A study on prothrombogenic and antithrombogenic biomarkers in deep vein thrombosis following meta-epiphyseal cancellous bone traumatization in major orthopedic surgeries

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Abstrak

Latar belakang: *Penelitian ini bertujuan untuk menunjukkan perbedaan rerata nilai rasio Kolagen I (Kol I)/*Nitric Oxide*(NO), Kolagen IV (Kol IV)/NO,* Tissue Factor *(TF)/NO, P-selectin (P-sel)/NO antara pasien dengan trombosis vena dalam (TVD) positif dan TVD negatif, pasca traumatisasi spongiosa meta-epifisis sendi panggul dan lutut pada operasi besar ortopedi.*

Metode: *Studi observasional kohort prospektif kasus operasi ortopedi tanpa tromboprofilaksis pada 69 pasien berumur > 50 tahun. Pemeriksaan kadar serum biomarker Kol I, Kol IV, TF, P-sel, dan NO dilakukan tiga kali yaitu sebelum operasi, 72 jam sesudah operasi, dan 144 jam sesudah operasi, masing-masing untuk melihat perbedaan rerata kadar biomarker dan rerata nilai rasio protrombogenik/antitrombogenik (Kol I/NO, Kol IV/NO, TF/NO dan P-sel/NO) pada 72 jam dan 144 jam sesudah operasi antara pasien TVD positif dan TVD negatif. Kejadian TVD dikonfirmasi pada 144 jam sesudah operasi dengan venografi (kecuali pada 8 kasus yang dikonfirmasi dengan USG* color Doppler *karena kontra indikasi zat warna).*

Hasil: *Kejadian TVD positif didapatkan pada 18 pasien (26,1%). Perbedaan rerata kadar antara TVD positif dan TVD negatif ditemukan pada Kol IV sebelum operasi (p = 0,022) dan pada NO 72 jam sesudah operasi (p = 0,014). Perbedaan rerata nilai rasio protrombogenik/ antitrombogenik antara TVD positif dan TVD negatif ditemukan pada rasio Kol IV/NO, TF/NO, dan P-sel/NO pada 72 jam sesudah operasi (p = 0,007; p = 0,028; p = 0,049), dengan median yang lebih rendah pada pasien dengan TVD positif. Sedangkan pada 144 jam sesudah operasi, perbedaan rerata nilai rasio hanya ditemukan pada rasio Kol IV/NO (p = 0,014) dengan nilai median yang lebih tinggi dari median pada 72 jam sesudah operasi.*

Kesimpulan: *Kejadian TVD pada traumatisasi spongiosa meta-epifisis pasca operasi besar ortopedi sendi panggul dan lutut dipengaruhi oleh keseimbangan protrombogenik dan antitrombogenik yang ditunjukkan dengan adanya perbedaan rasio Kol IV/NO, TF/NO dan Psel/NO pada 72 jam dan rasio Kol IV/NO pada 144 jam sesudah operasi antara TVD positif dan TVD negatif.* ***(Med J Indones. 2013;22:9-15)***

Abstract

Background: This study was aimed to show differences in the mean values of Collagen I (Col I)/Nitric Oxide (NO), Collagen IV (Col IV)/NO, Tissue Factor (TF)/NO, and P-selectin (P-sel)/NO ratios between patients with DVT and those without DVT, following hip and knee meta-epiphyseal cancellous bone traumatization in major orthopedic surgeries.

Methods: This is an observational prospective cohort study on 69 patients aged > 50 years, who had orthopedic surgery without thromboprophylaxis. Examination of serum Col I, Col IV, TF, P-sel, and NO biomarker levels were performed three times, i.e. before surgery, 72 hours and 144 hours after surgery. We looked for the differences in mean levels of biomarkers, and mean ratio values of the prothrombogenic/antithrombogenic (Col I/NO, Col IV/NO, TF/NO, P-sel/NO) at 72 hours and 144 hours post surgery between patients with DVT and those without. DVT events, which were confirmed at 144 hours post surgery by venography (with the exception of 8 cases where color Doppler ultrasound was done due to contrast usage contraindications).

