

Dynamic changes of coagulation and fibrinolytic biomarkers in peri-operative arthroplasty patients

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Abstract

Objective: To evaluate coagulation and fibrinolytic parameters after total joint arthroplasty (TJA) and provide evidence for optimization of timing of perioperative anticoagulation medicine.

Methods: The prospective study was conducted at the Jishuitan Hospital of Peking University from January to April in 2016, and comprised patients who were scheduled consecutively to undergo primary total knee arthroplasty (TKA) or total hip arthroplasty (THA). Blood samples were obtained at day 1 preoperatively and day 1, day 3 postoperatively. Antigenic levels of protein C (PC), endothelial protein C receptor (EPCR), tissue factor pathway inhibitor (TFPI), antithrombin III (AT-III), plasminogen activator inhibitor 1 (PAI-1) and tissue plasminogen activator (tPA) were measured with commercially available enzyme-linked immunosorbent assay kits.

Results: Postoperative levels of coagulation parameters TFPI and AT-III were increased compared to preoperative values (118.7±34.6 vs 70.0±20.5 µg/ml for AT-III, and 26.37±7.91 vs 16.68±8.92 µg/l for TFPI), while postoperative levels of coagulation parameters PC and EPCR were decreased (0.88±0.30 vs 2.03±0.66 µg/ml for PC, and 100.8±31.0 vs 199.4±57.4 µg/ml for EPCR). Postoperative levels of fibrinolytic parameter tPA was increased compared to preoperative values (2.87±0.83 vs 2.03±1.03 µg/l), while its specific inhibitor PAI-1 was decreased (0.88±0.30 vs 2.03±0.66 µg/l).

Conclusion: These results demonstrated the perturbation of the coagulation and fibrinolytic system of patients undergoing TJA. Hypercoagulation and hyperfibrinolysis were observed in postoperative patients, which suggested anticoagulant therapy is effective and necessary.

Keywords: Total joint arthroplasty, anticoagulation, fibrinolysis.

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Introduction

Total joint arthroplasty (TJA), including total knee arthroplasty (TKA) and total hip arthroplasty (THA), is an effective treatment in patients with severe joint diseases. It was well determined that patients with TJA had a high risk of postoperative bleeding events and venous thromboembolism (VTE).¹⁻³ TJA is associated with a risk of thromboembolism because patients undergoing this procedure had a hypercoagulable state frequently. According to previous studies, the incidence of VTE in patients with TJA could be as high as 15~30 %, which led to a mortality rate of 2 % because of threatening pulmonary embolism.¹⁻³

It was also shown that perioperative thrombotic events induced a hypercoagulable state and increased fibrinolytic activity after TKA.^{4,5} Understanding of the coagulable and fibrinolytic dysregulation will be important to have more therapies to prevent thrombotic events in TJA patients.

In 2013, the new guideline recommended that

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anticoagulant therapy should be used for at least 14 days after TJA to prevent VTE.² The use of anticoagulant agents (such as warfarin and low molecular weight heparin) protected patients from potential thrombotic events. The mechanisms for postoperative coagulation dysregulation are not fully understood. Protein C (PC) is a major component of the human anticoagulation system, which regulates thrombosis and limits inflammatory response.⁶ Protein C circulates in the blood in the form of inactive zymogen. During surgical stress, PC on the surface of endothelial cells is activated by binding thrombin and thrombomodulin.⁷ Endothelial cell protein C receptor (EPCR) can augment the thrombin-thrombomodulin-mediated PC activation.⁸ (AT-III), the inhibitor for serine proteases, is the most important physiological anticoagulant in the plasma.⁹ Tissue factor pathway inhibitor (TFPI), also known as an exogenous pathway inhibitor (EPA) and lipoprotein-related coagulation inhibitor (LACI), is a major inhibitor during the extrinsic coagulation pathway.¹⁰ TFPI is an endogenous anticoagulant protein produced primarily by endothelial cells and secondarily from liver and monocytes/macrophages.¹⁰ It was shown that TFPI can be combined with heparin sulfate on the surface of endothelial cells. Heparin injection could release TFPI from endothelial cells, so it was considered that the increased

