Dynamic changes of coagulation and fibrinolytic biomarkers in perioperaive 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 day1, 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

24 tissue plasminogen activator (tPA) were measured with commercially available

25 enzyme-linked immunosorbent assay kits.

26 **Results:** Postoperative levels of coagulation parameters TFPI and AT-III were

27 increased compared to preoperative values (118.7±34.6 vs 70.0±20.5 μg/ml for

28 AT-III, and 26.37±7.91vs 16.68±8.92 μg/l for TFPI), while postoperative levels

29 of coagulation parameters PC and EPCR were decreased (0.88±0.30 vs

30 2.03±0.66 μg/ml for PC, and 100.8±31.0 vs 199.4±57.4 μg/ml for EPCR).

31 Postoperative levels of fibrinolytic parameter tPA was increased compared to

32 preoperative values (2.87±0.83 vs 2.03±1.03 μg/l), while its specific inhibitor

33 PAI-1 was decreased (0.88±0.30 vs 2.03±0.66 μg/l).

34 **Conclusion:** These results demonstrated the perturbation of the coagulation and

35 fibrinolytic system of patients undergoing TJA. Hypercoagulation and

36 hyperfibrinolysis were observed in postoperative patients, which suggested

37 anticoagulant therapy is effective and necessary.

38 **Keywords**：Total joint arthroplasty, anticoagulation, fibrinolysis.

39

Introduction

41 Total joint arthroplasty (TJA), including total knee arthroplasty (TKA) and total

42 hip arthroplasty (THA), is an effective treatment in patients with severe joint

43 diseases. It was well determined that patients with TJA had a high risk of

44 postoperative bleeding events and venous thromboembolism (VTE) [1-3]. TJA is

45 associated with a risk of thromboembolism because patients undergoing this

46 procedure had a hypercoagulable state frequently. According to previous studies,

47 the incidence of VTE in patients with TJA could be as high as 15~30 %, which

48 led to a mortality rate of 2 % because of threatening pulmonary embolism [1-3].

49 It was also shown that perioperational thrombotic events induced a

50 hypercoagulable state and increased fibrinolytic activity after TKA [4, 5].

51 Understanding of the coagulable and fibrinolytic dysregulation will be

52 important to have more therapies to prevent thrombotic events in TJA patients.

53 In 2013, the new guideline recommended that anticoagulant therapy should be

54 used for at least 14 days after TJA to prevent VTE [2]. The use of anticoagulant

55 agents (such as warfarin and low molecular weight heparin) protected patients

56 from potential thrombotic events. The mechanisms for postoperative

57 coagulation dysregulation are not fully understood. Protein C (PC) is a major

58 component of the human anticoagulation system, which regulates thrombosis

59 and limits inflammatory response [6]. Protein C circulates in the blood in the form

60 of inactive zymogen. During surgical stress, PC on the surface of endothelial

61 cells is activated by binding thrombin and thrombomodulin [7]. Endothelial cell

62 protein   C   receptor   (EPCR)   can   augment   the

63 thrombin-thrombomodulin-mediated PC activation [8]. (AT-III), the inhibitor for

64 serine proteases, is the most important physiological anticoagulant in the plasma

65 [9]. Tissue factor pathway inhibitor (TFPI), also known as an exogenous pathway

66 inhibitor (EPA) and lipoprotein-related coagulation inhibitor (LACI), is a major

67 inhibitor during the extrinsic coagulation pathway [10]. TFPI is an endogenous

68 anticoagulant protein produced primarily by endothelial cells and secondarily

69 from liver and monocytes/macrophages [10]. It was shown that TFPI can be

70 combined with heparin sulfate on the surface of endothelial cells. Heparin

71 injection could release TFPI from endothelial cells, so it was considered that the

72 increased TFPI in plasma was correlated with the routine application of

73 low-molecular-weight heparin for preventive anticoagulation [10, 11].

74 complications, including allergic reactions, infectious agent contamination,

75 transfusion related acute lung injury and hemolytic complications [12-14].

76 Increased fibrinolytic activity in blood samples after TKA might be attributed to

77 increased fibrin degradation, which leads to excessive bleeding [15]. Tissue

78 plasminogen activator (tPA) is an important element of the fibrinolytic system [16].

79 tPA has a high affinity towards the fibrin-plasminogen complex within the

80 thrombus, which enables it to rapidly form into fibrin-tPA-plasminogen

81 complex. This process greatly facilitates the activation of plasminogen and

82 exerts a highly efficient and specific thrombolytic effect [17]. The production and

83 release of tPA was decreased in the injured vascular endothelial cells, which

84 induce thrombosis and decreased fibrinolytic activity [18]. Plasminogen activator

85 inhibitor 1(PAI-1) is a specific inhibitor of tPA, which is produced and released

86 primarily by vascular endothelial cells and partially by platelets [19, 20].

