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HYPERCOAGULABILITY IN PATIENTS WITH LUNG CANCER UNDERGOING CHEMOTHERAPY

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ABSTRACT

There is evidence that in the case of malignancies including lung cancer, that there is hypercoagulability. In spite of this, it is still not clear whether the course of chemotherapy alters the risk. This study aimed to investigate whether there was state of pre-thrombosis and hypercoagulability in patients with lung cancers and the underlying effect of chemotherapy during the treatment. Twelve lung cancer patients were recruited. Their stages and clinical performances were determined. The blood sample was taken before the chemotherapy, shortly after the first- and third-chemotherapy cycles, for the investigation of D-dimer, platelet count, PT (INR), ratios of APTT, and TT. The chemotherapy protocols vary from one patient to the others as well as between the 1st and the 3rd chemotherapy regimens although most of the protocols consist of carboplatin + gemcitabine or carboplatin + paclitaxel. From the thrombosis view of point, they were all asymptomatic and remained so during the period of investigation. Thrombosis is defined as an increase of D-dimer and hypercoagulability as finding one or more of PT (INR), ratio APTT, ratio TT <1.0. The trend of the result in the three sampling points was carried out by ANOVA, while Wilcoxon test for small samples did univariate analysis between two investigations. The result of PT, APTT, and TT indicating hypercoagulability showed that they remained unchanged until the third cycle of chemotherapy ($p>0.05$). The platelets of patients dropped significantly; median (range) 422 to 287 x 109/L before the chemotherapy to the end of the third cycle respectively. The D-Dimer of patients remained unchanged, however when it was investigated by univariate analysis in the group with D-Dimer >500 ng/mL, this group showed a decreased D-Dimer towards the end of the third cycle ($p <0.05$). This study demonstrated that there was hypercoagulability in patients with lung cancers before the chemotherapy until the 3rd cycle of chemotherapy. The course of chemotherapy did not alter hypercoagulability. However, in the group where pre-thrombosis had already happen as evidenced by high D-dimer (>500 ng/ mL), the chemotherapy showed benefit regarding of reduction of the D-dimer which may lead to the possible breakdown of the existing thrombus.

Key words: Lung cancer thrombosis, hypercoagulability, D-dimer, PT, APTT, TT

INTRODUCTION

Cancer is associated with a pre-thrombotic or hypercoagulable state involving the activation of the hemostatic mechanism. Hypercoagulability is an important and well-established risk factor for venous thrombosis contributing 2- to 4-fold increased risk and has been reported to be the most frequent cause of mortality in cancer.[1-4] Cancer patients with solid tumors commonly present laboratory coagulation tests with varying degrees of clotting activation indicating a subclinical hypercoagulable state.[5-8] Cancer cells produce procoagulant factors initiating coagulation activation that increases the risk of thrombosis. Hypercoagulability is commonly associated with cancer type including lung cancer.[6,9,10]

The highest risk of developing venous thrombosis in lung cancer patients is believed to occur in patients with adenocarcinoma rather than in squamous cell carcinoma.[11,12] Factors contributing to venous thrombosis include endothelial damage caused by chemotherapy.[13] The use of cytotoxic drugs can have a thrombogenic effect on endothelial lesion release of procoagulant products and cytokines observed after chemotherapy.[1,14,15] The thromboembolic complications during the first three months of treatment result in an annual rate of 11% have been reported in a retrospective study.[16]

Significant increase in fibrinopeptide A and a decrease in fibrinolytic activity with increased fibrinolytic inhibitor in patients with lung cancer could be associated with an enhanced tendency to develop thromboembolism after cytostatic chemotherapy have been observed.[17] Coagulation mechanism has been stated to play a role in the progression of the disease.[18] D-dimer, the lysis product of cross-linked fibrin indicates hyperfibrinolysis in response to clotting activation and fibrin formation.[8,15] D-dimer assays have been shown to have a high sensitivity and high predictive value for deep vein thrombosis (DVT) and a negative value for DVT exclusion.[8,19] It is a marker for hypercoagulability and has been used to determine the hypercoagulable state leading to thrombosis in myeloproliferative disease.[20,21]

