The diagnostic and prognostic value of first hour glycogen phosphorylase isoenzyme BB level in acute coronary syndrome

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Abstract

Background: Evaluating patients with symptoms suggestive of acute coronary syndrome (ACS) is a time consuming, expensive and problematic process in the emergency department. This study aimed to evaluate the diagnostic and prognostic value of glycogen phosphorylase isoenzyme-BB (GP-BB) in ACS.

Methods: A total of 72 patients (mean age 61.8 ± 11.6 years) with ACS were enrolled. The ELISA method for determining GP-BB level was performed and considered positive at > 10 ng/mL. Duration of angina, type of ACS, demographic features, myoglobin, creatinine kinase and troponin T (cTnT) were also assessed. The cTnT levels eight hours after pain onset was considered the gold standard test for the diagnosis of myocardial infarction.

Results: The most sensitive biomarker at first hour of admission was GP-BB (95.8%). However, the specificity of GP-BB was low (43.7%). Receiver operating characteristics curve analysis of the GP-BB level for predicting myocardial infarction revealed the area under the curve value as 0.82 (SE 0.04; 95% CI 0.78–0.85). Positive treadmill exercise test (60% vs 17%, p = 0.047), coronary artery disease (CAD; 59% vs 19%, p = 0.007), percutaneous coronary intervention (44% vs 27%, p = 0.031) and 30-day mortality and/or readmission (33% vs 5%, p = 0.028) were found to be higher in unstable angina (UA) patients having GP-BB (+).

Conclusions: GP-BB is considerably cardiosensitive at the first hour of admission in patients with ACS, but the specificity of GP-BB is lower and it is elevated in nearly half of the patients with UA. However, in this group, GP-BB predicts significant CAD and the combined end-point of mortality and re-hospitalization. (Cardiol J 2011; 18, 5: 496–502)

Key words: acute coronary syndrome, chest pain, diagnosis, glycogen phosphorylase, prognosis

Introduction

Chest pain is one of the commonest complaints among patients presenting to cardiology or emergency departments. It may be the initial (and sole) complaint of acute coronary syndrome (ACS) [1, 2]. Diagnosis of ACS is based on an assessment of risk factors, careful and rapid assessment of ECG, and measurement of cardiac enzymes [1, 3]. Cardiac troponins, creatinine kinase isoenzyme MB (CK-MB) and myoglobin, which are routinely used in the diagnosis of ACS, are not elevated in the initial hours, removing their value in an early diagnosis [1, 2].

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Human glycogen phosphorylase (GP), particularly its isoenzyme BB (GP-BB), has a distinct sensitivity to myocardial oxygen deficiency in cardiomyocytes [4, 5]. There is evidence that GP-BB is rapidly released into the circulation in the early phase of ACS as well as after perioperative myocardial infarction following coronary artery bypass grafting (CABG) [6].

GP in mammals is known to have three major isoenzymes: BB (brain), MM (muscle), and LL (liver). GP-BB is also found in heart muscle, including human myocardium, where it is, besides the MM subtype, the predominant isoenzyme. Ischemia induces glycogen breakdown. After changing from a structurally bound form into a soluble cytosolic form during phosphorolysis, the enzyme may penetrate as a dimer through the altered cell membrane into the extracellular space [5, 6]. This metabolic sequence reflects the strongly structural and functional coupling of GP-BB to the ischemia-sensitive process of glycogenolysis [5, 6]. Therefore, the specific mechanism of GP-BB release suggests some new aspects for the laboratory diagnosis of ACS.

In the present study, we aimed to (i) investigate the diagnostic performance of first hour GP-BB in identifying different states of myocardial ischemia in patients with ACS; (ii) compare the diagnostic performance of first hour GP-BB to that of serially measured myoglobin, CK-MB and cardiac troponin T (cTnT); and (iii) determine the prognostic value of GP-BB in patients with unstable angina (UA) whose cTnT level was normal.

