet count and aPTT values were different between groups, the clinical relevance of these findings is limited, given both parameters were in the normal range for each group.

Table 2 displays the respective aFXa activity, TGT, PCF and CEM parameters across the 12-h time interval. There were no intergroup differences in the aFXa activities achieved throughout the dosing interval, however, there was a trend toward a greater 4-h peak aFXa level in the controls compared with the ESRD group (0.64 vs. 0.53 IU mL^{-1}, respectively, $P = 0.06$). Despite achieving similar aFXa activities, the ESRD group tended to have prolonged TGT values across the 12-h time interval. The peak TGT at 4 h trended toward significant prolongation (13.0 vs. 10.8 min, respectively, $P = 0.06$), and reached statistical significance at 8 h (9.8 vs. 7.4 min, respectively, $P = 0.04$) and 12 h (8.1 vs. 5.0 min, respectively, $P = 0.008$). At the 12-h time point, the TGT was prolonged by more than 60% in the ESRD group compared with the controls. There were no intergroup differences in PCF or CEM across time.

Discussion

The intended mechanism of any anticoagulant drug is to inhibit the generation of thrombin. Therefore, the laboratory parameter TGT has the potential to become an important clinical monitoring parameter. It is evolving from merely a benchtop research assay into a clinically useful instrument to monitor the thrombosis and hemostasis status of patients. Thrombin generation time can be rapidly performed on whole blood [20], and can provide a detailed analysis of the critical interaction between platelets and thrombin.

The results of this study failed to find any differences in aFXa disposition over 12 h between normal volunteers and patients with ESRD. This was relatively unexpected given the previous reports of a correlation between enoxaparin clearance and GFR [3,4]. It should be noted, however, that other studies have reported similar pharmacokinetic parameters within these two populations [29]. Although a more formal pharmacokinetic discussion is beyond the scope of this paper, one potential explanation for this finding is that this was a single-dose study, which would not account for drug accumulation upon multiple dosing.

This study highlights the limitations of merely monitoring the peak aFXa activity concentration in patients with renal dysfunction. As shown in Table 2, if clinicians simply monitored the peak aFXa concentration at 4 h in ESRD patients, they may feel relatively confident that their enoxaparin dosing regimen was adequate. However, based on the respective TGT, the ESRD group is 20% more anticoagulated at 4 h; 32% more anticoagulated at 8 h; and 62% more anticoagulated at 12 h. In fact, the 12-h TGT of 8.1 min in the ESRD group is not much different than the 4-h peak TGT in the controls (10.8 min). These relative differences in TGT would be likely to become much more disparate upon repeated dosing. Not only do these data confirm previous *in vitro* reports from our laboratory [27,28], but they may help begin to explain why ESRD patients tend to have a greater incidence of bleeding episodes despite clinicians tailoring enoxaparin dosing to maintain aFXa activity in the target range of 0.5–1.2 IU mL^{-1}.

The exact mechanism of the enhanced TGT in the ESRD patients is difficult to explain, however, several potential mechanisms should be explored. First, the pharmacokinetics of enoxaparin are poorly understood. Enoxaparin is not a single molecular entity, but rather a conglomerate of small to medium chain heparin molecules less than 5000 Da. Thus, there is no available assay that directly measures the enoxaparin 'molecule' in plasma. Therefore, clinicians must rely on aFXa concentrations as a surrogate marker. The elimination of enoxaparin is thought to occur via non-saturable renal mechanisms [30–32], however, other investigations have suggested other metabolic pathways [33,34]. It is possible that enoxaparin, and other LMWH drugs, undergo metabolism to smaller, active heparin saccharide fragments that are undetectable by conventional aFXa assays [34]. These active metabolites may possess antithrombotic properties and may be renally excreted. Thus in ESRD, metabolite accumulation occurs, thereby resulting in prolonged TGT without an appreciable increase in the aFXa concentration. Secondly, enoxaparin is known to have other mechanisms besides the inhibition of factor Xa. These mechanisms include antifactor IIa (anti-thrombin) activity, a high

