GLYCOGEN PHOSPHORYLASE ISOENZYME BB IN EARLY DIAGNOSIS OF ACUTE CORONARY SYNDROME

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ABSTRACT

Objective: *To investigate the usefulness and diagnostic performance of glycogen phosphorylase BB (GPBB) to aid early diagnosis of acute coronary syndrome and to compare with cardiac-specific troponin I (cTnI), creatine kinase isoenzyme MB and myoglobin.*

Material and Method: *Non-traumatized 72 patients arriving at the emergency department within 3 hours after the onset of chest pain suggestive of acute coronary syndrome were included. The patients were classified as having acute myocardial infarction (AMI) or unstable angina pectoris (UAP). Blood samples were obtained on arrival (0 hours), 6 hours, and 24 hours. To determine the diagnostic performances of GPBB and other cardiac markers for ACS, sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and receiver operating characteristic (ROC) curves with areas under curves were calculated.*

Results: *Seventy two non-traumatic chest pain patients and 45 controls were enrolled. GPBB was the most sensitive biochemical cardiac marker of all tested in AMI and UAP patients on admission, whereas its specificity was low. In both AMI and UAP patients, the area under the GPBB ROC curve, was significantly greater than the areas under all other ROC curves on admission.*

Conclusion: *GPBB is a promising enzyme for the early laboratory detection of ischemic myocardial damage. Because the diagnostic specificity of GPBB for myocardial damage is low, a positive GPBB result should subsequently be confirmed by cTnI measurement. However, further studies on specificity and development of a fast, automated assay are necessary before GPBB can be recommended as a routine diagnostic tool.*

Key Words: *Acute coronary syndrome, glycogen phosphorylase BB, troponin I, creatine kinase MB, myoglobin.* ***Nobel Med 2012; 8(2): 65-72***

AKUT KORONER SENDROMUN ERKEN TANISINDA GLİKOJEN FOSFORİLAZ İZOENZİM BB

ÖZET

Amaç: Akut koroner sendromun erken tanısı için glikojen fosforilaz BB'nin (GPBB) tanısal performansını araştırmak ve kardiyak troponin I (cTnI), kreatin kinaz izoenzim MB ve miyoglobin ile karşılaştırmaktır.

Materyal ve Metod: Çalışmaya, akut koroner sendromu düşündüren bir göğüs ağrısının başlamasından sonraki 3 saat içinde acil servise başvuran travmaya uğramamış 72 hasta alındı. Hastalar akut miyokard infarktüsü (AMI) veya anstabil angina pektoris (UAP) olarak sınıflandırıldı. Kan örnekleri acil servise ilk gelişte (0. saat), 6. ve 24. saatlerde alındı. GPBB ve diğer kardiyak belirteçlerin tanısal performanslarını belirlemek için sensitivite, spesifisite, pozitif ve negatif prediktif değerler, pozitif ve negatif olasılık oranları ve ROC (receiver operating characteristic) eğrileri ile eğri altı alanları hesaplandı.

Bulgular: Çalışmaya non-travmatik göğüs ağrısı olan 72 hasta ile 45 kontrol alındı. GPBB, AMI ve UAP'li hastalarda acil servise ilk başvuruda en duyarlı biyokimyasal kardiyak belirteçti, fakat özgüllüğü düşüktü.

Hem akut miyokard infarktüsü hem de unstabil anjina pektorisli hastalarda glikojen fosforilaz BB ROC eğri altı alanları da diğerlerine göre anlamlı olarak daha büyüktü.

Sonuç: Glikojen fosforilaz BB, iskemik miyokardiyal hasarın erken laboratuvar tespiti için ümit verici bir enzimdir. Ancak tanısal özgüllüğü düşük olduğu için pozitif glikojen fosforilaz BB sonucunun daha sonra cTnI ölçümü ile doğrulanması gerekmektedir. Glikojen fosforilaz BB rutin bir tanı aracı olarak önerilmeden önce özgüllüğü üzerine daha ileri çalışmalar yapılması, hızlı ve otomasyona uygun ölçüm yöntemlerinin geliştirilmesi gereklidir.

