Very Early Risk Stratification Using Combined ECG and Biochemical Assessment in Patients With Unstable Coronary Artery Disease (A Thrombin Inhibition in Myocardial Ischemia [TRIM] Substudy)

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Background—The diagnostic capability of troponin T (TnT), troponin I (TnI), myoglobin, and creatine kinase (CK)-MB mass for detection of myocardial injury seems evident. Newer studies have found these sensitive markers to carry independent prognostic information in patients with unstable coronary artery disease as well. ST-segment depression in the admission ECG is known to be an important indicator of poor outcome in these patients. The present study investigates the prognostic capacities of the ECG in combination with biochemical admission measurements in 516 patients admitted to hospital with unstable coronary artery disease.

Methods and Results—Baseline ECG recordings and blood samples were collected for central analysis. The patients were followed up for 30 days, and predefined end points, ie, death, myocardial infarction, and refractory angina, were registered as end points. By univariate analysis, ST-segment depression, inverted T waves in ≥5 leads, TnT ≥0.1 μg/L, TnI ≥0.5 μg/L, myoglobin ≥40 μg/L, female sex, and age ≥65 years were predictors of death and myocardial infarction at 30 days. By multivariate analysis, female sex, ST-segment depression at randomization, or inverted T-waves in ≥5 leads were the only independent predictors of death or myocardial infarction. On the basis of baseline ECG ST-T changes and CK-MB mass/TnT/TnI/myoglobin levels, the patients were divided into 3 subgroups at high (14% event rate), intermediate (6%), and low (3%) risk of early death/myocardial infarction.

Conclusions—The present study found the combination of baseline values of TnT, TnI, CK-MB mass, and ST-T changes in the ECG to be effective for early risk stratification in patients with unstable coronary artery disease. *(Circulation, 1998;98:2004-2009.)*

Key Words: prognosis □ angina □ electrocardiography □ myoglobin □ creatine kinase □ troponin

It is generally accepted that unstable myocardial ischemia and infarction, registered as ECG ST-T–segment changes, are caused by an atherosclerotic plaque rupture, with concomitant platelet activation and thrombus formation.1,2 Despite optimal medical treatment, the group of patients with unstable coronary artery disease (ie, unstable angina and non–Q-wave myocardial infarction) carries a high risk of an adverse event within a few months of follow-up.3-5 Thus, it would be of importance to detect the individual patients at increased risk with intention to offer a more expedient treatment. In patients admitted with suspected acute myocardial infarction (AMI), ST-segment depression in the admission ECG has been shown to be an important indicator of high risk.6,7 The admission ECG, however, fails to identify many of the patients who experience a cardiac event during the early follow-up period. During the past years, novel sensitive biochemical markers for detecting minimal myocardial injury have been developed. Myoglobin has been shown to reach peak values early after onset of acute chest pain. Also, the new cardiospecific markers troponin T (TnT), troponin I (TnI), and creatine kinase (CK)-MB mass appear in blood relatively early after symptom onset8-12; in addition, new rapid bedside assays have been developed, making it possible to measure the release of cardiac enzymes within minutes after onset of symptoms. This information could facilitate decision-making in the emergency room and coronary care unit and expand the use of revascularization therapies to patients suspected of having AMI but without current ECG indications for use of thrombolysis or primary PTCA. In recent years, several studies have demonstrated that these sensitive biochemical markers are superior with regard to diagnostic and prognostic information in patients with unsta-
ble coronary artery disease, but none have explored the potential of combining the admission markers with the conventional ECG for early risk stratification. The aim of the present study is therefore to investigate the prognostic capabilities of a standard ECG combined with biochemical measurements obtained at admission in patients with unstable coronary artery disease.

Methods

Population
Sixty centers (1209 patients) participated in the TRIM (Thrombin Inhibition in Myocardial Ischemia) study that randomized patients with unstable angina/non-Q-wave MI to 4 different treatment regimens of either heparin or a specific thrombin inhibitor (Inogaran). Many of these centers took part in substudies on biochemical markers, ECG, etc. The main study did not prove any superiority of the study drug (Inogaran) over heparin with regard to ischemic events; thus, the patients in this substudy were considered as having received similarly effective treatment. From a total of 1209 patients, 516 patients from 22 centers were included in a predefined substudy of biochemical markers of myocardial damage and ECG.

