HbA1c/C-peptide ratio is associated with angiographic thrombus burden and short-term mortality in patients presenting with ST-elevation myocardial infarction

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Objectives **Angiographic high thrombus burden (HTB) is associated with increased adverse cardiovascular events in patients with ST-elevation myocardial infarction (STEMI). HbA1c and C-peptide are two interrelated bioactive markers that affect many cardiovascular pathways. HbA1c exhibits prothrombogenic properties, while C-peptide, in contrast, exhibits antithrombogenic effects. In this study, we aimed to demonstrate the value of combining these two biomarkers in a single fraction in predicting HTB and short-term mortality in patients with STEMI.**

Methods **1202 patients who underwent primary percutaneous coronary intervention (pPCI) for STEMI were retrospectively included in this study. The study population was divided into thrombus burden (TB) groups and compared in terms of basic clinical demographics, laboratory parameters and HbA1c/C-peptide ratios (HCR). In addition, short-term mortality of the study population was compared according to HCR and TB categories.**

Results **HCR values were significantly higher in the HTB group than in the LTB group (3.5 ± 1.2 vs. 2.0 ± 1.1; $P < 0.001$; respectively). In the multivariable regression analysis, HCR was determined as an independent predictor of HTB both as a continuous variable [odds ratio (OR): 2.377; confidence interval (CI): 2.090–2.704; $P < 0.001$] and as a categorical variable (OR: 5.492; CI: 4.115–7.331; $P < 0.001$). In the receiver operating characteristic (ROC) analysis, HCR predicted HTB with 73% sensitivity and 72% specificity, and furthermore, HCR's predictive value for HTB was superior to HbA1c and C-peptide. The Kaplan-Meier cumulative survival curve showed that short-term mortality increased at HTB. In addition, HCR strongly predicted short-term mortality in Cox regression analysis.**

Conclusions **In conclusion, HCR is closely associated with HTB and short-term mortality in STEMI patients.** ***Blood Coagul Fibrinolysis*** **34:385–395**

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Keywords: C-peptide, HbA1c, short-term mortality, ST-elevation myocardial infarction, thrombus burden

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Introduction

Acute coronary syndromes (ACS) mainly develop as a result of the restriction of coronary blood flow by acute thrombus formation, which occurs as a result of the contact of endothelial and subendothelial plaque components with the molecular and cellular components of the circulating coagulation cascade after erosion or rupture of the atherosclerotic plaque [1]. Although platelet activation and coagulation pathways have been studied in detail, the mechanisms of thrombus formation on eroded and ruptured plaques have not been fully elucidated and platelets are thought to play a pivotal role in arterial thrombus formation [2]. Excessive thrombus development is a strong predictor of adverse clinical outcomes in acute coronary syndromes [3]. Among the types of acute coronary syndromes, thrombotic processes are more aggravated in ST-elevation myocardial infarction (STEMI) than in others, leading to larger thrombus formations [4]. High thrombus burden is associated with increased infarct area, increased risk of re-infarction, no-reflow, stent thrombosis, and short- and long-term mortality in STEMI patients [3–5]. Therefore, estimating HTB in STEMI patients undergoing primary percutaneous coronary intervention (pPCI) prior to the interventional procedure may increase the accuracy of risk assessment and improve short- and long-term surveillance in these patients.

Diabetes mellitus is a prothrombogenic disease and although the cellular and molecular mechanisms underlying the increased thrombotic tendency in type 2 diabetes mellitus (T2DM) have not been fully elucidated, it is thought that the bioactive roles of HbA1c and C-peptide may play an important role in this situation [6–8]. However, it has been shown that these two markers can also affect important bioactive pathways in nondiabetic

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patients [9–11]. HbA1c is not only a diagnostic parameter of DM, but also a prognostic biomarker for cardiovascular disease for both diabetics and nondiabetics [12,13]. HbA1c elevation has been shown to increase platelet activation and aggregation [14,15]. Moreover, it has been shown that tight glycemic control, that is, lowering of HbA1c, reduces platelet reactivity in patients with acute coronary syndrome presenting with hyperglycemia [16].

C-peptide is a released bioactive molecule that is released from pancreatic beta cells into the circulation in equal molar amounts with insulin as a result of the breakdown of proinsulin [10]. Beyond showing pancreatic beta cell reserve, it has been shown to take an active role in many molecular pathways beyond the pancreatic beta cell reserve [11]. It has been shown that high doses of C-peptide can reduce thrombus formation [17]. C-peptide regulates the interaction of leukocytes, erythrocytes and platelets with the endothelium [18,19]. It increases the stimulation of nitric oxide production by platelets and endothelium and reduces reactive oxygen species (ROS) generation through AMP-activated protein kinase alfa (AMPKa) activation and vascular endothelial growth factor (VEGF) inhibition [19,20]. Therefore, the C-peptide exerts beneficial effects on homeostasis and the inflammatory process involved in triggering thrombus formation, which causes many acute complications of atherosclerosis [20].

Considering all these results, the ratio of HbA1C/C-peptide (HCR) obtained by combining the two biomarkers in a single fraction may better predict the thrombus burden in STEMI patients, due to the prothrombogenic effect of HbA1c and the antithrombogenic effect of C-peptide. In this context, we wanted to demonstrate the accuracy of this postulation in STEMI patients in this study and we also aimed to investigate the effect of HCR on short-term mortality in this patient group.

Materials and methods

Study population and data collection

1202 consecutive patients who underwent primary percutaneous coronary intervention (pPCI) for STEMI between January 2019 and December 2022 at four different tertiary centers were included in this study. Demographic, clinical, medical characteristics and short-term follow-up results of patients were obtained using hospital patient data and national health system database. The diagnosis of STEMI was made in the presence of two criteria: persistent angina for $\geq$20 min and ST segment elevation of $\geq$1 mm in $\geq$2 contiguous leads other than V2 to V3, or the presence of new left bundle branch block. In leads V2 to V3, 2 mm ST segment elevation in males and 1.5 mm in females was required for the diagnosis of STEMI [21]. Those who received thrombolytic therapy before the invasive procedure, those who did not undergo the invasive procedure within 12 h of the onset of symptoms, those with any systemic inflammatory and rheumatological disease, any hematological disease including anemia, malignancy, advanced kidney and/or liver failure, acute and chronic infection, patients with a history of blood transfusion in the last 3 months, patients with a previous history of coronary artery disease (CAD) using antiaggregant or anticoagulant, patients with severe valvular disease and valve surgery were excluded and patients with missing data were excluded from the study. The study protocol was approved by the Local Ethics and Research Committee and was conducted in accordance with the Declaration of Helsinki Principles.