Anahtar Kelimeler: Akut koroner sendrom, glikojen fosforilaz BB, troponin I, kreatin kinaz MB, miyoglobin. **Nobel Med 2012; 8(2): 65-72**

INTRODUCTION

The acute coronary syndrome (ACS) is a pathophysiologic continuum of myocardial ischemia that results from rupture of an atherosclerotic plaque, with subsequent platelet aggregation and thrombus formation. It can lead to clinical presentations ranging from entirely asymptomatic to unstable angina, frequently associated with minor myocardial damage, to acute myocardial infarction (AMI), with extensive tissue necrosis, to sudden cardiac death attributable to arrhythmias.[1-3]

Evaluation of patients who present to the hospital with a complaint of chest pain or other signs or symptoms suggestive of ACS is time-consuming, expensive, and problematic. The discharge of patients with missed diagnosis of acute cardiac ischemia is associated with increased mortality, thus underlining the significance of correct determination of acute infarction. The early diagnosis of ACS leads to early intervensions and, therefore, better clinical outcomes.[4,5]

Historically, coronary artery disease assessment has been mainly binary, using WHO criteria of symptoms, electrocardiography (ECG), and biochemical markers. Although ECG is the simplest diagnostic modality, up to 50% of AMI patients may have nondiagnostic ECGs on emergency department admission.[6] On the other hand, biochemical markers are the primary diagnostic tests used to evaluate patients with inconclusive electrocardiograms for possible ACS.[7] Measurements of cardiac enzymes allow a clear distinction between patients with and without myocardial infarction.[8] The National Academy of Clinical Biochemistry recommends the use of an early (within 6 hours' rise) and definite marker for establishing the diagnosis of myocardial infarction. The ideal biochemical marker is one that has high clinical sensitivity and specificity, appears early after AMI to facilitate early diagnosis, remains abnormal for several days after AMI, and can be assayed with a rapid turnaround time. Because there is currently no single marker that meets all of these criteria, a multi-analyte approach has the most merit.[9]

Myoglobin, creatine kinase isoenzyme MB (CKMB), cardiac-specific troponins (cTnT and cTnI) are biochemical markers which are widely used in diagnosing of patients with acute chest pain. However, CKMB and troponin levels do not start to rise for at least 4 hours after the onset of the event, which can cause a delay in the diagnosis and implementation of needed therapies. Also, CKMB levels are normal in one fourth to one half of patients with AMI at the time of emergency department presentation.

Although myoglobin can be detected in the serum as early as two hours after myocardial necrosis begins, it has low cardiac specifity and therefore requires supplementation with some other analyses such as troponins to support the myoglobin value.[10-13] →

Table 1: Clinical baseline characteristics and biochemistry of the study groups

	Acute Coronary Syndrome (n=72)	Control (n=45)
Age (years)	58 ± 11	55 ± 10
Male/Female	53 / 19	29 / 16
Body Mass Index (kg/m2)	27.6 ± 4.1	27.2 ± 4.0
Risk factors		
Diabetes (%)	12	7
Hypertension (%)	49#	4
Dyslipidemia (%)	50#	9
Smoking (%)	45#	20
Family history (%)	21	15
Biochemistry, mmol/L		
Total Cholesterol	4.95 ± 0.9	4.81 ± 0.7
HDL- Cholesterol	1.12 ± 0.22#	1.35 ± 0.19
LDL- Cholesterol	3.15 ± 0.65‡	2.83 ± 0.49
Triacylglycerol	1.77 ± 0.43#	1.36 ± 0.31
Total Cholesterol/HDL-C	4.47 ± 0.43#	3.58 ± 0.39
Apolipoprotein AI (g/L)	1.21 ± 0.21‡	1.33 ± 0.16
Apolipoprotein B (g/L)	1.21 ± 0.22‡	1.09 ± 0.15
Lipoprotein(a)* (g/L)	0.20 (0.02 - 1.41)†	0.11 (0.01-0.58)

HDL: High density lipoprotein; LDL: Low density lipoprotein; *Median (Range), †p< 0.05, ‡p< 0.01, #p< 0.001 (Significantly different from control group)

