Soluble P-Selectin, Von Willebrand Factor, And Adamts13 Levels As Risk Factors Of Deep Vein Thrombosis In Cancer Patients Undergoing Chemotherapy

Budi Setiawan (boedhi_smg73@yahoo.com)
Dr Kariadi General Hospital Medical Center

Cecilia Oktaria Permatadewi
Dr Kariadi General Hospital Medical Center

Baringin de Samakto
Dr Kariadi General Hospital Medical Center

Ashar Bugis
Dr Kariadi General Hospital Medical Center

Eko Adhi Pangarsa
Dr Kariadi General Hospital Medical Center

Damai Santosa
Dr Kariadi General Hospital Medical Center

Catharina Suharti
Dr Kariadi General Hospital Medical Center

Research

Keywords:

DOI: https://doi.org/10.21203/rs.3.rs-34208/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background There is a high number of deep vein thrombosis (DVT) incidence among cancer patients undergoing chemotherapy. Chemotherapy-induced vascular endothelial cell activation (VECA) is marked with increasing plasma levels of von Willebrand Factor (VWF) and soluble P-selectin (sP-selectin) leading to activation of endothelial cells and coagulation cascade. The biological role of a disintegrin-like and metalloproteinase with thrombospondin type 1, motif 13 (ADAMTS13) is to control the activity of VWF. The objectives of this study is to investigate the role of sP-selectin, VWF, and ADAMTS13 as risk factors for the incidence of DVT in cancer patients undergoing chemotherapy. Methods This prospective cohort study was conducted in Dr. Kariadi hospital, Semarang Indonesia, on 40 cancer patients. Soluble P-selectin, VWF, and ADAMS13 plasma levels were determined with enzyme-linked immunosorbent assay (ELISA) method, examined before and after chemotherapy. These patients were observed for the possibility of developing DVT during three months. Results Deep vein thrombosis was confirmed in 5 patients (12.5%) after a median period of 8 weeks. In patients with DVT, sP-selectin and VWF were significantly higher, while ADAMTS13 were significantly lower compared in cancer patients without DVT. Pre- and post-chemotherapy concentration of sP selectin, VWF, and ADAMTS13 could effectively predict the incidence of DVT in cancer patients undergoing chemotherapy. The levels of sP-selectin, VWF and ADAMTS13 pre-chemotherapy with cut-off point >106.7 ng/mL, >2.99 U/mL and <0.80 U/mL, respectively, had relative risk (RR) for DVT incidence being 16 (95% CI 2.06-124.25, p=0.001); 36 (95% CI 5.21-248.65, p=0.000) and 10.5 (95% CI 1.31-84.28, p=0.015), respectively, whereas the levels of sP-selectin, VWF and ADAMTS13 post-chemotherapy with cut-off point >111.7 ng/mL, >3.06 U/mL and <0.49 U/mL, respectively, had RR for DVT incidence being 8.7 (95% CI 1.01-74.39, p=0.045); 20.4 (95% CI 2.60-159.94, p=0.004) and 26.25 (95% CI 3.50-196.48, p=0.002), respectively. Pre-chemotherapy vWF levels (cut-off value >2.99 U/mL) was found to be independently predict DVT incidence with RR 11.1 (95% CI, 1.95-62.74, p=0.007). Conclusions Plasma levels of VWF more than 2.99 U/mL pre-chemotherapy was an independent risk factor for DVT incidence, which could be performed early and helpful for thromboprophylaxis therapy.

Introduction

Deep vein thrombosis (DVT) and its major complication, pulmonary embolism (PE), designated together as venous thromboembolism (VTE), are the leading causes of death, morbidity, late treatment, and increasing treatment costs. Moreover, DVT in cancers prompts the risk of recurring DVT, bleeding complications, long-term need of anticoagulant, and interruptions to chemotherapy programs. Cancer patients have 4–7 times higher risk of having DVT compared to those without cancers. The incidence of DVT also increases among cancer patients undergoing chemotherapy. Chemotherapy increases the risk of DVT by six times and recurrent DVT by two times.

Systemic chemotherapy may cause injuries on blood vessel walls due to its direct toxic effect on endothelial cells which prompts endothelial cells activation. The increasing activation of endothelial cells is considered as one of the causes of hemostatic activation. Biochemically, chemotherapy-induced
vascular endothelial cell activation (VECA) is indicated by the increasing the number of endothelial cells in circulation, von Willebrand Factor (VWF) in plasma and endothelial cell activation markers such as vascular cell adhesion molecule-1 (VCAM-1), soluble P-selectin (sP-selectin) and E-selectin.\textsuperscript{5,6}

An appropriate tool of DVT risk stratifications facilitates a more effective thromboprophylaxis therapy. Khorana risk score is a score to predict the risk of cancer-related thrombosis in patients undergoing chemotherapy.\textsuperscript{7} Various recent studies have been aimed at finding other biomarkers with high predictive value on DVT incidence.\textsuperscript{8,9,10,11}