Our study group has set the criteria that hypercoagulable state can also be measured using the ratios of patient' s prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT) against normal healthy subjects where the

arbitrary normal ratio is 1.0. When any of the two parameters slide to less than 1.0, then it is deemed as hypercoagulable due to increased procoagulant factors that shortened the clotting times. The evidence of thrombotic events in lung cancer in the Indonesian population is scarce and it is not known if the incidence is low.

The study aimed to determine the hemostatic changes and the risk of thrombosis in lung cancer patients undergoing chemotherapy in a small study.

METHODS

The study received ethical approval from the Health Research Ethical Committee (369/KOMET/FKUSU/2015), Faculty of Medicine, University of North Sumatera, Indonesia. It was conducted at the Department of Clinical Pathology, Faculty of Medicine, University of North Sumatera/Adam Malik Hospital Medan.

Inclusion criteria, patients diagnosed with lung cancer by histopathology, who have not undergone chemotherapy and have given informed consent were recruited. Exclusion criteria, lung cancer patients on on-going anticoagulant therapy were excluded.

Twelve lung cancer patients (all males and smokers) having met the above inclusion criteria were recruited after having given written informed consent. The mean age was 58.2 ± 7.3 years ranging between 37 and 65 years. Histologically, they were diagnosed as having non-small-cell lung cancer (Stage I n=1, Stage IV n=11) consisted of 9 adenocarcinomas and 3 squamous carcinomas.

The patients received three cycles of chemotherapy consisting of either gemcitabine + carboplatin (n=5) or paclitaxel + carboplatin (n=7). About 7.5 mL of blood from a clean venipuncture was obtained and drawn into 3.0 mL EDTA vacutainer tubes for full blood count analysis and another 4.5 mL into vacutainer tube containing 0.5 mL of 0.109M trisodium citrate. The citrated blood was centrifuged at 2500g for 25 minutes and the platelet poor plasma used for determination of PT, aPTT, TT, and D-dimer. Blood sampling was performed at pre-chemotherapy and the end of cycles one and three of chemotherapy.

Prothrombin Time (PT) INR, activated Partial Thromboplastin Time (aPTT) and Thrombin Time (TT) were determined in a Coagulation Analyzer (TecoCoatron A6, Germany), direct clotting time with thrombin, D-dimer (Dimex Quantitative kit, Teco). An arbitrary ratio of <1.0 in PT (INR), Aptt and TT was considered a hypercoagulable state. The normal range for D-dimer was less than 500 ng/mL.

The Statistical Package for Social Sciences (SPSS 22 IBM Corp) was used to perform statistical analysis. The paired sample and independent t-test for differences between groups were also performed. A p-value of <0.05 was considered a statistically significant.

RESULTS AND DISCUSSION

Twelve patients with non-small-cell lung cancer were evaluated for hypercoagulability or prethrombotic state when undergoing chemotherapy. PT (INR), aPTT, TT ratios, and D-dimer levels at post-chemotherapy in cycles 1 and 3 were not significantly different from its pre-chemotherapy results. The hypercoagulable state was present throughout pre- and chemotherapy cycles as evident by mean aPTT and TT ratios of<1.0. However, platelets showed a significant reduction (P=<0.01) by cycle 3. There was no significant difference in the parameters studied between cycles 1 and 3 of chemotherapy Table 1.

