P-Selectin as Predictor Venous Thromboembolism in Cancer Patients Undergoing Chemotherapy

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ABSTRACT

Background: Venous thromboembolism (VTE), consisting of deep vein thrombosis (DVT) and pulmonary embolism (PE), is the second-leading cause of death in patients with malignancy. P-selectin, the member of the selectin family of cell adhesion molecules, is found in the alpha granules of platelets and the Weibel-Palade bodies of endothelial cells. It is express on the cell surface on activation, mediates the adhesion of leukocytes, platelets, and cancer cells in inflammation, thrombosis, and cancer growth and metastasis. Recently, P-selectin has been investigated as a novel predictor for DVT. **Objectives:** To investigate the role of sP-selectin as predictors of DVT in cancer patients undergoing chemotherapy. **Patients and Methods:** This prospective cohort study was conducted in Dr. Kariadi hospital, Semarang, Indonesia, on 40 newly diagnosed cancer patients. Venous blood samples were drawn prior and after initial chemotherapy, for sP-selectin measurement with ELISA method. These patients were observed for the possibility of developing VTE during three-months period. **Results:** DVT occurred in 5 (12.5%) patients after a median period of 8 weeks. The most frequent cancer type was colorectal cancer (45%) and cervical cancer (15%). The cut-off point sP-selectin pre-and post- chemotherapy were 106,7 ng/ml and 111,7 ng/ml respectively. The median levels of sP-selectin in DVT patients pre-chemotherapy was 121.0ng/ml (IQR 107.5-230.6) and post-chemotherapy was 204.4ng/ml (IQR 110.9-278.3). In other hand, the median levels of sP- selectin pre-chemotherapy and post- chemotherapy in DVT negative patients were 82.0ng/ml (IQR 31.3-230.6) and 92.5 (IQR 40.9-278.3), respectively. With cut-off point sP-selectin level 111,7 ng/ml, the relative risk of DVT event was 8,7 (95% CI 1,017-74,39). **Conclusion:** In this study, high plasma levels of s P-selectin are predictive for venous thromboembolism in cancer patients undergoing chemotherapy.

Keywords: venous thromboembolism, sP-selectin, cancer, chemotherapy

Venous thromboembolism (VTE), consisting of deep vein thrombosis (DVT) and pulmonary embolism (PE), is the second-leading cause of death in patients with malignancy. The risk for VTE increased in hospitalized cancer patient and in those on active therapy. VTE results in a requirement for long-term anticoagulation, risk of bleeding complications, risk of recurrent events even with anticoagulation, delay or discontinuation of chemotherapy, consumption of healthcare resources, and a potential impact on patient quality of life.(1)(2)

The pathogenesis of VTE in cancer patients appears to be multifactorial. The most important clinical determinants for the risk of VTE are: (i) cancer-related factors (primary site, extensive disease, time interval from cancer diagnosis); (ii) patient-related factors (raised body mass index, reduced mobility, comorbidities, sepsis, previous VTE, abnormalities in blood counts); and (iii) treatment-related factors (chemotherapy, hormonal and biological therapy, surgery, indwelling catheters, erythropoietin stimulating agents).(3)

Among tumor sites, the very high risk for thrombosis were pancreas and stomach, the high risk were lung, lymphoma, gynecologic, bladder and testicular; and the lower risk were breast and prostate cancer.(4) There also appears to be a time-dependent variation in the VTE risk. During the first 3 months after cancer diagnosis, patients are at highest risk of VTE.(5)(6)

Virchow's triad consisting of stasis, endothelial injury and hypercoagulability has long been known as the major risk factor predisposing patients to VTE. This model is particularly illustrative for cancer and thrombosis. Elevation in coagulation activation and thrombin generation (prothrombin fragment 1+2, thrombin-antithrombin complex, and D-dimer), alteration in fibrinolysis (plasmin-antiplasmin complexes, PAI-1), decreased inhibitors (protein C, protein S, antithrombin) and activated protein C resistance are considered as markers of hypercoagulability in cancer. Venous stasis in cancer may due to decreased patient mobility, extrinsic compression by tumor or node and venous invasion by tumor. Endothelial injury in cancer may as a result from tumor invasion,

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surgery, radiation, central venous catheter and chemotherapy.(7)(8) Recent advances in the epidemiology, pathophysiology and management of thrombosis in cancer patients.