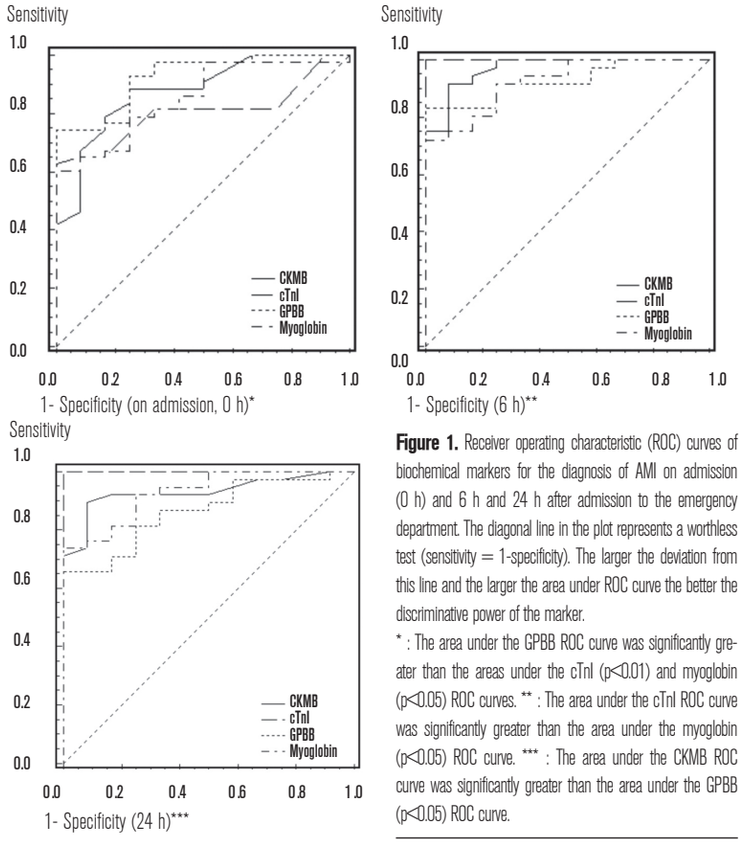


Figure 1. Receiver operating characteristic (ROC) curves of biochemical markers for the diagnosis of AMI on admission (0 h) and 6 h and 24 h after admission to the emergency department. The diagonal line in the plot represents a worthless test (sensitivity = 1-specificity). The larger the deviation from this line and the larger the area under ROC curve the better the discriminative power of the marker.
* : The area under the GPBB ROC curve was significantly greater than the areas under the cTnI ($p<0.01$) and myoglobin ($p<0.05$) ROC curves. ** : The area under the cTnI ROC curve was significantly greater than the area under the myoglobin ($p<0.05$) ROC curve. *** : The area under the CKMB ROC curve was significantly greater than the area under the GPBB ($p<0.05$) ROC curve.

CKMB: Creatine kinase isoenzyme MB, **cTnI**: Cardiac-specific troponin I
GPBB: Glycogen phosphorylase BB

Nowadays, there is intense search to find new serum biomarkers that are released very early during myocardial ischemic injury.[14] New markers are currently being investigated for early AMI diagnosis, including glycogen phosphorylase BB (GPBB), carbonic anhydrase III, free fatty acid binding protein, phosphoglyceric acid mutase isoenzyme MB, annexin V, ischemia-modified albumin (IMA), heart fatty acid-binding protein and myosin light chains.[15,16] Among the recently proposed new markers, GPBB is the most promising because it increases as early as 1 to 4 h from chest pain onset and its early release appears to be essentially dependent on ischemic myocardial injury. GPBB usually peaks 6 to 20 h after onset of chest pain and it returns to reference intervals within 1-2 days after MI.[16-18]

As a key enzyme of glycogenolysis, glycogen phosphorylase (GP) plays an essential role in the regulation of carbohydrate metabolism by mobilization of glycogen. It catalyses the first step in glycogenolysis in which glycogen is converted to glucose-1-phosphate, utilizing inorganic phosphate, and it is primarily associated with provision of an emergency glucose supply during periods of hypoxia and hypoglycemia. With the onset of tissue hypoxia, when glycogen is broken down and disappears, GPBB is converted from a structurally bound to a soluble form as a result of the breakdown of glycogen, and the enzyme becomes free to move around in the cytoplasm and to diffuse out of the cell if the cell membrane permeability is simultaneously increased.