Table 1. Platelets, PT (INR), APTT ratio, TT ratio, and D-dimer levels in cycles 1 and 3 compared to pre-chemotherapy and between cycles 1 and 3 in patients with lung cancer

	Platelets x10^9/L	PT (INR) ratio	APTT ratio	TT ratio	D-dimer ng/mL
Chemotherapy:					
Pre- mean (SD)	438.8 (121.3)	1.05 (0.3)	0.96 (0.1)	0.83 (0.1)	575.9 (468.2)
Range	281 – 723	0.81 – 1.82	0.80 – 1.22	0.70 – 1.10	175 - 1600
Cycle 1					
Mean (SD)	386.2 (151.8)	1.08 (0.3)	0.96 (0.1)	0.83(0.1)	745.5 (688.3)
Range	43 – 635	0.79 – 1.90	0.82 – 1.38	0.70 – 1.02	153 - 1800
p	0.36	0.38	0.78	0.19	0.52
Cycle 3					
Mean (SD)	266.8 (140.4)	1.02 (0.2)	0.96 (0.1)	0.83 (0.1)	378.3 (336.0)
Range	7 – 460	0.81 – 1.21	0.73 – 1.14	0.70 – 1.09	110 - 1300
p	<0.01	0.68	0.43	0.92	0.33
Cycle 1 vs. cycle 3					
p	0.05	0.42	0.39	0.36	0.09

PT = prothrombin time; APTT = activated partial thromboplastin time; TT = thrombin time

Cancer patients with D-dimer levels of greater than 500 ng/mL at pre-chemoteraphy, four (33.3%) patients (3 adenocarcinomas, 1 squamous cell carcinoma) had elevated D-dimer levels of mean 1127.5 ±407.1 ng/mL at pre-chemotherapy. The levels showed a significant reduction (p= 0.04) in cycle 3 to mean 256.8 ± 105.5 ng/mL. (Table 2) at pre-chemotherapy, only TT ratio was below 1.0 suggesting an elevated fibrinogen levels state that was also present post-chemotherapy. Moreover, platelets, PT (INR) and aPTT did not show any significant differences at pre and post-chemotherapy.

A hypercoagulable state was not evident by cycle 3 of chemotherapy except for raised fibrinogen level (TT ratio 0.90). The mean D-dimer levels at pre- and chemotherapy cycles 1 and 3 can be seen in Figure1.

x109/L in cycles 1 and 3, respectively. Incidentally the same patient had D-dimer levels of 1255 ng/mL and 1300 ng/mL on cycles 1 and 3, respectively. The four patients who had normal D-dimer levels at pre-chemotherapy and elevated D-dimer post-

Table 2. Parameters studied in lung cancer patients with D-dimer levels above 500 ng/mL at pre and during post-chemotherapy cycles

	Platelets x10^9/L	PT (INR) ratio	APTT ratio	TT ratio	D-dimer ng/mL
D-dimer >500 ng/mL (n = 4)					
Pre- mean (SD)	470.0 (196.3)	1.26 (0.41)	1.02 (0.18)	0.86 (0.18)	1127.5 (407.1)
Range	281 – 723	0.85 – 1.82	0.81 – 1.22	0.71 – 1.10	610- 1600
Cycle 1					
Mean (SD)	440.0 (84.8)	1.24 (0.44)	1.10 (0.19)	0.79(0.09)	838.8 (667.9)
Range	351 – 553	0.98 – 1.90	0.94– 1.38	0.71 – 0.91	345 - 1800
p	0.81	0.82	0.42	2	.60
Cycle 3					
Mean (SD)	312.8 (133.2)	1.16 (0.21)	1.04 (0.07)	0.90 (0.13)	256.8 (105.5)
Range	123 – 435	0.94 – 1.43	1.00 – 1.14	0.82 – 1.09	110 - 335
p	0.14	0.65	0.82	0.47	0.04
Cycle 1 vs Cycle 3 p	0.30	0.74	0.64	0.05	0.17
D-dimer <500 ng/mL at pre-but elevated post-chemotherapy (n=4)					
Pre-mean (SD)	405.8 (82.8)	0.92 (0.13)	0.85 (0.06)	0.87 (0.12)	317.5 (222.8)
Range	342 - 519	0.81 – 1.09	0.80 – 1.06	0.71 – 0.98	175 - 375
Cycle 1					
Mean (SD)	289.0 (187.2)	1.02 (0.11)	0.85 (0.05)	0.84 (0.12)	007.5 (988.8)
Range	43 – 452	0.84 – 1.11	0.82 – 0.90	0.73 – 1.02	325 - 2300
	0.38	0.10	0.93	0.51	0.32
Cycle 3					
Mean (SD)	105.0 (118.3)	1.06 (0.15)	0.91(0.17)	0.82 (0.11)	726.8 (411.2)
Range	7 – 460	0.87 – 1.19	0.73 – 1,14	0.75 – 0.99	150 - 1300
p	5	0.25	0.45	0.64	0.23
Cycle 1 vs. cycle 3 p	0.14	0.68	0.56	0.89	0.63