Several biomarkers have been identified as potentially predictive for VTE. Pre-chemotherapy elevation in leukocyte and platelet count and low haemoglobin levels are all predictive for chemotherapy associated VTE.(9) Other biomarkers, including soluble P-selectin, prothrombin fragment 1,2, factor VIII, C reactive protein and D-dimers have been studied as well.

P-selectin (CD62) is localized in the alpha granules of platelets and the Weibel-Palades bodies of endothelial cells, and belong to the selectin family of cell adhesion molecules. P-selectin mediates binding to specific carbohydrate-containing ligands, like PSGL-1 (P-selectin glycoprotein ligand-1) which is present on the majority of leukocytes and in smaller amounts of platelet. Soluble P-selectin (sP-selectin) is a circulating form of P-selectin, which results from shedding of the extracellular domain and maintains the requirements for ligand binding.(10) Plasma levels of sP-selectin are elevated in acute deep vein thrombosis. Furthermore, high levels of sP-selectin were associated with increased risk for recurrence of DVT(11)(12) and in cancer patients, high plasma levels of sP-selectin were predictive for VTE[13]. It is very interesting that cancer cells are able to enhance P-selectin expression on monocytes, macrophages, endothelial cells, and platelets. In the other hand, cancer cells also express CD24 on their surface which was identified to be a receptor for P-selectin. The interaction between P-selectin and CD24 on cancer cells allows their interaction with platelets, and their adherence to endothelium in the process of metastatic spread.(10)

The aim of the study is to investigate the role of sP-selectin as predictive parameter for the occurrence of VTE in cancer patients undergoing chemotherapy.

METHODS

Study setting

This cohort prospective study was performed in Dr. Kariadi hospital, the university hospital of Diponegoro University, Semarang, Indonesia. The research protocol was approved by the Review Board of the Dr. Kariadi hospital. Written informed consent was obtained from patients.

Patients and data collection

Between November 2016 and February 2017, 40 consecutive patients with active cancers were enrolled in the study. All patients were informed about the details of the study in an individual interview. The inclusion criteria for the study were as follows: (i) patients with newly diagnosed of cancer (ii) histological confirmation of the diagnosis (iii) age over 18 years (iv) willingness to participate and (v) written informed consent. Exclusion criteria were overt bacterial or viral infection within the last two weeks, venous or arterial thromboembolism within the last three months and continuous anticoagulation with vitamin K-antagonists or low molecular weight heparin (LMWH), and surgery or radiotherapy within the last two weeks.

Patients underwent a structured interview on their medical history, and data on tumor site, tumor histology and tumor stage were documented. Patients were given detailed written information about symptoms of VTE and were asked to report immediately to our center, if such symptoms occurred. A blood sample for determination of laboratory parameters was drawn before and after initial chemotherapy. The observation period started at the time of blood sampling. Patients were contacted every month via telephone to get information about the clinical course of their disease regarding occurrence of VTE. Observation period ended after 3-mo nths period, or until the occurrence of VTE, death, loss of follow-up or withdrawal of consent (which one of these came first).

Diagnosis of deep vein thrombosis

Color duplex sonography was performed at the Division of Radiology of Dr. Kariadi Hspital, Semarang, Indonesia. Patients with clinically suspected DVT and the pretest probability (Well scores $\geq$ 2) were performed color duplex ultrasonography. To avoid investigator-related variations of the results, color duplex sonography was performed in each patient by the same investigator. Diagnosis VTE was established when patient presented symptoms of VTE and positive findings either in duplex sonography or venography.