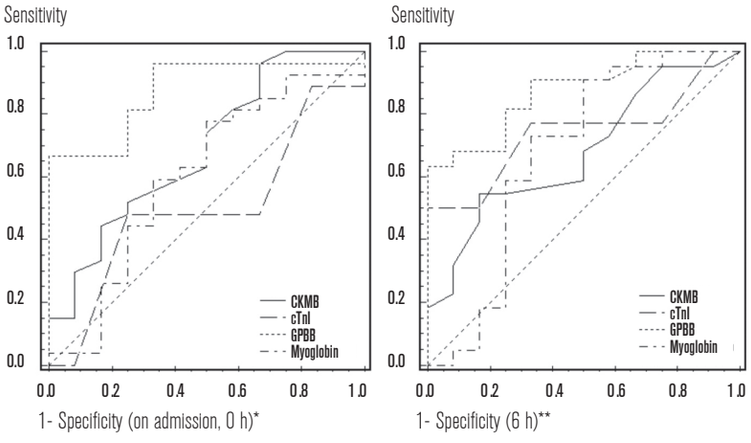
The early release of GPBB requires both a burst in glycogenolysis and concomitantly increased plasma membrane permeability, which is the case in ischemic myocardial damage. The efflux of GPBB into the extracellular fluid follows if ischaemia-induced structural alterations in the cell membrane become manifest.[18-20] The physiological form of GP is a dimer which is composed of two identical subunits. Three different GP isoenzymes have been described in human tissues, GPLL (liver), GPMM (muscle), and GPBB (brain). Brain and myocardium are the only known tissues with considerable GPBB content and, therefore, increases in GPBB should be highly specific for ischemic myocardial injury when damage to the brain and consequent disturbance of the blood-brain barrier can be excluded.[20]

The aim of the study was to investigate the usefulness and diagnostic performance of GPBB to aid early diagnosis of ACS and to compare with cTnI, CKMB and myoglobin.

MATERIAL and METHOD

Study Design

The study was designed as a prospective study of →



GPBB: glycogen phosphorylase BB, CKMB: creatine kinase isoenzyme MB

Figure 2. Receiver operating characteristic (ROC) curves of biochemical markers for the diagnosis of UAP on admission (0 h) and 6 h and 24 h after admission to the emergency department. The diagonal line in the plot represents a worthless test (sensitivity = 1-specificity). The larger the deviation from this line and the larger the area under ROC curve the better the discriminative power of the marker. * : The area under the GPBB ROC curve was significantly greater than the areas under the cTnI ($p<0.01$) and myoglobin ($p<0.01$) ROC curves. The area under the CKMB ROC curve was significantly greater than the area under the cTnI ($p<0.01$) ROC curve. ** : The area under the GPBB ROC curve was significantly greater than the area under the myoglobin ($p<0.05$) ROC curve. *** : The areas under the all ROC curves were not statistically significant each other.

Table 2: Characteristics of markers at different time points from admission in patients with acute coronary syndrome

Time after emergency department admission	Values above the cut-off, %*			
	cTnI	CKMB	Myoglobin	GPBB
AMI patients (n= 45)				
0 h (admission)	63	68	71	93
6 h after admission	100	91	83	92
24 h after admission	100	86	80	81
UAP patients (n= 27)				
0 h (admission)	48	30	30	82
6 h after admission	56	37	32	78
24 h after admission	56	30	28	78

*Percentage of measured values above the respective cut-off value according to ROC analysis, GPBB: glycogen phosphorylase BB, CKMB: creatine kinase isoenzyme MB, cTnI: cardiac-specific troponin I, AMI: acute myocardial infarction, UAP: unstable angina pectoris

diagnostic performances of GPBB, cTnI, CKMB and myoglobin. The study was approved by an ethics committee on human research.

Study Setting and Population

This study was performed at an urban academic hospital. Trained research assistants enrolled patients presenting to the emergency department with a primary complaint of non-traumatic chest pain during a 6-month period in 2007. 18 years and older patients who admitted within 3 hours after the onset of chest pain were eligible for enrollment. Patients with brain damage, renal, hepatic or malignant diseases were excluded. Volunteers who had no history of any cardiac symptoms and had a normal physical examination and normal electrocardiogram at rest and no elevation in cardiac enzymes comprised the control population.

Study Protocol

Immediately after emergency department admission (0 h), electrocardiogram was recorded and venous blood samples were collected in heparin-coated and non-additive tubes (BD Vacutainer, Becton Dickinson and Company, Plymouth, UK). Serum and plasma were immediately separated by centrifugation at 3000 g for 10 min. After CKMB activity, cTnI and myoglobin were measured without delay, heparinized plasma for measurement of GPBB was frozen in aliquots and stored at -80°C until analysis was performed. These cardiac markers were repeatedly measured on 6 and 24 h. Other biochemical parameters were measured on the day of blood collection.

The definite diagnosis of AMI was based on the recommendations of the American College of Cardiology/European Society of Cardiology.[21] a) Spontaneous ischaemic episode (usually) lasting >20 minutes, b) the evolution of new ST elevation in at least two contiguous leads, measuring >0.2 mV in leads V1-V3, or >0.1 mV in all other leads, c) elevation of troponin levels; for cTnI, diagnostic value of >0.1 ng/ml was used.