TFPI in plasma was correlated with the routine application of low-molecular-weight heparin for preventive anticoagulation.^{10,11}

Complications, including allergic reactions, infectious agent contamination, transfusion related acute lung injury and haemolytic complications.¹²⁻¹⁴ Increased fibrinolytic activity in blood samples after TKA might be attributed to increased fibrin degradation, which leads to excessive bleeding.¹⁵ Tissue plasminogen activator (tPA) is an important element of the fibrinolytic system.¹⁶ tPA has a high affinity towards the fibrin-plasminogen complex within the thrombus, which enables it to rapidly form into fibrin-tPA-plasminogen complex. This process greatly facilitates the activation of plasminogen and exerts a highly efficient and specific thrombolytic effect.¹⁷ The production and release of tPA was decreased in the injured vascular endothelial cells, which induce thrombosis and decreased fibrinolytic activity.¹⁸ Plasminogen activator inhibitor 1 (PAI-1) is a specific inhibitor of tPA, which is produced and released primarily by vascular endothelial cells and partially by platelets.^{19,20}

All of the above basic biomarkers are used for further clinical application. Throughout the coagulation process, a small quantity of activated coagulation factors could activate a large number of cascaded downstream coagulation factors, so the whole coagulation process shows a huge amplification phenomenon. Antithrombin-, protein C- or protein S- deficiency constitutes a major risk factor for venous thromboembolism.^{21,22} Understanding of the coagulable and fibrinolytic dysregulation would be important to have more therapies to prevent thrombotic events and bleeding complications in patients after TJA surgery. In this research, we evaluated the coagulation functions and fibrinolytic parameters during the perioperative period, which provide evidence for the time window of anticoagulation treatment after TKA surgery.

Patients and Methods

A total of 119 patients enrolled in the study were scheduled consecutively to undergo primary THA or TKA at the Jishuitan Hospital of Peking University from January to April in 2016. Only patients with surgical indications for THA or TKA participated in this study. TKA were performed in 48 patients and THA were performed in 71 patients. The surgical indications included instability, pain, deformity, and dysfunction of the hip or knee joint. Patients who meet any of the following criteria were considered not eligible for the study: (1) patients with cerebral or gastrointestinal bleeding within the previous 6 months; (2) ischaemic heart disease in the previous 6 months; (3) hepatic failure; (4) end-stage kidney disease; (5) coagulation or fibrinolysis

disorder; (6) any kind of cancer. Before their enrollment, all patients were provided a detailed explanation of the risks and alternatives to participation in this research and informed consent was provided according to the Declaration of Helsinki. This study was approved by the institutional review board of Beijing Jishuitan Hospital.

Blood samples were obtained from peripheral veins on the preoperative day, postoperative 24h and postoperative 72h respectively. Samples were centrifuged at 3000 rpm for 15 minutes at room temperature. Plasma samples were stored at -80°C. Protein levels of antithrombin III, protein C, endothelial protein C receptor, tissue factor pathway inhibitor, plasminogen activator inhibitor 1 and tissue plasminogen activator were measured from these plasma samples with enzyme linked immunosorbent assay (ELISA) kit (Elabscience, E-EL-H0432c for antithrombin III, E-EL-H1167c for protein C, E-EL-H0065c for endothelial protein C receptor, E-EL-H0165c for tissue factor pathway inhibitor, E-EL-H2104c for plasminogen activator inhibitor 1, E-EL-H2106c for tissue plasminogen activator). Incidences of high-risk pulmonary embolism and DVT were estimated through inquiring and vascular ultrasound with a 3-month follow-up.

