Beta-Trace Protein and Cystatin C as Predictors of Major Bleeding in Non-ST-Segment Elevation Acute Coronary Syndrome

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Background: Beta-trace protein (BTP) and cystatin C (CysC) are novel biomarkers of renal function. We assessed the ability of both to predict major bleeding (MB) in patients with non-ST-segment elevation acute coronary syndromes (NSTE-ACS), compared to other renal function parameters and clinical risk scores.

Methods and Results: We included 273 patients. Blood samples were obtained within 24 h of admission. The endpoint was MB. During a follow-up of 760 days (411–1,098 days), 25 patients (9.2%) had MB. Patients with MB had higher concentrations of BTP (0.98 mg/L; 0.71–1.16 mg/L vs. 0.72 mg/L, 0.60–0.91 mg/L, P=0.002), CysC (1.05 mg/L; 0.91–1.30 mg/L vs. 0.90 mg/L, 0.75–1.08 mg/L, P=0.003), higher CRUSADE score (39±16 points vs. 29±15 points, P=0.002) and lower estimated glomerular filtration rate (eGFR; 66±27 vs. 80±30 ml·min⁻¹·1.73 m⁻², P=0.02) than patients without MB; there was no difference in creatinine level between the groups (P=0.14). After multivariable adjustment, both were predictors of MB, while eGFR and creatinine did not achieve statistical significance. Among subjects with eGFR >60ml·min⁻¹·1.73m⁻², those with elevated concentrations of both biomarkers had a significantly higher risk for MB. Net reclassification indexes from the addition of BTP and CysC to CRUSADE risk score were 38% and 21% respectively, while the relative integrated discrimination indexes were 12.5% and 3.8%.

Conclusions: Among NSTE-ACS patients, BTP and CysC were superior to conventional renal parameters for predicting MB, and improved clinical stratification for hemorrhagic risk.

Key Words: Acute coronary syndrome; Beta-trace protein; Hemorrhage
found to be prognostically meaningful in different clinical scenarios.\textsuperscript{12–14} The potential role of BTP and CysC concentration, however, for the prediction of MB risk among patients with ACS has not been studied. Therefore, the aims of this study were: (1) to assess the importance of BTP and CysC in the prediction of MB risk in high-risk non-ST-segment elevation (NSTE) ACS; (2) to compare BTP and CysC with serum creatinine and estimated glomerular filtration rate (eGFR); and (3) to test whether BTP and CysC provide additional information to the in-hospital bleeding prognostication scheme of the CRUSADE (Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA guidelines) risk score.\textsuperscript{8}

Methods

Subjects and Study Design
From September 2006 to December 2008, we prospectively enrolled 273 consecutive patients with an established final diagnosis of high-risk unstable angina or NSTE myocardial infarction (NSTEMI). The diagnosis of high-risk NSTE-ACS was established on the basis of current criteria guidelines.\textsuperscript{15} Patients with evidence of hepatic dysfunction, concomitant neoplastic, infectious, connective tissue or inflammatory disease were excluded; those with deep vein thrombosis or pulmonary embolism and recent (<1 month) surgery or trauma were excluded; as were patients taking immunosuppressant agents. Further exclusion criteria were hospitalization for MI, unstable angina, acutely decompensated heart failure or pulmonary embolism in the last 3 months or any cardiac revascularization procedure 1 month before enrollment. Patients who refused or were incapable of giving informed consent were also excluded. Last, given that all blood samples were obtained within 24h of hospital admission, we could not include those patients who were admitted to hospital on the weekend (Friday 15.00 hours–Sunday 08.00 hours). During the entire hospitalization period, baseline clinical characteristics were prospectively recorded. All patients received standard management as recommended for NSTE-ACS.\textsuperscript{15} Risk of bleeding was calculated using CRUSADE risk score.\textsuperscript{8} Patients were classified into 5 categories as a function of CRUSADE risk score: very low, ≤20 points; low, 21–30 points; moderate, 31–40 points; high, 41–50 points; and very high, >50 points.\textsuperscript{8} The clinical management decisions about each patient were decided by the cardiologist responsible, who was unaware of the patient’s BTP and CysC levels. The study was approved by the local ethics committee, and informed consent was obtained from each patient at inclusion.