Soluble P-selectin (CD62) is located in platelets alpha granule and Weibel-Palade body (WPB) endothelial cell.\textsuperscript{12} Soluble P-selectin increases tissue factor (TF) expressions on monocytes and mediates TF transfer to platelets. Tissue factor is the main initiator of in vivo coagulation causing the activation of coagulation cascade.\textsuperscript{13} Plasma levels of sP-selectin increase in DVT. High levels of sP-selectin are also related to the increasing risk of recurrent DVT, and in cancer patients, high plasma levels of sP-selectin indication of DVT incidence.\textsuperscript{12}

A platelet-adhesive blood coagulation protein, VWF is synthesized mainly in the vascular endothelial cells and megakaryocytes, stored in WPB and platelets alpha granule in the form of "ultra large" VWF (UL-VWF). Von Willebrand factor is released into plasma in the form of UL-VWF, very active in interacting with platelets and collagen. In plasma, UL-VWF is rapidly depolymerazed into small sizes ranging from 500 to 20,000 kDa.\textsuperscript{14,15} Von Willebrand factor plays an important role in both primary and secondary hemostasis. This glycoprotein acts as a carrier for factor VIII and mediator for platelet adhesion to endothelial cells.\textsuperscript{16} Previous studies have shown that its number would increase in cancer patients with DVT, and it is related to cancer staging and metastasis. The increasing VWF antigen and the decreasing VWF-cleaving protease will prompt thrombogenesis in cancer patients.\textsuperscript{17}

The biological role of a disintegrin-like and metalloproteinase with thrombospondin type 1, motif 13 (ADAMTS13) is to regulate thrombosis by controlling VWF activities.\textsuperscript{9} The platelet adhesive nature of VWF depends on multimeric size and conformation in which large and long VWF multimers best react to platelets circulating in high shear stress and most thrombogenic. The VWF cleaving protease by ADAMTS13 determines the average size of circulating VWF by selectively cleaves the Tyr1605-Met1606 bond of VWF that becomes exposed under the influence of fluidic shear stress. ADAMTS13, therefore, has a significant systemic antithrombotic activity.\textsuperscript{15} The previous studies have indicated lower levels of ADAMTS13 in cancer patients than those without cancers, which lead to increasing risk of DVT. They also decreased in metastasis cancer patients.\textsuperscript{9}

Considering these data, the aim of this study was to investigate the role of sP-selectin, VWF, and ADAMTS13 as predictive biomarkers for the incidence of DVT in cancer patients undergoing chemotherapy.
Methods

Study setting

This prospective cohort study was performed in Dr. Kariadi hospital, the university hospital of Diponegoro University, Semarang, Indonesia. The study protocol was approved by the Ethical Committee of the Dr. Kariadi Hospital, and written informed consent was obtained from all study participants.

Patients and data collection

During November 2016 and February 2017, 40 consecutive patients with active cancers undergoing chemotherapy were enrolled in the study followed consecutively for 3 months. The inclusion criteria for the study were patients with newly diagnosed of cancer with histological confirmation, age over 18 years, willingness to participate, and undersigned a 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.

Before this study was conducted, all patients had been informed about study's details in an individual interview. Then, anamnesis on patients' cancer history, tumor site, tumor histology and tumor stage were documented. The patients who met the inclusion criteria were selected as the subjects of this study. Samples were examined twice, before and after chemotherapy, to see the plasma levels of sP-selectin, VWF and ADAMTS13. The patients were monitored for 3 months. In the first, second and third month, evaluations on DVT occurrence were conducted with Wells pretest probability model to assess DVT. If the Wells score was ≥ 2, color duplex sonography was performed. If there were no symptoms of DVT, and Wells score was < 2, color duplex sonography examination was conducted to determine DVT incidence at the end of the third month of chemotherapy.

Color duplex sonography

Color duplex sonography was performed at the Division of Radiology of Dr. Kariadi Hospital, Semarang, Indonesia. Patients with clinically suspected DVT and Wells score ≥ 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 at all time points.

Measurement of sP-selectin, VWF and ADAMTS13

Venous blood specimens were collected by sterile and atraumatic antecubital venipuncture, and were collected in citrate vacutainer tubes SST 5 ml, containing 0.5 ml of liquid anticoagulant. To obtain platelet-poor plasma, the citrated blood was centrifuged at 1000 g (3000 rpm) for 15 minutes. Plasma aliquots were stored at-20 °C until they were assayed to determine sP-selectin, VWF, and ADAMTS13 plasma levels in series. Samples were coded prior to laboratory analysis.
Measurements of sP-selectin levels were carried out using a recombinant human P-selectin Immunoassay/CD62P catalog number ADP3 (R&D Systems, Inc. 614 McKinley Place NE, Minneapolis, MN 55413, USA). Von Willebrand factor levels were measured by a VWF activity kit catalog number 885_BcSD20121001 (Sekisui Diagnostic, LLC 500 West Avenue, Stamford). ADAMTS13 levels were measured with a Quantikinine enzyme-linked immunosorbent assay (ELISA) Human ADAMTS13 Immunoassay catalog number DAD7130 (R&D System, 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 was completely administrated.