Continuous data were expressed as mean \pm standard deviation and compared with Student's t-test or one-way analysis of variance (ANOVA). Categorical data were presented as percentages and frequencies and compared with chi-square test. All statistical analyses were performed with SPSS 19.0 software. P-values < 0.05 were considered statistically significant.

Results

A total of 119 patients (30 male /89 female, mean age: 57.7 \pm 12.01 years, mean body mass index: 24.3 \pm 6.67 kg/m²) were enrolled in this study. The clinical characteristics of patients with TJA are shown in Table 1. Among the 119 patients, 42 suffered from hypertension, 8 patients had diabetes, 7 were diagnosed with coronary heart disease, 2 had cerebrovascular disease, 2 experienced joint replacement surgery and 3 were glucocorticoids users.

Table-1: SClinal characteristics in all TJA Patients.

	TJA (n=119)	THA(n=48)	TKA(n=71)
Age (years)	57.8 \pm 12.0	63.5 \pm 7.9	49.3 \pm 12.2
Sex (male, %)	25.2	35.4	18.3
BMI (kg/m ²)	24.3 \pm 1.3	24.1 \pm 1.8	24.5 \pm 1.4
Hypertension (%)	35.3	22.9	43.7
Diabetes (%)	7.6	2.1	11.3
Cerebrovascular disease (%)	1.7	2.1	1.4
Coronary heart disease (%)	5.9	0	9.9
Use of glucocorticoids (%)	2.5	6.3	0
Joint replacement (%)	1.7	2.1	1.4

Values presented as mean \pm SD or n (%); BMI, body mass index; TJA, total joint arthroplasty; THA, total hip arthroplasty; TKA, total knee arthroplasty; SD, standard deviation; TJA group includes both THA and TKA.

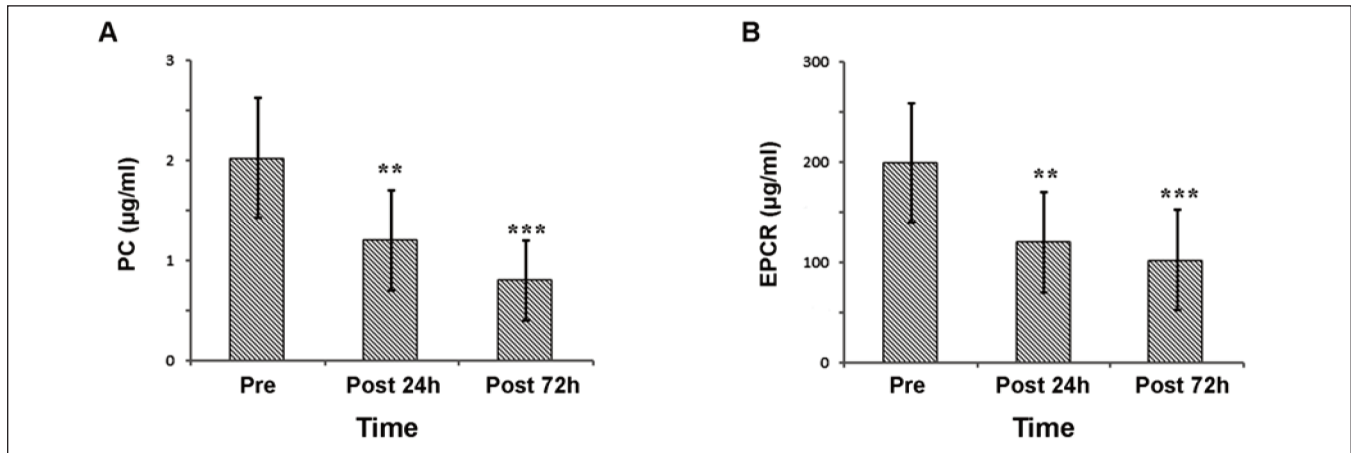


Figure-1: Changes of the Protein C (PC) and Endothelial cell protein C receptor (EPCR) levels in patients compared to healthy controls in the pre- and postoperative period. Both the pre- and postsurgical plasma samples were significantly different in comparison to the control. The boxes represent the mean data ranges. The perpendicular lines represent standard deviation. Statistical differences were observed in the plasma levels of these biomarkers preoperatively and on postoperative 24h, 72h. **denotes $p < 0.05$. *** denotes $p < 0.01$.