Biochemistry

All blood samples were obtained prior to coronary angiogram by venipuncture within 24h of hospital admission and aliquots of serum were immediately stored at −80°C until analyzed. The determination of both BTP and CysC were performed using a BN ProSpec analyzer (Dade Behring, Liederbach, Germany). The intra-assay and inter-assay coefficients of variation were 2.8% and 4.7%, respectively, for BTP, and 2.5% and 2.0%, respectively, for CysC.

Conventional measurements of renal function included serum creatinine and eGFR (calculated using the Cockroft-Gault (eGFR\textsubscript{CG}) formula: [(140–age)\times weight [kg]]/(serum creatinine [mg/dL]×72) (×0.85 for women)\textsuperscript{16} and the simplified Modification of Diet in Renal Disease equation (eGFR\textsubscript{MDRD}: 186.3×[plasma creatinine]\textsuperscript{−1.154}×[age]\textsuperscript{−0.203}×0.742 [if female]).\textsuperscript{17}

Follow-up and Endpoints

After hospital discharge, patients were followed during a median of 760 days (interquartile range [IQR], 411–1,098 days). All medical records were carefully reviewed, and the patients or their relatives were contacted by telephone to obtain the incidence of events during the follow-up.

The primary clinical endpoint was defined as the occurrence of MB, which was defined according to the Bleeding Academic Research Consortium Definition criteria\textsuperscript{2} as bleeding types 3–5: type 3a, overt bleeding plus hemoglobin drop of 3–5 g/dL, any transfusion with overt bleeding; type 3b, overt bleeding plus hemoglobin drop 5 g/dL, cardiac tamponade, bleeding requiring surgical intervention for control (excluding dental/nasal/skin/hemorrhoid), bleeding requiring i.v. vasoactive agents; type 3c, intracranial hemorrhage (does not include microbleeds or hemorrhagic transformation, does include intraspinal), subcategories confirmed by autopsy or imaging or lumbar puncture, intraocular bleed compromising vision; type 4, coronary artery bypass graft (CABG)-related bleeding (perioperative intracranial bleeding within 48 h, reoperation after closure of stenotomy for the purpose of controlling bleeding, transfusion of ≥5 U whole blood or packed red blood cells within a 48-h period, chest tube output ≥2L within a 24-h period); type 5, fatal bleeding (type 5a, probable; type 5b, definite).\textsuperscript{2}

We also examined the predictive value of MB for the composite of all cause death, MI or urgent revascularization. Death was ascertained from available medical records and death certificates. MI was defined as detection of rise in cardiac biomarkers of necrosis with at least 1 measurement above the 99th percentile upper reference limit, together with evidence of myocardial ischemia with at least one of the following: electrocardiographic changes indicative of new ischemia (new ST-T changes or new left bundle branch block), new pathological Q waves in at least 2 contiguous leads, imaging evidence of new loss of viable myocardium or new wall motion abnormality.\textsuperscript{18} At the end of the follow-up period, clinical status was obtained in all patients.

Statistical Analysis

Continuous variables were tested for normal distribution using the Kolmogorov-Smirnov test. Normally distributed data are presented as mean±SD and non-normally distributed data as median (IQR). Categorical variables are expressed as percentages. Patients were grouped according to BTP tertiles. Differences in baseline characteristics were compared using analysis of variance or the Kruskal-Wallis test for continuous variables and the chi-square test for categorical variables. Comparisons of both biomarkers between groups with and without events were performed using the Mann-Whitney U test. Correlations between both biomarkers and analytical parameters were assessed using the Spearman rank correlation. To compare operating characteristics of the various biomarkers for clinical events, we generated receiver operator characteristic (ROC) curves; the best prognostic cut-off was defined as the highest product of sensitivity and specificity, and sensitivity, specificity, positive predictive value and negative predictive value were consequently calculated. We calculated hazard ratios (HR) derived from the Cox regression analysis to identify predictors of clinical events (both MB and ischemic events) during follow-up. The independent effect of variables on all clinical events was calculated using Cox multivariate regression analysis, incorporating covariates with P<0.05 on univariate analysis. Renal function biomarkers were tested separately in multivariate models. Linearity assumption was tested using Martingale residuals. Log-cumulative hazard plots, time-dependent covari-
significant. Statistical analysis was performed using SPSS 15.0 (SPSS, Chicago, IL, USA) and SAS (version 9.2; SAS Institute, Cary, NC, USA).