Laboratory examination

After the patients were admitted to the hospital for coronary angiography, all blood samples were taken from peripheral venous blood. Parameters other than C-peptide, lipid panel, fasting plasma glucose, creatine kinase myocardial band (CK-MB) and troponin-I were obtained from peripheral venous blood samples obtained at the time of admission to the hospital. C-peptide, lipid panel, fasting plasma glucose were recorded from the results obtained after at least 8 h of fasting, and CK-MB and troponin-I values were obtained from the peak levels during hospitalization. Complete blood count (CBC) was evaluated with an automated blood cell counter (Coulter LH 780 Hematology Analyzer, Beckman Coulter Corp, Hileh, Florida, USA). Serum creatinine levels were measured using Architect Plusci 4100 meters (Abbott Laboratories, Abbott Park, Illinois, USA). Estimated glomerular filtration rate (eGFR) calculated from Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation formula.

Clinical definitions and measurements

Hypertension (HT) was defined as a systolic blood pressure $\geq$140 mmHg, diastolic blood pressure $\geq$90 mmHg, or current use of antihypertensive medication. Dyslipidemia was defined as the presence of one of four parameters of the lipid profile measured after at least 8 h of fasting or a history of lipid-lowering drug use: total cholesterol >200 mg/dl, low-density lipoprotein cholesterol (LDL-C) >130 mg/ dl, high-density lipoprotein cholesterol (HDL-C) <40 mg/dl in men and <50 mg/dl in women, and triglycerides >150 mg/dl. DM was defined as fasting serum glucose $\geq$126 mg/dl, hemoglobin A1c $\geq$6.5%, or the use of blood glucose lowering agents. Current smokers were defined as those who smoked for a certain period of time within the past year. Positive family history of (coronary artery disease) CAD was defined as confirmed evidence of premature CAD in a first degree relative (men <55 and women <65 years of age). Individuals without clinical signs of heart failure were classified as Killip class I, individuals with rales or crackles in the lungs, an S3 gallop, and elevated jugular venous pressure were classified as Killip class II, individuals with significant acute pulmonary edema were classified as Killip class III, individuals with evidence of cardiogenic shock or hypotension (measured as systolic blood pressure < 90 mmHg), and evidence of low cardiac output (oliguria, cyanosis, or impaired mental status) were classified as Killip class IV. Body mass index (BMI) was calculated as weight

(kg)/height^2 (m^2). Thrombus burden observed before percutaneous coronary intervention was defined as low thrombus burden (LTB) with grade 1–3, and high thrombus burden (HTB) with grade 4–5 [22]. Angiographic no-reflow (NR) was defined as thrombolysis in myocardial infarction (TIMI) flow less than grade III in the infarct-related artery without dissection or spasm after successful percutaneous coronary intervention in epicardial coronary arteries [23]. CAD severity was determined by the SYNTAX score using a web calculator (https://www.syntaxscore.org). Left ventricular ejection fraction (LVEF) was measured by two experienced cardiologists with the modified Simpson method by echocardiography (Philips Epiq 7 device, Andover, MA, USA) during hospitalization.

Angiographic evaluation of thrombus burden

Angiographic thrombus burden in the infarct-related artery was classified according to the following TIMI grades: Grade 0: absence of angiographic thrombus, Grade 1: possible thrombus (reduced contrast, irregular contour at the site of total, smooth convex contour, or hazy appearance occlusion region), Grade 2: the largest thrombus size is <half the vessel diameter, Grade 3: the largest size of the thrombus is >1/2 of the vessel diameter but <2 vessel diameter, Grade 4: the largest thrombus size is >2 vessel diameter, Grade 5: total occlusion of the vessel due to thrombus. Patients with total occluded infarct-related artery (Grade 5 thrombus burden) were reclassified and scored after restoration of antegrade flow via guidewire or predilatation via balloon. Based on final thrombus burden, patients with grade 1–3 thrombus burden were classified as LTB and grade 4–5 thrombus burden as HTB [22]. All angiographic images were digitized for quantitative analysis (DICOM viewer, MedCom GmbH, Darmstadt, Germany) and were evaluated by at least two experienced invasive cardiologists and the final decision was made.

Periprocedural medication

Before the invasive procedure, all patients were given a loading dose of dual antiplatelet before pPCI and standard dose intravenous bolus unfractionated heparin (70–100 U/kg) was administered when an additional dose was required to achieve an active clotting time of >250 s during percutaneous coronary intervention. The choice to use a glycoprotein IIb/IIIa receptor inhibitor is left to the discretion of the operator according to hospital protocol. Tirofiban was administered at a 25 μg/kg intravenous loading dose. Those who were given bolus tirofiban during the procedure received a tirofiban infusion of 0.15 μg/kg/min for at least 18 h after the procedure.

Primary endpoint and short-term outcomes

The primary endpoint was the development of all-cause mortality within 30 days in STEMI patients undergoing pPCI.