Results: DVTs were identified in 18 patients (26.1%). There were significant differences of mean levels in pre-surgical Col IV ($p = 0.022$) and 72 hours NO ($p = 0.014$) between patients with and without DVT. In addition, between the same two patient groups, significant differences were found in the mean values of the prothrombogenic/antithrombogenic ratios, i.e. Col IV/NO, TF/NO, and P-sel/NO at 72 hours post-surgery ($p = 0.007$, $p = 0.028$, and $p = 0.049$ respectively), with lower median values that were found in subjects with DVT. At 144-hours post surgery, the only significant ratio difference between the two groups was the mean values of Col IV/NO ratio ($p = 0.014$) with the median values that were higher than the median values at 72-hours post surgery .

Conclusion: The incidence of DVT following traumatization of the meta-epiphyseal cancellous bone after major orthopedic surgeries in hip and knee is influenced by the balance of prothrombogenic and antithrombogenic factors as shown by the significant differences in Col IV/NO, TF/NO and P-sel/NO ratios at 72-hours and Col IV/NO ratio at 144 hours after surgery between DVT positive and DVT negative patients. ***(Med J Indones. 2013;22:9-15)***

Keywords: *Collagen I, collagen IV, deep vein thrombosis, nitric oxide, orthopedic surgery, P-selectin, tissue factor*

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Deep vein thrombosis (DVT) is a condition where a total or partial blockage occurs due to blood clots in the deep veins of the lower extremities, namely the tibial, fibular, popliteal, femoral or ileofemoral vein segments. Deep vein thrombosis is characterized by the presence of venous hypertension, leg swelling, and venous valvular damage, which can progress into deadly pulmonary embolism (PE).[1] The Seventh American College of Chest Physicians (ACCP) Conference on Antithrombotic and Thrombolytic Therapy: Evidence Based Guidelines (2004), stated that patients who had undergone hip and knee arthroplasty or hip fracture surgery comprise the group with the highest incidence of DVTs. A preliminary study conducted by researchers in Indonesia showed that the incidence of DVT is 32.6% for the total arthroplasty (TA) group, 17.2% for the open reduction and internal fixation (ORIF) of the proximal femur fracture group, and 8.8% for the hemiarthroplasty (HA) group.[2]

DVTs are the end result of a balancing process between the prothrombogenic stimuli that form the thrombus and the other protective mechanisms that liquefy it.[3] The pathomechanism of the high incidence of DVT after major orthopaedic surgeries have not yet been fully elucidated. The significant traumatization to the meta-epiphyseal cancellous bone of the hip and knee is believed to be a contributing factor.[2] To investigate this effect, the prothrombogenic and antithrombogenic biomarkers that may represent the involved mechanisms in thromboses that follow major orthopaedic surgeries are assessed. Col I biomarker, 90% of which is found in the skeleton, and Col IV that is found in the basal lamina of subendothelial matrix layer of blood vessels, act as initiators of platelet activation in primary hemostasis, and also act when factor XII triggers secondary hemostasis.[4,5] Tissue factor is a biomarker, which is found in subendothelial tissues, platelets, and white blood cells and acts as a trigger for thrombus formation, and also as an amplifying nidus. In normal homeostasis, blood clots form early in the venous valve axillae.[6] Endothelial cell damage leads to an increase of Col and TF, which in turn activates the platelets.[7] This condition will trigger the inflammatory process during the first 6 hours after thrombus formation, by presenting circulating adhesion molecule P-selectin (P-sel) in the vein wall. The P-sel biomarker plays an important role in thrombus growth.[8] Biomarkers for thrombus enlargement will reach its peak in the 5th day and start to decline at the 14th day, with decreasing levels of pro-inflammatory mediators due to the down regulating mechanisms.[9,10] Antithrombogenic NO biomarker, which is found in the endothelium of the blood vessel walls serves to inhibit platelet adhesion and to keep the blood liquid.[11,12]

Serial monitoring of prothrombogenic (Col I, Col IV, TF, and P-sel) and antithrombogenic (NO) factors help us observe the whole DVT formation process. This study aimed to show differences in the mean values of Collagen I (Col I)/Nitric Oxide (NO), Collagen IV (Col IV)/NO, Tissue Factor (TF)/NO, and P-sel/NO ratios between patients with DVT and those without DVT, following hip and knee meta-epiphyseal cancellous bone traumatization in major orthopedic surgeries.