Table 1 Subject characteristics

Parameter	Control ($n = 8$)	ESRD ($n = 8$)	P-value
Race	4 black/4 white	6 black/2 white	ns
Sex	3 F/5 M	4 F/4 M	ns
Age (years)	29.8 ± 9.6	47.9 ± 6.9	0.0007
Weight (kg)	83.1 ± 19.2	79.3 ± 28	ns
Enoxaparin dose (mg)	82.5 ± 20.4	79.5 ± 27.3	ns
GFR (mL min^{-1})	95.0 ± 15.4	–	
Platelets (×10^{9})	285.5 ± 51.5	218.3 ± 62.0	0.03
Hemoglobin (g dL^{-1})	14.1 ± 1.38	13.0 ± 2.0	ns
Hematocrit (%)	41.5 ± 3.9	40.0 ± 6.0	ns
BUN (mg dL^{-1})	13.3 ± 1.9	37.8 ± 13.8	0.002
Serum creatinine (mg dL^{-1})	1.0 ± 0.2	9.6 ± 3.2	< 0.0001
aPTT (s)	30.8 ± 2.8	35.9 ± 4.8	0.02
PT (s)	9.9 ± 0.4	10.1 ± 1.8	ns
Kt/V	–	1.7 ± 0.3	

Where appropriate, data are presented as mean ± SD.
ESRD, end-stage renal disease; BUN, blood urea nitrogen aPTT, activated partial thromboplastin time; PT, prothrombin time; ns, not significant.

© 2006 International Society on Thrombosis and Haemostasis

Table 2 Least squares mean ± SD. Antifactor Xa, TGT, PCF and CEM estimates at each time interval

Hours postdose	Antifactor Xa Activity (IU mL^{-1})			TGT (min)			PCF (Kdynes)			CEM (Kdynes cm^{-2})		
	Control	ESRD	*P*	Control	ESRD	*P*	Control	ESRD	*P*	Control	ESRD	*P*
0	0.0	0.0	ns	4.3 ± 0.8	4.3 ± 1.3	ns	8.9 ± 0.9	8.5 ± 2.2	ns	21.2 ± 4.0	23.1 ± 6.7	ns
4	0.64 ± 0.12	0.53 ± 0.14	ns*	10.8 ± 1.8	13.0 ± 4.5	ns*	4.0 ± 1.9	3.3 ± 1.9	ns	11.9 ± 2.9	9.5 ± 6.1	ns
8	0.41 ± 0.14	0.38 ± 0.13	ns	7.4 ± 1.6	9.8 ± 3.1	0.04	6.6 ± 1.1	5.2 ± 2.6	ns	15.7 ± 3.0	13.3 ± 4.8	ns
12	0.23 ± 0.14	0.25 ± 0.09	ns	5.0 ± 0.9	8.1 ± 1.6	0.008	7.9 ± 0.8	6.7 ± 1.6	ns	17.9 ± 1.8	17.8 ± 3.7	ns

ESRD, end-stage renal disease; TGT, thrombin generation time; PCF, platelet contractile force; CEM, clot elastic modulus; ns, not significant. *$P = 0.06$.