Unstable angina pectoris was defined as ischemic chest pain at rest, undetectable cTnI values but with non-specific ECG changes (ST depression or T wave inversion or transient ST elevation) or continuing chest pain with or without non-specific ECG changes and without significant changes in cardiac enzymes. When needed, the diagnosis was supported by coronary angiographic findings.

Measurements of cTnI (intra-assay CV 3.0%, inter-assay CV 4.9%) and myoglobin (intra-assay CV 3.2%, inter-assay CV 3.1%) were done using a chemiluminescence system (ACS:180, Bayer HealthCare LLC, Tarrytown, NY USA). Determination of CKMB activity (intra-assay CV 3.4%, inter-assay CV 4.2%) was based on immunoinhibition assay in the Aeroset automatic analyzer (Dallas, USA) using commercial kit (Randox Lab. Antrim, UK).

Determination of GPBB concentrations in plasma was performed batchwise using a commercially available ELISA kit (intra-assay CV 3.2%, inter-assay CV 6.04%, Diacordon®; Diagenics, Woburn, MA, USA). →

Total cholesterol, high density lipoprotein-cholesterol (HDL-C) and triacylglycerol concentrations were determined by enzymic methods. LDL-C was calculated by the Friedewald formula.[22] Apo AI, apo B and Lp(a) levels were analyzed by immunonephelometric assay (Dade Behring, Newark, USA).

Additionally, the following conventional cardiovascular risk factors were defined:[23] Arterial hypertension (systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg on two or more occasions and/or use of anti-hypertensive drugs), dyslipidemia (total cholesterol ≥5.2 mmol/L and/or low density lipoprotein cholesterol (LDL-C) ≥3.38 mmol/L and/or use of cholesterol lowering drugs, diabetes (fasting glucose ≥6.99 mmol/L and/or use of pharmacological treatment), family history of cardiovascular disease (symptomatic coronary artery disease occurring in first degree male relatives aged <55 years or first degree female relatives aged <65 years), obesity (body mass index, BMI ≥30 kg/m^2), and smoking (regular smoking or quitting <3 months ago).

Statistical Analysis

Statistical analysis was performed using SPSS (Statistical Program for Social Sciences) package, version 10 for Windows (SPSS Inc., Chicago, IL., USA). The results of quantitative variables are presented as mean±standard deviation or as median and range. Qualitative variables are presented as percentages. To compare quantitative and qualitative variables between groups, Student's t-test or Mann-Whitney U and chi-square test were used, respectively. Because of the highly skewed distribution of serum Lp (a) concentrations, all values are given in medians and range and Mann Whitney-U test was applied. A p-value <0.05 was regarded as statistically significant.

To determine the diagnostic performances of cardiac markers for acute ischemic coronary syndromes, we calculated sensitivity (true positive test results of all patients with disease), specificity (true negative test results of all patients without disease), positive and negative predictive values (true positive test results of all positive test results, true negative test results of all negative test results), positive likelihood ratio [sensitivity/(1-specificity)], negative likelihood ratio [(1-sensitivity)/specificity] and receiver operating characteristic (ROC) curves with areas under curves.

ROC analysis for the calculation of cut-off values, areas under ROC curves, and the comparison of ROC curves, sensitivity, specificity, predictive values, likelihood ratios were performed using the software MedCalc Version 4.31 (MedCalc Software, Mariakerke, Belgium).

Table 3: Diagnostic performances of markers in diagnosis of acute myocardial infarction