Statistical analysis

Statistical analyses were performed using SPSS 26.0 software (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to evaluate whether the distribution of continuous variables was normal. Continuous variables were expressed as mean ± SD or median (interquartile range) and compared with Student's t or Mann–Whitney U tests according to distribution. Categorical variables were expressed as percentages and numbers and compared with the χ^2 test. In addition, univariable and multivariable regression analyses were performed to determine the independent predictors of HTB. The baseline variables for which evident significance ($P < 0.05$) was found by univariable analysis were included in the multivariable logistic regression analysis. To avoid multicollinearity, HCR was entered into two different multivariable models separately with the same cofounders as categorical and continuous variables. Receiver operating characteristic (ROC) curve analysis was used to calculate the best cut-off values of HCR, HbA1c and C-peptide for detecting patients with HTB. The best cut-off value obtained from the ROC curve of the HCR was taken as the cut-off value for categorizing the HCR values as high and low. To compare discriminant ability for HTB, ROC curves were compared pairwise between variables using the DeLong test using MedCalc 16 statistical software (MedCalc Software Ltd, Ostend, Belgium). Intra and interobserver variability was calculated using Cohen's kappa value. Univariate Cox proportional regression analysis was performed to identify predictors of short-term mortality in STEMI patients. Variables that were significant in the univariate Cox proportional regression analysis ($P < 0.05$) were included in the multivariate Cox proportional regression analysis. Four models were created to show the effects of possible confounders on the relationship between HCR level (as a continuous and categorical variable) and short-term mortality. The model 1 was unadjusted. Model 2 was adjusted for fasting plasma glucose, diabetes mellitus, insulin and oral antidiabetics usage. Based on model 2, model 3 was further adjusted for C-reactive protein, triglyceride, high-density lipoprotein cholesterol, peak CK-MB, peak Troponin- I, neutrophil count, platelet count, mean platelet volume and SYNTAX Score. Based on model 3, model 4 was further adjusted for C-peptide and HbA1c. Results of Cox regression analysis was presented as hazard ratio (HR) and 95% confidence interval (CI). At the same time, Kaplan–Meier survival curve was used to examine the difference in event-free survival rates between thrombus burden groups and HCR groups, and statistical significance was determined using the Log-rank test. A two-sided $P < 0.05$ was considered statistically significant.

Results

1202 patients who underwent pPCI due to STEMI were included in this study. The basic demographic, clinical

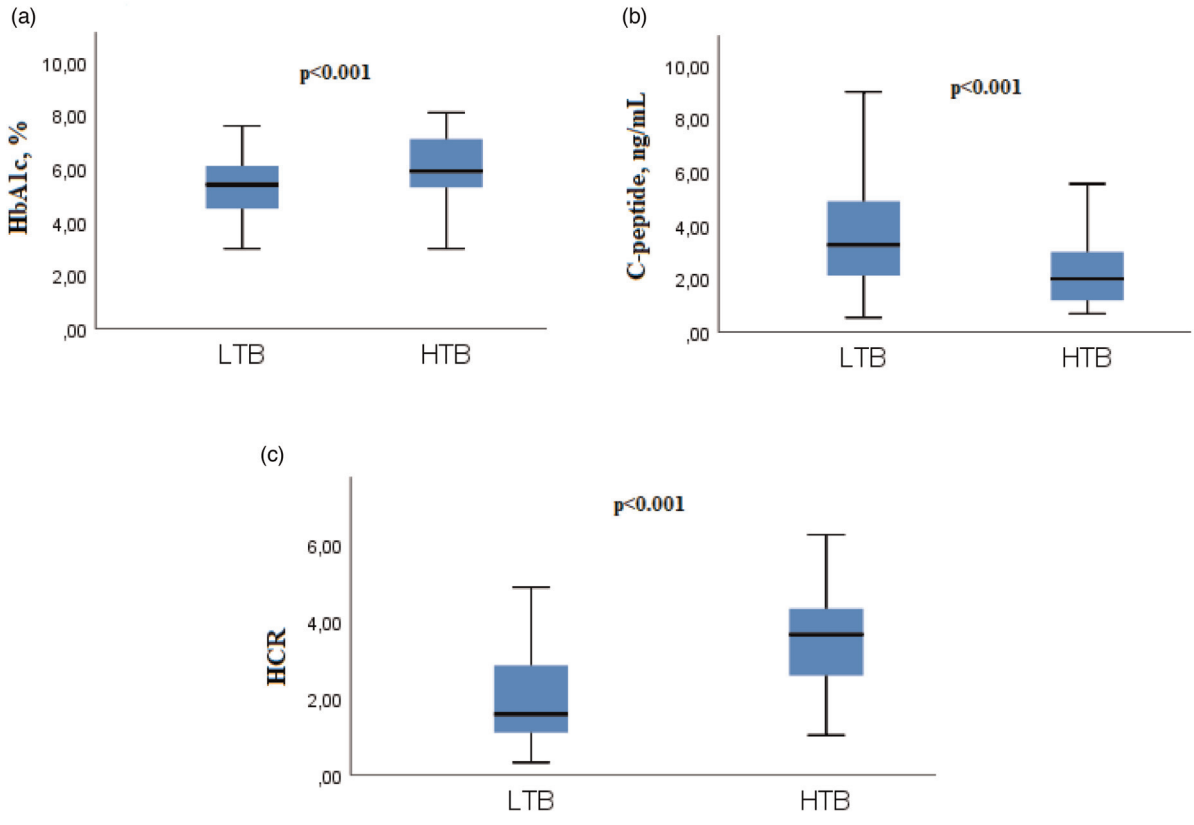
and laboratory characteristics of the subjects included in the study are given in Table 1 according to the categorization of the thrombus burden. The patients were divided into two groups according to their angiographic thrombus burden as those with LTB and those with high thrombus burden (HTB). 435 (36.2%) of the patients had HTB and 767 (63.8%) had LTB. There was no significant difference in demographic data such as age, gender and body mass index of the groups ($P > 0.05$, for all). Although there was no significant difference in the clinical histories of hypertension, dyslipidemia, smoking status, family history of CAD and atrial fibrillation ($P > 0.05$, for all), diabetes mellitus was significantly higher in the HTB group than in the LTB group (40.0% vs. 31.2%; $P = 0.002$). There was no significant difference in terms of hemodynamic properties of the groups ($P > 0.05$, for all). In HTB group, compared to LTB group, fasting blood glucose, triglycerides, C-reactive protein (CRP), neutrophil, mean platelet volume (MPV), peak CK-MB and peak Troponin-I levels were significantly higher, while high-density lipoprotein cholesterol (HDL-C) and platelet count were significantly lower ($P < 0.05$, for all). Although HCR and HbA1c were significantly higher in the HTB group than in the LTB group, C-peptide levels were lower ($P < 0.001$, for all) (Fig. 1). In addition, in the diabetic subgroup, HTB was observed

