Effects of anticoagulation on markers of activation of clotting following major orthopedic surgery

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SUMMARY

Introduction: This study examines makers of activation of clotting following three chemoprophylactic regimens used for prevention of postoperative venous thromboembolic disease (TED) following high-risk surgery for TED.

Methods: Patients having elective primary knee or hip replacement surgery received variable dose warfarin (target international normalized ratios 2.0–2.5), 1 mg warfarin daily starting 7 days preoperatively or aspirin 325 mg daily starting on the day of surgery. Twelve patients in each group were treated for 28 ± 2 days. Thrombin–antithrombin (T-AT) and prothrombin fragment F1 + 2 were measured at baseline and postoperative days 3 and 28 ± 2.

Results: Thrombin–antithrombin and F1 + 2 on postoperative day 3 were equal for the study groups. By days 28 ± 2, variable dose warfarin therapy group suppressed production of F1 + 2 ($P = 0.002$) with no difference in the T-AT accumulation. F1 + 2 for other patients overlapped the normal range.

Conclusion: The signals of activated clotting following surgery did not differentiate the three regimens on postoperative day 3. Variable dose warfarin was associated with suppression of F1 + 2 after 1 month of therapy, with no effect on accumulation of T-AT. Fixed low-dose warfarin started 7 days prior to surgery and aspirin are not inferior on postoperative day 3, but appear to be inferior over a longer treatment.

INTRODUCTION

Markers of activation of the clotting system have been used to investigate prothrombotic and thrombotic diseases and to test the efficacy of drugs used to treat those diseases [1]. Published articles involve the fields of cardiology, stroke, primary hypercoagulation syndromes, deep vein thrombosis, pulmonary embolus, peripheral vascular disease, antiphospholipid syndrome, breast cancer, and hemodialysis.

All such studies imply that the results from a blood sample drawn at a specific time of the day are representative of the patient's entire day and that the result derived on a single day is representative of several days for the patients under study. Unfortunately, most such studies give little information regarding the overlap of results within study groups or with nontreated controls. Only a few reports include repetitive assays of markers conducted over several time points [2–4]. Perhaps only the changes from baseline should be reported, as in Millenson *et al.* [5] and Koefoed *et al.* [6], very few studies ask how clearly separated are the results from either untreated controls or from baseline. To be useful, there should be clear biologically meaningful differences in the results when reporting upon these markers.

The current study examined the effect of three thromboembolic disease (TED) chemoprophylactic programs upon two signals of activation of the clotting system. This pilot study was performed in an attempt to detect distinguishing effects by these therapies upon two markers of activation of coagulation 3 days and again 28 ± 2 days following major orthopedic surgery. This current study was not designed to test the efficacy of these regimens in the prevention of TED.

MATERIALS AND METHODS

The current study was conducted in the midst of a larger prospective, randomized study comparing fixed low dose of warfarin to variable dose warfarin as chemoprophylaxis against TED among patients undergoing elective primary hip or knee replacement arthroplasty. [7] A concomitant addendum study was created, which allowed the examination of the same markers in a third group of patients receiving only aspirin following the same surgeries.

In the master protocol, patients planning elective primary unilateral hip or knee replacement arthroplasty were randomized to receive variable dose warfarin [postoperative target international normalized ratios (INR) 2.0–2.5] starting with **5** mg the night prior to surgery or to receive 1 mg warfarin starting 7 days preoperatively, continuing the same fixed low dose postoperatively. The addendum study *allowed* the study of a cohort of patients who received 325 mg of aspirin only for TED prophylaxis, with the aspirin therapy beginning the night of surgery. All patients received therapy for 28 ± 2 days. (Table 1) Inclusion criteria were patients over 20 years of age planning elective knee or replacements surgery. Exclusion criteria are demonstrated in Table 2. Twelve randomly selected patients participated in each arm of this substudy.

The hospital's Institutional Review Board approved both the primary and the addendum studies. The work was conducted in accordance with the Declaration of Helsinki. All patients signed informed consents for the primary study where appropriate, and all patients signed consent for the addendum study with its added laboratory investigations.

