RESEARCH ARTICLE

Predictive Value of Baseline Plasma D-dimers for Chemotherapy-induced Thrombocytopenia in Patients with Stage III Colon Cancer: A Pilot Study

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Abstract

Background: : Chemotherapy-induced thrombocytopenia (CIT) is an important cause of morbitity in patients with cancer. Aim: To investigate the effect of the baseline plasma D-dimer level, an important marker for thrombotic activity, on chemotherapy-induced thrombocytopenia in patients with stage III colon cancer. Materials and Methods: A total of 43 (28 men) eligible patients were divided into two groups according to whether they exhibited chemotherapy-induced thrombocytopenia: Group 1 (n=21) and Group 2 (n=22). Comparison was made using demographic, histopathologic, and laboratory variables. Additionally, baseline plasma D-dimer levels underwent receiver operation characteristics curve analysis, and areas under the curve were calculated. Sensitivity, specificity, and positive and negative likelihood rates were then determined. Results: The incidence of chemotherapy-induced thrombocytopenia had a significant correlation with baseline platelet count (r=0.568, P=0.031) and baseline plasma D-dimer levels (r=0.617, P=0.036). When the cut-off point for the latter was set as 498 ng/mL, the area under the curve was 0.89 (95% CI: 0.74-0.93), the sensitivity was 91.4%, the specificity was 89.7%, the positive likelihood rate was 3.64 and the negative likelihood rate was 0.24 for chemotherapy-induced thrombocytopenia diagnosis. Conclusions: The baseline level of plasma D-dimer could help to differentiate high-risk patients for chemotherapy-induced thrombocytopenia.

Keywords: Thrombocytopenia - plasma D-dimer - chemotherapy-related toxicity - predictive value

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Introduction

Chemotherapy-induced thrombocytopenia (CIT) is the most important cause of thrombocytopenia in patients with cancer (Hitron et al., 2011). However, the underlying etiopathogenic mechanisms of chemotherapy-related myelosuppression are not yet clear (Levin et al., 2010; Bartucci et al., 2011; Ten et al., 2011). Persistent and severe CIT not only has a risk of life-threatening hemorrhagic events but also may necessitate dose reduction and/or delay in schedules of chemotherapy treatment (Jelic et al., 2006; Sekhon et al., 2006; Arnold et al., 2011; Ten et al., 2011; Wu et al., 2012). Previous limited studies have demonstrated that various factors such as a platinum regimen, multiple high-risk chemotherapy treatments, multiple tumors, lung cancer, and baseline thrombocyte count, have a high predictive value for CIT (Ten et al., 2011). However, no specific factor to identify which patient will develop CIT has been defined.

Previous studies have reported the importance of the prothrombotic and fibrinolytic activation pathway in tumor progression and metastasis (Blackwell et al., 2000; Dirix et al., 2002; Antoniou et al., 2006; Kirwan et al., 2008). Plasma D-dimer is a plasmin-mediated fibrin degradation product and the most important marker for hemostatic activation. Additionally, plasma levels of D-dimer are increased in malignancies. Edwards et al. (1993) reported that plasma D-dimer levels were increased in patients with Dukes' C stage colorectal cancer. Based on this information, I hypothesized that the baseline level of plasma D-dimer may be help to differentiate high-risk patients for CIT. The main objective in this study was to determine whether the plasma D-dimer level in eligible patients with stage III colon cancer had a relationship with CIT. Therefore, the predictive value of the baseline plasma D-dimer level before first course of adjuvant chemotherapy on CIT was investigated in this study.

Materials and Methods

Study design and individuals

The study was planned as a retrospective cohort study with diagnostic accuracy. The subjects of the present study were selected from 117 patients with colon cancer who were treated at the outpatient department of my institution between May 2008 and April 2012.

A total of 43 (28 men) eligible patients with stage III colon cancer whose medical file information was complete

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and who did not meet the exclusion criteria were enrolled in this study. Subjects were categorized into two groups with or without CIT: Group 1 and Group 2, respectively.