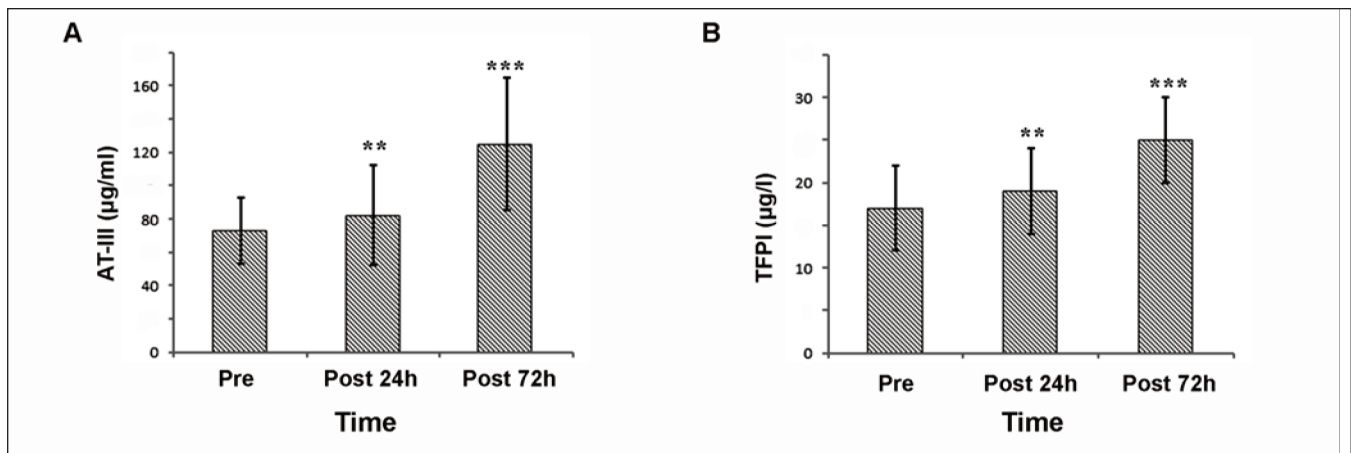


Figure-2: Changes of the Antithrombin III (AT-III) and Tissue factor pathway inhibitor (TFPI) levels in patients compared to healthy controls in the pre- and postoperative period. Both the pre- and postsurgical plasma samples were significantly different in comparison to the control. The boxes represent the mean data ranges. The perpendicular lines represent standard deviation. Statistical differences were observed in the plasma levels of these biomarkers preoperatively and on postoperative 24h, 72h. **denotes $p < 0.05$. *** denotes $p < 0.01$.