Patient recruitment

Informational letters were sent to patients preparing for elective primary knee or hip replacement surgery.

Table 1. Research design

ARM A	
Variable dose warfarin	5.0 mg beginning the night before surgery, followed by 5.0 mg the PM of surgery, and then variable daily dose, (target INR 2.0–2.5) until day 28 ± 2 follow-up.
ARM B	
Fondaparinux	2.5 mg daily starting 6 or more hours following surgery, but no later than 6 AM the next day, or 6–8 h after epidural catheter removal; until day 28 ± 2.
ARM C	
Fixed low-dose warfarin	1.0 mg daily beginning 7 days preoperatively and continued at 1.0 mg daily until day 28 ± 2 follow-up.

Table 2. Exclusion criteria

1	Abnormal platelet count, prothrombin time (PT) or partial thromboplastin time (PTT)
2	Surgery for acute fracture (< 4 weeks), septic joint, or extraction arthroplasty
3	History of TED or documented hypercoagulation syndrome
4	Increased risk of hemorrhage, as from active gastric ulcer or urinary tract bleed within the last year
5	Hemorrhagic stroke; brain, spinal, or ophthalmologic surgery in previous 6 months
6	Liver enzymes or bilirubin greater than 2 × normal
7	Decreased renal function with GFR <30 mL/min.
8	Cancer in last year, other than localized cancers of the skin
9	Requires chronic anticoagulation
10	Requires chronic platelet function suppressive therapy
11	Prior adverse reaction to any of the study drugs
12	Uncontrolled hypertension
13	BMI >42
14	Pregnancy

Interested patients who met the eligibility criteria were met by the clinical research coordinators (CRCs) in the prescreening unit for discussion of the protocol. After patients signed informed consent, the CRC's reviewed patients' laboratory data to determine continued eligibility and drew blood samples for baseline laboratories.

Surgical and postoperative care

Surgeries and postoperative therapies were conducted in a hospital specializing in orthopedic surgery. All patients had early ambulation and used pneumatic compression stockings while in hospital and elastic compression stockings following discharge until their follow-up visits on postoperative days 28 ± 2. The CRCs visited patients while in hospital. Following discharge, patients were called weekly to monitor for compliance and for complications.

Laboratory monitoring

Blood samples were taken for baseline on the day of consent, on operative day for patients receiving warfarin 7 days and on postoperative days 3 and 28 ± 2 for all patients. Blood samples for Thrombin–antithrombin (T-AT) and F1 + 2 were drawn into Becton Dickinson vacuum tubes containing 3.2% trisodium citrate at a 1 : 9 proportion to blood using minimal tourniquet time. The first withdrawn tube was discarded. The second aliquot was centrifuged at *2500 RCF* **for 8 min**. The supernatant plasma was then aliquoted into 1-mL plastic tubes and frozen at −70 °C for batch testing. T-AT (Enzygnost® TAT micro; Siemens Healthcare, Marburg, Germany) and Prothrombin fragment F1 + 2 (Enzygnost® F 1 + 2 monoclonal; Siemens Healthcare) were measured. Prothrombin In Vitamin K Absence/Antagonists II (PIVKA-II) (Asserachrom PIVKA-II, Diagnostica Stago S.A.S, Asnieres, France) assays were performed from the same supernatant plasma samples taken at baseline, on the day of surgery and postoperative days 3 and 28 ± 2 for patients receiving the warfarin therapies (Colorado Coagulation; Laboratory Corporation of America® Holdings, Englewood, CO, USA).

Patients receiving warfarin had prothrombin times (PT) reported as INR measured daily while in hospital. Patients discharged on variable dose warfarin had PT/INR measurements twice per week with target INR 2.0–2.5. Warfarin dosing was managed by the hospital Coumadin Hotline. Patients on 1 mg daily warfarin received INR measurements weekly.

Statistics

All tests were run in duplicate and the results averaged. Group comparison analysis was by the Kruskal–Wallis test in lieu of the one-way analysis of variance due to the data being nonparametric. Analysis for differences for specific nonparametric sample pairs used the Wilcoxon rank-sum test. Values reported as below the limit of detection for the assays reported were managed according to the methods of Crogan and Egeghy [8].