Time after emergency department admission		cTnI	CKMB	Myoglobin	GPBB
On admission 0 h (n = 45)	Sensitivity, %	62.8 (46.7-77)	68.2 (52.4-81.4)	70.5 (54.8-83.2)	93.2 (81.3-98.5)
	Specificity, %	100 (73.4-100)	94.4 (72.6-99.1)	75 (50.9-91.2)	78 (50.9-91.2)
	PV (+), %	100	96.8	86.1	89.1
	PV (-), %	42.9	54.8	53.6	83.3
	LR (+), %	11	12.27	2.82	3.73
	LR (-), %	0.37	0.34	0.39	0.09
	Areas under ROC curve	0.80 (0.67-0.89)	0.87 (0.82-0.97)	0.85 (0.74-0.92)	0.93 0.84-0.98
	Cut-off	0.1 ng/mL	25 U/L	73 ng/mL	14.3 ng/mL
6 h (n= 45)	Sensitivity, %	100 (91.7-100)	90.9 (78.3-97.4)	82.5 (67.2-92.6)	92.3 (79.1-98.3)
	Specificity, %	100 (73.4-100)	94.4 (72.6-99.1)	80 (56.3-94.1)	80 (56.3-94.1)
	PV (+), %	100	97.6	89.2	87.3
	PV (-), %	100	81	69.6	83.3
	LR (+), %	12	16.36	4.13	3.69
	LR (-), %	0.0	0.10	0.22	0.1
	Areas under ROC curve	1 (0.93-1)	0.96 (0.91-0.99)	0.92 (0.83-0.97)	0.94 (0.84-0.98)
	Cut-off	0.1 ng/mL	25 U/L	73 ng/mL	14.3 ng/mL
24 h (n= 45)	Sensitivity, %	100 (91.7-100)	86 (72.1-94.7)	80 (64.3-90.9)	81 (65.9-91.4)
	Specificity, %	100 (73.4-100)	94.4 (72.6-99.1)	80 (56.3-94.1)	85 (62.1-96.6)
	PV (+), %	100	97.4	88.9	87.2
	PV (-), %	100	73.9	66.7	65.2
	LR (+), %	12	15.49	4	3.24
	LR (-), %	0.0	0.15	0.25	0.25
	Areas under ROC curve	1 (0.93-1)	0.94 (0.85-0.98)	0.90 (0.83-0.97)	0.86 (0.75-0.93)
	Cut-off	0.1 ng/mL	25 U/L	82.5 ng/mL	14.3 ng/mL

PV(+): Positive predictive value; PV(-): Negative predictive value; LR(+): Positive likelihood ratio; LR(-): Negative likelihood ratio, ROC: Receiver operating characteristic, GPBB: glycogen phosphorylase BB, CKMB: creatine kinase isoenzyme MB

RESULTS

The clinical baseline characteristics and risk factors and lipid profile of the study groups are summarized in Table 1. The study subjects included 72 patients (53 men and 19 women, ages between 35 and 80 years) and 45 controls (29 male and 16 female, ages between 31 and 66 years). Although the presentation of conventional risk factors was higher in the patient group, significant differences were observed only in hypertension, dyslipidemic and smoking subjects. There were no significant differences in age, BMI, diabetes and family history between the groups. While LDL-C, triglyceride, apo-B and Lp(a) levels were elevated in the patient group, HDL-cholesterol and apo-AI levels were decreased.

According to the diagnosis established by clinical and electrocardiographic examination and the measurement of the serum cardiac markers, 45 patients had an AMI and 27 had UAP. Based on ROC analysis, optimized cut-off values for the AMI and UAP patients were calculated for the 0 h, 6 h, →

Table 4: Diagnostic performances of markers in diagnosis of unstable angina pectoris

Time after emergency department admission		cTnI	CKMB	Myoglobin	GPBB
On Admission 0 h (n = 27)	Sensitivity, %	48.1 (28.7-68)	29.6 (13.8-50.2)	29.6 (13.8-50.2)	81.5 (61.9-93.6)
	Specificity, %	75 (42.8-94.2)	94.4 (72.6-99.1)	75 (50.9-91.2)	78 (56.3-94.1)
	PV (+), %	81.3	88.9	61.5	81.5
	PV (-), %	39.1	47.2	44.1	75
	LR (+), %	1.93	5.33	1.19	3.26
	LR (-), %	0.69	0.75	0.94	0.25
	Areas under ROC curve	0.50 (0.33-0.66)	0.79 (0.64-0.89)	0.58 (0.43-0.72)	0.88 (0.75-0.95)
	Cut-off	0.1 ng/mL	25U/L	68.2 ng/mL	14.3 ng/mL
6 h (n= 27)	Sensitivity, %	55.6 (35.3-74.5)	37 (19.4-57.6)	32 (15-53.5)	78.3 56.3-92.5
	Specificity, %	83.3 (51.6-97.4)	94.4 72.6-99.1	75 (50.9-91.2)	78 50.9-91.2
	PV (+), %	88.2	90.9	61.5	78.3
	PV (-), %	45.5	50	46.9	75
	LR (+), %	3.33	6.67	1.28	3.13
	LR (-), %	0.53	0.67	0.91	0.29
	Areas under ROC curve	0.78 (0.62-0.89)	0.80 (0.64-0.90)	0.63 (0.47-0.77)	0.87 (0.73-0.95)
	Cut-off	0.1 ng/mL	25 U/L	66 ng/mL	14.3 ng/mL
24 h (n= 27)	Sensitivity, %	55.6 (35.3-74.5)	29.6 (13.8-50.2)	28 (12.1-49.4)	77.8 (57.7-91.3)
	Specificity, %	100 (73.4-100)	94.4 (72.6-99.1)	75 (50.9-91.2)	78 56.3-94.1
	PV (+), %	100	88.9	58.3	80.8
	PV (-), %	50	47.2	45.5	71.4
	LR (+), %	3.33	5.33	1.12	3.11
	LR (-), %	0.44	0.75	0.96	0.3
	Areas under ROC curve	0.78 (0.62-0.89)	0.79 0.64-0.89	0.63 (0.47-0.71)	0.79 0.65-0.89
	Cut-off	0.1 ng/mL	25 U/L	66 ng/mL	14.3 ng/mL