Inclusion Criteria and Diagnosis
The entry criteria were either a clinical suspicion of unstable angina pectoris or a non-Q-wave MI. The patients had to be included within 24 hours after the qualifying episode of chest pain. The clinical diagnosis had to be supported by either ECG changes compatible with myocardial ischemia or a history of previous coronary artery disease. Inclusion and exclusion criteria have been described in detail elsewhere. The main exclusion criteria were an indication for thrombolytic treatment (decided by the attending cardiologist), heart failure, ongoing arrhythmia, increased risk of hemorrhage, and allergy to heparin or the study drug.

On the basis of the clinical course and plasma levels of biochemical markers obtained within 6 hours after inclusion, the patients were retrospectively categorized as having unstable angina pectoris (n=309), non-Q-wave MI (n=190), and other diseases (n=17).

Data

ECG Analysis
A standard 12-lead ECG was obtained in all patients at their inclusion in the study. These ECGs were sent to and analyzed in the ECG core laboratory at Righospitalet, Copenhagen, before any other data on the patient were revealed. The ST-T changes were measured in millimeters at 60 ms after the J point, and the changes had to appear in at least 2 contiguous leads to be considered of significance. ST-segment elevation had to be at least 2 mm in lead V₁-V₂ and at least 1 mm in any other lead. ST-segment depression had to be a minimum of 1 mm in any lead. Inverted T waves had to be at least 1 mm as well. Lead AVR was not evaluated. ECG data from the case report form were not used in the present study.

Blood Sampling and Biochemical Analysis
At randomization, blood samples were drawn and analyzed for myoglobin, TnT, TnI, and CK-MB mass. The analysis was performed centrally at the Core Laboratory at University Hospital, Aarhus, Denmark. TnT was analyzed with an ESI 300 analyzer (Boehringer Mannheim GmbH). The method is based on a single-step sandwich principle with streptavidin-coated tubes (solid phase) and 2 monoclonal anti-human TnT antibodies. Based on previous reports and the manufacturer's recommendation, a cutoff value for TnT was set at 0.10 μg/L. The chosen cutoff value was supported by our own receiver operating characteristic (ROC) analysis (see below). TnI, CK-MB mass, and myoglobin were measured with an Opus Magnum (Behring Diagnostics Inc) based on the principle of 2-site immunoassay using polyclonal antibodies to recognize epitopes unique to Tnl/myoglobin/CK-MB mass. The discrimination from the manufacturer and previous studies. An alternative cutoff value was set at 0.5 μg/L on the basis of ROC analysis. Very little information is available regarding recommended cutoff values for myoglobin for early risk stratification in unstable coronary artery disease. The upper limit of normal is set by the manufacturer at 90 μg/L, which is also the discrimination limit used for diagnosing MI in our institution. However, the aim of the present study was not to diagnose MI but rather to identify patients with minor myocardial damage. Thus, a discrimination level at 40 μg/L was chosen and further evaluated by ROC analysis. The prognostic capability of CK-MB mass has previously been studied in patients with unstable coronary artery disease. In those studies, cutoff levels at 6.0 and 7.5 μg/L were used. In the present study, a discrimination level at 7.0 μg/L was chosen.

End Points and Follow-Up
The following events were registered as end points: death, MI/infarction, refractory angina (despite optimal treatment, leading to any kind of intervention), and recurrent angina. MI was defined as a diagnostic series of ECGs or at least 2 of the following: (1) typical ischemic chest pain, (2) diagnostic ECG, or (3) typical elevation of cardiac enzymes. Refractory angina was defined as recurrence of chest pain lasting ≥5 minutes despite maximal ongoing medical treatment, including intravenous nitroglycerin and oral β-blockers or calcium antagonists. The pain episodes had to be associated with transient ECG changes indicative of myocardial ischemia and leading to coronary angiography. Recurrent angina was defined as recurrent chest pain of ≥5 minutes’ duration typical of myocardial ischemia and responding to sublingual nitroglycerin but not fulfilling the criteria for refractory angina pectoris. After discharge, recurrent angina was defined as readmission because of anginal chest pain. Further details on end-point definition have been described elsewhere.

The patients were followed up for a period of 30 days after admission to hospital. After discharge, the patients returned to the outpatient clinic for a follow-up visit at 30 days after inclusion in the study. If a patient reached >1 end point during follow-up, the time of the first event was registered. The registration of end points was supervised by an independent end point committee that reviewed all end-point data and paid special attention to separating the index event from the predefined end points. In the present substudy, the composite of death and MI will be the main end points under discussion.

