Original Article

Prothrombin fragment F1+2 in plasma and urine during total hip arthroplasty☆☆☆

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ABSTRACT

Objective: To study the time course and correlation of prothrombin fragment (F1 + 2) in plasma and urine during THA.**Method:** F1 + 2 concentrations were recorded at four time-points during THA.**Results:** F1 + 2 increased during surgery. In contrast to urine, plasma F1 + 2 was temporarily reduced the first postoperative day. At day six, plasma F1 + 2 was significantly elevated indicating on-going thrombin activation, while urine F1 + 2 was normalized.**Conclusion:** The response in coagulation to operative trauma can be recorded with F1 + 2 both in plasma and urine. We were not able to demonstrate any correlation of F1 + 2 in plasma and urine later in the postoperative period.

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1. Introduction

When the body is subjected to tissue injury like total hip arthroplasty (THA), activation of coagulation is necessary to avoid bleeding, but the process of coagulation may lead to unsuitable thrombus formation and increased morbidity. This process is dynamic and is influenced by several factors related to the patients, comorbidities, drugs, type of surgery, and postoperative clinical care. Patients undergoing THA are at high risk for venous thromboembolism (VTE), and anticoagulation therapy is therefore recommended, in the perioperative period.^{1,2} Low molecular weight heparins (LMWH) is commonly applied preventing thromboembolism. The use of these drugs exposes patients to treatments with potentially harmful side effects. From the surgeon's point of view, blood loss and bleeding complications are important and whether to start before or after surgery is controversial. Preoperative initiation has been common, but concerns for bleeding complications have resulted in a shift to

postoperative start in recent years.^{3–5} A simple method to separate those at high risk for thrombus formation would have been advantageous. Several markers of thrombosis and fibrinolysis are used to investigate hemostatic response to surgery. Prothrombin fragments F1+2 fragment, produced during conversion of prothrombin to thrombin, has been proposed as potential useful clinical marker of thrombogenesis during THA,^{6,7} and can be measured by enzyme-linked immune sorbent assay (ELISA).⁸ This small molecule, with a half-life in plasma of 90 min, is excreted in urine, and both blood and urine levels of F1+2 are increased for several days after THA.⁹ Furthermore, studies have demonstrated increased levels of urine F1+2 in patients with venous thromboembolism.^{10,11} When more thrombin is generated, the patients are at higher risk of thromboembolic complications.^{12,13} We have earlier published data from a study on biomarkers of coagulation and fibrinolysis in plasma. The aim of this present study was to investigate how the perioperative blood F1+2 (bF1+2) and urine F1+2 (uF1+2) in patients undergoing a THA is expressed, and what the relationship between bF1+2 and uF1+2 is?

2. Patients and methods

This study was approved by the Regional Ethics Committee (08012d), registered in the Norwegian Biobank register (2058), and performed in accordance with the ethical standards of the Declaration of Helsinki. After signing informed consent,

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Table 1Patient characteristics (mean $\pm$ standard deviation) and *P* value.

Characteristic	Preoperative group	Postoperative group	<i>P</i> value
Number of patients	10	10	
Sex (% males)	30	50	
Age (years)	65.6 $\pm$ 6.9	71.2 $\pm$ 6.6	0.083
Height (cm)	168.0 $\pm$ 8.7	171.5 $\pm$ 9.4	0.397
Weight (kg)	73.8 $\pm$ 16.8	81.9 $\pm$ 15.8	0.282
BMI (kg/m ²)	26.1 $\pm$ 5.3	28.0 $\pm$ 0.6	0.453
ASA classification	1.9 $\pm$ 0.6	2.0 $\pm$ 0.7	0.722
Preop. hemoglobin	14.3 $\pm$ 0.9	14.0 $\pm$ 0.7	0.495
Preop. hematocrit	41.9 $\pm$ 3.5	40.7 $\pm$ 2.5	0.395
Preop. C-reactive protein	2.6 $\pm$ 3.0	3.9 $\pm$ 5.6	0.540
Preop. creatinine	58.6 $\pm$ 10.6	66.0 $\pm$ 11.0	0.142
Preop. blood plates	246 $\pm$ 93.1	246 $\pm$ 56.6	1.0

