

Urinary 11-Dehydro-Thromboxane B₂ as a Predictor of Acute Myocardial Infarction Outcomes: Results of Leukotrienes and Thromboxane In Myocardial Infarction (LTIMI) Study

Wojciech Szczeklik, MD, PhD; Edyta Stodólkiewicz, MD; Marcin Rzeszutko, MD, PhD; Marek Tomala, MD, PhD; Anton Chrustowicz, MD, PhD; Krzysztof Żmudka, MD, PhD; Marek Sanak, MD, PhD

Background—Urinary 11-dehydro-thromboxane (TX)B₂ has been described as a potential predictive biomarker of major adverse cardiovascular events (MACEs) in high cardiac risk patients. This part of LTIMI (Leukotrienes and Thromboxane In Myocardial Infarction) study aimed to evaluate the relationship between 11-dehydro-TXB₂ and MACEs in patients with acute myocardial infarction (AMI).

Methods and Results—LTIMI was an observational, prospective study in 180 consecutive patients with AMI type 1 referred for primary percutaneous coronary intervention. On admission and at follow-up visits (1 month, 1 year), 11-dehydro-TXB₂ was measured in urinary samples by using high-performance liquid chromatography–tandem mass spectrometry. The primary outcome was occurrence of composite MACEs during 1-year after AMI. Left ventricular ejection fraction was assessed in echocardiography on admission and at 1-year follow-up. Analyses of 11-dehydro-TXB₂ (pg/mg creatinine) were performed on log-transformed data and expressed as median with IQR (Q1–Q3). 11-Dehydro-TXB₂ level on admission was 7.39 (6.85–8.01) and decreased at 1 month (6.73, 6.27–7.12; $P<0.001$) and 1-year follow-up (6.37, 5.91–6.94; $P<0.001$). In univariate analysis, baseline 11-dehydro-TXB₂ was higher in patients with MACEs ($n=60$; 7.73, 7.07–8.60) compared with those without MACEs ($n=119$; 7.28, 6.68–7.79; $P=0.002$). In multivariate regression model, 11-dehydro-TXB₂ and 3 other variables (diabetes, multivessel disease, and left ventricular ejection fraction) were found to be best 1-year cumulative MACE predictors with odds ratio for 11-dehydro-TXB₂ of 1.58 (95% CI 1.095–2.33; $P=0.017$) and area under the curve (in receiver operating characteristic analysis of 0.8). Baseline 11-dehydro-TXB₂ negatively correlated with both left ventricular ejection fraction on admission ($R=-0.21$; $P=0.006$) and after 1 year ($R=-0.346$; $P<0.001$).

Conclusions—11-Dehydro-TXB₂ predicts 1-year cumulative MACEs in AMI patients and provides prognostic information on the left ventricular performance. (*J Am Heart Assoc.* 2016;5:e003702 doi: 10.1161/JAHA.116.003702)

Key Words: atherosclerosis • complication • inflammation • myocardial infarction • risk factor • thromboxane

Thromboxane (TX)A₂ is a potent platelet agonist and vasoconstrictor¹ produced mostly by platelets, which play a pivotal role in thrombogenesis at sites of vulnerable

plaque and provide a strong rationale for blocking their function in the setting of acute myocardial infarction (AMI).^{1,2}

However, platelets produce only 70% of TX in human body, and the remaining 30% is produced by extraplatelet sources such as monocytes and macrophages and may increase in acute inflammation.³ This unrecognized fact may translate into the unacceptable rates of clinical events such as myocardial infarction, cardiovascular death, and need for repeat revascularization, despite proper antiplatelet therapy.