Table 1 Distribution of basic demographic, clinical and laboratory data according to angiographic thrombus burden degrees in STEMI patients

Variables	Low thrombus burden (LTB) ($n = 767$)	High thrombus burden (HTB) ($n = 435$)	*P*
Demographics and Medical history			
Age, years	59.6 ± 12.1	60.4 ± 10.5	0.270
Sex – male, *n* (%)	523 (68.2)	309 (71.0)	0.304
Body mass index, kg/m^2	26.5 ± 2.0	26.4 ± 1.8	0.389
Diabetes mellitus, *n* (%)	239 (31.2)	174 (40.0)	0.002
Hypertension, *n* (%)	504 (65.7)	293 (67.4)	0.562
Dyslipidemia, *n* (%)	273 (35.6)	179 (41.1)	0.056
Smoking, *n* (%)	480 (62.6)	368 (84.6)	0.418
Family history of CAD, *n* (%)	70 (9.1)	38 (8.7)	0.820
Atrial fibrillation, *n* (%)	39 (5.1)	17 (3.9)	0.352
Hemodynamic characteristics			
SBP (mmHg)	134.3 ± 19.1	133.7 ± 19.3	0.562
DBP (mmHg)	79.0 ± 13.9	78.7 ± 14.2	0.727
Heart rate, beats/min	87.6 ± 14.5	87.4 ± 15.2	0.746
LVEF, (%)	49 (40–60)	50 (45–60)	0.290
Killip class ≥ II, *n* (%)	175 (22.8)	101 (23.2)	0.873
Laboratory data			
Fasting plasma glucose, mg/dl	117 ± 36	122 ± 45	0.017
Creatinine, mg/dl	0.80 (0.70–0.96)	0.80 (0.70–1.00)	0.106
Uric acid, mg/dl	5.1 ± 1.5	5.2 ± 1.7	0.116
LDH, U/l	250 (208–334)	250 (213–335)	0.608
Albumin, g/dl	4.2 ± 0.4	4.1 ± 0.5	0.205
Total cholesterol, mg/dl	180 (144–206)	178 (145–201)	0.554
Triglycerides, mg/dl	138 (98–205)	152 (108–216)	0.002
HDL-C, mg/dl	35 (29–41)	33 (28–38)	0.044
LDL-C, mg/dl	105 (82–131)	109 (86–132)	0.542
CRP, mg/dl	1.10 ± 1.02	1.25 ± 1.22	0.023
e-GFR, ml/min	88.9 ± 19.0	87.9 ± 20.3	0.407
WBC (×1000/mm^3)	7.9 ± 3.6	8.3 ± 4.0	0.082
Lymphocyte, (×1000/mm^3)	2.07 ± 0.98	1.99 ± 0.75	0.109
Monocyte (×1000/mm^3)	0.60 (0.49–0.82)	0.60 (0.44–0.80)	0.080
Neutrophil (×1000/mm^3)	7.2 ± 3.7	7.7 ± 3.9	0.030
Hemoglobin, g/dl	13.5 ± 1.7	13.7 ± 1.8	0.085
Hematocrit, %	42.6 ± 5.7	43.2 ± 5.4	0.074
Platelet count (×1000/mm^3)	245 (210–298)	242 (189–295)	0.045
RDW, %	12.7 ± 1.3	12.6 ± 1.4	0.148
MPV, fl	7.64 (6.83–8.61)	7.91 (7.05–8.75)	0.003
Peak CK-MB, ng/ml	116.5 ± 43.9	121.7 ± 41.7	0.046
Peak Troponin I, ng/ml	19.7 ± 7.1	20.8 ± 7.1	0.017
C-peptide, ng/ml	3.5 ± 1.7	2.4 ± 1.5	<0.001
HbA1c, %	5.2 ± 1.2	5.9 ± 0.9	<0.001
HCR	2.0 ± 1.1	3.5 ± 1.2	<0.001
Preprocedure medication			
β-blocker, *n* (%)	128 (16.7)	69 (15.9)	0.710
Statins, *n* (%)	213 (27.8)	136 (31.3)	0.200
ACEI/ARB, *n* (%)	244 (31.8)	157 (36.1)	0.130
CCB, *n* (%)	105 (13.7)	56 (12.9)	0.690
Insulin, *n* (%)	108 (14.1)	83 (19.1)	0.023
Oral anti-diabetics, *n* (%)	113 (14.7)	42 (9.7)	0.012

Values are mean ± SD, *n* (%), or median (interquartile range) unless otherwise stated. ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BMI, body mass index; CAD, coronary artery disease; CCB, calcium channel blocker; CHF, congestive heart failure; CK-MB, creatine kinase–myocardial band; CRP, C-reactive protein; DBP, diastolic blood pressure; e-GFR, estimated glomerular filtration rate; HCR: HbA1c/C-peptide ratio; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LDH, lactate dehydrogenase; LVEF, Left-ventricular ejection fraction; MPV, mean platelet volume; RDW, red cell distribution width; SBP, systolic blood pressure; WBC, white blood cell.

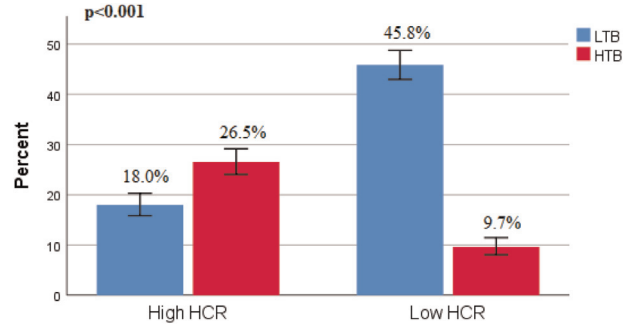
Fig. 1



Display of HbA1c, C-peptide and HCR values according to thrombus burden in box-plot chart. HCR, HbA1c/C-peptide ratio; HTB, high thrombus burden; LTB, low thrombus burden.