Exclusion criteria

The exclusion criteria were as follows: 1) patients with metastatic colorectal cancer; 2) patients diagnosed with deep vein thrombosis or/and pulmonary embolism by venous duplex/doppler ultrasound and/or computerized tomography before first course of adjuvant chemotherapy; 3) patients previously diagnosed with chronic renal insufficiency, sepsis, diabetes mellitus, multiple primary malignancies, rheumatologic diseases, bone or bone marrow metastasis, chronic liver diseases, splenomegaly, hematological malignancies, or plasma electrolyte abnormalities; 4) patients still receiving acetyl-salicylic acid and oral and/or parenteral antithrombotic or anticoagulant drugs; 5) patients receiving adjuvant chemotherapy or previously diagnosed with pseudo-thrombocytopenia, disseminated intravascular coagulation, idiopathic thrombocytopenic purpura, heparin-induced thrombocytopenia, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, or autoimmune hemolytic thrombocytopenia; 6) patients who had increased splenic size and chemotherapy-associated hepatic sinusoidal obstructive syndrome; and 7) patients receiving neoadjuvant chemo- and/or radiotherapy before analysis.

Study variables and outcome data assays

The following parameters and measurement methods were used throughout the study. The demographic, clinical, and histopathological variables of the study were age (years); sex; smoking habits; body mass index (BMI, kg/m^2); hemoglobin (g/dL); leukocyte (10^9/L); neutrophil (10^9/L), and platelet (10^9/L) counts; location of the primary tumor; pathological T stage (pT); histological grade of the tumor; pathological nodal status (pN); tumor size (centimeter); lymphovascular invasion; type of operation; and baseline plasma D-dimer level (ng/mL). The BMI was calculated using the Quetlet index (weight/height^2=kg/m^2).

Complete blood counts, including hemoglobin, leukocytes, neutrophils, and platelets, of the patients were obtained with an automated hematology analyzer (ABX-PENTRA 120 DX® Hematology Analyzer; ABX Diagnostics, France). The plasma D-dimer level was measured with the UV-kinetic method with an Olympus AU 2700® autoanalyzer (Olympus Diagnostics; Germany).

The results of only one blood sample which was taken before adjuvant chemotherapy were included in this study.

Definitions

Thrombocytopenia is defined as a platelet count below the normal range for the population. Grading of chemotherapy-related myelosuppression was based on the National Cancer Institute Common Terminology Criteria or Adverse Events. Furthermore, bleeding-associated CITs were classified in terms of severity according to the more widely used World Health Organization (WHO) Severity Grading System: grade 1, petechial, mucosal bleeding or retinal bleeding without vision impairment; grade 2, mild blood loss, including melena, hematemesis, hematuria, and hemoptysis; grade 3, gross blood loss including any bleeds requiring the transfusion of red cells; and grade 4, debilitating blood loss including retinal and cerebral bleeds associated with morbidity and fatal bleeds.

Treatment regimens

In the third or fourth week after the colon surgery, adjuvant systemic treatment was initiated for all of the patients. In this study, the treatment consisted of 85 mg of oxaliplatin per square meter of body surface area on day 1, intravenous bolus infusion with 400 mg of 5-fluorouracil per square meter on day 1, and 22-hour continuous infusion with 600 mg of 5-fluorouracil per square meter and 200 mg of calcium folinate per square meter on days 1 and 2 every 2 weeks for 12 cycles (FOLFOX4 regimen) or 85 mg of oxaliplatin per square meter of body surface area on day 1, 400 mg of calcium folinate per square meter on day 1, intravenous bolus infusion with 400 mg of 5-fluorouracil per square meter on day 1, and 46-hour continuous infusion with 2400 mg of 5-fluorouracil per square meter on days 1 and 2 every 2 weeks for 12 cycles (modified FOLFOX6 regimen; mFOLFOX6).