TXA₂ is an unstable compound with short plasma half-life of ≈30 seconds and therefore is difficult to measure.⁴ TXA₂ is rapidly inactivated to TXB₂ and further undergoes dehydrogenation to 11-dehydro-TXB₂.^{5,6} The latter is a stable metabolite excreted in urine, and its measurement provides a reliable estimate of the total in vivo production of TXA₂ including the extraplatelet sources.^{7,8} 11-Dehydro-TXB₂ has been found to be elevated in several atherothrombotic diseases and to correlate with major adverse clinical events.^{9–14} However, none of the

From the Departments of Medicine (W.S., M.R., M.S.) and Interventional Cardiology (E.S., M.T., K.Ż.), Jagiellonian University Medical College, Krakow, Poland; Noninvasive Cardiovascular Laboratory, John Paul II Hospital, Krakow, Poland (A.C.).

Accompanying Table S1, Figures S1 and S2, and Data S1 are available at <http://jaha.ahajournals.org/content/5/8/e003702/DC1/embed/inline-supplementary-material-1.pdf>

Correspondence to: Wojciech Szczeklik, MD, PhD, Department of Medicine, Jagiellonian University Medical College, ul Skawińska 8, 31–066 Krakow, Poland. E-mail: wojciech.szczeklik@uj.edu.pl

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studies have evaluated the association of 11-dehydro-TXB₂ with patients' long-term outcomes in AMI.

The LTIMI (Leukotrienes and Thromboxane In Myocardial Infarction) study was a prospective, observational study that aimed to assess the relationship between arachidonic acid derivatives and major adverse cardiovascular events (MACEs) in patients with AMI. We report TX biosynthesis evaluated by urinary 11-dehydro-TXB₂ excretion during AMI and after 1-year observation period, analyzing its impact on patient's prognosis.

Methods

Study Population

The LTIMI study was conducted between July 2011 and September 2014. The study personnel evaluated 254 consecutive patients with ST-segment elevation (STEMI) and non-ST-segment elevation AMI (NSTEMI) who were transferred to interventional cardiology reference center for urgent coronary angiography and percutaneous coronary intervention (PCI). Only patients with MI type I (atherothrombotic, spontaneous) and symptoms lasting no longer than 24 hours were eligible for the study. In the setting of NSTEMI, most of the patients underwent immediate invasive strategy (0–2 hours) or early invasive strategy (<24 hours of diagnosis); none underwent delayed invasive strategy (within 24–72 hours of diagnosis) in the setting of NSTEMI. All patients with STEMI received immediate PCI without previous fibrinolysis.

Type of AMI was based on the Joint European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Heart Federation Universal Definition^{15,16} by 2 cardiologists who were aware of all patients' clinical data including coronary angiography (CA) findings. Patients naive to antiplatelets were treated with 300 mg non-enteric-coated aspirin and 300 to 600 mg of clopidogrel in the ambulance or the emergency department. If the patients were taking aspirin on a long-term basis, they received a lower dose of 75 mg (not enteric coated); the same was true for clopidogrel (75-mg dose was used). Exclusion criteria were late presentation of AMI (>24 hours from first symptoms), AMI other than type I, cardiogenic shock, history of coronary artery bypass graft surgery (CABG), severe valvular heart disease, symptoms of acute infection, asthma, chronic obstructive pulmonary disease exacerbation, use of antileukotriene medications, chronic kidney disease, liver cirrhosis, malignancy, patient's refusal to participate in the study (<2% of patients), and noncompliance. Patients who received antiplatelet drugs other than aspirin and clopidogrel before or after PCI or were enrolled in any other interventional trial were excluded from the study. The latter was the most common exclusion criterion. A study flow chart is presented on Figure 1.

Finally, 180 patients who gave written informed consent were recruited to the study, and 171 completed the 1-year follow-up period. The study protocol complied with the Helsinki Declaration and was approved by the Jagiellonian University ethics committee.

Baseline Characteristics

Data on comorbidities, demographic characteristics, and history of presenting complaints were collected by research staff. Standard laboratory tests were performed including serial high-sensitivity troponin T measurements (hs-TnT, Roche Diagnostics). Blood and urine samples were collected from each participant before CA and stored immediately after centrifugation at –70°C for further analysis.