Results

Subjects and Renal Biomarkers
The subject group consisted of 273 patients with high-risk
NSTE-ACS (Table 1). Mean Global Registry for Acute Coronary Events (GRACE) risk score of the whole group was 130±38 points. During index hospitalization 242 patients (89%) had coronary angiography of them, 193 (80%) underwent revascularization (172 [89%], percutaneous coronary intervention [PCI]; 21 [11%], CABG).

The median plasma BTP concentration for the group as a whole was 0.74 mg/L (IQR, 0.60–0.95 mg/L), median CysC concentration was 0.91 mg/dL (IQR, 0.77–1.11 mg/L), median serum creatinine was 0.95 mg/dL (IQR, 0.83–1.12 mg/dL), mean eGFR C-G was 79 ± 23 ml·min⁻¹·1.73 m⁻² and mean eGFR MDRD was 77 ± 23 ml·min⁻¹·1.73 m⁻². A total of 199 patients (73%) had eGFR C-G >60 ml·min⁻¹·1.73 m⁻², while 215 (79%) had eGFR MDRD >60 ml·min⁻¹·1.73 m⁻². Plasma BTP was strongly correlated with CysC (r=0.75; P<0.001), and moderately with serum creatinine (r=0.50; P<0.001); a moderate inverse correlation was found with eGFR C-G (r=–0.58) and r=–0.53 for MDRD; both P<0.001.

Higher BTP was associated with more advanced age, and a greater prevalence of hypertension, previous stroke and previous bleeding. As expected, these patients also had higher concentrations of creatinine and CysC, whereas they had lower eGFR and hemoglobin (Table S1).

### Bleeding and Renal Biomarkers

During follow-up, a total of 25 patients (9.2%) had MB; 13 (52%) had type 3a, 7 (28%) type 3b, 2 (8%) type 3c, 1 (4%) type 4 and 2 (8%) type 5b. Location of MB was gastrointestinal in 10 (40%), intracranial in 4 (16%), cardiac tamponade in 4 (16%), pulmonary in 2 (8%), musculoskeletal in 2 (8%), urologic in 1 (4%), CABG-related bleeding in 1 (4%) and puncture site in 1 (4%). Prior to MB events, antiplaette therapy was aspirin plus clopidogrel in 18 patients (72%) and aspirin in 7 (28%). After MB, 4 patients (22%) discontinued double antiplatelet therapy and 1 (14%) discontinued simple therapy. Nine patients (36%) had MB during the first 30 days, 17 (68%) during the first year and 20 (80%) through the second year.

Patients with MB were older (76±9 vs. 67±12 years, P<0.001), had higher concentration of BTP (median, 0.98 mg/L; IQR, 0.71–1.16 mg/L vs. 0.72 mg/L; 0.60–0.91 mg/L; P=0.002), CysC (median, 1.05 mg/L; IQR, 0.91–1.30 mg/L vs. 0.90 mg/L, 0.75–1.08 mg/L; P=0.003) and lower hemoglobin (13±2.2 vs. 13.9±1.8 g/dL, P=0.038), eGFR C-G (66±27 vs. 80±30 ml·min⁻¹·1.73 m⁻², P=0.016) and eGFR MDRD (68±26 vs. 78±23 ml·min⁻¹·1.73 m⁻², P=0.045) than patients without hemorrhage. There was no difference, however, in the concentration of serum creatinine (median, 1.10 mg/dL; IQR, 0.84–1.38 mg/dL vs. 0.94 mg/dL, 0.83–1.10 mg/dL, P=0.135) between those with and without MB. Patients with MB also had higher CRUSADE (39±16 vs. 29±15 points, P=0.002; Table 1).

