Plasma Thrombin-activatable Fibrinolysis Inhibitor Levels Correlate with the Disease Activity of Ulcerative Colitis

Yavuz Beyazit¹, Abdurrahim Sayilir², Alpaslan Tanoglu³, Murat Kekilli³, Erdem Kocak¹, Fuat Ekiz¹ and Adnan Tas⁴

Abstract

Objective Patients with ulcerative colitis (UC) are at an increased risk for thromboembolic events, particularly in patients with extensive and active disease. To date, a few studies have been published on the role of thrombin-activatable fibrinolysis inhibitor (TAFI) in UC. However, there are no reports in the literature investigating the effect of UC treatment on plasma TAFI levels.

Methods The plasma TAFI antigen levels were quantitatively determined using ELISA kits for 20 UC patients at activation and remission, along with 17 healthy controls. The association between the TAFI levels and inflammatory markers was assessed to determine UC activation. To predict and determine the activation of UC, the Truelove-Witts index and the endoscopic activation index (EAI) were used for each subject.

Results The plasma TAFI levels were higher in UC patients at activation of the disease compared with the remission state and in healthy controls. Spearman’s correlation analyses revealed that the WBC (r: 0.586, p<0.001), hsCRP (r: 0.593, p<0.001) and EAI (r: 0.721, p<0.001) were significantly correlated with the TAFI levels. The overall accuracy of TAFI in determining UC activation was 82.5% with a sensitivity, specificity, NPV and PPV of 80%, 85%, 81% and 84.2%, respectively (cut-off value: 156.2% and AUC: 0.879).

Conclusion The present study demonstrates that the TAFI levels are elevated in the active state of UC. The assessment of TAFI levels in patients with UC in conjunction with other markers of inflammation may provide additional information for estimating UC activation and severity.

Key words: plasma thrombin-activatable fibrinolysis inhibitor, ulcerative colitis, treatment


Introduction

The hallmark of ulcerative colitis (UC) and Crohn’s disease (CD), the two major forms of inflammatory bowel disease (IBD), is chronic, uncontrolled inflammation of the intestinal mucosa. Apart from inflammation, coagulation and fibrinolysis also play significant roles in the pathophysiology of the disease. In both forms of the disease, a hypercoagulable state and a prothrombotic condition exist, whereas abnormalities in the coagulation system are partially responsible for the clinical picture. The regulation between coagulation and fibrinolysis plays an important role in preserving a balanced hemostatic process, and it is generally accepted that both have been found to be upregulated in active and inactive states of IBD (1, 2).

Thrombin-activatable fibrinolysis inhibitor (TAFI) is a single-chain glycoprotein of 58 kDa that is synthesized in the liver and circulates in human plasma as a zymogen. It is activated primarily by the thrombin/thrombomodulin complex. After being converted to the activated form (TAFIa), it cleaves C-terminal lysine residues from the fibrin surface, thereby decreasing its cofactor activity (3-5). Several studies have been performed to determine the TAFI levels in distinct disease states. Increased TAFI levels have been determined in conditions such as stable coronary artery disease,
venous thrombosis, peripheral arterial disease, type 2 diabetes and acute pancreatitis (3, 6-9), and decreased TAFI plasma levels have been described during acute promyelocytic leukemia and in chronic liver disease (10, 11). Apart from its well-known effects on suppressing fibrinolysis, TAFIa may also be involved in inflammation by inactivating the anaphylatoxins C3a and C5a, thrombin-cleaved osteopontin and bradykinin (5).

Although the correlation between UC and hypercoagulable states has been demonstrated in previous studies, the current literature regarding these factors and the role of fibrinolysis in patients with IBD is lacking and remains quite controversial (1). This study was undertaken in order to determine whether the TAFI levels are elevated in UC patients. Moreover, the correlation between the TAFI levels and disease activity with endoscopic, clinical and laboratory indices was also determined.

### Materials and Methods

#### Patients

Twenty-six patients with moderate or severe UC followed at the gastroenterology clinic of Ankara Training and Research Hospital were initially recruited for the study. After treatment with mesalazine and with or without immunosuppressive agents including steroids or azathiopurine only, 20 patients achieved remission (7 men, 13 women) and were included in the final analysis (Fig. 1). Seventeen healthy controls (7 men, 10 women) were recruited from healthy blood donors (11 individuals) and normal hospital personnel (6 individuals) who had no history of acute/chronic inflammatory disorders or drug use. The diagnosis of UC was obtained by means of standard clinical, radiological, endoscopic and histological criteria. The clinical disease activity was evaluated using the Modified Truelove-Witts Severity Index (Table 1) (12) at diagnosis and after remission. Moreover, according to the initial colonoscopic examination, the endoscopic activity index for each patient was calculated as suggested by Rachmilewitz et al. (13).