Statistical Analysis
All values are expressed as median (interquartile range). Intergroup comparisons of biochemical concentrations were performed by the Mann-Whitney U test. Event rates among subgroups were compared by log-rank survival analysis, and the results were presented as Kaplan-Meier plots. To evaluate independent prognostic value of the variables, a Cox regression model was constructed by use of backward elimination strategy. The Statsoft program Statistica was used for computer analysis. Separate analyses were performed for the composite of death and AMI and for the composite of death, AMI, and refractory angina.

| TABLE 1. Baseline Characteristics of the Study Population (n=516) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Risk Factor at Admission | Age, y, median (range) | 64 (31–80) | 64 (31–80) | 64 (31–80) | 64 (31–80) | 64 (31–80) | 64 (31–80) |
| Age ≥65 y, n (%) | 268 (52) | 268 (52) | 268 (52) | 268 (52) | 268 (52) | 268 (52) |
| Female sex, n (%) | 152 (29) | 152 (29) | 152 (29) | 152 (29) | 152 (29) | 152 (29) |
| Diabetes mellitus, n (%) | 76 (15) | 76 (15) | 76 (15) | 76 (15) | 76 (15) | 76 (15) |
| Systemic hypertension, n (%) | 194 (38) | 194 (38) | 194 (38) | 194 (38) | 194 (38) | 194 (38) |
| Previous MI, n (%) | 252 (49) | 252 (49) | 252 (49) | 252 (49) | 252 (49) | 252 (49) |
| Previous coronary angioplasty/bypass surgery, n (%) | 93 (18) | 93 (18) | 93 (18) | 93 (18) | 93 (18) | 93 (18) |
Results

Baseline Characteristics

Table 1 presents the baseline characteristics for the study population. In the 30-day follow-up period, 56 patients experienced ≥1 event(s). There were 9 deaths (1.7%), 33 AMIs (6.3%), and 21 cases of refractory angina (4.0%), and 242 patients (46.8%) experienced recurrent angina. CABG or PTCA was performed in 102 patients (19.5%).

ECG Assessments

An ECG was collected for each patient at inclusion in the study. Fourteen ECGs were either missing or of such poor quality that ST-segment analysis could not be performed. Twenty-two ECGs were nonevaluable because of bundle-branch block or left ventricular hypertrophy that precluded reliable ST-segment analysis. Thus, 9% of the ECGs were either missing or uninterpretable. Of the 470 patients with evaluable ECGs, 92 (19%) had minor ST elevations (median summed, 3.5 mm; range, 2 to 8 mm), 64 (14%) had ST-segment depression (median summed, 3.0 mm; range, 2 to 12.5 mm), and 280 (59%) had inverted T waves. ECGs without ST-T changes or confounding factors (n = 150 [32%]) were considered “normal.”

Biochemical Results: Determination of Cutoff Values

The patients were divided into 2 subgroups based on whether or not they reached a serious end point (death and AMI) within the 30-day follow-up period. The median concentration of each biochemical marker was calculated within each subgroup. Table 2 illustrates the concentration of biochemical markers among the patients who died or had an AMI within the follow-up period compared with the patients without end points. All of the 4 biochemical markers were significantly increased in the event group.

Figure 1 illustrates the spectra of sensitivities and specificities attained by the various cutoff levels of the 4 biochemical markers for prediction of death and AMI. The cutoff values with the best sensitivity and specificity were 0.10 μg/L for TnT, 0.5 μg/L for TnI, 40 μg/L for myoglobin, and 7.0 μg/L for CK-MB mass.

Prognostic Value of Biochemical Markers, ECG Changes, and Baseline Variables

Table 3 depicts the prognostic values of various risk factors regarding death and AMI during follow-up. All variables that were statistically significant at the 10% level by univariate analysis were entered into a Cox regression model using backward elimination strategy. Female sex, ST-segment depression at admission, and T-wave inversion in ≥5 leads were independent predictors of death/AMI by multivariate analysis.