On ROC analysis, BTP, CysC, eGFR C-G, eGFR MDRD and CRUSADE risk score had a similar ability to discriminate between patients with and without MB, while serum creatinine level had lower accuracy (Table 2).

### Table 2. Comparison of Ability to Predict MB

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>AUC</th>
<th>95% CI</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTP (mg/L)</td>
<td>0.69</td>
<td>0.63–0.74</td>
<td>0.97</td>
<td>0.56</td>
<td>0.80</td>
<td>0.22</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>0.68</td>
<td>0.62–0.73</td>
<td>0.96</td>
<td>0.72</td>
<td>0.63</td>
<td>0.16</td>
<td>0.96</td>
<td>0.806</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.59</td>
<td>0.53–0.65</td>
<td>1.09</td>
<td>0.52</td>
<td>0.74</td>
<td>0.17</td>
<td>0.94</td>
<td>0.009</td>
</tr>
<tr>
<td>eGFR C-G (ml·min⁻¹·1.73 m⁻²)</td>
<td>0.63</td>
<td>0.57–0.70</td>
<td>69</td>
<td>0.68</td>
<td>0.85</td>
<td>0.16</td>
<td>0.95</td>
<td>0.277</td>
</tr>
<tr>
<td>eGFR MDRD (ml·min⁻¹·1.73 m⁻²)</td>
<td>0.66</td>
<td>0.60–0.72</td>
<td>67</td>
<td>0.68</td>
<td>0.84</td>
<td>0.16</td>
<td>0.95</td>
<td>0.679</td>
</tr>
<tr>
<td>CRUSADE risk score (points)</td>
<td>0.67</td>
<td>0.61–0.72</td>
<td>34</td>
<td>0.64</td>
<td>0.68</td>
<td>0.17</td>
<td>0.95</td>
<td>0.803</td>
</tr>
</tbody>
</table>

†Comparison between BTP and other variables. AUC, area under the curve; CI, confidence interval; MB, major bleeding; NPV, negative predictive value; PPV, positive predictive value. Other abbreviations as in Table 1.

### Table 3. Cox Regression Risk Analysis for Prediction of MB

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Univariate HR (95% CI)</th>
<th>P-value</th>
<th>Multivariate HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTP &gt;0.97 mg/L</td>
<td>4.71 (2.14–10.37)</td>
<td>&lt;0.001</td>
<td>3.44 (1.49–7.95)</td>
<td>0.004</td>
</tr>
<tr>
<td>Cystatin C &gt;0.86 mg/L</td>
<td>3.98 (1.66–9.53)</td>
<td>0.002</td>
<td>3.06 (1.25–7.52)</td>
<td>0.02</td>
</tr>
<tr>
<td>Creatinine &gt;1.09 mg/dL</td>
<td>2.80 (1.28–6.13)</td>
<td>0.01</td>
<td>–</td>
<td>0.08</td>
</tr>
<tr>
<td>eGFR C-G &lt;60 ml·min⁻¹·1.73 m⁻²</td>
<td>3.46 (1.57–7.58)</td>
<td>0.002</td>
<td>–</td>
<td>0.07</td>
</tr>
<tr>
<td>eGFR MDRD &lt;60 ml·min⁻¹·1.73 m⁻²</td>
<td>2.31 (1.02–5.22)</td>
<td>0.045</td>
<td>–</td>
<td>0.10</td>
</tr>
<tr>
<td>Age &gt;75 years</td>
<td>3.80 (1.70–8.45)</td>
<td>0.001</td>
<td>2.59 (1.11–6.05)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hemoglobin (per g/dL)</td>
<td>0.76 (0.62–0.95)</td>
<td>0.01</td>
<td>–</td>
<td>0.20</td>
</tr>
<tr>
<td>CRUSADE risk score (per category)</td>
<td>1.52 (1.15–2.02)</td>
<td>0.004</td>
<td>–</td>
<td>0.38</td>
</tr>
</tbody>
</table>