Blood sampling and laboratory analysis

Venous blood specimens were collected by sterile and atraumatic antecubital venipuncture, and collected in in citrate vacutainer tubes SST 5ml, containing 0.5ml of liquid anticoagulant. To obtain platelet-poor plasma, the citrated blood was centrifuged at 1000g (3000 rpm) for 15 min. Plasma aliquots were stored at-20 °C until they were assayed for the determination of sP-selectin plasma levels in series. Samples were coded prior to laboratory analysis. P-selectin levels were measured using a recombinant human P-selectin

Immunoassay/CD62P catalog number ADP3 (R&D Systems, Inc. 614 McKinley Place NE, Minneapolis, MN 55413, USA) following the manufacturer´s instructions.

Blood samples were scheduled to be drawn at the following time-points: (i) baseline, before initial chemotherapy, and (ii) the day after initial chemotherapy completely administrated.

Statistical analysis

Continuous variables were summarized with mean (SD) or medians (25^{th}-75^{th} percentile), whereas categorical data were described by absolute frequencies and percentages. The correlation between two continuous variables were evaluated with Spearman rank correlation coefficient. Fisher exact test was performed if the number of cells with expected frequency less than 5. Independent t-tests were done to compare numeric variables between DVT and non DVT patients. The assumption of normality test of the data was checked before the t-test. Mann-Whitney were done when the data were not normally distributed. The cut-off-point of significance was p=0.05 with 95% confidence interval. The median follow-up time was calculated with the reverse Kaplan Meier method 33. A log rank test was used to compare the time until first thrombosis in these two groups.

RESULTS

Between November 2016 and February 2017, 40 patients with newly diagnosed cancer were enrolled in the study. One patient died before given chemotherapy on 6^{th} weeks. DVT occurred in 5 (12.5%) patients after a median period of 8 weeks. Positive findings in duplex sonography or venography established the diagnosis of DV

Table 1. Clinical characteristics of patients. n=40

Variable	N	%
Age		
<41	11	27.5
41-59	25	62.5
>59	4	10
Sex		
Male	22	55
Female	18	45
Body mass index		
Underweight	11	27.5
Normal	29	72.5
Site of cancer		
Rectal	10	25
Colon	8	20
Cervical	6	15
Pancreas	3	7.5
Lung	2	5
Gaster	2	5
Haematological	2	5
Others	7	17.5
Stage of cancer		
Localized	23	57.5
Distance metastatic	15	37.5
Unclassified	2	5

The mean age of patients was 47±11. The most frequent cancer type was colorectal cancer (45%) and cervical cancer (15%).

Plasma concentrations of sP-selectin in cancer patients with and without DVT

The plasma concentration of sP-selectin in cancer patients without DVT, pre- and post-chemotherapy administration (median, IQR) were: 82ng/ml, IQR (31.3-230.6) and 92.5ng/ml, IQR (40.9-278.3), respectively. The plasma concentrations sP-selectin in cancer patients with DVT, before and after chemotherapy (median, IQR) were: 121.0 ng/ml, IQR (107.5-230.6) and 204.4ng/ml, IQR (110.9-278.3), respectively. (Normal value sP-selectin: 0.99-47.7ng/ml).

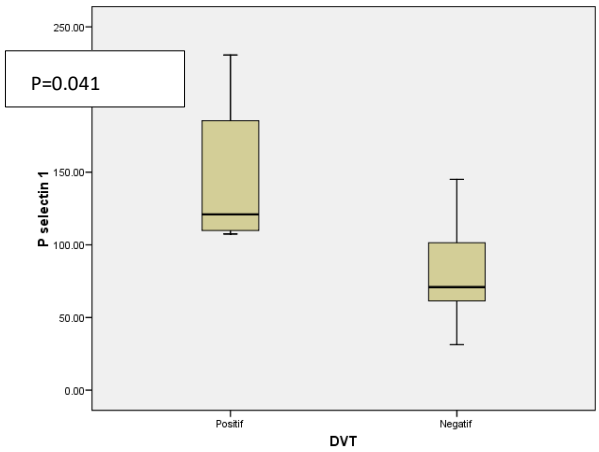

Figure 1.The plasma concentrations of sP-selectin levels pre-chemotherapyin 40 cancer patients with and without DVT. The difference is significant (p=0.041). Normal value sP-selectin: 0.99-47.7ng/ml.