The exclusion criteria for entry into the study were as follows: coronary artery disease, peripheral vascular disease, hematologic disorders, diabetes mellitus, neoplastic and hepatic diseases. None of the study participants had received any medication, including anticoagulant medications,  

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### Table 1. Criteria for Evaluating the Severity of UC Patients according to Truelove-Witts Criteria (Ref. 12).

<table>
<thead>
<tr>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;40</td>
<td>40-50</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>Normal</td>
<td>&gt;37.5</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>Normal</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>&lt;10</td>
<td>No</td>
</tr>
<tr>
<td>Sedimentation rate (mm/h)</td>
<td>&lt;30</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

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### Table 2. Demographic Features and Laboratory Values of Active UC Patients and Healthy Controls at Study Entrance.

<table>
<thead>
<tr>
<th>UC Patients (n:20)</th>
<th>Control group (n:17)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>42.6±15.8</td>
<td>49.5±12.3</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>13 (65%) /7 (35%)</td>
<td>10 (58.8%)/7 (41.2%)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13±1.8</td>
<td>14.4±0.7</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>21±17.5</td>
<td>12.3±4.9</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>6.62 (0.7-55.1)</td>
<td>2.4 (0.5-5)</td>
</tr>
<tr>
<td>TAFI (%)</td>
<td>164.4±14.1</td>
<td>125.3±23.6</td>
</tr>
</tbody>
</table>

TAFI: thrombin-activatable fibrinolysis inhibitor, CRP: C reactive protein, ESR: erythrocyte sedimentation rate, WBC: white blood cell, *Data are presented as median (range) or mean±SD.
Figure 2. Correspondence of the plasma TAFI levels between active stage and remission in each patient.

Table 3. Comparison of Plasma TAFI Levels and Other Inflammation Markers between Active and Remission Period of UC.

<table>
<thead>
<tr>
<th></th>
<th>Active state</th>
<th>Remission</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAFI (%)</td>
<td>164.8±14.1</td>
<td>136±25.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.4 (2.4-8.6)</td>
<td>3 (1.8-8.6)</td>
<td>0.245</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>6.62 (0.7-55.1)</td>
<td>2.5 (0.1-7.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>21±17.5</td>
<td>11.4±6.6</td>
<td>0.030</td>
</tr>
<tr>
<td>Platelet (×10³/mm³)</td>
<td>338 (242-914)</td>
<td>311 (227-541)</td>
<td>0.053</td>
</tr>
<tr>
<td>WBC (×10³/mm³)</td>
<td>10.4±2.3</td>
<td>7.9±2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

TAFI: thrombin-activatable fibrinolysis inhibitor, CRP: C reactive protein, ESR: erythrocyte sedimentation rate, WBC: white blood cell.

NSAIDs or oral contraceptive drugs, prior to hospital admission. The study was conducted in accordance with the guidelines of the Declaration of Helsinki. All study participants provided their informed consent, and the study was approved by the Ethics Committee of Ankara Training and Research Hospital, Ankara, Turkey.

Laboratory examinations

Fasting blood specimens were collected both at admission and at disease remission from the antecubital vein after overnight fasting without using any anticoagulants. Routine hematological and chemistry parameters with hemostatic parameters (PT, aPTT, INR and fibrinogen), the erythrocyte sedimentation rate (ESR), and high-sensitivity C-reactive protein (hsCRP) were determined.

TAFI Assay

For the analysis of the TAFI plasma levels, blood samples were collected in sodium citrate and rapidly centrifuged at 3,000 rpm for 15 minutes (Nuvefuge CN 180) at 4°C, frozen in small aliquots and stored at -80°C until further use. The TAFI antigen (TAFI-Ag) levels were quantitatively determined using ELISA kits (Imuclone, American Diagnostica Inc., Stamford, USA). Strip wells were precoated with polyclonal antibody. The plasma samples were then thawed, diluted and applied to the wells. After washing, tetramethylbenzidine (a peroxidase substrate) was added to the wells. The staining intensity was then calculated spectrophotometrically using a microplate reader at 450 nm. The assay was calibrated using reference plasma provided by the manufacturer. The results of TAFI-Ag are expressed as a percentage.