HR, P-value shown from the BTP model. Abbreviations as in Tables 1, 2.
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above the cut-offs had the highest rate (27.3% for BTP and 20.0% for CysC; P<0.001 for BTP and P=0.005 for CysC). In addition, among those patients with a CRUSADE risk score <40 points (207 patients, 76%), 13 (6%) had MB. Both BTP and CysC concentrations were also associated with MB risk in this patient subgroup (P=0.016 for BTP and P=0.004 for CysC; Figure 3).

Bleeding and Ischemic Complications

During the study period, there were 71 ischemic events (26%; 27 deaths, 29 MI and 41 urgent revascularizations). On multivariate Cox regression analysis (adjusted for age, hemoglobin and GRACE risk score), MB was an independent predictor of ischemic events during the follow-up, as were BTP and
In the present study of patients treated with contemporary ACS management, we found MB risk to be significant, extending for a considerable period of time from admission. Moreover, in accordance with previous studies, MB was related to ischemic outcome. Notably, both BTP and CysC were independently predictive of MB, even in this group of patients with relatively preserved renal function. Both novel biomarkers of renal function were superior to serum creatinine and eGFR equations for predicting bleeding, and both provided complementary information to the CRUSADE risk score in this clinical setting.

It is well known that renal function parameters play an important role in bleeding complications of patients with ACS. In the present study of patients treated with contemporary ACS management, we found MB risk to be significant, extending for a considerable period of time from admission. Moreover, in accordance with previous studies, MB was related to ischemic outcome. Notably, both BTP and CysC were independently predictive of MB, even in this group of patients with relatively preserved renal function. Both novel biomarkers of renal function were superior to serum creatinine and eGFR equations for predicting bleeding, and both provided complementary information to the CRUSADE risk score in this clinical setting.

### Discussion

Hemorrhage has become a major focus in modern ACS studies. This is because patients suffering MB in the course of ACS are at higher risk for ischemic complications, as well as suffering adverse outcome directly related to hemorrhage itself. Accordingly, major attention has been given to development of therapeutic strategies that mitigate risk for bleeding in the acute setting. Interestingly, understanding about the temporal association between ACS and MB has been largely limited to acute hospitalization; patients with ACS are frequently treated with drugs that increase the risk for bleeding for a considerable period of time following their index event. Thus, a better understanding of the risks for MB in those patients after ACS is desirable, and better methods for predicting risk are needed.

In the present study of patients treated with contemporary ACS management, we found MB risk to be significant, extending for a considerable period of time from admission. Moreover, in accordance with previous studies, MB was related to ischemic outcome. Notably, both BTP and CysC were independently predictive of MB, even in this group of patients with relatively preserved renal function. Both novel biomarkers of renal function were superior to serum creatinine and eGFR equations for predicting bleeding, and both provided complementary information to the CRUSADE risk score in this clinical setting.

It is well known that renal function parameters play an important role in bleeding complications of patients with ACS. Indeed, contemporary ACS registries show that the estimated risk of in-hospital MB increases by approximately 50% in patients with renal insufficiency, and in a graded manner with
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Several pathophysiological mechanisms have been described to explain why patients with renal dysfunction are at increased risk of bleeding, although the causal nature of this relation is still debated. Circulating uremic toxins are partly responsible for platelet dysfunction in patients with CKD, affecting both the activation, recruitment and aggregation processes as well as worsening the interaction between platelets and vascular wall. Also, these patients often have associated other risk factors for bleeding, such as advanced age or a higher prevalence of hypertension. Patients with renal failure are more susceptible to excess dosing of anti-thrombotic drugs but also typically have more diffuse and advanced arterial disease and therefore may be prone to higher risks of both thrombosis and bleeding. Finally, anemia (altered blood rheology) and erythropoietin deficiency are usually present in these patients and may facilitate bleeding complications.