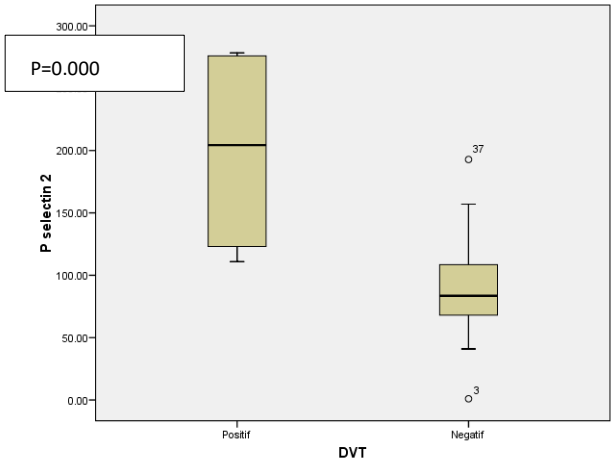

Figure 2.The plasma concentrations of sP-selectin levels post-chemotherapy in 40 cancer patients with and without DVT. The difference is significant (*p=0.000*). Normal value sP-selectin: 0.99-47.7ng/ml.

The cut-off point sP-selectin pre- and post-chemotherapy were 106,7 ng/ml and 111,7 ng/ml respectively. With the cut-off point P-selectin level > 111,7 ng/ml, the relative risk of DVT event was 8,7 (95% CI 1,017 – 74,39). (Table 2)

Table 2. Risk Estimate for the DVT occurrence with cut-off plasma levels sP-selectin111.7ng/ml in 39 cancer patients undergoing chemotherapy

	Value	Lower (95% CI)	Upper (95% CI)
Relative risk for sP-selectine 2(>111.7/≤111.7)	12.000	1.078	133.603
For cohort DVT = positive	8.700	1.017	74.390
For cohort DVT = negative	.725	.480	1.094
N of valid cases	39*		

The relative risk for DVT for 39 cancer patients undergoing chemotherapy with plasma levels sP-selectine >111.7ng/ml was 8.7 (95% CI 1.017-74.390). *one patient was died during the 6th week follow-up.

DISCUSSION

In the present study, we investigate the role of sP-selectine as a novel predictor DVT in cancer patients undergoing chemotherapy. Our finding demonstrates that plasma levels sP-selectine >111.7ng/ml, the relative risk for DVT is 8.7 (95% CI 1.017-74.390). Therefore, it means that high levels of sP-selectin are predictor for DVT in cancer patients undergoing chemotherapy.

Result from the Vienna CATS (Cancer And Thrombosis Study) demonstrates that elevated sP-selectin (cut-off level, 53.1ng/ml, 75th percentile of study population) was a statistically significant risk factor for VTE after adjustment for age, sex, surgery, chemotherapy, and radiotherapy (hazard ratio=2.6, 95% confidence interval, 1.4-4.9, p=.003).(13)

Our data indicate that, the levels of sP-selectin were high in all cancer patients, either in DVT or non-DVT, and, either pre- or post-chemotherapy. Virchow's triad (stasis, endothelial injury, and hypercoagulability) has long been used to illustrate the major risk factors predisposing patients to VTE. In our study, the increased levels of sP-selectin in all cancer patients reflected the hypercoagulability (prothrombotic) condition, which increased risk for thrombosis in cancer patients.

Moreover, in all cancer patients, either withDVT or without DVT, the levels of sP-selectin in post chemotherapy were higher than those in pre-chemotherapy, and the difference was significant. This finding indicated, that chemotherapy may increase the levels of sP-selectin, and therefore increase the risk for thrombosis.

In a population-based study of patients with a new diagnosis of VTE, there was a significantly increased risk of VTE in those who were receiving chemotherapy [OR 6.5, CI(2.11-20)].(14) Our study, which involving plasma levels sP-selectine >111.7ng/ml as cut-off point, the RRwas 8.7 (95% CI 1.017-74.390). Other study reported that chemotherapy increased the risk of VTE by 2-fold to 6-fold, with doxorubicin containing regimens.(3) The mechanism behind the risk of thromboembolic events with chemotherapy treatments are poorly understood. Many of these chemotherapies induce vascular damage, either directly or indirectly, thereby promoting local activation of the coagulation process. A number of more recent reports have demonstrated the role of P-selectin in hemostasis and thrombosis, including the demonstration that overexpression of P-selectin can induce a procoagulant state.