**Statistical analysis**

Continuous variables were summarized with mean (standard deviation [SD]) or median (25th-75th percentile) whereas categorical data were described by absolute frequencies and percentages. The correlation between two continuous variables was evaluated with Spearman rank correlation coefficient. Independent t-tests were done to compare numeric variables between DVT and non DVT patients. Test for normality of the data with Shapiro-Wilk test. Independent t-test was done when the data was normally distributed. Mann-Whitney was done when the data was not normally distributed. Receiver operating characteristic (ROC) curve analysis was performed to determine the cut-off point levels of sP-selectin, VWF, and ADAMTS13 for DVT incidence of cancer patients undergoing chemotherapy. The cut-off-point of significance was \( p = 0.05 \) with 95% confidence interval. Relative risk (RR) was determined with chi-square test between two comparative groups. All potential predictors were entered simultaneously in a multivariate logistic regression model that was reduced using a backward selection method. All test was two sided with \( p < 0.05 \) considered statistically significant. The Statistical Package for Social Science (IBM v 21; SPSS Inc., USA) was used for data analysis.

**Results**

During November 2016 and February 2017, 40 patients with newly diagnosed cancer were enrolled in the study. One patient died before given chemotherapy on 6th weeks. The median age of the patients was 49 (20–71) years old with male patients more dominant (55% vs 45%). The most frequent cancer types were colorectal cancer (45%) and cervical cancer (15%) (Table 1).
| Variable            | Patients n = 40 |
|---------------------|----------------|
| **Age**             | 49 (20–71)     |
| **Sex**             |                |
| Male, n (%)         | 22 (55)        |
| Female, n (%)       | 18 (45)        |
| **Body mass index** |                |
| Underweight, n (%)  | 11 (27.5)      |
| Normal, n (%)       | 29 (72.5)      |
| **Site of cancer**  |                |
| Rectal, n (%)       | 10 (25)        |
| Colon, n (%)        | 8 (20)         |
| Cervical, n (%)     | 6 (15)         |
| Pancreas, n (%)     | 3 (7.5)        |
| Lung, n (%)         | 2 (5)          |
| Gaster, n (%)       | 2 (5)          |
| Haematological, n (%)| 2 (5)         |
| Others, n (%)       | 7 (17.5)       |
| **Stage of cancer** |                |
| Localized, n (%)    | 23 (57.5)      |
| Distance metastatic, n (%) | 15 (37.5) |
| Unclassified, n (%) | 2 (5)          |
| **Hemoglobin (g/dL)** | 11 (6–16)   |
| **White blood cell (x10^9/L)** | 9.2 (3.9–99.1) |
| **Platelet (x10^9/L)** | 340 (51–766) |
| **D-Dimer (ng/mL)** | 815 (230–5050) |
| **DVT**             |                |
| Positive, n (%)     | 5 (12.5)       |
The cancer patients’ levels of sP-selectin were relatively high (normal value levels of sP-selectin 0.99–47.7 ng/mL) both before and after chemotherapy. The mean levels of sP-Selectin pre-chemotherapy and post-chemotherapy were $87.61 \pm 39.03$ ng/mL and $98.52 \pm 54.76$ ng/mL, respectively (Table 2). In patients with DVT, the levels of sP-selectin pre-chemotherapy and post-chemotherapy were higher than patients without DVT. The median levels of sP-selectin pre-chemotherapy and post-chemotherapy in the group of patients with DVT were $121.0$, IQR $107.5-230.6$ ng/mL and $204.4$, IQR $110.9-278.3$ ng/mL, respectively. In cancer patients with DVT, the levels of sP-selectin were significantly higher than those without DVT in both pre-chemotherapy and post-chemotherapy ($p = 0.041$; $p = 0.000$, respectively) (Table 3 and Fig. 1, A).

**Table 2:** sP-selectin (ng/mL), VWF (U/mL), and ADAMTS13 (U/mL) count at baseline, before initial chemotherapy, and the day after initial chemotherapy completely administrated (3 months).

| Biomarker | Pre-Chemoterapy | Post-Chemoterapy |
|-----------|-----------------|------------------|
|           | Mean ±SD | Median (IQR) | Mean ± SD | Median (IQR) |
| sP-Selectin | $87.61 \pm 39.03$ | $81.95 (31.3 – 230.6)$ | $98.52 \pm 54.76$ | $92.5 (40.9 – 278.3)$ |
| VWF | $1.57 \pm 0.87$ | $1.30 (0.37 – 3.75)$ | $1.83 \pm 0.89$ | $1.50 (0.72 – 3.97)$ |
| ADAMTS13 | $1.06 \pm 0.50$ | $0.86 (0.42-2)$ | $1.13 \pm 0.55$ | $0.94 (0.31-2)$ |

The levels of VWF in the cancer patients were relatively high (normal value levels of VWF 0.5–1.5 U/mL) pre-chemotherapy and post-chemotherapy. The mean levels of VWF pre-chemotherapy and post-chemotherapy were $1.57 \pm 0.87$ U/mL and $1.83 \pm 0.89$ U/mL, respectively (Table 2). In patients with DVT, the levels of VWF pre-chemotherapy and post-chemotherapy were higher than those without DVT. The median levels of VWF pre-chemotherapy and post-chemotherapy in the patients with DVT were $3.03$, IQR $2.97–3.75$ U/mL and $3.43$, IQR $3-3.97$ U/mL, respectively. In cancer patients with DVT, the levels of VWF were significantly higher than those without DVT in both pre-chemotherapy and post-chemotherapy ($p = 0.001$; $p = 0.002$, respectively) (Table 3 and Fig. 1, B).