According to these prior studies, we found renal dysfunction to be an independent predictor of MB, but only when estimated using BTP or CysC concentrations. The lack of sensitivity of conventional renal function parameters to detect a small decrease in kidney function, together with the fact that the majority of the present patients had normal or near-normal renal function, may explain these results. Indeed, BTP and CysC are particularly superior to eGFR or serum creatinine, in the “creatinine-blind” range; the present results might reflect that

**Figure 3.** Kaplan-Meier survival curves for major bleeding according to (A) β-trace protein (BTP) and (B) cystatin C (CysC) concentrations in patients estimated as being without high major bleeding risk according to CRUSADE risk score.
the risk of hemorrhage secondary to renal dysfunction is not fully encompassed by traditional renal function parameters or risk models. In contrast, BTP and CysC also participate in other physiological and pathological processes. For example, anti-thrombogenetic vascular properties have been attributed to BTP in the wound healing process in induced vessel injury after coronary angioplasty, while CysC is known to modulate neutrophil chemotactic activity and may inhibit prothrombotic activity of proteolytic substances secreted by activated neutrophils. In this, we found both markers to predict ischemic complications as well.

Several risk stratification models have been developed to predict MB in patients with ACS and those undergoing PCI; impaired renal function is a common predictor of hemorrhage in this setting. For example, in an analysis of more than 24,000 patients with ACS, Moscucci et al demonstrated that advanced age, female gender, history of bleeding and renal insufficiency were independently associated with a higher risk of bleeding. In an analysis of the CRUSADE Quality Improvement Initiative database, Subherwal et al also identified independent baseline predictors of major in-hospital bleeding among community-treated NSTEMI patients, including creatinine clearance. At present, clinical practice guidelines recommend the use of the CRUSADE risk score to assess the risk of bleeding in NSTE-ACS, but the CRUSADE risk score does not use novel renal function biomarkers such as BTP or CysC. We found that not only do BTP and CysC predict MB in NSTE-ACS patients, but the prognostic information provided by these biomarkers adds to that of the CRUSADE risk model.

Limitations of the present study include the small sample size, which makes it difficult to draw firm conclusions based on the results; a study with a larger sample size and more registered MB events would provide more power. For the prediction of outcomes, the number of covariates included in multivariate models was >1 for each 10 events. Therefore, it remains possible that the models were over-adjusted, and consequently the present results could fail to be replicated in future samples. Among the present study cohort, we found that conventional renal function parameters and CRUSADE risk score did not predict MB events beyond CysC and BTP. But, because several previous studies have demonstrated that these parameters strongly predict MB in this clinical setting, we cannot exclude the possibility that the present results may represent a false negative (type II) error due to over-adjustment of covariates in the context of a relatively small number of subjects with NSTE-ACS. Another limitation of the study is its single-center nature. Only patients admitted to University Hospital Virgen de la Arrixaca, which is equipped to perform coronary angiography and coronary revascularization, were included; the applicability of the present results should therefore be viewed with caution in centers with other types of patients and medical facilities, and should be considered as hypothesis-generating. Single-center studies, however, offer the advantage of evaluating homogeneous patient groups and care processes, unlike multicenter studies, which often differ in the availability of logistical resources and management habits. Finally, we do not have post-discharge measurements of renal function; such follow-up data would be expected to provide further information about the bleeding risk in the present patients.

Conclusions
Among high-risk NSTE-ACS patients, BTP and CysC concentrations were associated with higher long-term MB risk and improved the initial prognostic stratification compared to stan-
standard renal parameters as well as the CRUSADE risk score. Further research is required to gain insight into the true significance of increased BTP and CysC concentrations for prediction of MB and thus to assess their inclusion in the hemorrhagic risk models. Given that the spectrum of ACS treatment, involving varying degrees of anticoagulation intensity, is now growing, it is worth envisioning strategies to tailor medical therapies to risk predicted by such models.

Disclosures
Conflict of Interest: None declared.

References

Supplementary Files
Supplementary File 1
Table S1. Subject Characteristics as a Function of BTP Tertiles
Please find supplementary file(s) at: