Therapeutic Significance of a D-dimer Cut-off Level of >3 μg/ml in Colorectal Cancer Patients Treated with Standard Chemotherapy plus Bevacizumab

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Received January 17, 2010; accepted March 30, 2010

Objective: The risk of venous thromboembolism has been reported to increase when receiving bevacizumab. Many cancer patients are reported to have elevated D-dimer levels. It is not clear what D-dimer level might indicate an increased risk of venous thromboembolism in the colorectal cancer patients treated with bevacizumab-containing chemotherapy.

Methods: The D-dimer levels and any event concurrent with an elevated D-dimer level were evaluated in patients receiving bevacizumab. The D-dimer cut-off level was determined using the receiver-operating characteristic analysis. The selection criteria were as follows: histologically proven metastatic and unresectable colorectal adenocarcinoma; no prior chemotherapy containing bevacizumab; D-dimer test performed repetitively on the baseline and during bevacizumab administration; no venous thromboembolism identified at the baseline; and enhanced computed tomographic scan performed every 2 months.

Results: Sixty-nine patients were included. The chemotherapy regimens with bevacizumab included the regimen of 5-fluorouracil, leucovorin and oxaliplatin (FOLFOX), the regimen of 5-fluorouracil, leucovorin and irinotecan (FOLFIRI), and leucovorin-modulated 5-fluorouracil. The median baseline D-dimer level was 1.2 μg/ml. The appropriate D-dimer cut-off level was 3 μg/ml with the negative predictive value of 98% and relative risk of 6.9. Twenty-one of 69 patients showed elevated D-dimer levels of >3 μg/ml, with 11 patients for unknown reasons, 6 with tumor progression, 3 with venous thromboembolism and 1 with sepsis. In the remaining 48 patients whose D-dimer levels were ≤3 μg/ml, only one patient developed a venous thromboembolism.

Conclusions: A D-dimer cut-off level of 3 μg/ml might be a useful indicator level to exclude venous thromboembolism or show an increased risk for venous thromboembolism in colorectal cancer patients treated with bevacizumab-containing chemotherapy.

Key words: colorectal neoplasms – bevacizumab – venous thromboembolism – fibrin fragment D – ROC curve

INTRODUCTION

Venous thromboembolism (VTE), including deep venous thrombosis and pulmonary embolism, is a major complication of cancer, and one of the leading causes of death in cancer patients (1). Furthermore, the risk of VTE increases in cancer patients receiving bevacizumab (Avastin; Genentech, Inc., South San Francisco, CA, USA), a recombinant humanized monoclonal antibody against vascular endothelial growth factor (2). Bevacizumab is effective for patients with...
metastatic colorectal cancer (CRC) in combination with intravenous fluorouracil (FU)-based chemotherapy (3–5). Therefore, appropriate diagnosis of VTE is essential during the treatment for CRC patients.

D-dimer is a degradation product of cross-linked fibrin, and the D-dimer test is a diagnostic aid for VTE in outpatient settings, especially for non-cancerous patients, because of the high sensitivity and clinical usefulness in excluding VTE (6,7). However, the D-dimer level is also elevated when a systematic activation of coagulation is observed in patients with disseminated intravascular coagulation, hematoma, malignancy itself and tumor progression and so on.

It was reported so far that the D-dimer level was elevated in patients with CRC (8,9). Yoshikawa et al. (10) reported that 7.0 μg/ml was the D-dimer cut-off value for ruling out VTE in CRC patients treated with classical FU-based chemotherapy in an adjuvant setting. However, no previous report has identified the D-dimer level that might indicate an increased risk of VTE in metastatic CRC patients treated with bevacizumab in combination with intravenous FU-based standard chemotherapy.

The current study used the receiver-operating characteristic (ROC) analysis to determine the appropriate D-dimer cut-off level for the increased risk of VTE in CRC patients treated with bevacizumab in combination with intravenous FU-based chemotherapy.

**PATIENTS AND METHODS**

**PATIENTS AND SELECTION CRITERIA**

The current study retrospectively reviewed the medical charts of 209 consecutive patients with histologically confirmed metastatic or unresectable colorectal adenocarcinoma, who had been treated with standard chemotherapy plus bevacizumab, at the National Cancer Center Hospital East in Japan between June 2007 and November 2008. Sixty-nine patients who met the following selection criteria were included in the current study. The selection criteria included that D-dimer test was performed repetitively at the physicians’ request both at the baseline and during bevacizumab administration, and enhanced chest/abdominal computed tomographic (CT) scan was performed every 2 months basically to evaluate tumor progression and identify VTE. Written general consent that included research uses of clinical data had been obtained from all patients, and the study was performed in accordance with the Declaration of Helsinki and Japanese ethical guidelines for epidemiological research. We obtained an institutional review board (IRB) waiver to conduct this study from the chairperson of the IRB. Any patients who had been treated with bevacizumab as prior chemotherapy or who had VTE identified before bevacizumab administration were excluded.