87 All of the above basic biomarkers are used for further clinical application.

88 Throughout the coagulation process, a small quantity of activated coagulation

89 factors could activate a large number of cascaded downstream coagulation

90　factors, so the whole coagulation process shows a huge amplification

91　phenomenon. Antithrombin-, protein C- or protein S- deficiency constitutes a

92　major risk factor for venous thromboembolism [21, 22]. Understanding of the

93　coagulable and fibrinolytic dysregulation would be important to have more

94　therapies to prevent thrombotic events and bleeding complications in patients

95　after TJA surgery. In this research, we evaluated the coagulation functions and

96　fibrinolytic parameters during the perioperative period, which provide evidence

97　for the time window of anticoagulation treatment after TKA surgery.

98

Patients and Methods

100　A total of 119 patients enrolled in the study were scheduled consecutively to

101　undergo primary THA or TKA at the Jishuitan Hospital of Peking University

102　from January to April in 2016. Only patients with surgical indications for THA

103　or TKA participated in this study. TKA were performed in 48 patients and THA

104　were performed in 71 patients. The surgical indications included instability,

105　pain, deformity, and dysfunction of the hip or knee joint. Patients who meet any

106　of the following criteria were considered not eligible for the study: (1) patients

107　with cerebral or gastrointestinal bleeding within the previous 6 months; (2)

108　ischemic heart disease in the previous 6 months; (3) hepatic failure; (4)

109　end-stage kidney disease; (5) coagulation or fibrinolysis disorder; (6) any kind

110　of cancer. Before their enrollment, all patients were provided a detailed

111　explanation of the risks and alternatives to participation in this research and

112 informed consent was provided according to the Declaration of Helsinki. This

113 study was approved by the institutional review board of Beijing Jishuitan

114 Hospital.

115 Blood samples were obtained from peripheral veins on the preoperative day,

116 postoperative 24h and postoperative 72h respectively. Samples were centrifuged

117 at 3000 rpm for 15 minutes at room temperature. Plasma samples were stored at

118 -80 °C. Protein levels of antithrombin III, protein C, endothelial protein C

119 receptor, tissue factor pathway inhibitor, plasminogen activator inhibitor 1and

120 tissue plasminogen activator were measured from these plasma samples with

121 enzyme linked immunosorbent assay (ELISA) kit (Elabscience, E-EL-H0432c

122 for antithrombin III, E-EL-H1167c for protein C, E-EL-H0065c for endothelial

123 protein C receptor, E-EL-H0165c for tissue factor pathway inhibitor,

124 E-EL-H2104c for plasminogen activator inhibitor 1, E-EL-H2106c for tissue

125 plasminogen activator). Incidences of high-risk pulmonary embolism and DVT

126 were estimated through inquiring and vascular ultrasound with a 3-month

127 follow-up.

128 Continuous data were expressed as mean ± standard deviation and compared

129 with Student's t-test or one-way analysis of variance (ANOVA). Categorical

130 data were presented as percentages and frequencies and compared with

131 chi-square test. All statistical analyses were performed with SPSS 19.0 software.

132 P-values<0.05 were considered statistically significant.

133

Results

A total of 119 patients (30 male /89 female, mean age: 57.7±12.01 years, mean body mass index: 24.3±6.67 kg/m^2) 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 were in presence of diabetes, 7 diagnosed with coronary heart disease, 2 diagnosed with cerebrovascular disease, 2 experienced joint replacement surgery and 3 were glucocorticoids users. 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 (Fig. 1A), while the change of the expression of EPCR is consistent with PC (Fig. 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 (Fig. 2A). The postoperative TFPI expression demonstrated an increasing trend when compared with the preoperative level, which indicated an antithrombosis tendency (Fig. 2B).

As a physiological activator for human fibrinolysis system that is synthesized by the endothelial cells, tPA could transfer plasminogen into plasmin and

156 dissolve thrombus. Compared to the preoperative state, the expression of PAI-1

157 was significantly reduced while that of tPA was increased (Table 2, Fig. 3),

158 which indicated the state of hyperfibrinolysis following TJA.

159 Follow up at 3-month revealed that the morbidity of DVT was 5% (6/119), all

160 ultrasound result were intravenous thrombus. No patient experienced high-risk

161 pulmonary embolism. It illustrated that anticoagulation therapy is essential and

162 effective.

163

Discussion

165 As mentioned previously, prevalence of DVT following TJA was high despite

166 the wide use of anticoagulation measures. The factors of DVT formation

167 included slow blood flow, vascular wall injury and hypercoagulatable state.

168 Under a normal physiologic state, coagulation, hemostatic function and

169 anticoagulation, fibrinolytic function of the body are in a dynamic equilibrium.