The levels of ADAMTS13 in the cancer patients were relatively normal (normal value levels of ADAMTS13 0.72–1.17 U/mL) pre-chemotherapy and post-chemotherapy. The mean levels of ADAMTS13 pre-chemotherapy and post-chemotherapy were $1.06 \pm 0.50$ U/mL and $1.13 \pm 0.55$ U/mL, respectively (Table 2). In patients with DVT, the levels of ADAMTS13 pre-chemotherapy and post-chemotherapy were lower than those of the patients without DVT. The median levels of ADAMTS13 pre-chemotherapy and post-chemotherapy in the patients with DVT were $0.74$, IQR $0.45 – 0.81$ U/mL and $0.42$, IQR $0.31 – 0.52$ U/mL, respectively. In cancer patients with DVT, the levels of ADAMTS13 were significantly lower than
patients without DVT in both pre-chemotherapy and post-chemotherapy (p = 0.007 and p = 0.001, respectively) (Table 3 and Fig. 1,C).

**Table 3**: The differences plasma levels of sP-selectin, vWF, ADAMTS13 in positive and negative DVT groups.

|                      | DVT                | p         |
|----------------------|--------------------|-----------|
|                      | Positive           | Negative  |           |
| Sp-selectin          |                    |           |           |
| Pre-chemotherapy     | 121.0 (107.5-230.6)| 70.9 (31.3-145.1)| 0.041*§  |
| (ng/mL) median (IQR)|                    |           |           |
| Post-chemotherapy    | 204.4 (110.9-278.3)| 87.0 (40.9-192.8)| 0.000*§  |
| (ng/mL) median (IQR)|                    |           |           |
| VWF                  |                    |           |           |
| Pre-chemotherapy     | 3.03 (2.97-3.75)   | 1.19 (0.37-2.97)| 0.001*§  |
| (U/mL) median (IQR) |                    |           |           |
| Post-chemotherapy    | 3.43 (3-3.97)      | 1.44 (0.72-3.21)| 0.002*§  |
| (U/mL) median (IQR) |                    |           |           |
| ADAMTS13             |                    |           |           |
| Pre-chemotherapy     | 0.74 (0.45 – 0.81) | 0.94 (0.42 – 1.08)| 0.007*§  |
| (U/mL) median (IQR) |                    |           |           |
| Post-chemotherapy    | 0.42 (0.31 – 0.52) | 0.95 (0.46 – 1.08)| 0.001*§  |
| (U/mL) median (IQR) |                    |           |           |

*) significant; § Mann Whitney U test

In this study, the cut-off points levels of sP-selectin pre-chemotherapy and post-chemotherapy for DVT incidence in the cancer patients were 106.7 ng/ml and 111.7 ng/ml, respectively. With the cut-off points of pre-chemotherapy sP-selectin levels > 106.7 ng/mL and and post-chemotherapy > 111.7 ng/mL, it was established that RR for DVT incidence were 16 (95% CI 2.06-124.25, p = 0.001) and 8.7 (95% CI 1.01–74.39, p = 0.045), respectively (Table 4).
Table 4
Risk Estimation for the DVT occurrence with cut-off plasma levels of sPselectin, VWF and ADAMTS13 pre and post-chemotherapy.

|                      | Cut-off Point | AUC  | RR  | P         | 95% CI      |
|----------------------|---------------|------|-----|-----------|-------------|
| **sP-selectin (ng/mL)** |               |      |     |           |             |
| Pre-chemotherapy     | > 106.7       | 0.949| 16  | 0.001*§   | 2.06-124.25 |
| Post-chemotherapy    | > 111.7       | 0.929| 8.7 | 0.045*§   | 1.01-74.39  |
| **VWF (U/mL)**       |               |      |     |           |             |
| Pre-chemotherapy     | > 2.99        | 0.996| 36  | 0.000*§   | 5.21-248.65 |
| Post-chemotherapy    | > 3.06        | 0.986| 20.4| 0.004*§   | 2.60-159.94 |
| **ADAMTS13 (U/mL)**  |               |      |     |           |             |
| Pre-chemotherapy     | < 0.80        | 0.877| 10.5| 0.015*§   | 1.31-84.28  |
| Post-chemotherapy    | < 0.49        | 0.993| 26.25| 0.002*§   | 3.50-196.48 |

*) significant; § Chi Square test

The cut-off points of VWF levels pre-chemotherapy and post-chemotherapy for DVT incidence in the cancer patients were 2.99 U/mL and 3.06 U/mL, respectively. With the cut-off points of pre-chemotherapy VWF levels > 2.99 U/mL and post-chemotherapy > 3.06 U/mL, it was established that RR for DVT incidence were 36 (95% CI 5.21-248.65, p = 0.000) and 20.4 (95% CI 2.60-159.94, p = 0.004), respectively (Table 4).