Statistical analyses

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 18 software program (SPSS Inc., Chicago, USA). Continuous variables were tested for normality by the Kolmogorov-Smirnov test. Values are presented as the mean ± standard deviation or, in the case of non-normally distributed data, as the median and range. Comparisons of the percentages between different groups of patients were carried out using the chi-squared test. All normally distributed data were analyzed using unpaired or paired Student’s t-tests. Data found to be non-normally distributed were analyzed using the Mann-Whitney U test for independent subgroups and the Wilcoxon test for dependent subgroups. Spearman’s correlation analysis was performed between TAFI, WBC, PLT, CRP, ESR and disease activity indices. Receiver operating characteristic (ROC) curve analyses was used to identify the optimal cutoff values of TAFI, CRP, ESR, WBC, PLT, and fibrinogen with maximum sensitivity and specificity for the differentiation of active UC disease from remission. A p value of <0.05 was considered to be statistically significant.

Results

Twenty patients with UC and 17 healthy controls were enrolled in the study. There were 7 (35%) men and 13 (65%) women in the UC group and 7 (41.2%) men and 10 (58.8%) women in the control group. The mean age of UC patients and controls were 42.6±15.8 years and 49.5±12.3 years, respectively. There were no significant differences between the ages of the study participants. The demographic
features and laboratory values of the UC patients and controls are summarized in Table 2.

A significant elevation in the TAFI levels was observed in active UC patients compared to healthy controls (164.4±14.1 vs. 125.3±23.6, p<0.001). Fig. 2 shows the correspondence of the TAFI levels between active stage and remission in each patient. Higher plasma TAFI, WBC, ESR and CRP levels were observed in active UC patients compared with remission (Table 3).

The ROC curve analyses suggested that the optimum TAFI cut-off point for determining active UC was 156.2%, with a sensitivity, specificity, NPV and PPV of 80%, 85%, 81% and 84.2%, respectively (AUC: 0.879). The overall accuracy of TAFI in the determination of active UC was found to be 82.5% (Table 4, Fig. 3). Spearman’s correlation analyses revealed that the WBC (r: 0.586, p<0.001), CRP (r: 0.593, p<0.001) and EAI (r: 0.721, p<0.001) were significantly correlated with the TAFI levels (Fig. 4).

**Discussion**

Plasma TAFI levels have been reported to be elevated in several disease conditions, including acute pancreatitis, type 2 diabetes, rheumatoid arthritis, coronary heart disease, deep venous thrombosis and neoplastic diseases (3, 6-9, 14). In addition, several reports suggested alterations in the TAFI levels in IBD, in which the degree of intestinal inflammation and changes in the TAFI levels appears to be correlated (15, 16). However, the impact of this correlation remains controversial, with challenging results either with an increase or a decrease in the TAFI levels.

The present study demonstrated that patients with active UC have elevated plasma TAFI levels compared with healthy controls and a significant decrease occurs after treatment. The plasma TAFI levels were found to have comparably high sensitivity, specificity and predictive values for active UC. Moreover, we provided additional data regarding the association between TAFI and EAI scores, which are typically used for disease activity evaluation. The overall accuracy of plasma TAFI levels for estimating the disease activity in UC was found to be compatible with the CRP, ESR, WBC and fibrinogen values.

Coagulation and inflammation play pivotal roles in the pathophysiology of UC and CD, the two major forms of IBD. A growing body of evidence suggests a tight mutual network in which inflammation, coagulation and fibrinolysis play closely related roles (17). As inflammatory processes occur, proteases that originate from inflammatory infiltrate cells become activated as well as proteases involved in the coagulation and fibrinolytic pathways (15, 18). For this reason, it is not surprising to see IBD patients frequently suffering from thromboembolic events, with a three-fold increased risk of developing deep venous thrombosis and pulmonary embolism compared to the general population (19). This hypercoagulable state may lead to the development and progression of inflammation and be related to UC progression (20). In this context, concerning the role of TAFI in coagulation and inflammation, our study showed a marked elevation of TAFI in active UC in correlation with the inflammatory markers and EAI.