Methods

Study population

This prospective study was conducted in 80 patients admitted with a chief complaint of chest pain to our emergency department. From the 80 patients initially entered into the study, eight were later excluded (two because a diagnosis of ACS could not be confirmed, and six because they were lost from further follow-up). Inclusion criteria were: patients presenting within two hours of symptom onset and having typical chest pain. Patients were excluded if they had renal insufficiency or any renal disease impairing renal clearance, were < 18 years of age, or had atypical chest pain. Patients were also excluded who: had undergone percutaneous transluminal coronary angioplasty or CABG within the previous 30 days; had prior ACS within the previous 30 days; or had chronic muscle disease, pulmonary thromboembolism or pericarditis.

The protocol of this study was approved by the Local Ethics Committee, and written informed consent was obtained from every participant in this study.

All patients underwent a comprehensive inquiry regarding the degree of angina pectoris, risk factors and past history. A complete blood count, biochemical profile and an initial 12-lead ECG were obtained. Subjects underwent serial ECG and cardiac biomarkers follow-up every four hours. All subjects were managed medically in conformity with ACC/AHA ST elevation myocardial infarction (STEMI), non-STEMI (NSTEMI) and UA guidelines [1–3]. All patients received treatment with p.o. aspirin (300 mg), i.v. unfractionated heparin (bolus of 60 U/kg bodyweight up to a maximum of 5,000 U followed by continuous infusion of 7 U/kg per hour titrated to an activated partial thromboplastin time of 60–70 s), or 1 mg/kg enoxaparin subcutaneously every 12 hours, and clopidogrel (loading dose of 300 mg followed by 75 mg daily). Fibrinolytic therapy was administered to STEMI patients, and they underwent coronary angiography within five days. NSTEMI patients and UA patients with high-risk factors underwent coronary angiography within 24 hours. UA patients with low-risk factors initially received medical therapy and were then directed to the treadmill exercise stress test (TMET). If the TMET was positive, the patient was referred for coronary angiography. Patients were re-evaluated 30 days later for mortality and/or readmission.

Definition of acute coronary syndrome

Patients with ACS were classified into the following:

1. STEMI: defined as having ST-segment elevation ≥ 1 mm in two contiguous leads (or ≥ 2 mm in V1 to V3 leads) or new left bundle branch block together with chest pain for > 30 min and/or evidence of myonecrosis with elevated cTnT level (> 0.1 ng/mL).

2. NSTEMI: defined as no ST-segment elevation on ECG despite elevated cTnT level (> 0.1 ng/mL) and chest pain for more than 30 min.

3. UA: defined as ischemic chest pain lasting more than 30 min with no evidence of myonecrosis or ST elevation.

Coronary angiography and definition of coronary artery disease

Selective coronary angiography was performed in 32 (43%) patients under local anesthesia via the femoral artery using the Judkins method [7]. All
coronary angiograms were evaluated by two experienced cardiologists who were unaware of the laboratory results of the patients. The severity of each lesion was assessed by quantitative coronary angiography (QCA). Coronary artery disease (CAD) was defined as ≥ 50% luminal diameter stenosis in one or more major epicardial vessel. Ten (13.8%) patients had previous coronary artery bypass surgery. In these patients, CAD was defined as ≥ 50% luminal diameter stenosis of a nongrafted major coronary artery or 50% luminal diameter stenosis of the vein or arterial bypass graft [8].

Biochemical and laboratory analysis

Levels of conventional cardiac markers, namely myoglobin, CK-MB and cTnT, were measured by in vitro quantitative electrochemiluminescence immunoassay (ECLIA), sandwich test-specific antibody system and myoglobin STAT (Short Turn-Around Time), troponin STAT and CK-MB STAT kits. Normal reference levels for myoglobin, CK-MB and cTnT were accepted as 0–72 ng/mL, 0.0–5.0 ng/mL and 0.0–0.1 ng/mL, respectively. At the fourth, eighth and twelfth hours, repeat ECGs and cardiac biomarker samples were obtained. For GP-BB measurements, 2.5 cc of venous blood was drawn into EDTA-tubes. The tubes were centrifuged at 3,000 rpm for 7 min and plasma was stored at –20°C. An ELISA for GP-BB was performed using the Diacordon GP-BB (E-051) (DIAGENICS®) kit. Levels of GP-BB were considered positive at values exceeding 10 ng/mL [9]. The glomerular filtration rates of the patients were calculated by the modification of diet in renal disease formula [10], using age, gender, serum creatinine, and ethnicity variables.

Statistical analysis

Distribution of data was assessed using one-sample Kolmogorov-Smirnov test. Data was demonstrated as mean ± SD for normally distributed continuous variables, median and interquartile range (IQR) for skew-distributed continuous variables, and frequencies for categorical variables. Groups were compared by ANOVA method or χ² test. SPSS 15.0 statistical analysis software (SPSS Inc., Chicago, IL, USA) was used to evaluate variables and tests. A p value < 0.05 was considered significant. The eighth-hour cTnT level was accepted as the gold standard for the diagnosis of myocardial infarction. Cardiospecificity and sensitivity levels for initial, fourth-hour and eighth-hour CK-MB and myoglobin, and first-hour GP-BB levels, were calculated according to the reference cardiospecificity and specificity levels for eighth-hour cTnT level by using the Pearson χ² test. Receiver operating characteristics (ROC) curve analysis was performed for the diagnostic value of GP-BB levels in ACS.

Results

Basal clinical characteristics

Of the 80 patients initially entered into the study, eight were later excluded (two because a diagnosis of ACS could not be confirmed, and six because they were lost from further follow-up). Seventy-two subjects were enrolled in the study. Fifty-four (70%) were male and the mean age was 62 ± 12 (range 37–85 years, median 61). Diabetes mellitus was present in 18 (25%) patients, and a history of hypertension was present in 35 (49%). Thirty-seven (51%) patients had a history of smoking.

The mean time period between symptom onset and admission was 62 ± 22 (mean ± SD) minutes (minimum 25 min, maximum 102 min). Among all patients, 14 (19%) had a normal ECG on admission, 11 (15%) had an acute ST segment elevation, 18 (25%) had ST depression, 18 (25%) had T wave inversion, seven (10%) had pathologic Q waves, and four (6%) had any type of dysrhythmia. Sixty-three patients complained of chest pain only, five patients had both chest and back pain, two patients had chest pain together with dyspnea, and two patients suffered from chest pain, nausea and vomiting.

The final diagnosis of subjects was as follows: 48 (67%) had UA, 13 (18%) had NSTEMI, and 11 (15%) had STEMI (four anterior, four inferior, one inferolateral, one inferior with right ventricular involvement and one posterior myocardial infarction). Biomarker levels and the number and percentage of patients with elevated biomarkers in different types of final diagnosis at first and fourth hours are shown in Table 1.

GP-BB in patients with elevated cTnT (NSTEMI or STEMI)

In the patients with NSTEMI, GP-BB was elevated in all patients. Ten (91%) of 11 STEMI patients had an elevated GP-BB level at the first hour. Using a cTnT level > 0.1 ng/mL at the eighth hour as the gold standard for the diagnosis of acute myocardial infarction (AMI), cardiosensitivity and cardiospecificity values for myoglobin, CK-MB, cTnT and GP-BB at the first and fourth hours were calculated. The results obtained are set out in Table 2. The most sensitive biomarker of AMI at first hour was GP-BB (95.8%). However, the specificity of GP-BB for AMI was lowest when compared to other biomarkers (43.7%). ROC curve analysis re-
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revealed the area under the curve (AUC) value of the GP-BB level in predicting myocardial infarction to be 0.82 (SE 0.04; 95% CI 0.78–0.85) (Fig. 1).

**GP-BB in patients with normal eighth hour cTnT (UA)**

Of the 48 patients with normal eighth hour cTnT, 27 (56%) had elevated GP-BB levels on admission. GP-BB levels in UA patients (median 11.2, IQR 2.1–21.2) were lower than in patients with NSTEMI (median 61.3, IQR 35.1–78.4) and STEMI (median 44.2, IQR 19.4–68.1), respectively (Fig. 2).

When patients with UA were further stratified into two groups according to their GP-BB levels (≥ 10 ng/mL or < 10 ng/mL), positive treadmill exercise test (60% vs 17%, p = 0.047), CAD (59% vs 19%, p = 0.007), percutaneous coronary intervention (44% vs 27%, p = 0.031) and 30-day mortality and/or readmission (33% vs 5%, p = 0.028) were found to be higher in GP-BB (+) patients with UA (Table 3). As a final diagnosis, non-cardiac chest pain (initially thought to be ischemic) was present in 28 (33%) patients and the negative predictive value of GP-BB (+) was 61%.

**Discussion**

The main findings of the present study are as follows: (i) GP-BB was the most sensitive biomarker...
ker to detect myocardial infarction when compared to myoglobin, CK-MB, and cTnT at first hour; (ii) the specificity of GP-BB was the lowest and it was found to be elevated in nearly half of the patients with UA; (iii) UA patients with higher GP-BB levels had higher frequency of CAD and a poorer prognosis than those with a normal first hour GP-BB level.

Table 3. Comparison of clinical characteristics and prognostic factors between GP-BB (+) and GP-BB (−) patients with unstable angina.

<table>
<thead>
<tr>
<th></th>
<th>GP-BB (+)</th>
<th>GP-BB (−)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.9 ± 12.1</td>
<td>60.8 ± 12.4</td>
<td>NS</td>
</tr>
<tr>
<td>Male gender</td>
<td>20 (74%)</td>
<td>16 (76%)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking</td>
<td>13 (48%)</td>
<td>10 (47%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 (51%)</td>
<td>11 (52%)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6 (22%)</td>
<td>6 (28%)</td>
<td>NS</td>
</tr>
<tr>
<td>GFR (MDRD formula) [mL/min]</td>
<td>79.2 ± 16.1</td>
<td>78.1 ± 19.7</td>
<td>NS</td>
</tr>
<tr>
<td>ST-T alterations on ECG</td>
<td>20 (74%)</td>
<td>14 (67%)</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary angiography:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Performed initially</td>
<td>12 (44%)</td>
<td>9 (42%)</td>
<td>NS</td>
</tr>
<tr>
<td>Performed because of positive TMET</td>
<td>9 (60%)</td>
<td>2 (17%)</td>
<td>0.047</td>
</tr>
<tr>
<td>Coronary artery disease (% of group)</td>
<td>16 (59%)</td>
<td>4 (19%)</td>
<td>0.007</td>
</tr>
<tr>
<td>PCI (% of group)</td>
<td>12 (44%)</td>
<td>3 (27%)</td>
<td>0.031</td>
</tr>
<tr>
<td>CABG (% of group)</td>
<td>4 (15%)</td>
<td>1 (9%)</td>
<td>NS</td>
</tr>
<tr>
<td>30-day mortality and/or readmission:</td>
<td>9 (33%)</td>
<td>1 (5%)</td>
<td>0.028</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>2 (7%)</td>
<td>0 (0%)</td>
<td>NS</td>
</tr>
<tr>
<td>30-day readmission</td>
<td>7 (26%)</td>
<td>1 (5%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

GFR — glomerular filtration rate; MDRD — modification of diet in renal disease; ECG — electrocardiogram; TMET — treadmill exercise test; PCI — percutaneous coronary intervention; CABG — coronary artery bypass grafting

Figure 1. Receiver operating characteristics curve analysis for glycogen phosphorylase isoenzyme-BB level in predicting acute myocardial infarction; AUC — area under the curve; SE — standard error; CI — confidence interval.

Figure 2. Comparison of glycogen phosphorylase isoenzyme-BB (GB-BB) levels in different groups of acute coronary syndrome; UA — unstable angina; NSTEMI — non-ST elevation myocardial infarction; STEMI — ST elevation myocardial infarction.
Glycogen phosphorylase (glycogen phosphorylase; 1,4-D-glucan: orthophosphate D-glucosyltransferase; GP-BB) is a glycolytic enzyme that plays an essential role in the regulation of carbohydrate metabolism [11, 12]. It catalyzes the first step of glycogenolysis, in which glycogen is converted to glucose 1-phosphate (it mobilizes glycogen, which is connected to the delivery of glucose) [11, 12]. These conditions appear during ischemia and hypoglycemia. GP-BB is connected to glycogen in a macromolecular complex that is structurally bound to the sarcoplasmic reticulum (the sarcoplasmic reticulum glycogenolysis complex) [13, 14].

The degree of GP-BB association with this complex depends above all on the metabolic state of the individual. During hypoxia, bounded GP-BB dissociates and turns to a soluble form of GP-BB which can easily escape from the cell through diffusion [13, 14]. The early release of GP-BB into the blood is a common result of the combination of escalated glycogenolysis and increased permeability of cell membranes which is typical for myocardial ischemia and necrosis [12–14].

Mair et al. [15] investigated the diagnostic value of GP-BB and other cardiac biomarkers obtained immediately at presentation from 48 consecutive patients with UA or temporary ST-T changes. They demonstrated that a considerably higher proportion of GP-BB levels turned positive in UA patients with temporary ST-T changes in their ECG compared to other markers. There are studies showing an elevation of GP-BB in the first 1–4 hours after ACS [5, 16, 17]. It has also been shown that if early reperfusion was established in patients, GP-BB would rise even earlier [5]. In a study by Rabitzsch et al. [17] in 27 patients with myocardial infarction, GP-BB levels were measured during the first ten hours every hour, and thereafter every eight hours. Additionally, GP-BB levels were monitored after reperfusion was obtained through fibrinolytic treatment of the patients. They reported that in patients with AMI, GP-BB was the most sensitive cardiac marker from the initiation of chest pain to the first four hours, compared to myoglobin, CK-MB and cTnT [17]. Peetz et al. [11] investigated GP-BB in the early diagnosis of myocardial injury. A total of 61 patients admitted to the emergency department with ACS were tested for other cardiac biomarkers (CK-MB, myoglobin, and cTnT) as well as GP-BB. It was demonstrated that in patients with STEMI at the first hour, both the sensitivity of GP-BB (100%) and the specificity (96%) were highest. In UA/NSTEMI patients, a very high rate of elevated GP-BB was observed (94% at three hours) compared to myoglobin (67%), CK-MB (55.0%) and cTnT (33.8%) [11].

Our study found that GP-BB was the most sensitive biomarker to detect myocardial infarction when compared to myoglobin, CK-MB, and cTnT at the first hour. This result is consistent with the literature. However, the low specificity of GP-BB is a major concern, and it was found to be elevated in nearly half of the patients with UA. Such low specificity agrees with previous studies: the reason is the high levels of GP-BB in patients with UA. To date, no study has been published on the prognostic value of GP-BB. In this study, we attempted to establish whether or not there is a relationship between GP-BB and short-term prognosis, by following patients for one month. We found that UA patients with higher GP-BB levels had higher frequency of CAD and a worse prognosis than those with normal first hour GP-BB level.

**Limitations of the study**

The major limitations of the present study are the relatively small number of patients and that the results are based on a single center. Because the study population was highly selected, there was a higher probability of ACS, which might improve the diagnostic properties of biomarkers, especially myoglobin. GP-BB was measured only at the first hour of admission, and the follow-up time was relatively short. Cardiac TnT was selected (rather than regular cTnI or SENSITIVE troponin I — ‘ultra’ troponin) because of availability. Defining CAD is another limitation, as stenosis < 50% may be the etiology of UA and ACS.

**Conclusions**

GP-BB is considerably cardiosensitive at the first hour of ACS, but the specificity of GP-BB is lower and it is elevated in nearly half of the patients with UA. However, in this group, GP-BB has an important prognostic role. It predicts significant CAD and the combined endpoint of mortality and re-hospitalization. Further studies are needed with a larger number of subjects to better understand GP-BB’s short-term and long-term prognostic significance for ACS.

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References