The cut-off points of ADAMTS13 level pre-chemotherapy and post-chemotherapy for DVT incidence in the cancer patients were 0.80 U/mL and 0.49 U/mL, respectively. With the cut-off points pre-chemotherapy ADAMTS13 levels < 0.80 U/mL and post-chemotherapy < 0.49 U/mL, it was established that RR for DVT incidence were 10.5 (95% CI 1.31–84.28 p = 0.015) and 26.25 (95% CI 3.50-196.48, p = 0.002), respectively (Table 4).
Finally, in a stepwise manner, multiple logistic regression analysis was performed using DVT incidence as dependent variable vs. three possible biomarkers (sP-selectine, vWF, and ADAMTS pre- and post-chemotherapy) starting with a full model and removing non-significant variable one by one. The potential independent variable were categorized as dichotomy using pre-determined cut off value. The final result showed that VWF levels $> 2.99$ U/mL pre-chemotherapy was the only predictor of DVT incidence, with a RR of 11.1 (95% CI, 1.95–62.74, $p = 0.007$)

Discussion

This study found that the levels of sP-selectin and VWF in cancer patients with DVT pre-chemotherapy and post-chemotherapy were higher than patients without DVT. Furthermore, the levels of sP-selectin and VWF were getting higher after chemotherapy. In DVT cancer patients, the levels of sP-selectin and VWF were significantly higher than patients without DVT in both pre-chemotherapy and post-chemotherapy ($p = 0.041; p = 0.000$ and $p = 0.001; p = 0.002$, respectively).

In the patients with DVT, the levels of ADAMTS13 pre-chemotherapy and post-chemotherapy were lower than patients without DVT. The levels of ADAMTS13 were significantly lower than patients without DVT in both pre-chemotherapy and post-chemotherapy ($p = 0.007$ and $p = 0.001$, respectively).

It is known that sP-selectin and VWF are stored in platelets alpha granules and WPB bodies endothelial cells.$^{12,14}$ The study conducted by Payne H et al. on mice showed that endothelial activation and WPB exocytosis were immunity mechanism which initiated DVT. In this process, WPB fused with plasma membrane and released its constituents such as VWF and sP-selectin, in membrane, which allowed cell recruitments. It corresponded with the increasing levels of sP-selectin and VWF in the patients with DVT.$^{18}$

Soluble P-selectin and VWF levels in the cancer patients with DVT, which were already high before chemotherapy and increased higher after chemotherapy showed that immune systems and inflammations, played significant roles in DVT pathogenesis.$^1$ Cancers and chemotherapy treatments can cause inflammatory conditions.$^{19}$ In cancers, there are inflammatory stimulates in the tumor microenvironment.$^{20,21}$ Inflammatory stimulates in cancers and chemotherapy activate intracellular signal pathways which activate the production of inflammatory mediators.$^{22,23}$ Blood vessel invasions by cancer cells, diagnostic and therapeutic interventions, such as central venous catheter insertion, also cause injuries in cancer patients' blood vessels. This leads to endothelial cells damage which prompts endothelial cells activation resulting in inflammatory responses.$^{19}$

Systemic chemotherapy may cause injuries on blood vessel walls due to its direct toxic effect on endothelial cells which prompts endothelial cell activation. The increasing activation of endothelial cells is considered as one of the causes of increasing hemostatic activation.$^{4,5}$ Biochemically, chemotherapy-induced VECA is indicated by increasing endothelial cells in circulation, VWF in plasma and endothelial cell activation markers such as VCAM-1, sP-selectin and E-selectin.$^{5,6}$ The rising endothelial activities as a result of inflammatory induce various changes in endothelial cells and leukocytes and platelets which
encourage procoagulant and prothrombotic surfaces in blood vessel walls that increase the risk of DVT.\textsuperscript{6,24}

The adhesion process of leukocytes to blood vessel endothelial cell is a feature of inflammatory proces.\textsuperscript{6} There are leukocyte rolling, adhesion, and movement to the injured spots controlled by selectin, integrin, and other adhesive molecules. Some adhesive molecules such as P-selectin, involved in inflammation, contribute in thrombosis.\textsuperscript{15} The interaction between P-selectin and its main receptor in leukocytes, P-selectin glycoprotein ligand 1 (PSGL-1), causes neutrophil and macrophage recruitment; together with other mediators, they induce leucocytes to produce procoagulant microparticles. Subsequently, P-selectin prompts rising TF expressions in monocytes and mediates TF transfer to platelets. Tissue factor is the main initiator of in vivo coagulant, causing the activation of coagulant cascade extrinsic pathways.\textsuperscript{13} The study conducted by Ay et al. has showed that an elevated levels of sPselectin is a useful parameter to identify patients who are at risk of thrombotic events, showing an odds ratio of 2.6 for patients with serum levels of sPselectin > 53.1 ng/dl after adjusted for age, sex, surgery, chemotherapy and radiotherapy.\textsuperscript{25}