TX Measurements

11-Dehydro-TXB₂ was measured at the presence of identical deuterated standard added to the urine sample before high-performance liquid chromatography–tandem mass spectrometry measurement, as previously described.¹⁷ Results are expressed in picograms per milligram creatinine as natural logarithms. Detailed methods are described in Supplementary Materials.

Transthoracic Echocardiography

Standard transthoracic echocardiography was performed in all patients on admission because of AMI and at 1-year follow-up. Apical, parasternal, and subcostal views were used according to current guidelines¹⁸ with use of the Vivid 7 ultrasound system (GE Vingmed Ultrasound A/S). Left ventricular ejection fraction (LVEF) was calculated by using Simpson's method. Global left ventricular dysfunction was defined as LVEF <55%. For statistical comparisons, a patient's LVEF was stratified as slightly impaired (45–54%), moderately impaired (30–44%), or severely impaired (<30%). Two experienced cardiologists-echocardiographers assessed images independently, unaware of 11-dehydro-TXB₂ results.

CA and PCI

CA was performed by using the standard Seldinger technique, with the patient under local anesthesia, via puncture of the femoral or radial artery, with the use of typical diagnostic and guiding catheters. Arterial access was selected at the discretion of the operator. All patients were given the same type of low-osmolar and nonionic iodinated contrast media (Visipaque 320 GE Healthcare Inc). Immediately after CA, the results were analyzed and patients were qualified for immediate PCI with angioplasty and stent implantation of the culprit