METHODS

This observational, consecutive prospective cohort study was conducted on patients aged over 50 years old who were admitted into two private hospitals in Jakarta from May 2010 through September 2011 for major hip and knee surgery (TA, HA, and ORIF) without thromboprophylaxis. The required sample size of 69 patients was determined by comparative analytic unpaired categorical formula, by using the DVT's incidence of 32.6% for Indonesia,[2] and of 70% for Western countries.[13] The excluded criteria were strictly enforced in an effort to reduce the multiple confounding factors in the hosts. Excluded were patients with a history of DVTs, pulmonary embolism, malignancy, estrogen hormone consumption, total paralysis due to stroke, central venous catheter usage, major surgery within the preceding 3 months, inflammatory bowel disease (IBD), nephrotic syndrome, hemostatic disorders, smoking, sepsis, or with body mass index (BMI) $\geq$ 35. The other confounding factors, which could not be excluded as in some comorbidities would be taken in count in their relationship to the level of biomarkers and the value of prothrombogenic/ antithrombogenic ratios, and subsequently to the incidence of DVT. Any antithrombotic therapy (chemical or mechanical) must be stopped one week prior to surgery.

All surgical procedures were performed by a single orthopaedic surgeon according to international standard operating procedures of the company producing the implant, which emphasizes tissue-preservation techniques. Collection of serum for Col I, Col IV, TF, P-sel, and NO were serially performed three times. The first blood collection was done just before the surgery, and was used to compare mean levels of the biomarkers in the subjects subdivided by demographic/ comorbidity parameters, as well as in patients with and without DVTs. The second blood collection was done 72-hours after the surgery, and the third 144-hours after surgery. They were used to compare mean biomarker levels between the DVT positive and DVT negative patient groups. After that, the mean levels of prothrombogenic/antithrombogenic (Col I/NO, Col IV/

NO, TF/NO, P-sel/NO) ratios 72-hours and 144-hours after surgery were compared in the DVT positive and DVT negative patient groups. Subjects were evaluated by venography (considered to be the gold standard) at 144-hours post surgery to look for the presence of DVTs. However, doppler color ultrasound (USG) were used in diagnosing lower limb DVT as alternative in cases of contrast usage contraindications. Doppler color ultrasonography is considered to have 95.6% sensitivity compared to venography.[14] Deep vein thrombosis positive subjects were then treated with oral anticoagulant drugs.

This study used bivariate analysis (t-test or Mann-Whitney test) to compare the mean levels of biomarkers at 72-hours and 144-hours after surgery between the DVT positive and DVT negative groups. The same tests were also used to compare the mean values of prothrombogenic/antithrombogenic (Col I/NO, Col IV/NO, TF/NO, P-sel/NO) ratios at 72-hours and 144 -hours after surgery between the DVT positive and DVT negative groups.

To show the interference of confounding factors to the biomarkers and to the incidence of DVTs, we used t-test or Mann-Whitney test for the differences of mean levels of biomarkers before surgery to subjects subdivided by demographics and comorbidities. We used also Chi-square or Fisher test for the relationship of subjects subdivided by demographics and comorbidities to the presence of DVTs. A p level of < 0.05 was considered to be significant. This study was approved by the ethics committees of both hospitals.

RESULTS

Of the 69 study subjects, venography (or ultrasound color Doppler in 8 cases) at 144 hours after surgery revealed 18 patients with DVT (26.1%). The demographic data for the study patients (Fable 1) was remarkable for the predominance of female gender (75.4%), those aged > 70 years (68.1%), and BMI < 25 kg/m^2 (71%). Hypertension and Diabetes Mellitus (DM) were the most frequent comorbidities. Bone Mass Densitometry (BMD) values with T score < -2.5 (osteoporosis) were found in 52.2% of subjects. There were two times more patients with fractures compared to those with OA.

Table 2 shows the results of t-test or Mann-Whitney test, which compared different subject demographic and comorbidity subcategories for each pre-surgical biomarker level, as well as the relationship of those demographic and comorbidity subcategories to the presence of DVT at 144-hours post-surgery. This table aims to show the role of confounding factors in the study samples.

Col IV levels differed significantly in patients with and without Osteoarthrosis (OA), Chronic Kidney Disease (CKD), and hypertension. Tissue factor levels differed significantly in female compared to male patients, while NO levels also differed significantly in patients with OA compared to whom with fracture. There were no significant differences in Col I and P-sel levels for each of the demographic/comorbidities tested. However, when the subject demographic and comorbidity subgroups were evaluated for DVTs using Chi-square or Fishers test, only female and OA patient subgroups had a significanlty high OR (7.77 and 3.65) for devoloping DVTs.

Further analysis was then conducted to look for biomarker level differences before the surgery, 72-hours and 144-hours after surgery between patients with and without DVT (Table 3). Table 3 shows the t-test or Mann-Whitney test results for the level differences between patients with and without DVT, before surgery, at 72-hours and at 144-hours after surgery. Only Col IV levels before the surgery and NO levels at 72-hours after surgery were found to be significantly different between patients with and without DVT, while none of biomarkers at 144-hours after surgery differed.

Subsequently, we analyzed the ratios of each prothrombogenic biomarker (Col I, Col IV, TF, and

Table 1. Distribution of subjects by demographics and comorbidities

Demographics and Comorbidites n = 69	n (%)
Female / Male	52 (75.4%) / 17 (24.6)
Age > 70 / 50-70 years	47 (68.1%) / 22 (31.9%)
BMI ≥ 25 / < 25 kg/m2	20 (29%) / 49 (71%)
OA / Fracture	23 (33.3%) / 46 (66.7%)
CKD / non CKD	2 (2.9%) / 67 (97.1%)
Stroke / non stroke	8 (11.6%) / 61 (88.4%)
CD / non CD	5 (7.2%) / 64 (92.8%)
DM / non DM	16 (23.2%) / 53 (76.8%)
Hypertension / non hypertension	31 (44.9%) / 38 (55.1%)
Osteoporosis / no osteoporosis	36 (52.2%) / 33 (47.8%)

OA = Osteoarthrosis, BMI = Body mass index, CKD = Chronic kidney disease, CD = Cardiac dysfunction, DM = Diabetes mellitus, n = Total sample

Table 2. Pre-surgical biomarker-level mean difference between subjects subdivided by demographics and comorbidities, and relationship of demography and comorbidity to DVTs at 144-hours after surgery

Demographics and Comorbidites	Col I	Col IV	TF	P-Sel	NO	DVT
n = 69	p	p	p	p	p	OR
Female/Male	0.928^{++}	0.063^{++}	0.018^{+}	0.471^{+}	0.344^{++}	7.77*
Age (> 70/50-70)	0.395^{++}	0.155^{++}	0.346^{+}	0.292^{+}	0.137^{++}	1.90*
BMI (≥ 25/< 25)	0.863^{++}	0.075^{++}	0.450^{+}	0.606^{++}	0.331^{++}	1.32*
OA/Fracture	0.268^{++}	0.009^{++}	0.728^{+}	0.584^{++}	0.007^{++}	3.65*
CKD/non CKD	0.334^{++}	0.045^{++}	0.567 $^{++}$	0.381^{++}	0.773^{++}	- **
Stroke/non stroke	0.978^{++}	0.107^{++}	0.197^{+}	0.561^{++}	0.168^{++}	0.93*
CD/non CD	0.611^{++}	0.096^{++}	0.650^{+}	0.523^{+}	0.105^{++}	2.00*
DM/non DM	0.509^{++}	0.130^{++}	0.074^{+}	0.249^{++}	0.678^{++}	2.05*
H/non H	0.772^{++}	0.006^{++}	0.258^{+}	0.385^{++}	0.884^{++}	1.31*
O/non O	0.349^{++}	0.207^{++}	0.268^{+}	0.616^{+}	0.243^{++}	0.65*

OA = Osteoarthrosis, BMI = Body mass index, CKD = Chronic kidney disease, CD = Cardiac dysfunction, DM = Diabetes mellitus, H = Hypertension, O = Osteoporosis, Col I = Collagen I, Col IV = Collagen IV, TF = Tissue factor, P-sel = P-selectin, NO = Nitric oxide, OR = Odds ratio, DVT = Deep vein thrombosis, n = Total sample, + = T-test, ++ = Mann-Whitney test, * = Chi square test, ** = Fisher test