20 patients above 50 years that underwent primary cemented THA due to osteoarthritis were included. The patient characteristics are given in Table 1. International Normalization Ratio (INR) without thromboprophylaxis is normally approximately 1.0. In patients on anticoagulants, a level of 1.8 is generally accepted for performing spinal anesthesia and major orthopedic surgery. With this background we calculated the sample size using previously published data on F1+2 during THA surgery¹⁴ to detect an 80% difference in the increase with or without thromboprophylaxis. We calculated that 10 patients in each group would have the power of 80% with an alpha of 0.05. The patients were assigned to either 5000 international units (IU) dalteparin (Fragmin, Pharmacia and Upjohn, Stockholm, Sweden) subcutaneously or placebo (saline in 10 patients) injected 12 h before surgery. Randomization was prepared in blocks (of 10), and treatment group assignment was concealed by the hospital staff. (Fig.1) The syringes with 5000 IU dalteparin and placebo with the same volume in each syringe were prepared by a study nurse who otherwise was not engaged in the

study, according to randomized strata. The injection was blinded to the investigator, hospital staff, and the patient. All patients had 5000 IU dalteparin subcutaneously 6 h after surgery and each day until the 35th postoperative day. Patients with allergy to LMWH, bleeding disorders, renal failure, hepatic disease, active treatment for malignancy, ongoing antithrombotic treatment, and history of deep venous thrombosis (DVT) or pulmonary embolism (PE) and patients experiencing major operations, traumas, stroke, or cardiac infarction the last 3 months before surgery were excluded. In the hospital's written patient information, patients were advised to stop antiplatelet medication, that is, non-steroid anti-inflammatory drugs (NSAID) and high-dose aspirin, 1 week before surgery.

All patients received spinal anesthesia, and Cephalothin (Keflin; EuroCept Pharmaceuticals BV, Kortenhoef, The Netherlands) at 2 g $\times$ 4 was given intravenously as prophylaxis against infection. Voluven and Ringer's acetate (Fresenius KABI, Bad Homburg, Germany) were used as plasma substitutes. Blood and urine samples were obtained at the following time points: (T1) before surgery, (T2) at 6 h after surgery and before injection of dalteparin, (T3) at day one after surgery, and (T4) at 6 days after surgery.

2.1. Laboratory analyses

Blood samples were kept on ice until it was separated by centrifugation at 2500g for 20 min at 18 °C and stored at –80 °C. Urine samples were snap-frozen and stored at –80 °C until analysis. Prothrombin fragment F1+2 was measured in citrated plasma and urine by enzyme-linked immuno sorbent assay (ELISA) using a commercial kit (Enzygnost F1+2 micro; Dade Behring, Marburg, Germany), following manufacturer's instructions. Lower limit of F1+2 detection was 0.04 nmol/L.

Fig. 1. CONSORT flow diagram.

Fig. 2. Blood F1+2 (pmol mL⁻¹) in patients injected with dalteparin preoperatively (group1) and 6 h postoperatively (group 2). Timepoint are: T1 before surgery, T2 at 6 h after surgery and before injection of dalteparin, T3 at day one after surgery, and T4 at 6 days after surgery.

Fig. 3. Urine F1+2 (pmol mL⁻¹) in patients injected with dalteparin preoperatively (group1) and 6 h postoperatively (group 2). Timepoint are: T1 before surgery, T2 at 6 h after surgery and before injection of dalteparin, T3 at day one after surgery, and T4 at 6 days after surgery.

Table 2

Pooled blood and urine F1+2 (pmol mL^{-1}). Timepoint are: T1 before surgery, T2 at 6 h after surgery and before injection of dalteparin, T3 at day one after surgery, and T4 at 6 days after surgery. Values are mean $\pm$ standard deviation.

Time point	Blood F1+2	Urine F1+2
T1	212 $\pm$ 83	14 $\pm$ 22
T2	564 $\pm$ 151 ^a	102 $\pm$ 89 ^a
T3	283 $\pm$ 172 ^b	93 $\pm$ 84 ^c
T4	356 $\pm$ 111 ^c	49 $\pm$ 73 ^d
P value (ANOVA)	<0.001	<0.001

All in relation to time T1.

^a $P < 0.001$.

^b $P < 0.105$.

^c $P = 0.001$.

^d $P = 0.134$.

2.2. Statistical analyses

Statistical analyses were performed using SPSS II software Version 19 (IBM Inc., USA). Data are presented by mean, 95% confidence interval (CI) and standard deviation (SD). Independent samples *t*-test is used to compare descriptive variables. Time dependent changes were analysed by two-way analysis of variance (ANOVA). When significant differences were indicated, we used the LSD post hoc test. The level of significance was set at $p = 0.05$. Correlations were performed by Pearsons correlation coefficient.