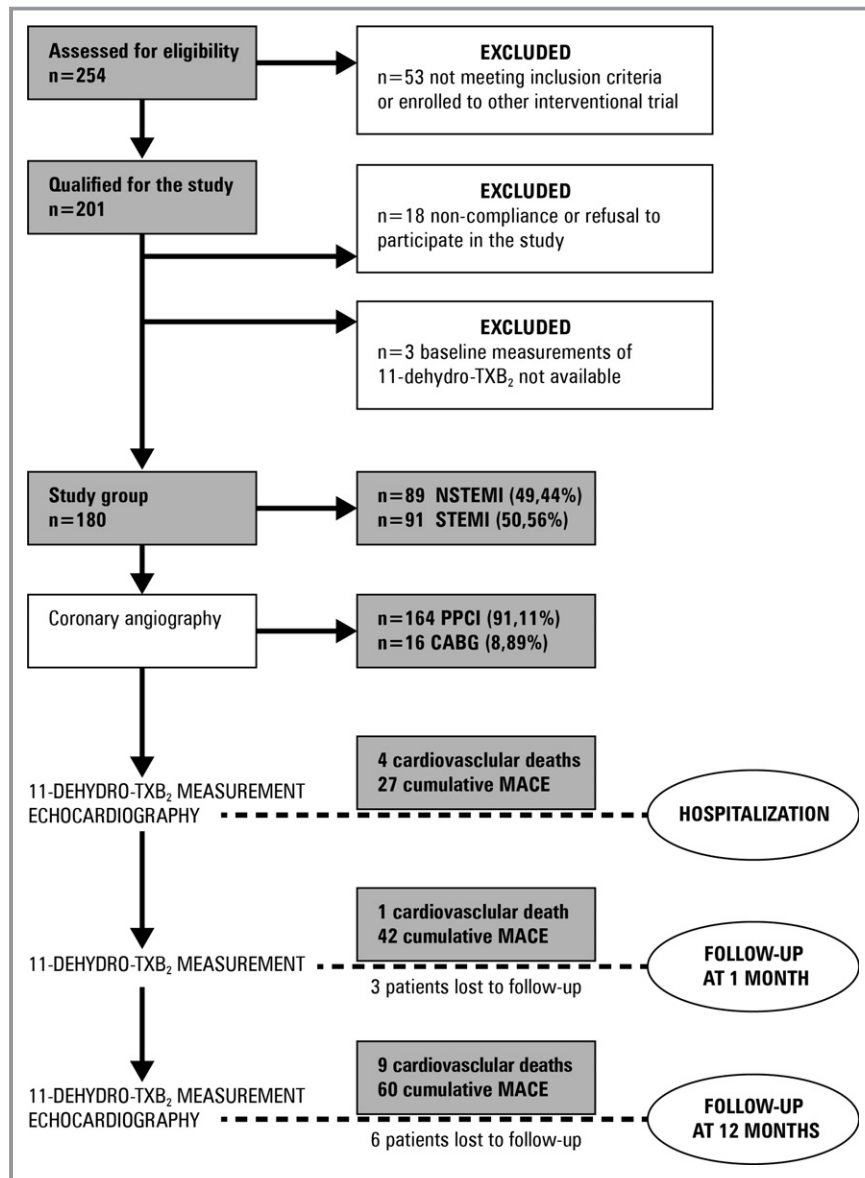


Figure 1. Study flow chart. CABG indicates coronary artery bypass graft surgery; NSTEMI, non-ST-elevation myocardial infarction; PPCI, primary percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction.

vessel or referred for urgent CABG. The type of stent and its diameter and length were selected on the basis of angiographic measurements assisted with digital quantitative angiography (Axiom ZEE angiograph, computer software VC21B; Siemens). Control angiography was performed after stent implantation to document and assess obtained result.

Final Outcomes Measurements and Follow-up

Study outcome measurements

The primary outcome was occurrence of a composite MACE that occurred during a 1-year follow-up period after AMI. This composite MACE consisted of 8 events: recurrent myocardial

infarction, stroke/transient ischemic attack, cardiogenic shock, pulmonary edema, nonfatal cardiac arrest, need for rescue PCI or CABG, and cardiovascular death. If 2 MACEs occurred in 1 patient (eg, recurrent MI and death in follow-up), it was counted as 1 cumulative MACE. Definitions are available in Supplemental Materials.

Secondary outcomes included (1) composite MACE at the following time points: discharge from hospital and at 1-month follow-up; and (2) LVEF measured on admission and at 1-year follow-up.

Two cardiologists-echocardiographers adjudicated all events independently and were blinded to the 11-dehydro-TXB₂ results while performing the clinical follow-up.

Follow-up

Patients were observed for a 1-year period after enrollment in the study. Two visits were scheduled in the outpatient clinic, at 1 month and 1 year after AMI. The data on the occurrence of MACEs were collected by the research team and verified based on either hospital discharge notes or family member interrogation. Urine samples were collected at both visits for 11-dehydro-TXB₂ analyses.

Statistical Analysis

Categorical variables were presented as counts (percentages), whereas continuous variables were reported as mean±SD or median with IQR (Q1–Q3) depending on their distribution. Urinary 11-dehydro-TXB₂ data were naturally log-transformed to approximate normal distribution for further analysis and expressed throughout the text as median with IQR (Q1–Q3). Assumption of normality was verified by using the Kolmogorov–Smirnov test. Categorical variables were compared between MACE and non-MACE groups by using χ^2 test or Fisher's exact test, and continuous variables were compared by using Student *t* test or Mann–Whitney *U* test as appropriate. Correlations between variables were analyzed by using Pearson's or Spearman's rank correlation, as appropriate for the data distribution. For analysis of 11-dehydro-TXB₂ in urine at different time points during follow-up, 1-way ANOVA for repeated measures and the post-hoc Tukey test were used.

For comparisons of LVEF with 11-dehydro-TXB₂ levels, the Kruskal–Wallis test was used with post-hoc Mann–Whitney comparison adjusted with the Bonferroni correction.