Combined ECG and Biochemical Evaluation

TnT was the best of the 4 markers for prediction of risk by univariate analysis. Figure 2 illustrates risk stratification of patients based on initial ECG evaluation followed by a single blood sample for biochemical analysis. Patients with ST-segment depression at admission had a 14% risk of subse-
**Discussion**

Risk stratification in patients with unstable coronary artery disease is a major clinical issue with important therapeutic implications. Very early identification of the high-risk patient is crucial, because a substantial number of cardiac events occur within the first few days after admission. Previous studies have proved the importance of the admission standard ECG in identifying patients at high risk for subsequent adverse events. However, the combination of the ECG and other indicators of myocardial ischemia might improve prognostic sensitivity and specificity.

In the present study of a population of patients admitted with unstable coronary artery disease, the independent and combined prognostic value of a single standard 12-lead ECG and a single blood assessment of biochemical markers obtained at admission was evaluated. ST-segment depression at admission, as well as T-wave inversion in ≥5 leads, was found to be a strong individual predictor of poor outcome. New sensitive biochemical markers of ischemia have been found to carry independent prognostic information in patients with unstable coronary syndromes, and through recent years, new, rapid methods of analyzing these markers have been introduced. Thus, a biochemical assessment of a patient can be available within a very short time after admission to hospital. In the present study, TnT, TnI, myoglobin, and CK-MB mass were all markedly increased among the patients who subsequently died or experienced an MI within a follow-up period of 30 days after admission to hospital. By univariate analysis, elevated admission levels of TnT, TnI, and CK-MB mass were significant predictors of poor outcome, together with other baseline variables. However, neither the biochemical markers nor various baseline characteristics were found to carry independent prognostic information when entered into a multivariate regression model with ECG data.

Most of the newer studies of prognostic ability of biochemical markers have used serial blood sampling and peak values. However, in the GUSTO IIa population of patients with acute myocardial ischemia, Ohman et al found increased mortality within 30 days among patients with admission TnT values >0.10 μg/L. Compared with our population, the GUSTO IIa study population was a selected high-risk group, with 72% of the patients having Q-wave MI. The GUSTO IIa results are in concordance with a study by Stubbs et al, who found that elevated admission TnT values in patients with AMI defined a subgroup at increased risk of subsequent cardiac events. The only study of patients with MI ruled out in which the admission sample of TnT was used was reported by Wu et al, who evaluated the prognostic value of a single admission blood sample in a small population of 131 patients admitted with unstable angina pectoris. The study showed that TnT on admission could provide independent prognostic information, although it could be claimed that multivariate analysis including other variables was not performed. Although in the present study, elevated admission TnT did not carry independent prognostic information by multivariate analysis, it provided significant information regarding the patients without ST-segment depression in the standard 12-lead ECG or with noninterpretable ECGs. Previous reports based on the present

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**TABLE 3. Prognostic Values of Different Risk Factors Regarding Death or AMI Within 30 Days**

<table>
<thead>
<tr>
<th>Risk Factor at Admission</th>
<th>Log Rank, P (Univariate)</th>
<th>Cox Regression, P (Multivariate)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST-segment depression</td>
<td>0.0002</td>
<td>0.0009</td>
</tr>
<tr>
<td>Inverted T waves in ≥5 leads</td>
<td>0.0005</td>
<td>0.01</td>
</tr>
<tr>
<td>Abnormal ECG at admission</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Inverted T-waves</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>ST-segment elevation</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>TnT ≥0.10 μg/L</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>TnI ≥2.0 μg/L</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>≥0.5 μg/L</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Myoglobin ≥40 μg/L</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td>CK-MB mass ≥7.0 μg/L</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Female sex</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Age ≥65 y</td>
<td>0.0005</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Systemic hypertension</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Previous MI</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Previous coronary</td>
<td>0.18</td>
<td></td>
</tr>
</tbody>
</table>

*Only variables with a statistical significance of P<0.10 by univariate analysis were evaluated.

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Figure 2. Suggested triage for evaluation of patients admitted with unstable angina/non-Q-wave MI. Inv. indicates inversion.
study population found peak TnT values >0.10 μg/L within the first 6 hours to be independent predictors of death and MI. In that report, ECG changes were not found to be important by multivariate analysis. The ECG data in that study, however, were based on the local investigators’ reports in the case report form and not on blinded analysis in an ECG core laboratory. Considerable differences between on-site evaluation of ECG data and the blinded evaluation performed in a core laboratory have recently been reported. Thus, the results from the present study must acknowledge the importance of baseline ECG ST-T changes.