PT = prothrombin time; APTT = activated partial thromboplastin time; TT = thrombin time

Cancer patients with D-dimer levels below 500 ng/mL at pre but elevated post-chemotherapy. However, four patients (33%) had elevated D-dimer levels of between 150, and 1300 ng/mL by cycles 1 and 3 of chemotherapy the D-dimer levels at pre-chemotherapy was between 175 and 375 ng/mL. However, no statistical significance in D-dimer levels could be seen between pre- and post-chemotherapy states. No significant differences in PT (INR), aPTT, and TT ratios were seen but a hypercoagulable state (aPTT and TT ratios of <1.0) was still present at pre- and post-chemotherapy. Platelets showed a mean reduction post-chemotherapy but did not reach statistical significance when compared with pre-chemotherapy state. One patient with stage IV squamous cell carcinoma had a reduction of platelets level from 519 x109/L to 43 and 7 chemotherapy can be seen in Figure 1.

D-dimer levels <500ng/mL at pre- and post-chemotherapy (Table 3), four patients (33.3%, adenocarcinoma 3 and squamous cell carcinoma 1). No statistically significant differences could be seen in parameters studied between pre- and post-chemotherapy states. D-dimer levels were not elevated but PT (INR), aPTT, and TT ratios remain below 1.0 suggesting that hypercoagulable states were still present post-chemotherapy. The mean D-dimer levels in this group of patients can be seen in Figure 1.

Cancer is a known risk factor for venous thrombosis contributing to 2- to 4-fold increased risk and has been reported to be the most frequent cause of mortality.[1-4] Cancer can confer a pre-thrombotic or hypercoagulable state including lung cancer through activation of the coagulation or fibrinolytic pathways, the vascular endothelium and platelets,[5-7,9,20] even

Table 3. Lung cancer patients with normal D-dimer levels before and post-chemotherapy

	Platelets x10^9/L	PT (INR) ratio	APTT ratio	TT ratio	D-dimer ng/mL
D-dimer levels <500 ng/mL pre- and post-chemo (n = 4)					
Pre- mean (SD)	434.0 (76.9)	0.93 (0.06)	0.96 (0.02)	0.76 (0.06)	351.5 (122.0)
Range	341 – 519	0.88 – 1.00	0.93 – 0.98	0.70 – 0.81	230 - 457
Chemo- Cycle 1					
Mean (SD)	429.5 (152.7)	1.00 (0.17)	0.97 (0.08)	0.76 (0.11)	265.3 (132.9)
Range	277 – 635	0.79 – 1.19	0.89 – 1.06	0.68 – 0.92	153 - 458
p	0.93	0.36	0.90	1.00	0.52
Cycle 3					
Mean (SD)	290.0 (148.5)	0.88 (0.08)	0.87 (0.09)	0.77 (0.06)	218.0 (29.9)
Range	118 -460	0.79 – 0.96	0.78 – 0.98	0.70 – 0.82	190 - 257
p	0.21	0.29	0.09	0.19	0.07
Cycle 1 vs. cycle 3		0.22	0.33	0.80	0.59
p	0.37				

PT = prothrombin time; APTT = activated partial thromboplastin time; TT = thrombin time

in the absence of apparent thrombosis. Chemotherapeutic agents or tumor-derived products causes direct endothelial injury leading to a loss of antithrombotic properties may play a role in enhancing venous thromboembolism risk in cancer.[13]

The highest risk of developing venous thrombosis in lung cancer patients is believed to occur in patients with adenocarcinoma than in squamous cell carcinoma.[9,10] D-dimer assays have been shown to have a high sensitivity, high predictive value for deep vein thrombosis (DVT) and the negative value for DVT exclusion.17 It is a marker for hypercoagulability and has been used to determine hypercoagulable state leading to thrombosis in myeloproliferative disease.[18,19]

In our study of twelve patients, four (33.3%) had elevated D-dimer levels at pre-chemotherapy, but by post-cycle-3 the levels had returned to the normal state with only evidence of hypercoagulability in raised fibrinogen level indicated by TT ratio of <1.0, fibrinogen also known as an acute phase reactant protein.[21] In the next four patients (33.3%) who were hypercoagulable at pre-chemotherapy state had normal D-dimer at pre- but elevated levels at post-cycles-1 and 3. Together with high D-dimer levels, the hypercoagulable state was still present post-chemotherapy. The remaining four patients had normal D-dimer levels at pre- and post-chemotherapy but hypercoagulable state was still evident in these patients. [22-23]

The risk of pre-thrombotic state in non-small-cell-lung cancer is leaning more towards adenocarcinoma (ratio 3:1) as suggested.9,10 The patients with elevated D-dimer levels at pre-chemotherapy had better hemostatic profile outcome during treatment as shown in our study. Chemotherapy contributed to elevated D-dimer in 33.3% of lung cancer patients where hypercoagulability associated with pre-thrombotic state is still relevant in non-small-cell lung cancer. A more extensive study is required to confirm these findings.

CONCLUSION AND SUGGESTION

Elevated D-dimer levels indicated hypercoagulability or pre-thrombotic state and 33.3% of lung cancer patients were seen at pre-chemotherapy state, but by cycle-3 of post-chemotherapy the levels returned to a normal state with raised fibrinogen levels seen. In the other 66.7%, the hypercoagulable state was present at pre- and post-chemotherapy. Moreover, chemotherapy contributed to elevated D-dimer levels in 33.3% of these patients suggesting hypercoagulable states was evident in lung cancer patients. The hemostatic profile for pre-thrombotic or hypercoagulable states depended on D-dimer levels before and after the effects of treatment.

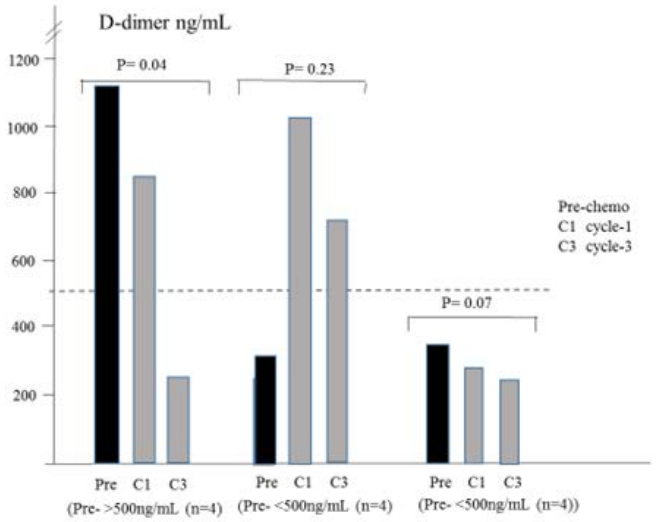

Figure 1. Lung cancer patients with D-dimer levels at pre-chemotherapy of greater (left) and below 500ng/mL. Pre-chemo levels of <500 ng/mL (center) but with elevated levels post-chemo and normal levels throughout chemo (right).

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