Associations between 11-dehydro-TXB₂ and MACEs after PCI were estimated as an odds ratio (OR) and corresponding 95% CI by using univariate logistic regression analysis.

The associations of 11-dehydro-TXB₂, cardiac biomarkers, inflammatory parameters, and composite MACE were tested after division into quartiles according to the 11-dehydro-TXB₂ distribution. The diagnostic performance of 11-dehydro-TXB₂ for the prediction of cumulative MACEs was evaluated by using receiver operating characteristic curve analysis, and the optimal threshold was based on the point of combined best sensitivity/specificity results.

Finally, the association between 11-dehydro-TXB₂ and cumulative MACE incidence was evaluated with use of the multiple logistic regression. Explanatory variables were chosen in stepwise approach on the basis of the Akaike information criterion based on the prespecified baseline clinical and laboratory characteristics. Variables included age, sex, body mass index, smoking (pack-years), past history of MI, diabetes mellitus, hypertension, time from symptoms to PCI, multivessel disease, LVEF during hospitalization, maximal

TnT level, C-reactive protein (CRP), glomerular filtration rate, and 11-dehydro-TXB₂.

All statistical analyses were performed by using R software, version 3.1.2 (R Development Core Team [2009]. R: A language and environment for statistical computing. R Foundation for Statistical Computing). *P*-values <0.05 were considered statistically significant.

Results

Patients

One hundred eighty patients with type I AMI (50.6% with STEMI and 49.4% with NSTEMI) who underwent CA on hospital admission were found to be eligible for the study (Figure 1). Time from symptom onset to the PCI was 9.23±6.71 hours. Mean age of the study cohort was 66.48±11.87 years, and men outnumbered women (69.4% versus 30.6%). Detailed demographic parameters and cardiovascular risk factors are shown in Table 1. On admission, all patients had elevated hs-TnT, 52.8% presented with increased leukocyte count >10⁴/μL, and 46.1% had high-sensitivity CRP elevated above the normal range. In 164 patients (91.1%), PCI was performed, and 16 patients (8.9%) required surgical revascularization (CABG). The majority of the patients (n=117; 65%) had at least 2-vessel disease, and mean LVEF measured on admission was 44.7±13.8%.

Aspirin and clopidogrel were administered to all patients at least 30 minutes before the urine sample collection. In the studied group, 54 (30%) patients received aspirin on a long-term basis before admission, and 11 (6.1%) additional patients were taking clopidogrel. By discharge from the hospital, all patients were taking aspirin, and 94.4% were taking clopidogrel (except 10 patients scheduled for CABG).

Follow-up MACEs

At 1-year follow-up, the composite MACE appeared in 60 patients (33.3%), and of this group, 14 (7.8%) patients died. All MACEs with their time occurrence are listed in Table S1. Patients with MACEs compared with patients without MACEs more frequently had multivessel disease, diabetes, and peripheral artery disease, whereas there were fewer current smokers. Additionally, in the MACE group, patients presented with higher admission levels of glucose (7.85 [6.27–10] versus 6.8 [5.8–8.3] mg/L; *P*=0.012) and lower triglyceride levels (1.05 [0.81–1.56] versus 1.3 [0.94–1.67] mmol/L; *P*=0.044). Comparisons of clinical and laboratory data are shown in Tables 1 and 2.

The baseline LVEF was 44.71±13.79% and increased at 1-year follow-up to 55.68±10.62% (*P*<0.001), being lower at both time points in patients with MACEs.

Table 1. Baseline Characteristic of the Study Group by MACE and Non-MACE Subgroup

Characteristic	Overall (n=180)	MACE (n=60)	Non-MACE (n=120)	P Value
Demographic characteristics				
Age, y (%)				
<55	36 (20)	8 (13.3)	28 (23.3)	0.126
55–75	98 (54.4)	32 (53.3)	66 (55)	
>75	46 (25.6)	20 (33.3)	26 (21.7)	
Female sex	55 (30.6)	22 (36.7)	33 (27.5)	0.277
Body mass index, kg/m ² (%)				
Normal weight (18.5–24.99)	60 (33.3)	19 (31.7)	41 (34.2)	0.877
Overweight (25–29.99)	76 (42.2)	25 (41.7)	51 (42.5)	
Obesity (>30)	44 (24.4)	16 (26.7)	28 (23.3)	
Medical history				
Hypertension, n (%)	128 (71.1)	46 (76.7)	82 (68.3)	0.323
Diabetes mellitus, n (%)	61 (33.9)	33 (55)	28 (23.3)	<0.001
Dyslipidemia, n (%)	147 (86.5)	50 (89.3)	97 (85.1)	0.608
Myocardial infarction, n (%)	48 (26.7)	21 (35)	27 (22.5)	0.108
TIA/stroke, n (%)	13 (7.2)	5 (8.3)	8 (6.7)	0.762
PAD/ Claudication, n (%)	37 (20.6)	18 (30)	19 (15.8)	0.043
Family history of CAD, n (%)	78 (43.3)	29 (48.3)	49 (40.8)	0.425
Current smoker, n (%)	68 (37.8)	16 (26.7)	52 (43.3)	0.044
Clinical evaluation				
Diagnosis				0.792
NSTEMI, n (%)	89 (49.4)	31 (51.7)	58 (48.3)	
STEMI, n (%)	91 (50.6)	29 (48.3)	62 (51.7)	
CAD, n (%)				<0.001
1-Vessel disease	63 (35)	9 (15)	54 (45)	
2-Vessel disease	59 (32.8)	19 (31.7)	40 (33.3)	
3-Vessel disease	58 (32.2)	32 (53.3)	26 (21.7)	
LVEF during AMI, n (%)				0.002
Normal (≥55)	55 (30.6)	11 (18.3)	44 (36.7)	
Mildly impaired (45–54)	45 (25)	14 (23.3)	31 (25.8)	
Moderately impaired (30–44)	57 (31.7)	20 (33.3)	37 (30.8)	
Severely impaired (<30)	23 (12.8)	15 (25)	8 (6.7)	

AMI indicates acute myocardial infarction; CAD, coronary artery disease; LVEF, left ventricular ejection fraction; MACE, major adverse cardiovascular event; NSTEMI, non-ST-elevation myocardial infarction; PAD, peripheral artery disease; STEMI, ST-elevation myocardial infarction; TIA, transient ischemic attack.

Urinary TX Metabolite Excretion

All analyses were performed on log-transformed data. The highest level of 11-dehydro-TXB₂ was on admission, 7.39 (6.85–8.01), and decreased in the follow-up after 1 month to 6.73 (6.27–7.12) and after 1 year to 6.37 (5.91–6.94) pg/mg creatinine. The decline was statistically significant between each follow-up level (1 month and 1 year) compared with the admission sample (*P*<0.001; Figure 2).

Patients taking aspirin and/or clopidogrel on a long-term basis had lower admission 11-dehydro-TXB₂ (7.12 [6.68–7.59] versus 7.57 [6.94–8.15] pg/mg creatinine; *P*=0.005).

11-Dehydro-TXB₂ levels were higher in STEMI patients than in those with NSTEMI (7.59 [6.97–8.14] versus 7.24 [6.55–7.7] pg/mg creatinine, respectively; *P*=0.013). There were no differences in 11-dehydro-TXB₂ levels adjusted for age and sex.