Von Willebrand factor has a role in both primary and secondary hemostasis. This glycoprotein acts as a carrier for factor VIII and a platelet adhesion mediator to endothelial cells.\textsuperscript{16} Von Willebrand factor is an adhesive protein capable of attaching to tumor cells and platelets, which can cause microthrombi formations. Platelet-tumor cell aggregation also facilitate the metastasis process by making it easier for tumor cells to adhere and migrate through vessel walls.\textsuperscript{26} The previous study has shown that the numbers will increase in cancer patients with DVT and are related to cancer stage and metastasis. The increasing VWF antigen and the decreasing VWF-cleaving protease will prompt thrombogenesis in cancer patients.\textsuperscript{17} High VWF levels were significantly associated with the risk of developing DVT in cancer patients.\textsuperscript{27}

The rise of VWF in circulation causes increasing ADAMTS13 activities, which results in decreasing levels of ADAMTS13. Mechanistically, this association is hypothesized that DVT related to cancers can be based on platelet aggregations mediated by VWF. Bauer et al. have recently showed that melanoma cells could activate vascular endothelial cells and push them to release ULVWF followed by platelets aggregation in vitro. They further explained that a combination of VWF release and decreased ADAMTS13 in tumor tissues tend to prompt procoagulant environment.\textsuperscript{28} The decrease in ADAMTS-13 activities, which regulate size and, therefore, affect VWF cleansing from circulation, may result in a rise in plasma VWF. Although the mechanism prompting a decrease in plasma ADAMTS-13 in cancer patients has not been fully understood, various oncogenes have been found to regulate the expression of extracellular proteinases, including matrix-degrading metalloproteinases, which can directly disrupt ADAMTS13 activities.\textsuperscript{26}

The result of this study also showed that the cut-off points of sP-selectin and VWF levels were high both pre-chemotherapy and post-chemotherapy and the low levels of ADAMTS13 pre-chemotherapy and post-
chemotherapy posed high relative risk for DVT incidence. The levels of sP-selectin, VWF and ADAMTS13 pre-chemotherapy with cut-off point > 106.7 ng/mL, > 2.99 U/mL and < 0.80 U/mL, respectively had RR for DVT incidence being 16 (95% CI 2.06–124.25, p = 0.001); 36 (95% CI 5.21–248.65, p = 0.000) and 10.5 (95% CI 1.31–84.28, p = 0.015) respectively, whereas the levels of sP-selectin, VWF and ADAMTS13 during post-chemotherapy with cut-off point > 111.7 ng/mL, > 3.06 U/mL and < 0.49 U/mL, respectively, had RR for DVT incidence being 8.7 (95% CI 1.01–74.39, p = 0.045); 20.4 (95% CI 2.60–159.94, p = 0.004) and 26.25 (95% CI 3.50–196.48, p = 0.002), respectively.

The data above indicated that the cut-off points of sP-selectin, VWF, and ADAMTS13 levels both pre-chemotherapy and post-chemotherapy could be used to predict the incidence of DVT in the cancer patients undergoing chemotherapy. Multiple logistic regression analysis is performed to determine the strongest biomarker as a predictor of DVT incidence pre- and post-chemotherapy.

Multiple logistic regression analysis showed that VWF levels pre-chemotherapy was the strongest biomarker as a predictor of DVT incidence pre-chemotherapy. Cancer patients whose initially have VWF levels > 2.99 U/mL will have 11-fold increased risk of developing DVT (RR 11.1, 95% CI 1.95–62.74, p = 0.007) independently to other coagulation marker such as sP-selectin and ADAMTS13. The data above indicated that the cut-off points of VWF levels > 2.99 U/mL effectively predict the incidence of DVT in cancer patients undergoing chemotherapy.

Deep vein thrombosis has long been considered as a blood clotting disorder. The new understanding of DVT mechanism with some evidence and research data above shows the important role of immune systems and inflammations in the pathogenesis of DVT. Deep vein thrombosis is a process related to immunity and inflammation, not just the coagulation process that causes thrombosis. Local endothelial activations and the release of WPB present in endothelial cells have a very important role in initiating DVT. In addition to vascular systems, the immune system is emerging as a pivotal player in the pathophysiology of DVT.\textsuperscript{1,29}

The paradigm above opens the way to new thoughts on studies about new potential thromboprophylaxis strategies, or suggests a role for anti-inflammatory, which could be used for DVT prevention with a lower risk of bleeding complications than conventional therapeutic approaches.\textsuperscript{1,29}

The limitation of this study is that sP-Selectin, VWF, ADAMTS13 and color duplex sonography serial examinations were not conducted monthly. They can be used to see the changes in the levels of sP-selectin, VWF and ADAMTS13 every chemotherapy cycle, so that the changes in biomarker levels and the incidence of DVT can be observed regularly. It will allow us to see the changes in biomarker levels affecting DVT incidence more accurately.