PV(+): Positive predictive value; PV(-): Negative predictive value; LR (+): Positive likelihood ratio;
LR (-): Negative likelihood ratio, ROC: Receiver operating characteristic, GPBB: glycogen phosphorylase BB, CKMB: creatine kinase isoenzyme MB

and 24 h. Based on the cut-off values, GPBB showed the greatest number of data points above the cut-off values on admission to emergency department in the AMI patients. However, 24 h after admission, the number of samples with concentrations above the cut-off value was lower for markers with a short half-life (myoglobin and GPBB) than for markers with a long half-life (cTnI and CKMB) (Table 2). In the unstable angina pectoris (UAP) patients; GPBB again showed the greatest number of data points above the cut-off values 0 h, 6 h, and 24 h after admission to emergency department (Table 2). In the AMI patients, the diagnostic performances for each marker at different time points from admission are shown in Table 3. On admission (0 h), GPBB showed the highest sensitivity (93.2%), whereas its specificity was low (78%). However, cTnI, CKMB and myoglobin had significantly lower sensitivities (62.8%, 68.2% and 70.5%, respectively) on admission. At 6 hours from admission, cTnI and CKMB were significantly better than myoglobin and GPBB with regard to sensitivity and specificity. As expected, cTnI showed the highest sensitivity (100%) and specifity (100%) both 6 h and 24 h after admission.

In UAP patients, GPBB showed fairly high sensitivity on admission (0 h) and 6 h and 24 h after admission (81.5%, 78.3% and 77.8%, respectively). Although GPBB showed higher specificity (78%) compared to cTnI (75%) and myoglobin (75%), it showed lower specificity compared to CKMB (94.4%) on admission. CKMB had the highest specificity on admission and 6 h later, whereas cTnI had the highest specificity 24 h later (Table 4). ROC curves of all investigated cardiac markers and the calculated area under curves are shown in Figure 1. ROC curve and ROC area under curve calculations proved the superior diagnostic performance of GPBB for detecting acute ischemic coronary syndromes (AMI or UAP) at hospital admission (0 hour) compared with CKMB, myoglobin and cTnI. The area under the GPBB ROC curve (0.93) was significantly greater than the areas under all other ROC curves on admission (Figure 1) in AMI patients. The potential usefulness of GPBB for diagnosing and monitoring UAP was also demonstrated by Figure 2. GPBB ROC curve was significantly greater than the areas under all other ROC curves on admission and after 6 h and 24 h (Figure 1).

DISCUSSION

Diagnostic strategies in patients with acute chest pain have to be reliable and simple. The objective is to reduce mortality and morbidity by initiating the best therapy.[24] Biochemical markers play a pivotal role in the diagnosis and management of patients with ACS. Since most patients with chest pain today are seen in the emergency department within 6 h of symptoms, a biochemical marker that is consistently abnormal during this time period would be valuable for the efficient triaging of patients to the appropriate levels of care.[12] No currently used cardiac-specific serum marker meets all the criteria for an "ideal" marker of AMI and no test is both highly sensitive and highly specific for acute infarction within 6 hours following the onset of chest pain. Therefore in patients presenting to the emergency department with chest pain or other symptoms suggestive of acute cardiac ischemia, a diagnosis of AMI can not be excluded on the basis of a single cardiac marker value obtained within a few hours after symptom onset.[25,26]