Table 2. Baseline Laboratory Parameters Characteristic of the Study Group on Admission

Parameter	Normal Values	Overall (n=180)	MACE (n=60)	Non-MACE (n=120)	P-Value
hs-TnT on admission, µg/L	<0.014	0.14 (0.06–0.33)	0.19 (0.05–0.46)	0.14 (0.06–0.28)	0.14
hs-TnT max, µg/L		1.25 (0.29–4.65)	0.92 (0.41–4.96)	1.28 (0.24–4.51)	0.88
CK-MB on admission, U/L	0–24	21 (15–42.3)	23 (16.8–42.3)	20.5 (15–40.8)	0.41
CK-MB max, U/L		66.5 (26–187)	52 (29.8–154.8)	71.5 (23–206.5)	0.76
CK on admission, U/L	0–190	182 (104–341.3)	188.5 (110.75–369.3)	179 (103–335)	0.4
CK max, U/L		623 (215–1756)	643.5 (282.3–1445.8)	605 (201.5–1816.5)	0.7
hs-CRP, mg/L	≤3.0	2.8 (1.1–6.4)	3.95 (1.21–7)	2.5 (1.05–5.7)	0.16
WBC, ×10 ³ /µL	3.8–10	10.1 (8.1–12.2)	10.3 (8.4–13.7)	10.1 (7.9–11.9)	0.26
RBC, ×10 ⁶ /µL	4.2–6.0	4.7 (4.4–4.97)	4.59 (4.1–4.9)	4.8 (4.5–4.99)	0.07
Hb, g/dL	14–18	14.2 (13.2–15.1)	14 (12.8–15.2)	14.4 (13.4–15.1)	0.28
PLT, ×10 ³ /µL	140–440	221.5 (181.8–263.3)	218.5 (164–257.3)	222.5 (188.5–264.3)	0.43
GFR, mL/min per 1.73 m ²	>60	74.8 (56.6–87.8)	67.3 (55.42–84.9)	78 (57.1–88.2)	0.08
Creatinine, µmol/L	62–106	84 (76.8–103)	86.5 (78–104.8)	83.5 (75–101)	0.33
Glucose, mmol/L	3.4–5.6	7 (6–8.6)	7.9 (6.27–10)	6.8 (5.8–8.3)	0.012
TC, mmol/L	3.1–5.0	4.7 (4.06–5.52)	4.42 (3.64–5.22)	4.85 (4.18–5.6)	0.15
LDL, mmol/L	<3.0	3.01 (2.4–3.9)	2.67 (2.0–3.6)	3.09 (2.5–3.9)	0.1
HDL, mmol/L	>1.0	1.2 (1.01–1.39)	1.2 (1.04–1.45)	1.2 (1.01–1.37)	0.3
TG, mmol/L	<1.7	1.2 (0.9–1.6)	1.1 (0.8–1.6)	1.3 (0.9–1.7)	0.044

CK indicates creatine kinase; CK-MB, creatine kinase isoform; GFR, glomerular filtration rate; Hb, hemoglobin; HDL, high-density lipoprotein; hs-TnT, high-sensitivity troponin T; hs-CRP, high-sensitive C-reactive protein; LDL, low-density lipoprotein; MACE indicates major adverse cardiovascular event; PLT, platelets; RBC, red blood cell; TC, total cholesterol; TG, triglycerides; WBC, white blood cell.

TX Correlations

Baseline 11-dehydro-TXB₂ correlated weakly with maximal peri-AMI levels of cardiac damage biomarkers (creatine kinase