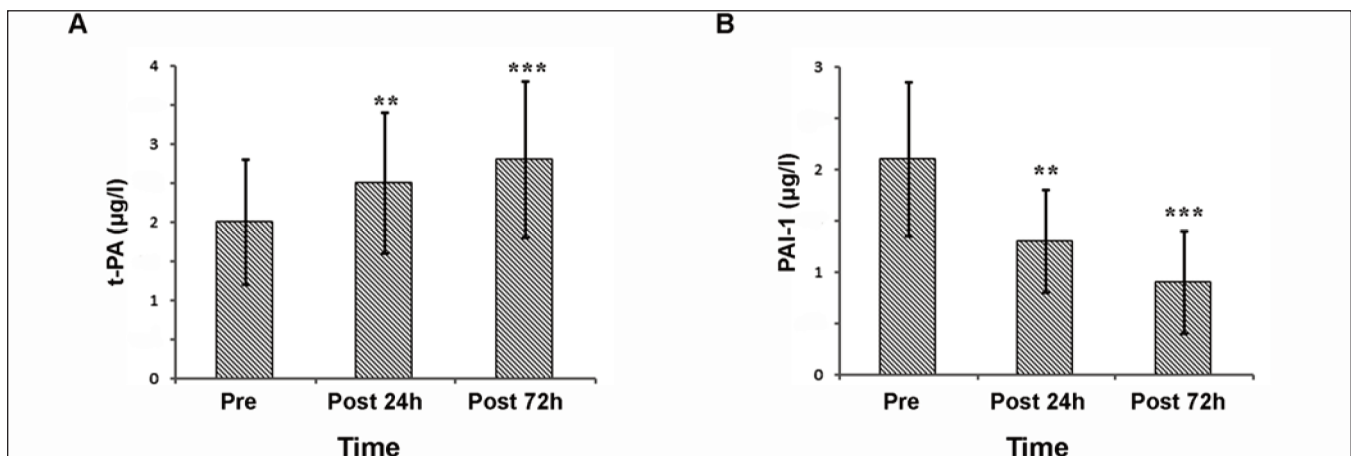


Figure-3: Changes of the Tissue plasminogen activator (tPA) and Plasminogen activator inhibitor 1 (PAI-1) levels in patients pre- and postoperative period compared to healthy controls. Both the pre- and postsurgical plasma samples were significantly different in comparison to the control. The boxes represent the mean data ranges. The perpendicular lines represent standard deviation. Statistical differences were observed in the plasma levels of these biomarkers preoperatively and on postoperative 24h, 72h. **denotes $p < 0.05$. *** denotes $p < 0.01$.

Table-2: Preoperative and Postoperative Values for coagulation and fibrinolytic biomarkers.

	Pre	24h	72h	p-value (Pre vs 24h)	p-value (Pre vs 72h)
PC (µg/ml)	2.03±0.66	1.20±0.36	0.88±0.30	0.0176	0.00315
EPCR (µg/ml)	199.4±57.4	118.9±36.3	100.8±31.0	0.0446	0.00955
AT-III (µg/ml)	70.0±20.5	81.4±24.9	118.7±34.6	0.0129	0.00432
TFPI (µg/l)	16.68±8.92	20.14±7.94	26.37±7.91	0.0268	0.00677
tPA (ng/ml)	2.03±1.03	2.53±0.86	2.87±0.83	0.0409	0.00675
PAI-1 (ng/ml)	2.03±0.66	0.88±0.30	1.20±0.36	0.0201	0.00917

PC, Protein C; EPCR, Endothelial cell protein C receptor; AT-III, Antithrombin III; TFPI, Tissue factor pathway inhibitor; tPA, Tissue plasminogen activator; PAI-1, Plasminogen activator inhibitor 1.

The levels of PC and its receptor EPCR were measured in TJA patients before and 24h, 72h after the procedure, respectively (Table 2). PC was significantly decreased in postoperative 24h and 72h groups compared to preoperative group (Figure 1A), while the change of the expression of EPCR is consistent with PC (Figure 1B). The reduced expression levels of both PC and its receptor validated the hypercoagulable state in TJA patients postoperatively. It was suggested that reduced bioactivity of the PC anticoagulation system was involved in the pathogenesis of thrombosis event in TJA patients. The level of another important physiological anticoagulant factor AT-III was also investigated (Table 2). An increasing level of AT-III expression was found at the postoperative state (Figure 2A). The postoperative TFPI expression demonstrated an increasing trend when compared with the preoperative level, which indicated an antithrombosis tendency (Figure 2B).