3. Results

All 20 patients completed the study. Patient characteristics were similar in the two groups and are shown in Table 1. There

were significant increases in blood and urine F1+2 following surgery, but no significant differences between the two groups of preoperative versus postoperative anticoagulation ($P = 0.790$ and 0.805 , respectively; Figs. 2 and 3). The values for the two groups, then, were pooled (Table 2). While blood F1+2 was reduced to non-significant levels at day 1 as compared to preoperative levels, urine levels were still significantly elevated. However, high blood levels of F1+2 at day 6 after surgery indicated on-going thrombin activation, while urine F1+2 was non-significantly elevated at this time. These observations were reflected in a poor statistical correlation between blood and urine F1+2 (Pearsons correlation 0.366, Fig. 4). None of the patients experienced thromboembolic events or serious bleeding events.

4. Discussion

This study was undertaken in stable patients undergoing a standardized major musculoskeletal injury. We measured F1+2 changes in blood and urine in the perioperative period to assess alterations in coagulation when thromboprophylaxis with 5000 IU dalteparin was initiated 12 h before versus 6 h after surgery. We found significant coagulatory responses during surgery for the two groups in both plasma and urine, but our laboratory recordings did not show any significant differences between the patients that received anticoagulation before as compared to those who received it after the operation. This lack of effect of heparins on plasma F1+2 has been shown in earlier studies.¹⁰ However, clinical studies have shown that the rate of thromboembolic and bleeding events differs depending on the timing and dose of LMWHs.¹⁵ An explanation might be that the thrombin generation caused by the operative trauma masks the remaining effect of dalteparin left 12 h

Fig. 4. Pooled correlations between blood F1+2 and urine F1+2. Timepoint are: T1 before surgery, T2 at 6 h after surgery and before injection of dalteparin, T3 at day one after surgery, and T4 at 6 days after surgery.

after injection. As no group difference was found, we pooled the patients, and measured F1+2 both in plasma and urine at selected time points. A corresponding and significant increase in F1+2 both in plasma and urine was caused by the surgical trauma as expected and in accordance with the view that THA surgery is a strong activator of thrombus formation. The day after surgery, plasma levels of F1+2 decreased significantly, in contrast to the concentration measured in urine. This fibrinolytic shutdown has been explained by the complex interaction of pro and anticoagulants, and the dynamics of dilution, blood loss and consumption of haemostatic factors. Why F1+2 in urine are not affected by this process is difficult to explain, but these findings are in agreement with the available literature.¹⁶ Fluids and cell products are continuously transported between intracellular, intramural and intravascular spaces, and the distribution of fluids and substances in this dynamic system over time is difficult to follow. A delayed production of urine after surgery is common, and a condensation of waste products and uF1+2 could then be likely. Bezeaud et al. identifying prothrombin derivative in urine, showed that 20% of radio labelled F1 and F1+2 appeared in urine after 90 min.⁸ The low uF1+2 levels measured at day 6, after the initial response to the operative trauma, may reflect this limited excretion. There was only a slight correlation between F1+2 in blood and urine. In contrast to urine, blood F1+2 reflect the degree of intravascular coagulation, which is the most important identifier for the perioperative management of patients at risk for thromboembolic or bleeding events.

Our study has some limitations. We had few patients and samples were collected at few time points. F1+2 have a half-life of 90 min in plasma, and even if these patients are handled in a very standardized manner during surgery, individual responses to administration of drugs and fluids could influence the concentration in plasma and elimination from the circulation. We have not adjusted for dilution (hematocrit, albumin, BMI) or possible differences in excretion due to variation in glomerular filtration rates (creatinine clearance). There is also a potential for contamination by other drugs or substances excreted in the urine, which can bind to and influence on F1+2 in the urinary bladder. The fact that there are so many variables to control for when measuring F1+2 in urine, make it doubtful that this biomarker is suitable as a simple clinical test. Further studies with larger samples, more frequent measurements and with correction for possible confounders would be useful.

5. Conclusion

Both blood F1+2 and urine F1+2 increased as a result of surgery with no significant differences whether patients had started prophylaxis before or after the operation. There were poor correlation between F1+2 in blood and urine, and this indicate that measurements of urine F1+2 is not precise to reflect intravascular coagulation following tissue injury.

Conflict of interests

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article. They received no financial support.

Contributions

(I) Conception and design: Both authors; (II) Administrative support: None; (III) Provision of patients: Pål Borgen; (IV) Collection and assembly of data: Pål Borgen; (V) Data analysis and interpretation: Both authors; (VI) Manuscript writing: Both authors; (VII) Final approval of manuscript: Both authors.

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