The finding of increased TAFI levels in our cohort is consistent with a previous report by Saibeni et al. (16), who initially showed increased plasma levels of TAFI in IBD patients. They speculated that TAFI may play a key role in regulating the crosstalk between coagulation, fibrinolysis and inflammation. Although UC patients with active and inactive states were reported to be different in the study by Saibeni et al., a unique point of our study is that we demonstrated the transition of TAFI levels from active stage to remission stage in each UC patient. Contrary to these find-

**Table 4. Overall Accuracy and ROC Analyses of TAFI and Other Inflammation Markers to Differentiate Activation of UC from Remission.**

<table>
<thead>
<tr>
<th>Marker</th>
<th>AUC</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
<th>Overall accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAFI (cut off: 156.2)</td>
<td>0.879</td>
<td>80</td>
<td>85</td>
<td>81</td>
<td>84.2</td>
<td>82.5</td>
</tr>
<tr>
<td>Fibrinogen (cut off:3.05)</td>
<td>0.600</td>
<td>60</td>
<td>58.8</td>
<td>55.6</td>
<td>63.2</td>
<td>59.4</td>
</tr>
<tr>
<td>CRP (cut off:3.78)</td>
<td>0.826</td>
<td>75</td>
<td>85</td>
<td>77.3</td>
<td>83.3</td>
<td>80</td>
</tr>
<tr>
<td>ESR (cut off:11.5)</td>
<td>0.635</td>
<td>65</td>
<td>65</td>
<td>65</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>PLT (cut off:314,000)</td>
<td>0.644</td>
<td>65</td>
<td>55</td>
<td>61.1</td>
<td>59.1</td>
<td>60</td>
</tr>
<tr>
<td>WBC (cut off:9.005)</td>
<td>0.794</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

TAFI: thrombin-activatable fibrinolysis inhibitor, CRP: C reactive protein, ESR: erythrocyte sedimentation rate, WBC: white blood cell, AUC: area under curve, PPV: positive predictive value, NPV: negative predictive value
ings, Koutroubakis et al. (1) reported a decrease in the TAFI levels versus an increase in plasminogen activator inhibitor-1 (PAI-1) in IBD patients. The authors speculated that this was due to activation of the TAFI pathway, leading to an imbalance in fibrinolysis. Moreover, decreased TAFI levels might represent a compensatory anticoagulant mechanism in IBD patients attempting to overcome the procoagulant effect of PAI-1. Aiming to resolve this conflict, a more recent report by Owczarek et al. (15) investigated the role of activated TAFI levels in IBD patients. Although the authors showed that the TAFI levels were increased in CD patients compared to UC patients, the lack of a control group made it difficult to draw a definitive conclusion as to whether the TAFI levels are altered in IBD patients.

The fibrinolytic system has been widely investigated in IBD patients (21). Although hyperfibrinolysis and hypofibrinolysis have been demonstrated in patients with UC and CD, the role of the fibrinolytic system in patients with IBD remains controversial (22, 23). In this context, TAFI is an important inhibitor of this system, which cleaves the lysine binding sites of partially degraded fibrin, interfering with the interaction between plasminogen and fibrin (6). Furthermore, TAFIa plays an important role in modulating inflammation with its ability to downregulate pericellular plasminogen activation and inactivation of bradykinin, thrombin-cleaved osteopontin and the anaphylatoxins C3a and C5a (24). TAFIa is currently recognized as the second physiological substrate for the thrombin/thrombomodulin complex. This dual role in regulating inflammatory processes and hemostatic situations makes TAFI an interesting molecule in the search for a simple, noninvasive and reliable method for the assessment of disease activity and therapeutic management in IBD patients. In this study, we prospectively evaluated the plasma TAFI levels of UC patients before and after remission for the first time. TAFI was shown to be a reliable and simple factor to predict the disease activity.

We also studied other inflammation markers, including the WBC, CRP, sedimentation and fibrinogen, which are risk factors in the development of disease activation in relation to TAFI. Excluding fibrinogen levels, all other inflammation markers were found to be elevated in active UC patients, although only the WBC and CRP levels were correlated with the TAFI levels.

There are some limitations associated with this study. The most obvious limitation is that we did not measure other fibrinolysis parameters, such as PAI-1, D-dimer, tissue type plasminogen activator and plasma plasmin inhibitor, which would aid in the understanding of the present data regarding the function of the fibrinolytic system in IBD. Another limitation of the present study is the relatively small patient population and steroid use in a majority of UC patients to achieve remission. Although there is little known about the effect of steroids on the plasma TAFI levels, according to previous data that blood coagulation is promoted by steroids, the plasma TAFI levels in UC patients may be ef-
lected by steroid treatment. Lastly, the current study does not address the issue of changes in the TAFI levels over time.

In conclusion, our results showed that plasma TAFI levels are elevated in active UC patients and return to a normal range after remission. The crucial value of TAFI in determining disease activity in conjunction with other inflammatory markers and EAI was also demonstrated. These results suggest that TAFI may represent an alternative indicator of active UC and may play a key role in regulating the cross-talk between coagulation, fibrinolysis and inflammation.

The authors state that they have no Conflict of Interest (COI).

References