binding affinity for antithrombin III, and inhibition of thrombin via the prothrombinase enzyme complex [30], as well as release of heparan sulfate or tissue factor pathway inhibitor from the endothelium [34]. Although the relative contributions of these mechanisms to anticoagulation appear to be non-significant in patients without renal dysfunction [30], they may play a larger role in patients with ESRD. Third, it is possible that the ESRD subjects in this study had acquired platelet dysfunction from their baseline azotemic state. There has been a preponderance of reports documenting altered platelet function in this population. This was likely not the case in the current study, given our subject characteristics. The ESRD subjects were adherent to 4-h hemodialysis treatments three times weekly, and they had very good urea solute removal adequacy. Kt/V_{urea}, a measurement of overall dialysis urea clearance, was 1.7, which is well above the recommended Kt/V_{urea} threshold of 1.3 [35]. Further, the mean blood urea nitrogen (BUN) concentration was 37.8 mg dL^{-1}, which would not be considered 'uremic' by current clinical or laboratory standards. These subjects were not anemic (hemoglobin 13.0 g dL^{-1}; hematocrit 40%), and all subjects were receiving erythropoietin therapy. The mean platelet count was normal (218 000 mL^{-1}). Moreover, the ESRD group had nearly identical PCF and CEM values compared with controls at baseline, suggesting their platelet function was not impaired. The amalgamation of these findings makes the altered platelet function theory unlikely. Lastly, it is possible that chronic unfractionated heparin (UFH) exposure in the ESRD group may sensitize this population to the effects of enoxaparin. The baseline aPTT was significantly higher in the ESRD group compared with controls (36 vs. 31 s, respectively, $P = 0.02$) despite the fact that our study was conducted at least 24 h after UFH exposure during hemodialysis. Given the relatively short plasma half-life of UFH, it is unlikely that any UFH would still be in the body to affect the aPTT one day following dialysis. Although the aPTT was significantly prolonged in the ESRD group, this apparently did not alter the baseline aFXa activity or the baseline TGT in the ESRD group relative to controls. To determine if UFH exposure may explain the enhanced TGT response in the ESRD patients, we are currently enrolling peritoneal dialysis patients who are UFH naïve, and comparing these results to those observed in hemodialysis patients. Clearly further study as to the exact mechanism(s) of the enhanced TGT response in ESRD patients is warranted.

These results question the validity of current recommendations to monitor and maintain 4-h peak aFXa activity between 0.5 and 1.2 IU mL^{-1} in patients with renal dysfunction. National clinical practice guidelines [1] and the manufacturer of enoxaparin [5] have widely recommended clinicians monitor aFXa activity to avoid overdosing and consequently to prevent adverse bleeding events in patients with renal dysfunction. In fact the manufacturer of enoxaparin recently changed the dosing recommendations for patients with a creatinine clearance estimate less than 30 mL min^{-1}. These new guidelines recommend an enoxaparin dose of 1 mg kg^{-1} s.c. every 24 h. These dosing guidelines were based in part on published pharmacokinetic data that demonstrated reduced aFXa activity clearance and increased bleeding incidence in patients with renal dysfunction. Another recently published study [36] that used sophisticated pharmacokinetic modeling methods suggested an enoxaparin dose of 0.66 mg kg^{-1} s.c. every 12 h will maintain peak aFXa concentrations within the goal range of 0.5–1.2 IU mL^{-1} in patients with severe renal failure.

This study, and other data collected in our laboratory [27], suggests that at a given peak aFXa activity level, the corresponding TGT value appears to be approximately 20–60% more prolonged in the ESRD group relative to the controls. This may suggest that the currently accepted target range of aFXa activity in patients with renal dysfunction may be too liberal. To better delineate the role of using the Hemodyne instrument to monitor LMWH therapy in the ESRD population vs. the traditional aFXa assay, further large-scale clinical trials are needed that specifically evaluate clinical efficacy as well as bleeding events at a given TGT value.

In conclusion, this is the first *in vivo* study to document the utility of using TGT as a monitoring parameter for enoxaparin in ESRD subjects. We have convincingly shown that despite achieving similar aFXa activity concentrations across a 12-h dosing period, patients with ESRD have significantly prolonged TGT values relative to controls. These data document that at a given aFXa level, ESRD subjects are more anticoagulated relative to the normal controls without renal dysfunction. These data corroborate our previous *in vitro* experiments. Thrombin generation time may become an important monitoring parameter for ESRD patients who receive enoxaparin therapy. Further large-scale studies are needed to confirm these results.

© 2006 International Society on Thrombosis and Haemostasis

Conflict of interest disclosure

Marcus E. Carr, Jr., MD, PhD, is the founder and Chairman of the Board of Hemodyne, Inc. No other authors have a conflict of interest.