\section*{Conclusion}

Our study demonstrated that (i) plasma levels of sP-selectin and VWF were high and ADAMTS13 were low in cancer patients with DVT (ii) plasma levels of VWF more than 2.99 U/mL is an independent risk
factor for the incidence of DVT in cancer patients undergoing chemotherapy, which indicated VWF could be an early and helpful marker for thromboprophylaxis therapy in cancer patients undergoing chemotherapy.

Further research is needed to understand the important role of immune system and inflammation in the pathogenesis of DVT and thus opens the way to new thoughts on new potential thromboprophylaxis strategies, or suggest a role for anti-inflammatory, which could be used for DVT prevention with a lower risk of bleeding complications in comparison to conventional therapeutic approaches.¹,²⁹

**Abbreviations**

DVT
Deep vein thrombosis
PE
Pulmonary embolism
VTE
Venous thromboembolism
VECA
Vascular endothelial cell activation
VWF
von Willebrand factor
VCAM-1
Vascular cell adhesion molecule-1
sP-selectin
soluble P-selectin
WPB
Weibel-Palade body
TF
Tissue factor
UL-VWF
Ultra large VWF
ADAMTS13
A disintegrin-like and metalloproteinase with thrombospondin type 1, motif 13
LMWH
Low molecular weight heparin
ELISA
Enzyme-linked immunosorbent assay
SD
Standard deviation
ROC
Declarations

Ethical Approval and Consent to participate

This single-centre observational study was approved by Ethical Committee of the Dr. Kariadi Hospital.

Consent for publication

All authors have consented for the publication.

Availability of supporting data

Database of all patients and statistical analysis are available upon request and authorization from Dr. Kariadi Hospital.

Competing interest

All authors stated that they all have no conflicts of interest in relation to this work.

Funding: Not applicable

Authors’ contributions:

Budi Setiawan : Conceptualizing and design the study, reviewed data, wrote the first manuscript, analyzed the data and reviewed the final manuscript

Cecilia Oktaria Permatadewi: Collected data, cleaned the data, and reviewed the manuscript

Baringin de Samakto: Collected data, cleaned the data, and reviewed the manuscript

Ashar Bugis : Collected data, cleaned the data, and reviewed the manuscript

Eko Adhi Pangarsa : Supervision the study, critically appraised the manuscript

Damai Santosa: Supervision the study, critically appraised the manuscript

Chatarina Suharti : Conceptualization, supervision the study, critically appraised the manuscript.

All authors contributed, read and approved the final manuscript hereby submitted for publication
Acknowledgments

Our sincere gratitude to Mika Lumban Tobing, MD, and Suyono, MD, from the Department of Internal Medicine, Dr. Kariadi Hospital, Diponegoro University, Semarang, Indonesia for the data support. We thank Darminto, MD from the Clinical Epidemiology Unit of Diponegoro for the statistical support.

Authors detail:

Budi Setiawan and Eko Adhi Pangarsa is currently work as PhD candidate from Faculty of Medicine, Diponegoro University/Dr. Kariadi Hospital, Semarang, Indonesia

Cecilia Oktaria Permatadewi, Baringin de Samakto, Ashar Bugis are resident physician in Department of Internal Medicine, Faculty of Medicine Diponegoro University/Dr. Kariadi Hospital, Semarang, Indonesia

Damai Santosa is chief of Division of Hematology-Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Diponegoro University/Dr. Kariadi Hospital, Semarang, Indonesia

Catharina Suharti is professor of medicine in Division of Hematology-Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Diponegoro University/Dr. Kariadi Hospital, Semarang, Indonesia

References

1. Budnik I, Brill A. Immune Factors in Deep Vein Thrombosis Initiation. *Trends Immunol.* 2018;39(8):610-623.
2. Elyamany G, Alzahrani AM, Bukhary E. Cancer-Associated Thrombosis: An Overview. *Clin Med Insights Oncol.* 2014;8:129-137.
3. Prandoni P, Lensing AW PA. Recurrent deep vein thrombosis and bleeding complications during anticoagulant treatment in patients with cancer and venous thrombosis. *Blood.* 2002;100(10):3484-3488.
4. Weitz I, Liebman HA. Chemotherapy-Induced Hemostatic Activation and Thrombosis in cancer. In: Korana AA, Charles W. Francis, eds. Cancer-Associated Thrombosis: New Findings in Translational Science, Prevention, and Treatment. 1st Edition. New York: Informa Healthcare USA, Inc; 2008:65-75.
5. Kirwan CC, Mccollum CN, Mcdowel G, Byrne GJ. Investigation of Proposed Mechanisms of Chemotherapy-Induced Deep vein thrombosis: Endothelial Cell Activation and Procoagulant Release Due to Apoptosis. *Clin Appl Thromb.* 2015;2(5):420-427.
6. Granger DN, Senchenkova E. Inflammation and the Microcirculation. San Rafael (CA): Morgan & Claypool Life Sciences; 2010. [Internet]. Available at: https://www.ncbi.nlm.nih.gov/books/NBK53380/#ch8
7. Connolly GC, Francis CW. Cancer-associated thrombosis. *Am Soc Hematol.* 2013;2013:684-691.
8. Shahidi M. Thrombosis and von Willebrand Factor. *Adv Exp Med Biol.* 2017;906:285-306.
9. Pepin M, Kleinjan A, Hajage D, Buller HR, Di Nisio M et al. ADAMTS-13 and von Willebrand factor predict deep vein thrombosis in patients with cancer. *J Thromb Haemost*. 2016 Feb;14(2):306-15.