more frequently in subjects using insulin (19.1% vs. 14.1%, $P=0.023$), while the frequency of LTB was significantly higher in subjects using oral antidiabetic (14.7 vs. 9.7, $P=0.012$). When HCR was taken as a categorical variable, the frequency of HTB was significantly higher in high HCR than in low HCR levels (26.5% vs. 9.7%, $P<0.001$) (Fig. 2). In addition, high SYNTAX score, no-reflow development, distal embolization and thrombus aspiration were significantly higher in the HTB group than in the LTB group ($P<0.05$, for all) (Table 2). In the multivariable logistic regression analysis, when HCR was taken as a continuous variable (model 1), diabetes mellitus, neutrophil count, MPV, antidiabetics usage, HbA1c, C-peptide, and HCR were determined as independent potential predictors for HTB ($P<0.05$, for all) also when HCR was taken as a categorical variable (model 2), CRP, HDL-C, neutrophil count, MPV, antidiabetics usage, SYNTAX score, HbA1c, C-peptide, and HCR were determined as independent potential predictors for HTB ($P<0.05$, for all) (Table 3). When ROC curve analysis was performed, the optimal cut-off value of HCR to predict HTB was >2.76 with 73% sensitivity and 72% specificity [area under the curve (AUC): 0.813, 95% confidence interval (CI): 0.788–0.838, $P<0.001$] (Fig. 3). When HbA1c, C-peptide and HCR were pairwise compared, HCR was found to be superior capability to HbA1c and C-peptide to predicting the HTB [difference between area under the curves (ΔAUC): 0.160; 95% CI, 0.124–0.196; $P<0.0001$ &

Fig. 2



Frequency of high thrombus burden (HTB) and low thrombus burden (LTB) at high and low HCR values in bar graph. The frequency of high thrombus burden at high HbA1c/C-peptide ratio (HCR) values (>2.76) was significantly higher than at low HCR values (26.5% vs. 9.7%; $P<0.001$).

Table 2 Periprocedural and angiographic features of the patients according to thrombus burden

Variables	Low thrombus burden (LTB) ($n=767$)	High thrombus burden (HTB) ($n=435$)	*P*
Door to balloon time, min	57.3 ± 8.1	56.6 ± 8.1	0.145
SYNTAX score	13.7 ± 5.1	14.5 ± 5.3	0.010
Angiographic access route			
Radial access, *n* (%)	563 (73.4)	337 (77.5)	0.118
Femoral access, *n* (%)	204 (26.6)	98 (22.5)	
Infarct-related artery			
LAD and/or its branches, *n* (%)	305 (39.8)	201 (46.2)	0.127
Cx and/or its branches, *n* (%)	236 (30.8)	116 (26.7)	
RCA and/or its branches, *n* (%)	216 (28.2)	110 (25.3)	
LMCA, *n* (%)	10 (1.3)	8 (1.8)	
No-reflow, *n* (%)	93 (12.1)	82 (18.9)	0.001
Stent type			
DES, *n* (%)	715 (93.2)	392 (90.1)	0.055
BMS, *n* (%)	52 (6.8)	43 (9.9)	
Maximal stent diameter, mm	3.24 ± 0.39	3.22 ± 0.39	0.426
Maximal stent length, mm	30.3 ± 6.4	29.7 ± 6.5	0.162
Procedure type			
Only PTCA, *n* (%)	25 (3.3)	22 (5.1)	0.242
Direct stenting, *n* (%)	9 (1.2)	7 (1.6)	
PTCA+stenting, *n* (%)	733 (95.6)	406 (93.3)	
Occlusion localization			
Proximal, *n* (%)	310 (40.4)	185 (42.5)	0.751
Mid, *n* (%)	295 (38.5)	159 (36.6)	
Distal, *n* (%)	162 (21.1)	91 (20.9)	
Thrombus aspiration, *n* (%)	31 (4.0)	82 (18.9)	<0.001
Distal embolization, *n* (%)	9 (1.2)	16 (3.7)	0.003

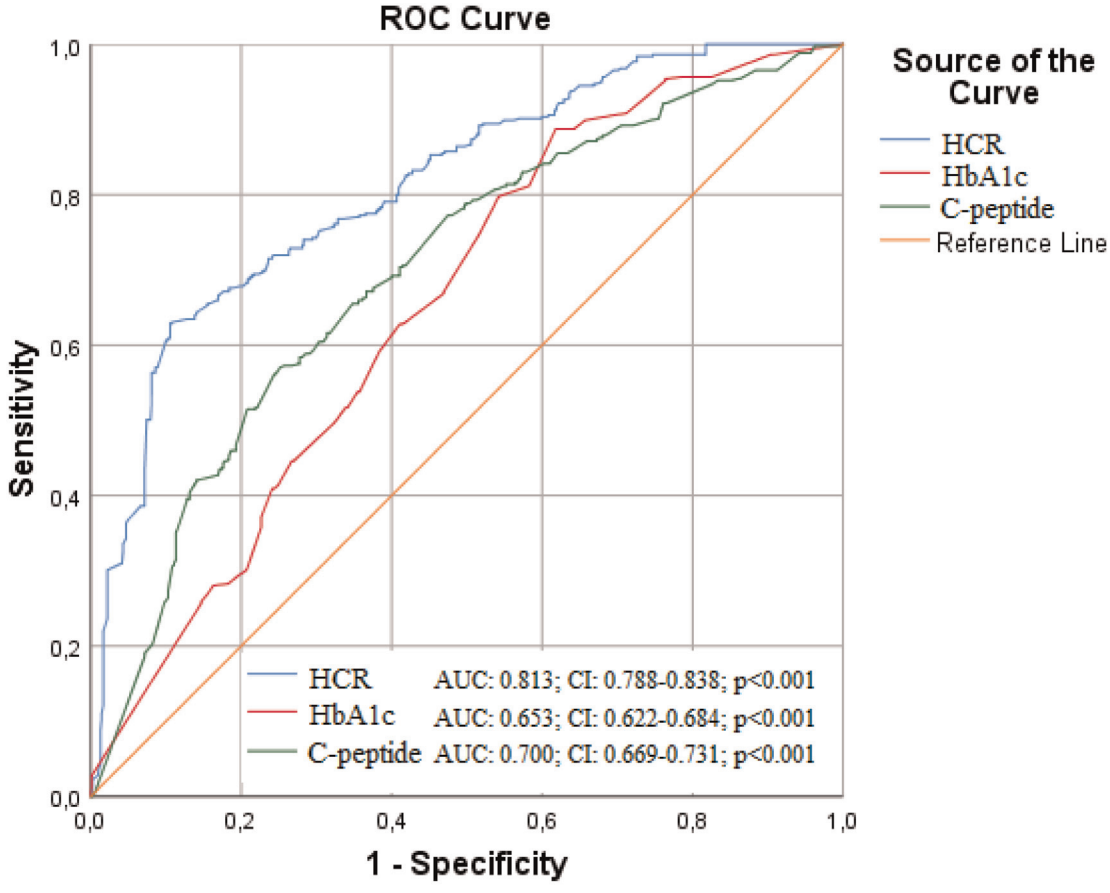
Values are mean ± SD or *n* (%) unless otherwise stated. BMS, bare-metal stent; Cx, circumflex artery; DES: drug-eluting stent, LAD, left anterior descending artery; LMCA, left main coronary artery; PTCA, percutaneous transluminal coronary angioplasty, RCA, right coronary artery.