The process of myocardial injury and infarction weakens the myocyte membrane wall and permits the intracellular macromolecules, collectively referred to as biochemical cardiac markers or biomarkers, to diffuse into the peripheral circulation.[13] GPBB has →

a high sensitivity to myocardial oxygen deficiency in cardiomyocytes and is rapidly released into the circulation from injured myocardial cells, reflecting the burst in glycogenolysis associated with myocardial ischemia and AMI.[18] Furthermore, experimental studies and clinical observations also demonstrated that increases in GPBB only occur in response to clinical settings in which cardiac work is increased and glycogen is mobilized if concomitant myocardial damage with ischemia-induced plasma membrane injury occurs.[20,27]

In this study comparing the sensitivity and specificity for myocardial infarction of various biochemical cardiac markers, GPBB was found to be the most efficient for the early diagnosis of AMI. Really, the most striking feature of GPBB is its high early diagnostic sensitivity for AMI during the early hours after the onset of chest pain. There were distinct differences in the sensitivity of GPBB (93.2%) in comparison with myoglobin (70.5%), CKMB (68.2%) and cTnI (62.8%) on admission. In particular, GPBB was markedly more sensitive on admission. However, this high early sensitivity requires the development of a rapid assay suitable for "stat" determination in the routine emergency laboratory.

Our study confirms that the sensitivity of conventional markers, including myoglobin, CKMB, and cTnI, is not sufficient during the early hours after the onset of chest pain.

The sensitivities of CKMB activity and TnI were lower compared with GPBB (on admission) and they were particularly efficient for the late diagnosis. Similarly, Rabitzsch et al. demonstrated GPBB was the most sensitive marker for detection of AMI during the first 4 h after onset of chest pain.[20] Furthermore, GPBB was found to be sensitive for the detection of perioperative ischemic myocardial damage and infarction in patients undergoing coronary artery bypass grafting, and GPBB more accurately reflected ischemic myocardial damage than CKMB.[28] Apart from the highest sensitivity, GPBB also showed the highest negative predictive value and ROC area under curve and the lowest negative likelihood ratio compared to other cardiac biomarkers. These findings are in agreement with those obtained by Rabitzsch et al. But GPBB showed lower specificity.[20] The cause of lower specificity may be due to the great number of control subjects with other diseases showing an increase of GPBB levels. Moreover, leukocytes, spleen, kidneys, bladder, testes, intestine and aorta of healthy people could be potential sources of GPBB in certain pathologic circumstances.

The ROC curves of GPBB, myoglobin, CKMB, and cTnI for the discrimination between patients with and without acute ischemic coronary syndromes AMI and UAP) are shown in Figure 1 and 2. The discriminatory power of GPBB to detect acute ischemic coronary syndromes in patients at admission to the emergency department is superior to all other markers tested. This is emphasized by the significantly greater area under GPBB ROC curve compared to the areas under all other ROC curves on admission.

The application of GPBB is not restricted to conventional myocardial infarction. An early release of GPBB was reported in patients with UAP too.[29] The high number of GPBB positive patients with UAP found in this study shows the potential suitability of GPBB for diagnosis of ischemia. Because we found an early release of GPBB in the blood of patients with UAP, it could be useful for early risk stratification in these patients. Also, Mair et al. suggested that the early release of GPBB in patients with UAP may help to identify high risk patients with UAP even on admission to an emergency department and GPBB concentrations could help to guide decisions about patient management.[29] However, the clinical importance, in particular for prognosis, needs to be analyzed in future studies.

This study is limited by the fact that we measured GPBB concentrations and other cardiac markers only on arrival (0 hours), 6 hours, and 24 hours after arriving at the emergency department because of our financial limitations. It would be more descriptive to follow these markers hourly after admission and in a larger patient population. Also, GPBB levels should be compared with other serum biochemical cardiac markers.

CONCLUSION

There is general agreement that both 'early' and 'definitive' biochemical markers of myocardial damage are necessary. Currently, myoglobin is the marker that most effectively fits the role as an 'early' marker, whereas 'definitive' markers are cardiac troponins. According to the results of our study, GPBB is basically suitable as a marker for the early diagnosis of ACS. However, because the diagnostic specificity of GPBB is low, we would recommend a combination of cTnI and GPBB measurement which combines cardiac-specificity with high early sensitivity for ischemic myocardial damage to be used in the early evaluation of patients with chest pain. However, further studies on specificity and diagnostic performance and development of a fast, automated assay are necessary before GPBB can be recommended as a routine diagnostic tool.

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