The chemotherapy regimen was administered to patients with adequate counts of neutrophils (>$4x10^9$/L), leukocytes (>$1.5x10^9$/L), and platelets (>$120x10^9$/L).

Ethics

The protocol for this retrospective study was compatible with the local ethical guidelines. The study was approved by the Academic Committees in both centers.

Statistical analyses

The data are expressed as the mean±standard deviation or the median and interquartile range (25-75%). The distribution of variables was analyzed with the Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, independent Student's t test. Nonparametric variables were analyzed with the Mann-Whitney U test. However, qualitative parameters were analyzed with the Chi-square test and Fisher's test. The Kruskal-Wallis test was used for comparisons between clinical and demographic variables.

Additionally, plasma D-dimer levels were analyzed with receiver operating characteristics (ROC) curve analysis, and the area under the curve (AUC) was calculated. The sensitivity, specificity, and positive and negative likelihood rates (LRs) of the plasma D-dimer level were determined.

The relationships between the cut-off level of plasma D-dimer and other study variables were determined using Spearman's correlation tests and analysis of variance (ANOVA). Additionally, the relationships between clinical and demographic variables (such as age, sex, smoking habits, BMI, type of treatment regimen, type of surgery, and histopathological findings) and cut-off level of plasma D-dimer measurement were determined using a Pearson correlation test. The dependent variable for the multiple logistic regression analysis was the presence of CIT (patients with CIT vs. free of CIT). The independent variables were age, sex, BMI, smoking habits, treatment

type, histopathological findings, and plasma D-dimer level. Both adjusted and crude odds ratios (ORs) were calculated with 95% confidence intervals (95%CI) to assess the influence of various independent variables on the presence of CIT.

A significance value of P<0.05 was accepted as statistically significant. All of the analyses were performed using the Statistical Program for Social Sciences (SPSS) version 15.

Results

Compliance with treatment

A total of 493 chemotherapy cycles were administered (median 11.1 cycles, range 6-12). The median interval between cycles was 19 days (range 14-28). Five patients discontinued adjuvant treatments (1 patient in Group 1 because of acute myocardial infarction during the 7th cycle, 1 patient in Group 1 because of acute myocardial infarction during the 9th cycle, 1 patient in Group 1 because of persistent cardiac arrhythmia during the 8th cycle, 1 patient in Group 2 because of new metastatic lesions in the liver during the 6th cycle, and 1 patient in Group 2 because of new metastatic lesions in the liver during the 7th cycle). The treatment cycles in Group 1 did not differ from Group 2 (median 10.8 cycles, range 6-12 in Group 1 vs. median 11.2 cycles, range 7-12; P=0.097). In 26 patients (67 cycles, 14%), chemotherapy was delayed by one week due to myelotoxicity, including all grades of neutropenia, anemia, and thrombocytopenia. Dose reduction was necessary in these same 26 patients after the appearance of myelosuppressive toxicity.

Toxicity

CIT was determined in 21 patients (49%) and 53 cycles (11%) in this study. The mean duration of CIT was 11 days (range 6-19). All of the 21 patients with CIT had various levels of chemotherapy-induced anemia (CIA) and chemotherapy-induced neutropenia (CIN). These results were consistent with those reported in previous studies. CIT has been reported to occur in 45% to 77% of patients receiving oxaliplatin-based regimens (FOLFOX4, mFOLFOX6, and mFOLFOX7) for the treatment of colorectal cancer (Andre et al., 2004; Colucci et al., 2005).

For the patients with CIT (Group 1, n=21) and without CIT (Group 2, n=22), the baseline demographic and clinical features, histopathological findings, and laboratory values are shown in Table.

The baseline thrombocyte counts of Group 1 were significantly different from those of Group 2 (204±67x10^9 vs. 272±136x10^9/L, respectively) (P=0.035). The incidence of the CIT in patients who were treated with FOLFOX4 regimen in Group 1 did not differ from those who were treated with the mFOLFOX6 regimen in the same Group (n=12, 57% vs. n=9; 43%, respectively) (P=0.161).