10. Gibson NS, Sohne M, Gerdes VEA, Meijers JCM, Buller HR. Clinical usefulness of prothrombin fragment 1 + 2 in patients with suspected pulmonary embolism. *Thromb Res.* 2010;125(1):97-99.

11. Barnes DM, Wakefield TW, Rectenwald JE. Novel Biomarkers Associated with Deep Venous Thrombosis: A Comprehensive Review. *Biomark Insights*. 2008;3(734):93-100.

12. Gremmel T, Ay C, Seidinger D, Pabinger I, Panzer S, Koppensteiner R. Soluble p-selectin, D-dimer, and high-sensitivity C-reactive protein after acute deep vein thrombosis at the lower limb. *J Vasc Surg*. 2011;54:48S-55S.

13. Ay C, Jungbauer L V, Sailer T, Tengler T, Koder S, Kaider A et al. High Concentrations of Soluble P-Selectin Are Associated with Risk of Deep vein thrombosis and the P-Selectin Thr715 Variant. *Clinical Chemistry*. 2007;53(7):1235–1243.

14. Kokame K, Matsumoto M, Soejima K, Yagi H, Ishizashi H, Funato M, et al. Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity. *Proc Natl Acad Sci U S A*. 2002;99(18):11902-11907.

15. Feng Y, Li X, Xiao J, Li W, Liu J, Zeng X, et al. ADAMTS13: more than a regulator of thrombosis. *Int J Hematol*. 2016;104(5):534-539.

16. Franchini M, Frattini F, Crestani S, Bonfanti C, Lippi G. Von Willebrand factor and cancer: A renewed interest. *Thromb Res.* 2013 Apr;131(4):290-292.

17. Goldenberg BN, Kahn SR, Solymoss S. Markers of coagulation and angiogenesis in cancer-associated deep vein thrombosis. *J Clin Oncol*. 2003;21:4194-4199.

18. Payne H, Brill A. Stenosis of the Inferior Vena Cava: A Murine Model of Deep Vein Thrombosis. *J. Vis. Exp.* 2017;130:e56697.

19. Boccaccio C, Paolo M. Comoglio. Oncogenesis, Cancer and Hemostasis. In: Khorana AA, Francis CW, eds. Cancer-Associated Thrombosis: New Findings in Translational Science, Prevention, and Treatment. 1st Edition. New York: Informa Healthcare USA, Inc; 2008:1-15.

20. Karin M. NF-KB as a Critical Link Between Inflammation and Cancer. *Cold Spring Harb Perspect Biol* 2009;1:a000141

21. Xia Y, Shen S, Verma IM. NFkB, an active player in human cancer. *Cancer Immunol Res.* 2014 September;2(9): 823–830.

22. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. *Ontarget*. 2018;9(6):7204-7218.

23. Aggarwal BB. Nuclear factor-κ B: The enemy within. *Cancer cell*. 2004 Sep;6(3):203-8.

24. Mo J, Zhang D, Yang R. Expression of P-selectin , VCAM-1, and PSGL-1 in traumatic deep venous thrombosis. *Int J Clin Exp Pathol*. 2016;9(3):3403-3409.

25. Ay C, Simanek R, Vormittag R, Dunkler D, Alguel G, Koder S, et al. High plasma levels of soluble P-selectin are predictive of deep vein thrombosis in cancer patients: results from the Vienna Cancer
26. Guo R, Yang J, Liu X, Wu J, Chen Y. Increased von Willebrand factor over decreased ADAMTS- activity is associated with poor prognosis in patients with advanced non- small cell lung cancer. *J Clin Lab Anal*. 2018;32:e22219.

27. Obermeier HL, Riedl J, Ay C, et al. The role of ADAMTS - 13 and von Willebrand factor in cancer patients: Results from the Vienna Cancer and Thrombosis Study. *Res Pract Thromb Haemost*. 2019 May 6;3(3):503-514.

28. Bauer AT, Suckau J, Frank K, Desch A, Goertz L, Wagner AH, et al. von Willebrand factor fi bers promote cancer-associated platelet aggregation in malignant melanoma of mice and humans. *Blood*. 2015 May 14; 125(20): 3153–3163.

29. Branchford BR, Carpenter SL. The Role of Inflammation in Deep vein thrombosis. *Front Pediatr*. 2018 May 23;6:142.

**Figures**
Figure 1

The plasma concentrations of sP-selectin (A), VWF (B) and ADAMTS13 (C) levels of pre-chemotherapy and post-chemotherapy in 40 cancer patients with and without DVT. The difference was significant (p<0.05).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- COVERLETTER.docx