Table 3 Univariable and multivariable regression analysis to identify independent predictors of high thrombus burden in STEMI patients

			Multivariable analysis	
	Univariable analysis		Nagelkerke R^2 in final step: 0.445	
Model 1	OR (95% CI)	*P*	OR (95% CI)	*P*
Diabetes Mellitus	1.473 (1.152–1.882)	0.002	1.380 (1.020–1.867)	0.037
Fasting plasma glucose	1.004 (1.001–1.001)	0.018	1.002 (0.999–1.006)	0.189
CRP	1.127 (1.016–1.251)	0.023	1.110 (0.973–1.267)	0.121
Triglycerides	1.001 (1.000–1.002)	0.029	1.001 (1.000–1.002)	0.057
HDL-C	0.973 (0.986–0.999)	0.041	0.988 (0.971–1.005)	0.179
Peak CK-MB	1.003 (1.000–1.006)	0.047	1.002 (0.999–1.006)	0.207
Peak troponin I	1.020 (1.004–1.037)	0.017	1.012 (0.992–1.033)	0.251
C-peptide	0.637 (0.585–0.693)	<0.001	0.684 (0.620–0.755)	<0.001
HbA1c	1.657 (1.482–1.854)	<0.001	1.313 (1.146–1.504)	<0.001
HCR (as a continuous variable)	2.607 (2.311–2.941)	<0.001	2.377 (2.090–2.704)	<0.001
Neutrophil count	1.034 (1.003–1.066)	0.030	1.045 (1.007–1.085)	0.020
Platelet count	0.999 (0.997–1.000)	0.150	-	-
MPV	1.140 (1.051–1.236)	0.002	1.152 (1.044–1.270)	0.005
Insulin usage	1.439 (1.051–1.970)	0.023	1.770 (1.199–2.614)	0.004
Oral anti-diabetics usage	0.602 (0.412–0.879)	0.009	0.567 (0.356–0.902)	0.017
SYNTAX score	1.030 (1.007–1.053)	0.010	1.022 (0.993–1.051)	0.136
Model 2				
Diabetes mellitus	1.473 (1.152–1.882)	0.002	1.219 (0.898–1.654)	0.204
Fasting plasma glucose	1.004 (1.001–1.001)	0.018	1.003 (1.000–1.007)	0.085
CRP	1.127 (1.016–1.251)	0.023	1.173 (1.036–1.329)	0.012
Triglycerides	1.001 (1.000–1.002)	0.029	1.001 (1.000–1.002)	0.098
HDL-C	0.973 (0.986–0.999)	0.041	0.983 (0.967–1.000)	0.047
Peak CK-MB	1.003 (1.000–1.006)	0.047	1.002 (0.999–1.005)	0.244
Peak troponin I	1.020 (1.004–1.037)	0.017	1.011 (0.991–1.031)	0.294
C-peptide	0.637 (0.585–0.693)	<0.001	0.686 (0.625–0.753)	<0.001
HbA1c	1.657 (1.482–1.854)	<0.001	1.438 (1.264–1.635)	<0.001
HCR (as a categorical variable)	7.015 (5.385–9.138)	<0.001	5.492 (4.115–7.331)	<0.001
Neutrophil count	1.034 (1.003–1.066)	0.030	1.043 (1.004–1.082)	0.029
Platelet count	0.999 (0.997–1.000)	0.150	-	-
MPV	1.140 (1.051–1.236)	0.002	1.152 (1.047–1.267)	0.004
Insulin usage	1.439 (1.051–1.970)	0.023	1.790 (1.225–2.617)	0.003
Oral Anti-Diabetics usage	0.602 (0.412–0.879)	0.009	0.569 (0.363–0.890)	0.013
SYNTAX Score	1.030 (1.007–1.053)	0.010	1.031 (1.003–1.061)	0.032

Values are mean ± SD, *n* (%), or median (interquartile range) unless otherwise stated. CK-MB, creatine kinase–myocardial band; CRP, C-reactive protein; HCR, HbA1c/C-peptide ratio; HDL-C, high-density lipoprotein cholesterol; MPV, mean platelet volume.