**STUDY DESIGN**

This is a retrospective study to determine the appropriate D-dimer cut-off level for the increased risk of VTE in CRC patients treated with standard chemotherapy plus bevacizumab, using the ROC analysis. Every D-dimer level during the treatment of bevacizumab-containing chemotherapy and any events concurrent with elevated D-dimer level were reviewed. The events include VTE, progressive disease, sepsis and so on. Patients with an elevated D-dimer level of no apparent cause were classified as ‘unknown reason’. The D-dimer cut-off level was determined using the ROC analysis with the maximum D-dimer level in each patient.

**PLASMA D-DIMER MEASUREMENTS**

The plasma D-dimer latex immunoassay (LIA) was performed using a commercial immunoassay kit (LIAS AUTO D-dimer NEO, Sysmex, Japan) measured on a Sysmex CA-1500 System, a fully automated coagulation analyzer. The assays were performed according to the manufacturer’s instructions. The measurable D-dimer LIA value ranges from 0.5 to 30 μg/ml, and the upper limit of normal is 1.0 μg/ml. D-dimer assays in the current study were performed and interpreted by independent operators without any knowledge of the clinical course of the patients.

**STATISTICAL ANALYSIS**

The sensitivity and specificity were calculated for every potential D-dimer cut-off level. The results were summarized with the ROC analysis to evaluate the performance of every potential D-dimer cut-off level. A D-dimer cut-off level was determined when it maximized the average of the observed sensitivity and specificity.

**RESULTS**

**PATIENT CHARACTERISTICS**

Sixty-nine patients with a total of 817 D-dimer tests were included. The patient characteristics are shown in Table 1. The standard chemotherapy regimens that were administered concurrently with 2.5 mg/kg bevacizumab per week through central venous catheter inserted into the subclavian vein included mFOLFOX6 (oxaliplatin 85 mg/m², l-leucovorin 200 mg/m², 5-FU bolus 400 mg/m² followed by infusional 5-FU 2400 mg/m² 46 h every 2 weeks; n = 43), FOLFIRI (irinotecan 150 mg/m², l-leucovorin 200 mg/m², 5-FU bolus 400 mg/m² followed by infusional 5-FU 2400 mg/m² 46 h every 2 weeks; n = 25) and leucovorin-modulated 5-FU (l-leucovorin 250 mg/m², 5-FU bolus 500 mg/m² weekly; n = 1). The median baseline D-dimer level was 1.2 μg/ml (range 0.2–5.0). The median number of D-dimer tests per patient was 10 times (range 2–32). Thirty-nine of 69 (57%) patients showed elevated D-dimer levels of >1 μg/ml before
receiving bevacizumab, and 62 of 69 (90%) patients showed elevated D-dimer levels of \( >1 \mu g/ml \) while receiving bevacizumab.

### VTE Cases

Four out of the 69 patients developed VTE (5.8%). The clinical course of the D-dimer level and the characteristics of the four patients are described in Fig. 1. Only one case of VTE (Case 3) with a D-dimer level of 7.9 \( \mu g/ml \) at that time was identified in the left femoral vein with swelling of the left lower limb, whereas the remaining three cases of VTE were found by CT scan in the superior vena cava or subclavian vein without any symptoms, with a D-dimer level of 7.6, 2.0 and 3.3 \( \mu g/ml \), respectively. The onset of VTE detected ranged from days 42 to 439 after the first administration of bevacizumab.

### ROC Analysis

The sensitivity and specificity were 100% and 11%, respectively, when the upper limit of normal (1 \( \mu g/ml \)) was set as the D-dimer cut-off level (Fig. 2). The positive and negative predictive values were 6.5% and 100%, respectively, and the accuracy was only 16%.

A graph of the ROC analysis was plotted to investigate the D-dimer cut-off level from 0.5 to 41 \( \mu g/ml \) at 0.5 \( \mu g/ml \) intervals. When 3 \( \mu g/ml \) was set as the D-dimer cut-off level, the sensitivity and specificity were 75% and 72%, and the average of the sensitivity and specificity showed the maximum score among all the potential D-dimer cut-off levels. Hence, the recommended D-dimer cut-off level for the increased risk of VTE might be 3 \( \mu g/ml \) (Fig. 2). The positive and negative predictive values were 14% and 98%, and the accuracy improved to 72%, respectively (Fig. 2). Twenty-one patients showed an elevated D-dimer level of \( >3 \mu g/ml \) while receiving bevacizumab, and VTEs were identified in three patients. In the remaining 48 patients who showed less elevated D-dimer levels of \( \leq 3 \mu g/ml \), only one patient developed VTE. The relative risk of VTE incidence had a 6.9-fold increase among patients who showed elevated D-dimer levels of \( >3 \mu g/ml \) during the administration of bevacizumab (Fig. 2).