RESULTS

Table 3 demonstrates patient demographics and the transfusions given. Patients taking 1 mg warfarin daily for 7 days or 5 mg the night prior to day of surgery had normal INR values on the day of surgery. Figure 1 demonstrates the INR values for the patients taking the fixed low-dose warfarin regimen and for

those taking the variable dose warfarin for weeks 1, 2, 3, and 4.

Figure 2 demonstrates the PIVKA II results. The results are in keeping with the expected physiologic effect of the variable warfarin dose upon the PIVKA II levels. Patients taking fixed low-dose warfarin had minimal measurable increase of PIVKA II compared to baseline on days 3 and 28 ± 2. This occurred despite there being no change in the prothrombin times. (INR day 3 for fixed low-dose warfarin patients: mean 1.09, median 1.1, range 1.0–1.2).

Figure 3 demonstrates the T-AT complex results for each study group over the course of the study. There was a surge of the T-AT complex for each study group on postoperative day 3, with no differences between the study groups. By postoperative day 28 ± 2, that surge returned to baseline.

Figure 4 demonstrates the prothrombin fragment F1 + 2 results for each study group over the course of the study. There was trend upward of the F1 + 2 on day postoperative day 3 without distinction among the three study groups. However, patients taking variable dose warfarin had significantly lower F1 + 2 results than those of the two other study groups for F1 + 2 on postoperative days 28 ± 2 by the Kruskal–Wallis test (chi-squared 20.39, with 2 df, $P < 0.001$).

Figure 5 gives further detail using box plot of the F1 + 2 results on postoperative days 28 ± 2. Variable dose warfarin was more effective than aspirin (Wilcoxon two-sample test, $Z = 3.79$, two-sided $P = 0.0001$) and more effective than fixed low-dose warfarin (Wilcoxon two-sample test, $Z = 3.7263$ with two-sided $P = 0.002$) for suppression of F1 + 2 production.

DISCUSSION

Even though previous evidence-based guidelines published by the American College of Chest Physicians (ACCP) and the current American Academy of Orthopaedic Surgeons (AAOS) considered aspirin, as well as adjusted dose warfarin, low molecular weight heparin, or factor Xa inhibitors as chemoprophylaxis against TED following hip or knee replacement surgery, when constructing the current study, the control arm we considered aspirin to be a control arm as **we** expected *there* to be little suppression of TAT or F1 + 2 production by aspirin compared to warfarin following large joint replacement surgery [6, 9–12].

There is a very sparse medical literature examining the effect of aspirin therapy upon the generation of TAT or F1 + 2. In the Boston Area Anticoagulation Trail for Atrial Fibrillation, aspirin had no effect on levels of F1 + 2 [13]. Meta-analysis of aspirin therapy for DVT prophylaxis demonstrated no or minimal benefit of using aspirin for DVT prophylaxis following
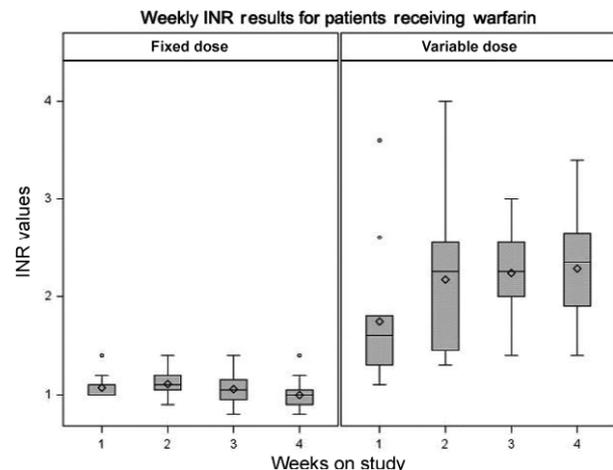

Figure 1. International Normalized Ratios for the patients taking the fixed low dose warfarin and for those taking the variable dose warfarin for weeks 1, 2, 3 and 4. Box plot demonstrates the range from first to third quartiles, plus outliers. The symbol within the box represents the mean value.