Fig. 3



Pairwise comparison of ROC curves for predicting thrombus burden

Compared Variables	ΔAUC	95% CI	p
HbA1c & C-Peptide	0.047	0.003-0.090	0.032
HCR & HbA1c	0.160	0.124-0.196	<0.0001
HCR & C-Peptide	0.113	0.074-0.151	<0.0001

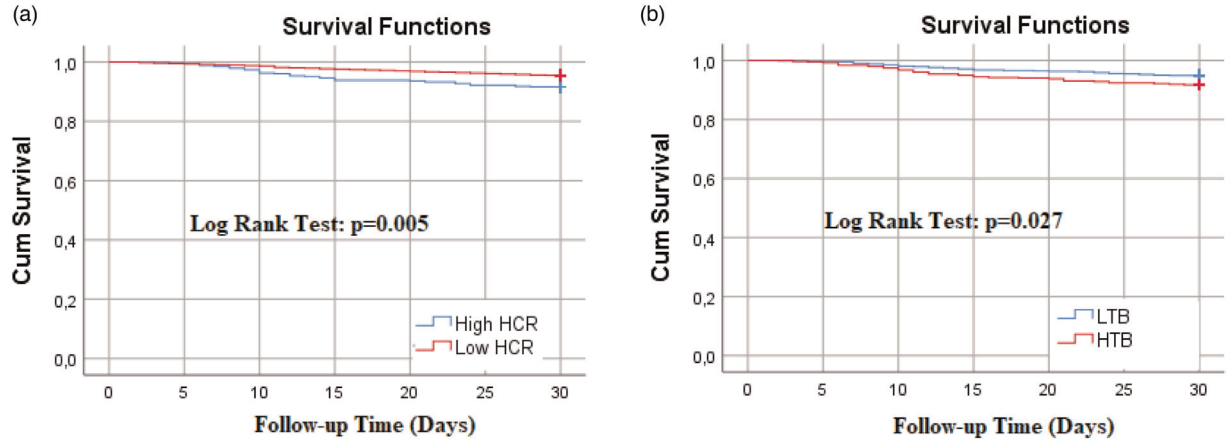
Receiver operating characteristic (ROC) curve analysis demonstrates the ability of the HbA1c, C-peptide and HCR to predict the high thrombus burden (HTB). HCR predicted HTB with 73% sensitivity and 72% specificity at the best cut-off value (2.76). When HbA1c, C-peptide and HCR were pairwise compared, HCR was found to be superior capability to HbA1c and C-peptide to predicting the HTB (ΔAUC: 0.160; 95% CI, 0.124–0.196; $P < 0.0001$ & ΔAUC: 0.113; 95% CI, 0.074–0.151; $P < 0.0001$; respectively). ΔAUC, difference between area under the curves; AUC, area under the curve; CI, confidence interval; HTB, high thrombus burden; LTB, low thrombus burden.

ΔAUC: 0.113; 95% CI, 0.074–0.151; $P < 0.0001$; respectively] (Fig. 3). Kaplan–Meier cumulative survival curves showed an increased risk of short-term mortality in those with high HCR and high thrombus burden (log-rank test: $P = 0.005$, log-rank test: $P = 0.027$; respectively) (Fig. 4a,b). The Cox proportional regression analysis for short-term mortality related with the HCR levels are presented in Table 4. Even after adjustment for multiple confounding factors (model 4), HCR was found to be an independent predictor of short-term mortality in STEMI patients, both as continuous and categorical variables [hazard ratio (HR) = 1.430, 95% CI: 1.218–1.678, $P < 0.001$ and HR = 1.926, 95% CI: 1.213–3.057, $P = 0.005$, respectively].

Discussion

The main finding of our study is that the HbA1c/C-peptide ratio was determined as an independent potential risk factor for high thrombus burden in STEMI patients regardless of the presence of diabetes mellitus, and its predictive value of high thrombus burden was superior to HbA1c and C-peptide alone. Moreover, high HCR was identified as an independent risk factor for

Fig. 4



Kaplan–Meier survival curves of short-term mortality in STEMI patients according to HbA1c/C-peptide ratio (a) and thrombus burden (b) categories. HCR, HbA1c/C-peptide ratio; HTB, high thrombus burden; LTB, low thrombus burden.

short-term mortality in STEMI patients, and high HCR predicts short-term mortality in these patients.

Acute coronary syndromes mainly occur as a result of endothelial and subendothelial substrates resulting from rupture or erosion of the atherosclerotic plaque, interacting with cellular and molecular prothrombotic substrates in the bloodstream, triggering an aggregation and coagulation cascade, forming thrombus and restricting antegrade coronary flow [1]. Among the acute coronary syndromes, an increased thrombotic process is observed in STEMI more than the others, and as a result, the clinical presentation becomes more noisy and serious [24]. Platelet activation and aggregation is the main determinant of thrombus formation and in patients with STEMI often results in complete occlusion of the coronary artery by thrombus [2,24]. In STEMI patients, high thrombus burden is associated with the development of re-infarction, no-reflow, increased myocardial infarct area, larger myocardial damage, stent thrombosis, and increased short- and long-term cardiovascular mortality [3–5,25]. For these reasons, due to the pivotal role of thrombus burden as the ultimate effector of coronary occlusion and ischemic injury, identifying modifiable risk factors for high thrombus burden and developing preventive strategies for it may contribute to surveillance of these patients by reducing the incidence of these adverse cardiovascular events.

Table 4 Hazard ratios based on Cox regression models to estimate the effects of HbA1c/C-peptide ratio (HCR) on short-term mortality in STEMI patients

	Short-term mortality	
	Hazard ratio (95% confidence interval)	*P*-value
HCR as a continuous variable		
Model 1	1.432 (1.221–1.679)	<0.001
Model 2	1.432 (1.221–1.679)	<0.001
Model 3	1.430 (1.218–1.678)	<0.001
Model 4	1.430 (1.218–1.678)	<0.001
HCR as a categorical variable		
Model 1	1.913 (1.205–3.036)	0.006
Model 2	1.913 (1.205–3.036)	0.006
Model 3	1.926 (1.213–3.057)	0.005
Model 4	1.926 (1.213–3.057)	0.005

Model 1, unadjusted; model 2, adjusted for fasting plasma glucose, diabetes mellitus, insulin and oral antidiabetics usage; model 3, further adjusted for C-reactive protein, triglyceride, high-density lipoprotein cholesterol, peak CK-MB, peak Troponin- I, neutrophil count, platelet count, mean platelet volume, SYNTAX score; model 4, further adjusted for C-peptide and HbA1c.