Table 3. Comparison analysis of biomarker levels between DVT and non-DVT, before surgery, at 72-hours and at 144-hours after surgery

Biomarkers n = 69	DVT vs non-DVT		
	Before surgery	72-hours after surgery	144-hours after surgery
	p	p	p
Col I	0.499^{++}	0.101^{++}	0.243^{++}
Col IV	0.022^{++}	0.891^{++}	0.647^{++}
TF	0.827^{++}	0.838^{++}	0.558^{+}
P-sel	0.675^{+}	0.523^{+}	0.271^{++}
NO	0.325^{++}	0.014^{++}	0.249^{++}

Col I = Collagen I, Col IV = Collagen IV, TF = Tissue factor, P-sel = P-selectin, NO = Nitric oxide, DVT = Deep vein thrombosis, n = Total sample, + = T-test, ++ = Mann-Whitney test

P-sel) to the antithrombogenic biomarker (NO) at 72-hours after surgery, and 144-hours after surgery to look for differences in patients with and without DVT. The results are shown in table 4.

Table 4 shows the t-test or Mann-Whitney test results for the biomarker ratios between DVT positive and negative patients. Significant differences at 72-hours after surgery between patients with and without DVT were noted in the Col IV/NO, TF/NO, and P-sel/NO ratios (p = 0.007, p = 0.028 and p = 0.049 respectively). At 144-hours after surgery the only ratio found to differ significantly between patients with and without DVT was the Col IV/NO ratio (p = 0.014).

DISCUSSION

From the 69 consecutive subjects in the study, 18 patients (26.1%) were diagnosed to have DVT 144-hours after surgery. Some of the confounding factors that could not be excluded, and statistically related to the occurrence of DVT were female gender (p = 0.029) and OA diagnosis (p = 0.020). Col IV level was the only preoperative biomarker that significantly differed between patients with and without DVT (p = 0.022).

The relationship between postmenopausal female gender and increased risk of DVT was in accordance with several other studies.[13,15,16] The decrease of natural estrogen production in postmenopausal females is one

Table 4. Comparison analysis of biomarker ratios at 72-hours and 144-hours after surgery between DVT positive and negative patients

Ratios n = 69	DVT		p
	Positive n = 18 (26.1%) median/min-max	Negative n = 51 (73.9%) median/min-max	
72-hours after surgery			
Col I / NO	25.39/1.39-135.78	35.05/10.10-130.96	0.172++
Col IV / NO	19.75/4.11-145.58	38.51/8.90-168.92	0.007++
TF / NO	55.50/5.28-148.23	78.24/18.08-208.19	0.028++
P-sel / NO	11.72/1.95-30.80	15.33/1.00-40.44	0.049++
144-hours after surgery			
Col I / NO	30.71/2.73-221.89	32.12/9.56-253.18	0.967++
Col IV / NO	34.44/6.47-75.62	40.48/8.64-151.57	0.014+
TF / NO	89.89/19.39-164.50	81.72/17.23-195.15	0.429+
P-sel / NO	20.09/4.60-49.32	20.51/1.00-61.49	0.642+

Col I = Collagen I, Col IV = Collagen IV, TF = Tissue factor, P-sel = P-selectin, NO = Nitric oxide, DVT = Deep vein thrombosis, n = Total sample, Med = Median, Min = Minimum, Max = Maximum, + = T-test, ++ = Mann-Whitney test

possible explanation, and women comprise 75% of our subjects. Estrogen is known to have a protective role against thromboses. Low levels of estrogen stimulate the production of microparticles that result in elevated levels of TF, which was found also in our subjects, which in turn may also increase the risk of cerebro- and cardio-vascular accidents.[17]

There is no known direct relationship between OA and DVT, but it is known that patients with OA have lower levels of Col IV. The decreased levels of Col IV in OA are associated with the degree of inflammation and synovial lining thickness. This may occur due to the down-regulation effect of TNF-α and IL-1 in inflammatory conditions that suppress the local expression of Col IV.[18] As such, further investigation is needed to determine whether OA causes DVT through proinflammatory mechanisms. Another possible explanation on the relationship between OA and DVT in our study is the problem of sample distribution, in that 95% of our OA patients were postmenopausal women (22/23). Estrogen decline due to menopause is known to decrease NO, increase proinflammatory cytokines, increase TF, and increase the incidence of CVA. Therefore, the association between pre-surgical Col IV level to DVT as shown in our results can also be explained by the estrogen decline in OA patients who were mostly postmenopausal women.