One out of 40 patients died during follow-up. The clinical presentation of this patient were shock and sudden death. This patient died possibly due to pulmonary embolism.

In conclusion, our study demonstrated that: (i) high plasma levels of soluble P-selectin are predictive for venous thromboembolism in cancer patients; (ii) the levels of sP-selectin were high in cancer patients, and the levels in post-chemotherapy were significantly higher than those in pre-chemotherapy. Further studies are needed whether combination of high levels of sP-selectin and predictive model for calculating risk of chemotherapy associated thrombosis (e.g. Khorana predictive model) increase the benefit of prophylactic anticoagulation for cancer patients undergoing chemotherapy.

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REFERENCES

1. Mandala M, Clerici M, Corradino I, Vitalini C, Colombini S, Torri V, et al. Incidence, Risk Factors and Clinical Implications of Venous Thromboembolism in Cancer Patients Treated Within The Context of Phase I Studies: The "SENDO" Experience. Ann Oncol. 2012;23(6):1416–21.
2. Elting L, Escalante C, Cooksley C, Avritscher E, Kurtin D, Hamblin L, et al. Outcomes and Cost of Deep Venous Thrombosis Among Patients with Cancer. Arch Intern Med. 2004;164(15):1653–61.
3. Dutia M, White R, Wun T. Risk Assessment Models for Cancer-Associated Venous Thromboembolism. Cancer. 2012;118(14):3468–76.
4. Khorana A. Cancer-Associated Thrombosis:Updates and Controversies. ASH Education Book. 2012. 626-630 p.
5. Blom J, Vanderschoot J, Oostindier M, Osanto S, Van Der Meer F, Rosendaal F. Incidence of Venous Thrombosis in A

Large Cohort Of 66329 Cancer Patients: Results of A Record Linkage Study. J Thromb Haemost. 2006;4:529–35.
6. Ferroni P, Roseli M, Guadagni F. Impact of Chemotherapy in Cancer-Related Venous Thromboembolism. Looking For Prediction. J Cardiol Clin Res. 2013;1(1002):1–3.
7. Hicks L, Selby R. Recent Advances in The Epidemiology, Pathophysiology and Management of Thrombosis in Cancer Patients. Lab Med. 2004;35(8):492–6.
8. Mullard A, Innes H. Venous Thromboembolism in Malignancy. Clin Med (Northfield Il). 2014;14(5):532–4.
9. Khorana A, Kuderer N, Culakova E, Lyman G, Francis C. Development And Validation of A Predictive Model for Chemotherapy Associate Thrombosis. Blood. 2008;111(10):4902–7.
10. Gremmel T, Ay C, Seidinger D, Pabinger I, Panzer S, Koppensteiner R. Soluble P-Selectine, D-Dimer, And Hih-Sensitivity C-Reactive Protein After Acute Deep Vein Thrombosis of The Lower Limb. J Vasc Surg. 2011;54(48S–55S).
11. Rectenwald J, Myers DJ, Hawley A, Longo C, Henke P, Guire K, et al. D-Dimer, P-Selectin, and Microparticles: Novel Markers to Predict Deep Venous Thrombosis. A Pilot Study. Thromb Haemost. 2005;94(6):1312–7.
12. Kyrle P, Hron G, Eichinger S, Wagner O. Circulating P-Selectine and The Risk of Recurrent Venous Thromboembolism. Thromb Haemost. 2007;97:880–3.
13. Ay C, Simanek R, Vormittag R, Dunkler D, Alguel G, Koder S, et al. High Plasma Levels of Soluble P-Selectin are Predictive for Venous Thromboembolism in Cancer Patients: Results From The Vienna Cancer and Thrombosis Study (CATS). Blood. 2008;112:2703–8.
14. Khorana A. Cancer and Thrombosis: Implications of Published Guidelines for Clinical Practice. Ann Oncol. 2009;20(10):1619–30.