Diabetes mellitus is a prothrombotic disease that produces a hypercoagulable state [6–8]. It mainly contributes to this situation by increasing endothelial dysfunction and platelet activation and aggregation [26]. Although various mechanisms are involved in platelet hyperactivation and aggregation in these patients, elevated glycosylated hemoglobin (HbA1c) plays an important role in these patients [13–16]. It has also been shown that HbA1c is an important prognostic marker in nondiabetics [9]. It has been shown that HbA1c triggers this prothrombotic process by various mechanisms. It has been suggested that HbA1c contributes to this interaction by inhibiting the production of nitric oxide (NO), which inhibits platelet activation and adhesion of platelets to the vascular endothelium by increasing cytoplasmic cyclic guanosine monophosphate, mediated by β-adrenoceptors in platelets or in surrounding endothelial cells [15]. Lowering HbA1c has also been shown to reduce platelet hyperreactivity in patients with poor glycemic controlled acute coronary syndrome [16]. In a meta-analysis, it has been shown that HbA1c levels increase the risk of thrombotic processes such as target vessel revascularization requirement and nonfatal myocardial infarction in patients undergoing PCI [27]. In addition, HbA1c increases the contribution of

erythrocytes to the thrombotic process as a result of decreased deformability of glycosylated erythrocytes due to increased oxidative stress [28,29]. In a recent small cohort study by Shah *et al.* [30], it was shown that increased glycated hemoglobin level was positively correlated with thrombus score in both diabetic and nondiabetic patients, and these findings were consistent with the results of our study.

C-peptide is a pancreatic reserve marker that is released from beta cells of the pancreas in equal molar amounts with insulin as a result of cleavage of proinsulin, and plays a bioactive role in many molecular pathways [10,11]. Since its half-life is longer than insulin, it is currently a useful and widely used method used in clinical practice to determine the cause of hypoglycemia, to manage diabetes treatment, to confirm whether type 1 or type 2 diabetes is present if the diagnosis is not certain, monitoring pancreatic beta cell function, detection of absolute insulin deficiency, identification of patients with maturity-onset diabetes of the young and to monitor some pancreatic tumors [31]. Contrary to HbA1C, C-peptide has been well demonstrated in studies to show antithrombotic properties by making positive contributions to microvascular blood flow and blood hemorheology by a number of mechanisms [32–34]. It has been shown that high doses of C-peptide can reduce thrombus formation [17]. NO is a molecule that plays a pivotal role in inhibiting the activation and aggregation of platelets, and studies have shown that it mediates many bioactive effects of C-peptide [35]. It has been shown that NO production induced by C-peptide may be beneficial for cardiovascular complications by reducing the interaction of erythrocytes and leukocytes with endothelium in vitro [19,20]. It has been shown that by stimulating Na^+, K^+-ATPase activity and nitric oxide (NO) production, it can increase the microvascular blood flow of the skin in T1DM patients [36]. In contrast to HbA1c, C-peptide has been shown to improve erythrocyte deformability by increasing Na^+, K^+-ATPase activity [33]. In an in vitro study involving immobilized endothelial cells, it was shown that C-peptide can inhibit the adhesion of platelets to the endothelium in the presence of erythrocytes [37]. In another study, C-peptide was shown to induce NO production in both endothelium and platelets by stimulating adenosine triphosphate (ATP) production in erythrocytes [38]. It has been shown that healthy human erythrocytes were incubated with C-peptide can induce NO production in bovine pulmonary artery endothelial cells compared to untreated cells [39]. C-peptide has been demonstrated in several studies to reverse NO depletion and the consequences of its deficiency in many tissues [18,31–34]. Endothelial dysfunction is associated with decreased NO production and increased ROS production, leading to impaired blood flow and predisposition of blood vessels to atherogenesis and thrombosis [40]. C-peptide has been shown to reduce hyperglycemia-induced endothelial apoptosis and reduce the formation of NADPH oxidase-induced reactive oxygen specimens (ROS) in the human aorta [41]. In addition, C-peptide has been shown to reduce ROS generation through AMPKα activation and VEGF inhibition [42,43]. In contrast to C-peptide, ROS production is increased in chronic hyperglycemic conditions determined by HbA1c [44]. It has been shown by Scalia *et al.* [45] that C-peptide inhibits endothelial cell adhesion molecules and induces NO production. Haidet *et al.* [46] showed that C-peptide reduces the activation of monocytes.

Another result of our study was that the incidence of high thrombus burden was significantly higher in diabetic patients receiving insulin therapy compared to those using oral antidiabetic drugs. Considering that insulin therapy is usually initiated in cases where pancreatic reserves and thus C-peptide production are decreased and glycemic control is not sufficient, this supports our postulation regarding the effect of the balance between HbA1c and C-peptide on thrombus burden. As a matter of fact, a number of studies support this suggestion [47,48]. C-peptide increases NO production in endothelial cells by inducing low O_2-induced ATP release in erythrocytes, and it has been shown that hyperglycemia-induced C-peptide resistance in erythrocytes is reversed by the oral antidiabetic metformin [49].

When all these results are evaluated together, the balance between the prothrombogenic milieu created by HbA1c and the antithrombogenic effects of C-peptide, on the contrary, is closely related to the thrombus burden in patients with STEMI. Moreover, the marker obtained by proportioning Hba1c and C-peptide in a single fraction has a better predictive value than the prothrombogenic-antithrombogenic balance represented by Hba1c and C-peptide alone in predicting susceptibility to high thrombus formation and short-term mortality.

Our study had some limitations. First of all, our study design was retrospective and studied on a relatively small sample. Second, the assessment of thrombus load was based solely on visual assessment, although it was evaluated by at least two independent cardiologists. Third, Hba1c and C-peptide assessments were made on only one measurement. Fourth, only STEMI patients from acute coronary syndromes were studied. Fifth, although consecutive patients were tried to be included, patients who were not included in the study due to lack of data may affect the results, so bias cannot be completely ignored. Finally, long-term follow-up was not performed.

Conclusions

The ratio of HbA1c/C-peptide strongly predicted thrombus burden in STEMI patients independent of diabetes. It also predicted well short-term mortality in this patient group. In this context, the ratio of HbA1c/C-peptide can be used as an easily obtainable, inexpensive and easily

reproducible prothrombotic biomarker in risk stratification of STEMI patients. Studies with larger cohorts are needed to confirm the results of our study.

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Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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