Biomarker levels that were statistically different in patients with and without DVT were Col IV before the surgery (p = 0.022) and NO at 72-hours after surgery (p = 0.014) (Table 3). This fact suggests an association between Col IV levels before surgery to DVT events, which is likely due to the association between OA to DVT, as lower levels of Col IV have been found in OA patients, similar to our study results (Table 2). Therefore, a number of confounding factors that were present in our study might affect the relation between biomarker levels and the occurance of DVTs.

The difference in NO levels at 72-hours after surgery between patients with and without DVT events is also of interest. NO works as an antithrombotic compound (endogenous inhibitor) to inhibit platelet aggregation and adhesion, inhibit thrombus enlargement, as well as to prevent the interaction of monocytes with the endothelium.[19] The increasing levels of NO at 72-hours after surgery may indirectly indicate the growing thrombus formation that occurs after surgery, as well as the growing of the antithrombotic compounds as the body's counter mechanism.

Prothrombogenic biomarker (Col I, Col IV, TF, and P-sel) results at 72-hours and 144-hours after surgery were similar between patients with and without DVT (Table 3). However, the increase of antithrombotic NO levels at 72-hours after surgery might indicate the occurrence of DVT, which indirectly shows high prothrombogenic and antithrombogenic activity that might occur at 72-hours after surgery.

As none of the biomarkers could show dominant role in the occurance of DVTs, except for the antithrombogenic NO at 72-hours after surgery, we evaluated the biomarker ratios to look for significant difference between DVT positive and negative patients. The prothrombogenic (Col I, Col IV, TF, P-sel) to antithrombogenic (NO) ratio data at 72 and 144-hours after surgery could better reveal the difference in prothrombogenic and antithrombogenic balance between DVT positive and negative patients.

At 72-hours after surgery, Col IV/NO, TF/NO, and P-sel/NO ratios were each significantly different between patients with and without DVT ($p = 0.007$; $p = 0.028$ and $p = 0.049$ respectively) (Table 4). These results indicate an important role of NO as an antithrombogenic biomarker in the process of DVT formation, especially 72-hours after major orthopedic surgery.

At 144-hours after surgery, only the Col IV/NO ratio differed significantly in patients with and without DVT. The median of Col IV/NO ratio of the DVT positive patients at 144-hours after surgery was higher than corresponding ratios at 72-hours after surgery. This fact implies that in DVT patients, Col IV at 144-hours after surgery were still active doing its prothrombogenic role, while the antithrombogenic NO levels were declining.

This study suggests that the balance of prothrombogenic/antithrombogenic biomarkers might play a role in DVT occurence following traumatization of meta-epiphyseal cancellous after major orthopedic surgeries in hip and knee, with the dominant role of NO as the antithrombogenic factors trying to counter-balance the prothrombogenic effects of Col IV especially 72-hours until 144-hours after surgery. However, various confounding factors related to the study subjects could affect the biomarker levels and the occurrence of DVTs.

In conclusion, the incidence of DVT following traumatization of the meta-epiphyseal cancellous bone after major orthopedic hip and knee surgeries is influenced by the balance of prothrombogenic and antithrombogenic factors as shown by the differences in Col IV/NO, TF/NO and Psel/NO ratios between DVT positive and DVT negative patients. At 72-hours after surgery, Col IV plays a bigger role as a trigger in the occurrence of DVT than Col I, while NO plays a central role in the defensive mechanism against thrombus formation.

Therefore, we suggest to conduct another study with a more balanced subject demographic and comorbidities, particularly with regards to gender, age, presence of OA, DM and hypertension in order to minimize the effects of confounding factors on the biomarkers. We also suggest to do a study, which uses additional prothrombogenic and antithrombogenic biomarkers to give better insight into the pathomechanism of thromboses after traumatization of meta-epiphyseal cancellous bone. Further, a study on the role of the Col I as a factor in the high incidence of DVT following major orthopedic surgery is of high importance.

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