Acknowledgements

Supported by grant 1R41 HL77964-01 from the National Institutes of Health (National Heart, Lung and Blood Institute), and General Clinical Research Center grant M01 RR00065, NCRR, NIH.

References

1 Hirsch J, Raschke R. Heparin and low-molecular weight heparin. The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004; **126**: 188S–203S.
2 Gerlach AT, Pickworth KK, Seth SK, Tanna SB, Barnes JF. Enoxaparin and bleeding complications: a review in patients with and without renal insufficiency. *Pharmacotherapy* 2000; **20**: 771–75.
3 Chow SL, Zammit K, West K, Dannenhoffer MA, Lopez-Candales A. Correlation of antifactor Xa concentrations with renal function in patients on enoxaparin. *J Clin Pharmacol* 2003; **43**: 586–90.
4 Becker RC, Spencer FA, Gibson M, Rush JE, Sanderink G, Murphy SA, Ball SP, Antman EM, TIMI 11A Investigators. Influence of patient characteristics and renal function on factor Xa inhibition pharmacokinetics and pharmacodynamics after enoxaparin administration in non-ST segment elevation acute coronary syndromes. *Am Heart J* 2002; **143**: 753–9.
5 Package insert. *Lovenox*® (enoxaparin sodium). Bridgewater, NJ: Sanofi-Aventis Pharmaceuticals, July 2004.
6 Matthiasson SE, Lindblad B, Stjernquist U, Bergqvist D. The haemorrhagic effect of low molecular weight heparins, dermatan sulphate and hirudin. *Haemostasis* 1995; **25**: 203–11.
7 Laposata M, Green D, Van Cott EM, Barrowcliffe TW, Goodnight SH, Sosolik RC. College of American Pathologists Conference XXXI on laboratory monitoring of anticoagulant therapy. *Arch Pathol Lab Med* 1998; **122**: 799–807.
8 Greaves M. Limitations of the laboratory monitoring of heparin therapy. *Thromb Haemost* 2002; **87**: 163–4.
9 Harenberg J. Is laboratory monitoring of low-molecular weight heparin therapy necessary? Yes. *J Thromb Haemost* 2004; **2**: 547–50.
10 Bounameaux H, DeMoerloose P. Is laboratory monitoring of low-molecular weight heparin therapy necessary? No. *J Thromb Haemost* 2004; **2**: 551–4.
11 Lawson JH, Kalafatis M, Stram S, Mann KG. A model for the tissue factor pathway to thrombin. I. An empirical study. *J Biol Chem* 1994; **269**: 23357–66.
12 Butenas S, van't Veer C, Mann K. 'Normal' thrombin generation. *Blood* 1999; **94**: 2169–78.
13 Mann KG. Thrombin formation. *Chest* 2003; **124**(Suppl. 3): 4S–10S.
14 Mann KG, Brummel K, Butenas S. What is all that thrombin for? *J Thromb Haemost* 2003; **1**: 1504–14.
15 Brummel KE, Paradis SG, Butenas S, Mann KG. Thrombin functions during tissue factor-induced blood coagulation. *Blood* 2002; **100**; 148–52.
16 Hoffman M, Monroe DM. A cell-based model of hemostasis. *Thromb Haemost* 2001; **85**: 958–65.
17 Monroe DM, Hoffman M, Roberts HR. Platelets and thrombin generation. *Arterioscler Thromb Vasc Biol* 2002; **22**: 1381–9.
18 Allen GA, Wolberg AS, Oliver JA, Hofffman M, Roberts HR, Monroe DM. Impact of procoagulant concentration on rate, peak and total thrombin generation in a model system. *J Thromb Haemost* 2004; **2**: 402–13.
19 Roberts HR, Monroe DM, Oliver JA, Chang JY, Hoffman M. Newer concepts of blood coagulation. *Haemophilia* 1998; **4**: 331–4.
20 Carr ME, Martin EJ, Kuhn JG, Spiess MD. Onset of force development as a marker of thrombin generation in whole blood: the thrombin generation time (TGT). *J Thromb Haemost* 2003; **1**: 1977–83.