Additionally, the incidence of CIA in Group 1 did not differ from Group 2 (n=14; 67% vs. n= 12; 55%, respectively) (P=0.103). Similarly, the incidence of CIN in Group 1 did not differ from Group 2 (n=13; 62% vs. n=13; 59%, respectively) (P=0.118).

Table 1. Comparison of Baseline Demographic, Histopathological, Clinical, and laboratory Characteristics of Patients with CIT and Not-CIT in Study

Variables	Patients with CIT	Patients with not-CIT	P*
Patients (n)	21	22	-
Age (year)	50 ± 11	49 ± 12	0.214
Sex		0.168	
Male	13	15	
Female	8	7	
BMI (kg/m2)			
All patients	30.1 ± 1.7	30.6 ± 1.6	0.147
Male	30.4 ± 1.9	30.7± 1.4	
Female	30.8 ± 1.6	30.6 ± 1.5	
Smoking (n, %)			0.183
Present	14	12	
Absent	7	10	
Tumour localization			
Recto-sigmoid and sigmoid colon	9	12	0.146
Transverse colon	5	3	
Ascending colon	5	4	
Caecum	2	3	
Operation type			
Segmental resection	7	8	0.271
Right hemicolectomy	4	5	
Left hemicolectomy	10	9	
Nodal status, pN (n, %)			0.242
N1a	4	4	
N1b	6	5	
N2a	7	8	
N2b	4	5	
Tumor stage, pT (n, %)			0.293
T1	4	3	
T2	3	5	
T3	7	8	
T4a	5	4	
T4b	2	2	
Lenfovascular invasion			0.235
Presence	10	11	
Absence	8	9	
Unknown	3	2	
Tumour grades			0.244
Grade 1	5	6	
Grade 2	8	9	
Grade 3	8	7	
Baseline neutrophil count (x109)	5.4±1.6	5.3±1.4	0.299
Baseline leukocyte count (x109)	8.8±4.7	8.7±3.3	0.234
Baseline hemoglobin level (g/dl)	11.1±3.2	11.3±2.8	0.211
Baseline thrombocyte count (x109)	206±63	279±141	0.036*
CIA (n, %)	14 (67)	12 (55)	0.103
CIN (n, %)	13 (62)	13 (59)	0.118
Myelotoxicity (CIN + CIA; n, %)	14 (67)	12 (55)	0.091
Treatment regimens			0.157
FOLFOX4	14	12	
mFOLFOX6	7	10	
Serum D-dimer level (ng/mL)			
mean	643	482	0.027*
(range)	(465-945)	(428-744)	

*A two tailed p value of <0.05 was considered statistically significant; BMI; Body mass index, CIA; Chemotherapy-induced anemia, CIN; Chemotherapy-induced neutropenia

The incidence of CIT had a significant negative correlation with baseline platelet counts (r=-0.568, P=0.031), but it had no significant correlation with age, sex, smoking habits, BMI, location of primary tumor, pT, pN, lymphovascular invasion , histological grade of tumor, tumor size, or type of operation (r=0.237, P=0.403; r=0.162, P=0.207; r=0.241, P=0.418; r=0.256, P=0.402; r=0.247, P=0.293; r=0.351, P=0.175; r=0.236, P=0.078;

r=0.217, P=0.101; r=0.235, P=0.098; r=0.284, P=0.261, and r=0.219, P=0.418, respectively).

No grade 2-4 bleeding was observed in any of the patients with CIT during the study period. This lack may be related to the small number of patients in the present study. Grade 1 bleeding was determined in 4 (19%) patients with CIT, and the thrombocyte count of these patients was lower than $75x10^9$/L (mean $54x10^9$/L; range 46-67).