Table 3. Patient demographics and transfusions given

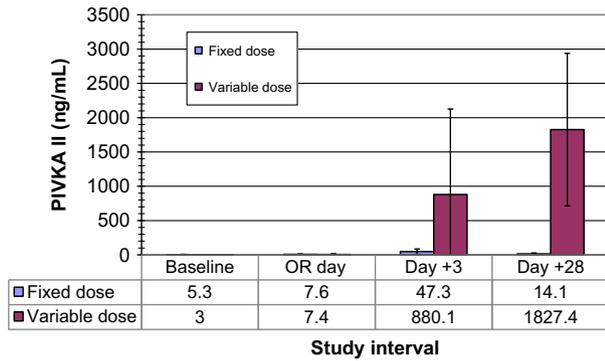| Study arm (*n*)	Sex M/F	Joint arthroplasty performed	AGE (years) Mean Median (Range)	Patients transfused 1 unit *vs.* 2 units
Variable Dose Warfarin (12)	5/7	Hip 5 Knee 7	62.4 63 (49–71)	4 received 1 unit 2 received 2 units
Fixed Dose Warfarin (12)	4/8	Hip 7 Knee 5	55.8 59 (45–64)	4 received 1 unit 0 received 2 units
Aspirin Only (12)	5/7	Hip 5 Knee 7	70.9 72 (59–78)	3 received 1 unit 1 received 2 units

Figure 2. Prothrombin in Vitamin K Absence II results for the patients taking the fixed low dose or variable dose warfarin, at baseline, on operating room (OR) day and after 3 and 28 ± 2 days following surgery. (ng/mL, mean ± 1 SD in graph and mean in the table below).
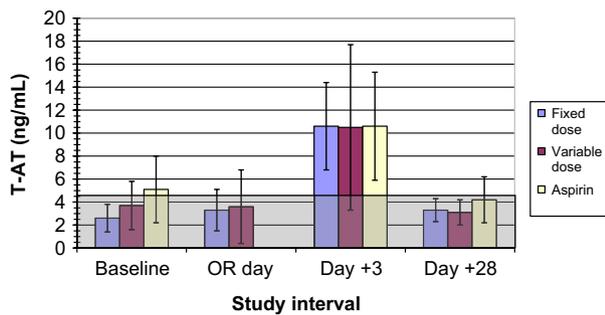

Figure 4. Prothrombin fragment F1 + 2 (F1 + 2) for each study group at baseline, on operating room day and after 3 and 28 ± 2 days following surgery. (pmol/L, mean ± 1 SD).

Figure 3. Thrombin–antithrombin complex for each study group at baseline, on operating room (OR) day and after 3 and 28 ± 2 days following surgery. (ng/mL, mean ± 1 SD).

Figure 5. Box plot of the prothrombin fragment F1 + 2 (F1 + 2) results on postoperative day 28 ± 2 for each study group, first to third quartiles, and outliers. The symbol within the box represents the mean value.