In the present study, TnI levels were elevated among the patients who died or had MI during the follow-up period, and by univariate analysis, TnI levels ≥0.2 μg/L as well as levels ≥0.5 μg/L were associated with an increased risk of the composite of death and AMI. Antman and coworkers found that TnI levels above a cutoff value of 0.4 μg/L obtained in a single blood test at admission were significantly associated with mortality in 1404 patients from the TIMI IIIB trial. Galvani et al found increased cardiac events among unstable angina patients with TnI values ≥3.1 μg/L at admission or 8 hours later. Except for the study by Antman, serial blood sampling for the assessment of peak values seems to be necessary for obtaining independent prognostic information from TnI. A major problem in comparing the various trials on TnI is the lack of standardization of assays and the use of different discriminator levels.

Previous studies have proved myoglobin to be an early sensitive and specific marker of MI; however, it has not yet been proved useful for risk stratification of patients with unstable angina pectoris. Even though the present study found elevated myoglobin concentrations among the patients who subsequently suffered a cardiac event, myoglobin was not found to carry independent prognostic information by univariate or multivariate analysis. These findings are in accordance with the results reported by de Winter and coworkers, who found elevated TnT but not myoglobin and CK-MB mass to predict future cardiac events in patients with acute chest pain in whom AMI had been ruled out.

In the present study, CK-MB mass values ≥7.0 μg/L were associated with an increased risk of death/AMI by univariate analysis. This is in accordance with the findings by Ravkilde et al, who found CK-MB mass values to be associated with poor outcome in serial analysis; however, once the ECG changes were considered, the biochemical markers did not add any additional information. The present study, however, revealed possibilities for baseline CK-MB mass measurements as a supplement to the admission ECG for early risk stratification.

**Study Limitations**

The present study is a substudy in a multicenter trial in which the patients were selected on the basis of several inclusion and exclusion criteria. The lack of standardization of assays

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**TABLE 4. Death or AMI Within 30 Days Among Patients Without ST-Segment Depression or Inverted T Waves in ≥5 Leads at Admission**

<table>
<thead>
<tr>
<th>Patients With No ST-Segment Depression/T-Wave Inversion in ≥5 Leads or Nonevaluable ECG, n = 339*</th>
<th>≥Cutoff Level (Event Rate), n (%)</th>
<th>&lt;Cutoff Level (Event Rate), n (%)</th>
<th>P (Log-Rank Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission TnT (cutoff 0.10 μg/L)</td>
<td>8/130 (6)</td>
<td>6/209 (3)</td>
<td>0.07</td>
</tr>
<tr>
<td>Admission TnI (cutoff 0.5 μg/L)</td>
<td>8/130 (6)</td>
<td>6/209 (3)</td>
<td>0.07</td>
</tr>
<tr>
<td>Admission myoglobin (cutoff 40 μg/L)</td>
<td>8/137 (6)</td>
<td>6/202 (3)</td>
<td>0.1</td>
</tr>
<tr>
<td>Admission CK-MB mass (cutoff 7.0 μg/L)</td>
<td>8/124 (6)</td>
<td>6/215 (3)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

See also Figures 2 and 3.

*Fourteen patients had missing ECGs; 162 patients had ST-segment depression/T-wave inversion in ≥5 leads at admission.
for determination of TnI and CK-MB mass and the use of different discriminator levels is a major problem when results from various trials are compared.

Conclusions
It can be concluded that the combination of admission values of troponin T, troponin I, CK-MB mass, and ECG ST changes are advantageous for early risk stratification in patients with unstable coronary artery disease. By multivariate analysis, only ST-segment depression in the baseline ECG carried independent prognostic value; however, admission TnT, TnI, and CK-MB mass added useful information.

Acknowledgment
The TRIM study was funded by ASTRA. The present substudy was supported by grants from the Beckett Foundation, Asta Floridan Boldings Mindelegat, and Murermester Laurits Peter Christensen og hustru Kirsten Sigrid Christensens fond. Statistisk support was financed through a grant from the Danish Medical Research Council. Astra Hassle AB has been very helpful in providing baseline and follow-up data. We gratefully appreciate the work of technican Hanne Høegh in data sampling. Without the enthusiastic work of all physicians and nurses at the TRIM study sites, the study could not have been completed.

References
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doi: 10.1161/01.CIR.98.19.2004

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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