D-dimer evaluation

The median value of the baseline plasma D-dimer level in all patients was 492 ng/mL (range: 426-945). The median baseline plasma D-dimer level of Group 1 were significantly different from those of Group 2 (643 ng/mL, range: 465-945 for Group 1 vs. 482 ng/mL, range: 428-744 for Group 2) (P=0.027). The incidence of CIT had a significant negative correlation with plasma D-dimer levels (r=-0.607, P=0.038), although the incidence of CIA and CIN had no correlation with plasma D-dimer levels (r=-0.213, P=0.402, and r=-0.245, P=0.179, respectively).

Higher plasma D-dimer levels had a significant correlation with tumor localization (for right-sided tumor), pN (for all nodal statues), positive lymphovascular invasion, and histological grade of tumor (for grade 2 and 3 tumors) (r=0.596, P=0.041; r=0.548, P=0.039; r=0.604, P=0.032; and r=0.5987, P=0.044, respectively).

The cut-off point for baseline plasma D-dimer levels was defined as 498 ng/mL. For plasma D-dimer levels, the AUC was 0.89 (95%CI: 0.74-0.93), the sensitivity was 92.4%, the specificity was 89.9%, the positive LR was 3.54 and the negative LR was 0.25 for CIT diagnosis. Similarly, for the plasma D-dimer level, the AUC was 0.93 (95%CI: 0.72-0.95), the sensitivity was 93.9%, the specificity was 91.7%, the positive LR was 3.46 and the negative LR was 0.24 for grade 2 CIT diagnosis, and these values for grade 3 CIT were 94.8%, 92.9%, 3.66, and 0.22, respectively.

It was concluded that the relation between plasma D-dimer levels and the presence of CIT in early-stage colon cancer is independent of other study variables (age, sex, smoking habits, BMI, location of primary tumor, pT, histological grade of tumor, pN, tumor size, lenfovascular invasion, and type of operation) (P=0.035; OR=3.24; 95%CI 1.19-7.57)

Discussion

In this present study, I demonstrated that the plasma D-dimer level significantly predicted CIT, especially grade 2 and grade 3 CIT, in patients with stage III colon cancer who had been treated with adjuvant FOLFOX4 or mFOLFOX6 regimens.

Patients with malignancy may present with various circulatory markers of prothrombotic and hemostatic activation which may be related with tumor growth and distant metastasis (Antoniou et al., 2006; Sekhon et al., 2006; Kirwan et al., 2008). In clinical oncology practice, physicians have observed hemostatic disorders with or without arterial or venous thrombotic events in patients with malignancies. The relationship between malignancy and the coagulation system of blood is still interest. However, the mechanism underlying this relationship is not sufficiently understood.

Coagulation markers, such as plasma D-dimer, have been associated with thrombotic events in traumas, malignancies, and many systemic inflammatory diseases. Several small studies have reported that plasma D-dimer levels increase in early-stage and advanced malignancies of the lung, breast, and colorectal region (Oya et al., 2001; Antoniou et al., 2006; Sekhon et al., 2006; Kirwan et al., 2008). Additionally, limited previous studies have demonstrated that plasma D-dimer levels predict the prognosis and response of systemic treatment in patients with breast, lung, and ovary cancer (Antoniou et al., 2006; Sekhon et al., 2006; Kirwan et al., 2008; Yoshiki et al., 2010; Komurcuoglu et al., 2011; Yamamato et al., 2012). However, no study has evaluated the relationship between plasma D-dimer levels and CIT.

It is not yet clear how chemotherapeutics drugs affect thrombocyte production and whether platelet precursors represent a direct target of these agents. Some authors speculate that a direct toxic effect to precursors of platelets might induce apoptosis of this population. In my opinion, the high thrombotic and fibrinolytic activity in patients with cancer may be considered the negatively affect the circulating precursors of platelets, except for disseminated intravascular coagulation or pure myelosuppression.

The most important limitation of this present study is that it is composed of narrowly selected patients. In this study, the preliminary conclusion is that the plasma D-dimer level can predict CIT. However, these results should be clarified with large series studies that include a sufficient number of patients and this hypothesis must be based on molecular evidence. Additionally, the true predictive value of measuring D-dimer level should be confirmed by prospective studies.

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