### Reasons for Elevated D-dimer Level

Twenty-one of the 69 (30%) patients showed elevated D-dimer levels of \( >3 \mu g/ml \) while receiving bevacizumab. The reasons for the elevated D-dimer levels were as follows: 11 patients of unknown reasons (52%), 6 with tumor progression (29%), 3 with VTEs (14%) and 1 with sepsis (4.8%).

### DISCUSSION

The current study is the first report to indicate that what D-dimer level might show an increased risk of VTE in metastatic or resectable CRC patients treated with standard chemotherapy plus bevacizumab.

The current study identified 39 patients (57%) who showed a positive D-dimer value of \( >1 \mu g/ml \) (range 0.2–5.0) before the administration of bevacizumab, which is consistent with previous reports (8,9). Sixty-two out of 69 (90%) patients showed elevated D-dimer levels of \( >1 \mu g/ml \) while receiving bevacizumab, thus indicating a D-dimer cut-off level of 1 \( \mu g/ml \) in CRC patients receiving bevacizumab not to be useful for evaluating VTE risk.

VTE was identified in four patients (5.8%) during the administration of bevacizumab and developed in the subclavian vein or superior vena cava in three asymptomatic patients. The incidence was close to that of 7.3% (96 out of 1315 patients) in a meta-analysis containing four randomized trials of bevacizumab therapy for CRC (2). There would be several factors to promote emboli formation in these cases. First, cancer patients themselves are at significantly increased risk of developing VTE, particularly while receiving systemic chemotherapy. The incidence of high-grade VTEs was reported as high as 4.2% in cancer patients with chemotherapy without bevacizumab (2). Second, the use of bevacizumab was significantly associated with an increased risk of developing VTE in cancer patients, and the incidence was reported to increase from 4.2% to 6.3% with a relative risk of 1.38 (2). Third, central venous catheter itself could cause central venous catheter-related activation of coagulation cascade. More than 80% of indwelling central venous
catheters were associated with measurable thrombin activity at the time of removal (11), and most of the VTE in the current study was identified in the subclavian vein or superior vena cava. These data might indicate that central venous catheter itself might contribute to the VTE formation in patients treated with chemotherapy including bevacizumab.

The ROC analysis showed that when the D-dimer cut-off level was set at 3 μg/ml, the accuracy and specificity improved to 72% with a negative predictive value of 98% and relative risk of 6.9. These findings might suggest that VTE could be mostly excluded when D-dimer level was ≤3 μg/ml, in terms of the high negative predictive value. Whereas the positive predictive value was only 14%; therefore, few VTE could be appropriately diagnosed with D-dimer test alone. The reason could be that D-dimer level elevated above 3 μg/ml in characteristic of not only VTE but also other situations such as tumor progression. In the current study, there were 6 patients with tumor progression
out of the 21 patients shown elevated D-dimer levels of >3 μg/ml. Indeed, Blackwell et al. (12) showed that maximum D-dimer levels since baseline occurred at the time of disease progression in 51 of 61 patients (84%) and concluded that D-dimer levels were good predictors of disease progression in patients with metastatic CRC.

Yoshikawa et al. (10) showed that 7.0 μg/ml, which is a much higher cut-off level than that in the current study, might be a D-dimer cut-off value for ruling out VTE in curatively resected CRC patients treated with classical FU-based chemotherapy without bevacizumab in the adjuvant setting. The normal upper limit of the D-dimer reagent used in their study was 1.2 μg/ml, which is higher than that in the current study. The difference of D-dimer reagents, chemotherapy regimens and target patient populations might influence the D-dimer cut-off level, although further investigation is required to clarify these points.

The current study has the following potential limitations. First, the study selected the patients with the D-dimer level measured before and during the administration of bevacizumab to evaluate clinical course of the D-dimer level. There could be a selection bias; however, this may be minor because the D-dimer test was performed routinely in our clinical practice and the incidence of VTE in the current study was similar to the previous report (2) described above. Second, the sample size could be too small to determine the D-dimer cut-off level. However, a preliminary ROC analysis with a smaller sample size of 39 patients with two identified cases of VTEs reproducibly showed the same D-dimer cut-off level of >3 μg/ml with increased risk (relative risk = 5) (13). Therefore, the D-dimer cut-off level of >3 μg/ml might be a useful indicator, although the efficacy need to be validated using another large-sized cohort.

In conclusion, a D-dimer cut-off level of 3 μg/ml might be a useful indicator level to exclude VTE or show an increased risk for VTE in CRC patients treated with standard chemotherapy plus bevacizumab.

Acknowledgements

We thank Kenichi Yoshimura, medical statistician in the Translational Research Center of Kyoto University Hospital, for his valuable statistical advice.

Conflict of interest statement

None declared.

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