21 Carr ME. Development of platelet contractile force as a research and clinical measure of platelet function. *Cell Biochem Biophy* 2003; **38**: 55–78.
22 Carr ME, Martin EJ, Kuhn JG, Seremetis SV. Effects of recombinant factor VIIa on platelet function and clot structure in blood with deficient prothrombin conversion. *Thromb Haemost* 2003; **89**: 803–11.
23 Carr ME, Angchaisuksiri P, Carr SL, Martin EJ. Effect of non-heparin thrombin antagonists on thrombin generation, platelet function, and clot structure in whole blood. *Cell Biochem Biophys* 2003; **39**: 89–99.
24 Carr ME, Martin EJ. Evolving techniques for monitoring clotting in plasma and whole blood samples. *Clin Lab* 2004; **50**: 539–49.
25 Carr ME, Martin EJ, Kuhn JG, Ambrose H, Fern S, Bryant PC. Monitoring of hemostatic status in four patients being treated with recombinant factor VIIa. *Clin Lab* 2004; **50**: 529–38.
26 Brophy DF, Martin EJ, Gehr TWB, Carr ME. A hypothesis-generating study to evaluate platelet activity in diabetics with chronic kidney disease. *Thromb J* 2005; **29**: 3–10.
27 Brophy DF, Martin EJ, Gehr TWB, Carr ME. Enhanced anticoagulant activity of enoxaparin in patients with end-stage renal disease as measured by thrombin generation time. *Am J Kidney Dis* 2004; **44**: 270–7.
28 Brophy DF, Martin EJ, Gehr TWB, Best AM, Carr ME. Antifactor Xa activity correlates to thrombin generation time, platelet contractile force and clot elastic modulus following *ex vivo* enoxaparin exposure in patients with and without renal dysfunction. *J Thromb Haemost* 2004; **2**: 1299–304.
29 Brophy DF, Wazny LD, Gehr TWB, Comstock TJ, Venitz J. The pharmacokinetics of subcutaneous enoxaparin in end-stage renal disease. *Pharmacotherapy* 2001; **21**: 169–74.
30 Nobel S, Peters DH, Goa KL. Enoxaparin: a reappraisal of its pharmacology and clinical applications in the prevention and treatment of thromboembolic disease. *Drugs* 1995; **49**: 388–410.
31 Anonymous. Pharmacology in animals and humans. In: Barrowcliffe TW, Johnson EA, Thomas DP, eds. *Low Molecular Weight Heparin*. Chichester, UK: Wiley & Sons, 1992: 101–23.
32 Cornelli U, Fareed J. Human pharmacokinetics of low molecular weight heparins. *Semin Thromb Hemost* 1999; **25**(Suppl. 3): 57–61.
33 Frydman AM, Bara L, Le Roux Y, Woler M, Chauliac F, Samama MM. The antithrombotic activity and pharmacokinetics of enoxaparine, a low molecular weight heparin, in humans given 20 to 80 mg. *J Clin Pharmacol* 1988; **28**: 609–18.
34 Brieger D, Dawes J. Characterisation of persistent anti-Xa activity following administration of the low molecular weight heparin enoxaparin sodium (Clexane). *Thromb Haemost* 1994; **72**: 275–80.
35 National Kidney Foundation, *Kidney Disease Outcome Quality Initiative (K/DOQI) Clinical Practice Guidelines for Hemodialysis Adequacy*: Update 2000. Available at: http://www.kidney.org/professionals/kdoqi/guidelines_updates/doqi_uptoc.html#hd (accessed July13, 2005).
36 Hulot JS, Montalescot G, Lechat P, Collet JP, Ankri A, Urien S. Dosing strategy in patients with renal failure receiving enoxaparin for the treatment of non-ST-segment elevation acute coronary syndrome. *Clin Pharmacol Ther* 2005; **77**: 542–52.

© 2006 International Society on Thrombosis and Haemostasis