170 Any alteration from either side would result in a pathological state or

171 occurrence of disease. Strengthened coagulation, hemostatic function, reduced

172 anticoagulation and fibrinolytic function would lead to thrombosis. Coagulation

173 is induced by multiple procoagulant factors such as endothelial injury and tissue

174 factor release, which is activated in a cascade manner by series of

175 coagulation-related cytokines [23, 24]. The studies of the pathogenesis of

176 postoperative thrombotic events in TJA patients were of great importance for

177 the prevention of thrombotic events after joint replacement. The changes of

178 coagulation and fibrinolysis system were observed in TJA patients before,

179 during and after the operation. Our research found that human body was in a

180 hypercoagulable and hyperfibrinolytic state after TJA. It was observed that the

181 expression of PC and its specific receptor EPCR were both decreased after the

182 operation, which implied the hypercoagulatable state in TJA patients

183 postoperatively. The plasma PAI-1 can promptly bind with tPA in the 1:1 ratio,

184 which then deactivates tPA [25]. Therefore, increase of PAI-1 can be observed at

185 the pre-thrombotic state and during thrombosis. The expression of tPA was

186 increased, while that of its inhibitor PAI-1 was decreased after the operation.

187 It was reported that surgical stress led to reduced regional release of AT-III

188 through impairing the endothelial cell function, which activated the intrinsic and

189 extrinsic coagulation pathways, consumed a lot of AT-III and eventually

190 weakened the anti-thrombotic function [26]. It has been shown that the extrinsic

191 coagulation pathway is mainly regulated by TFPI, a Kunitz-type serine protease

192 inhibitor [27]. Our results were opposite to the previous results. The following

193 reasons were considered. Direct anticoagulant effect of antithrombin was slow

194 and weak, but its anticoagulant effect can be enhanced for 2000 times after

195 combining with heparin. Physiologically, antithrombin enhanced the

196 anticoagulation of vascular endothelium by binding heparan sulfate on the

197 surface of endothelial cells. All of our patients received low-molecular-weight

198 heparin anticoagulation immediately after the operation. It might be due to the

199 use of anticoagulants, the expression of physiological anticoagulant substances

200 such as AT-III and TFPI were increased after surgery.

201 At present, conventional heparin, low-molecular-weight heparin, as well as

202 vitamin K antagonists are proved to be effective for the prevention and

203 treatment of thrombosis, which also have limitations [28]. Currently, rivaroxaban,

204 dabigatran and apixaban have been licensed to be used in patients after TJA

205 operation[28]. Current evidence suggested that rivaroxaban reduced the incidence

206 of symptomatic VTE compared with enoxaparin, whereas drug-related bleeding

207 events were also increased [29]. Thus, new oral anticoagulants have become the

208 focus of current research.

209

210 **Conclusion**

211 The reduced expression of PC and EPCR after TJA indicated PC

212 anticoagulation system was inhibited and a hypercoagulatable state was present.

213 Meanwhile, increased expression of a fibrinolytic system marker tPA and

214 decreased expression of PAI-1 suggested a state of hyperfibrinolysis was

215 observed following TJA. Multiple cytokines such as TFPI and tPA are all

216 secreted by endothelial cells, which indicated that the changes may correlate

217 with the injuries of vascular endothelial cells. Moreover, the investigation of

218 new anticoagulants was also closely related to the pathophysiological process of

219 postoperative coagulation, which was worthy of our further investigation and

220 tracking.

221

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223 **Conflict of Interest:** None to declare.

224 **Funding Disclosure:** None to declare.

225

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Table 1: Clinal characteristics in all TJA Patients.

	TJA (n=119)	THA(n=48)	TKA(n=71)
Age (years)	57.8±12.0	63.5±7.9	49.3±12.2
Sex（male，%）	25.2	35.4	18.3
BMI（kg/m^2）	24.3±1.3	24.1±1.8	24.5±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

305 Values presented as mean±SD or n (%).

306 BMI, body mass index; TJA, total joint arthroplasty; THA, total hip

307 arthroplasty; TKA, total knee arthroplasty; SD, standard deviation; TJA group

308 includes both THA and TKA.