total hip or knee arthroplasty [14, 15]. *In vitro* activation of clotting with measurement of TAT and fibrinopeptide A showed no suppression by aspirin when patients ingested the drug before the blood draw [16]. Aspirin did not suppress thrombin generation among patients with coronary artery disease, nor among those receiving aspirin alone in the Stroke Prevention Atrial Fibrillation III study [17, 18]. Aspirin had some effect suppressing F1 + 2 in the antiphospholipid syndrome, but without consistency among the patients studied [19]. It was effective for slowing production of TAT and F1 + 2 in the setting of shed blood from acute bleeding time laceration [20].
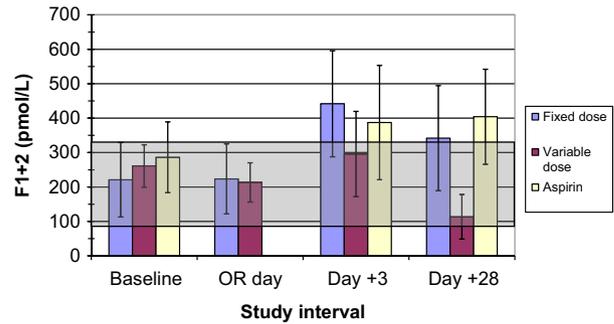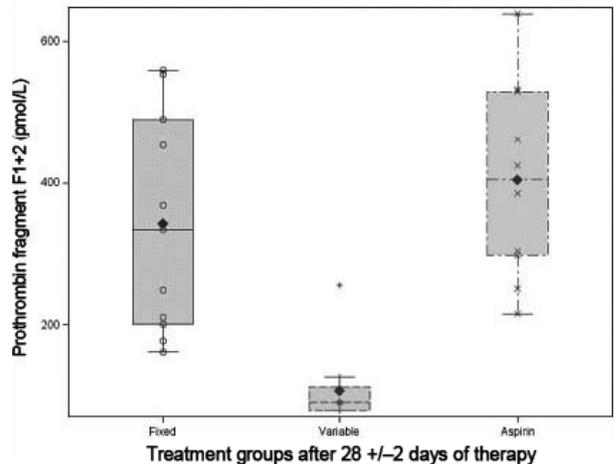
In the current study results, there were overlapping 1 standard deviation bars for the three study groups on postoperative day 3, suggesting there are no or minimal biologic differences at that point. Differences for F1 + 2 generation were not seen until *4th week of therapy* and only *for those receiving* full therapeutic doses of warfarin. There was slight activity of fixed low-dose warfarin as expressed in the generation of low titer PIVKA II, but this did not translate into changes of the TAT or F1 + 2. Even then, after

4 weeks of therapy, the patients receiving aspirin or fixed low-dose warfarin had values that overlapped the normal values.

In other studies, the suppression of production of F1 + 2 becomes tightly clustered only when there is significant downward pressure on thrombin generation created by high doses of warfarin. The results of increasing INR on the levels of F1 + 2 were hyperbolic and were tightly bunched below the normal range only when INR exceeded 4.0 [21, 22].

Based upon the current outcomes, there are two possible conclusions: (i) The regimens studied are generally equally effective postoperatively, or (ii) the assays are not informative as to how these regimens actually work, especially within 3 days from surgery.

For the former, it may be hypothesized that if no thromboprophylaxis was administered, the markers of activation of clotting would have been significantly greater on day 3 and beyond. To study the first possibility, we would need to include patients having the same surgery with the same duration of follow-up, but no DVT prophylaxis. We do not have access to such patients because such a study would be considered inappropriate by the IRB. It would be interesting to study the effects of low molecular weight *heparin* or *selective* Xa or IIa inhibitors at the same time points following the same surgeries.

For the second interpretation, distinctions should be drawn when considering what may be occurring in the systemic circulation *vs.* the microcirculation. This may be particularly important when drawing distinction between the venous and the arterial systems, or for circulating venous blood *vs.* shed blood. In the current study, the magnitude of surgical trauma and the ensuing release of tissue factor-ladened circulating microparticles may overwhelm any drug effects on the suppression of circulating plasma markers TAT and F1 + 2 by the drugs used in this study. It is also likely that the changes in the affected microcirculation are far more complex than reflected in these markers alone and depend significantly on effects of circulating microparticles, lipids, and cell adhesion molecules p-selectin and PSGL-1 [23–26]. Aspirin did not suppress release of tissue factor procoagulant activity, with no change of TAT or F1 + 2 [27, 28].

Finally, we note there are slight increases of the F1 + 2 without concomitant increase of the T-AT for patients receiving aspirin and very low-dose warfarin after 28 ± 2 days of therapy. This slight discrepancy in explainable as the rates of the accumulation and of the clearance of F1 + 2 and T-AT are not identical under some *in vivo* conditions [29–31]. The ratio favors F1 + 2 [29]. In the current study, it would appears that rate of generation of the F1 + 2 is not enough to change the final accumulated concentration of the T-AT as the rate thrombin generation would appear to be not very great.

ACKNOWLEDGEMENTS

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