As a physiological activator for human fibrinolysis system that is synthesized by the endothelial cells, tPA could transfer plasminogen into plasmin and dissolve thrombus. Compared to the preoperative state, the expression of PAI-1 was significantly reduced while that of tPA was increased (Table 2, Figure 3), which indicated the state of hyperfibrinolysis following TJA.

Follow up at 3-month revealed that the morbidity of DVT was 5% (6/119), all ultrasound result were intravenous thrombus. No patient experienced high-risk pulmonary embolism. It illustrated that anticoagulation therapy is essential and effective.

Discussion

As mentioned previously, prevalence of DVT following TJA was high despite the wide use of anticoagulation measures. The factors of DVT formation included slow blood flow, vascular wall injury and hypercoagulatable state. Under a normal physiologic state, coagulation, haemostatic function and anticoagulation, fibrinolytic function of the body are in a dynamic equilibrium. Any alteration from either side would result in a pathological state or occurrence of disease. Strengthened coagulation,

haemostatic function, reduced anticoagulation and fibrinolytic function would lead to thrombosis. Coagulation is induced by multiple procoagulant factors such as endothelial injury and tissue factor release, which is activated in a cascade manner by series of coagulation-related cytokines.^{23,24} The studies of the pathogenesis of postoperative thrombotic events in TJA patients were of great importance for the prevention of thrombotic events after joint replacement. The changes of coagulation and fibrinolysis system were observed in TJA patients before, during and after the operation. Our research found that human body was in a hypercoagulable and hyperfibrinolytic state after TJA. It was observed that the expression of PC and its specific receptor EPCR were both decreased after the operation, which implied the hypercoagulatable state in TJA patients postoperatively. The plasma PAI-1 can promptly bind with tPA in the 1:1 ratio, which then deactivates tPA.²⁵ Therefore, increase of PAI-1 can be observed at the pre-thrombotic state and during thrombosis. The expression of tPA was increased, while that of its inhibitor PAI-1 was decreased after the operation.

It was reported that surgical stress led to reduced regional release of AT-III through impairing the endothelial cell function, which activated the intrinsic and extrinsic coagulation pathways, consumed a lot of AT-III and eventually weakened the anti-thrombotic function.²⁶ It has been shown that the extrinsic coagulation pathway is mainly regulated by TFPI, a Kunitz-type serine protease inhibitor.²⁷ Our results were opposite to the previous results. The following reasons were considered. Direct anticoagulant effect of antithrombin was slow and weak, but its anticoagulant effect can be enhanced for 2000 times after combining with heparin. Physiologically, antithrombin enhanced the anticoagulation of vascular endothelium by binding heparan sulfate on the surface of endothelial cells. All of our patients received low-molecular-weight heparin anticoagulation immediately after the operation. It might be due to the use of anticoagulants, the expression of physiological anticoagulant substances such as AT-III and TFPI were increased after surgery.

At present, conventional heparin, low-molecular-weight heparin, as well as vitamin K antagonists are proved to be effective for the prevention and treatment of thrombosis, which also have limitations.²⁸ Currently, rivaroxaban, dabigatran and apixaban have been licensed to be used in patients after TJA operation.²⁸ Current evidence suggested that rivaroxaban reduced the incidence of symptomatic VTE compared with enoxaparin, whereas drug-related bleeding events were also increased.²⁹ Thus, new oral anticoagulants have become the focus of current research.

Conclusion

The reduced expression of PC and EPCR after TJA indicated PC anticoagulation system was inhibited and a hypercoagulatable state was present. Meanwhile, increased expression of a fibrinolytic system marker tPA and decreased expression of PAI-1 suggested a state of hyperfibrinolysis observed following TJA. Multiple cytokines such as TFPI and tPA are all secreted by endothelial cells, which indicated that the changes may correlate with the injuries of vascular endothelial cells. Moreover, the trial of new anticoagulants was also closely related to the pathophysiological process of postoperative coagulation, which was worthy of our further investigation and tracking.

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Conflict of Interest: None.

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