309 ---

310 **Table 2: Preoperative and Postoperative Values for coagulation and**

311 **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

312   PC, Protein C; EPCR, Endothelial cell protein C receptor; AT-III, Antithrombin

313   III; TFPI, Tissue factor pathway inhibitor; tPA, Tissue plasminogen activator;

314   PAI-1, Plasminogen activator inhibitor 1.

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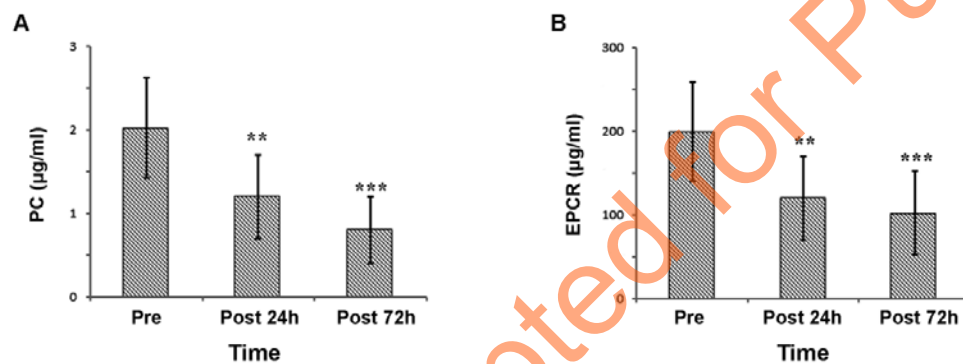316   --

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319   **Figure 1: Changes of the Protein C (PC) and Endothelial cell protein C**

320   **receptor (EPCR) levels in patients compared to healthy controls in the pre-**

321   **and postoperative period. Both the pre- and postsurgical plasma samples**

322   **were significantly different in comparison to the control. The boxes**

323   **represent the mean data ranges. The perpendicular lines represent**

324   **standard deviation. Statistical differences were observed in the plasma**

325   **levels of these biomarkers preoperatively and on postoperative 24h, 72h.**

326   ****denotes P <0.05. *** denotes P <0.01.**

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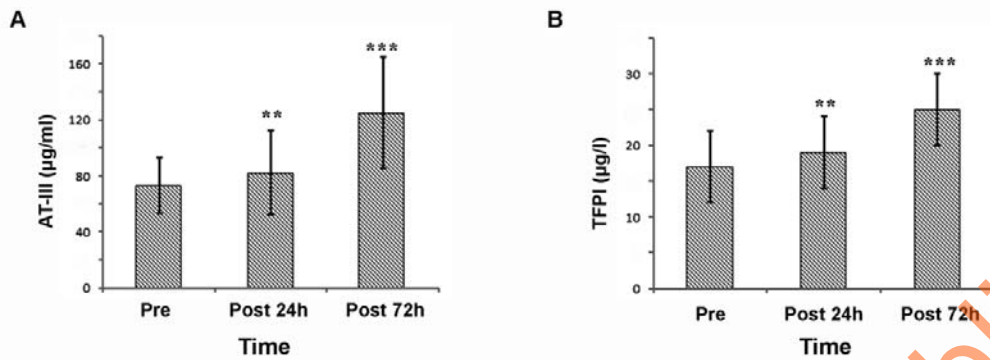
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Figure 2. Changes of the Antithrombin III (AT-III) and Tissue factor pathway inhibitor (TFPI) levels in patients compared to healthy controls in the pre- and postoperative period. Both the pre- and postsurgical plasma samples were significantly different 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.**

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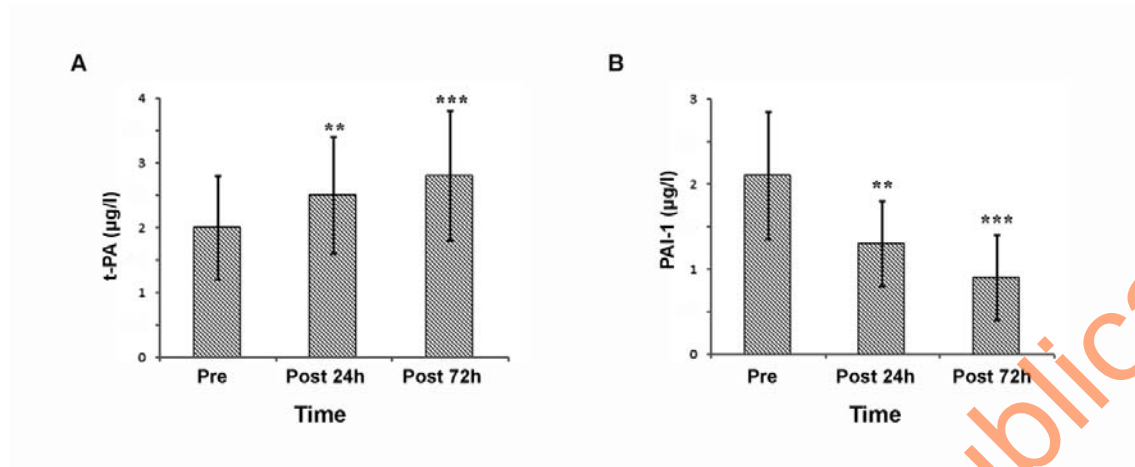341 --

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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 <0.0 . *** denotes P <0.01.

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