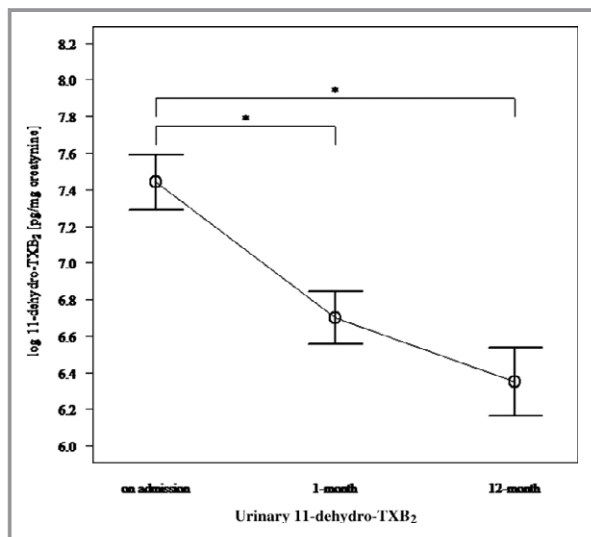


Figure 2. Urinary 11-dehydro-TXB₂ excretion on admission (baseline) and at 1-month and 1-year follow-up. Average (points) and SD (whiskers) of 11-dehydro-TXB₂ on admission, at 1-month and 1-year follow-up expressed in log-transformed values. **P*<0.001.

maximum [*R*=0.17; *P*=0.03], hs-TnT [*R*=0.19; *P*=0.01]). 11-Dehydro-TXB₂ also correlated with inflammatory parameters (white blood cell count >10×10³/µL [*R*=0.23; *P*=0.002], high-sensitivity CRP [*R*=0.31; *P*<0.001]). Both white blood cell count (9.46; 7.92–11.09 in Q1 versus 11.52; 9.34–14.51 ×10³/µL in Q4; *P*=0.007) and high-sensitivity CRP (1.22; 0.55–3.54 in Q1 versus 6.29; 1.29–23.4 mg/L in Q4; *P*<0.001; Figure S1) were significantly elevated in the highest quartile of 11-dehydro-TXB₂ level.

Primary Outcomes Analysis

Baseline mean 11-dehydro-TXB₂ measured during AMI was higher in patients who experienced MACEs at 1-year follow-up (7.73 [7.07–8.60]) compared with those who did not (7.28 [6.68–7.79]; *P*=0.002). Between the quartiles of 11-dehydro-TXB₂, the incidence of cumulative MACEs increased by 266% in Q4 compared with the first quartile (OR 3.66 [95% CI 1.47–9.13]) (Figure 3; receiver operating characteristic curve—Figure S2).

In the multivariate analysis based on logistic regression, 11-dehydro-TXB₂ and 3 other variables (diabetes, LVEF during AMI, and multivessel disease) were found to be the best predictors of 1-year cumulative MACEs (Table 3). In this model, the increase in natural logarithm of 11-dehydro-TXB₂

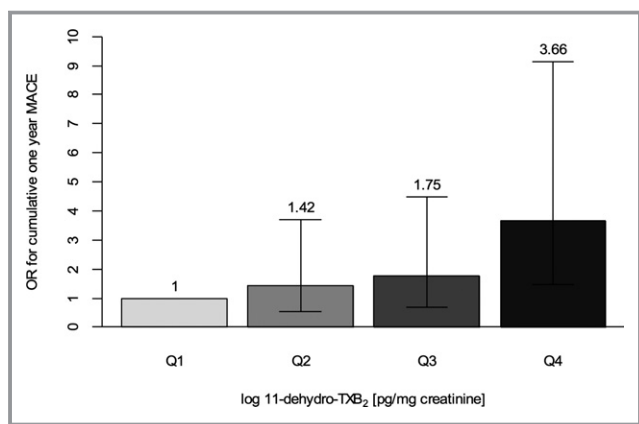


Figure 3. Cumulative MACEs risk in 1 year following AMI according to 11-dehydro-thromboxane (TX)₂ quartiles on admission. The ranges of quartiles intervals (Q1–Q4) expressed as log-transformed values of 11-dehydro-TXB₂ in pg/mg creatinine. Ranges of log 11-dehydro-TXB₂ quartiles are as follows: Q1: <6.85; Q2: 6.85 to 7.39; Q3: 7.40 to 8.01; Q4: >8.01 (pg/mg creatinine). Top bars are the ORs for cumulative MACEs at 1 year from AMI, whiskers indicate CIs. AMI indicates acute myocardial infarction; MACE, major adverse cardiovascular event; OR, odds ratio.

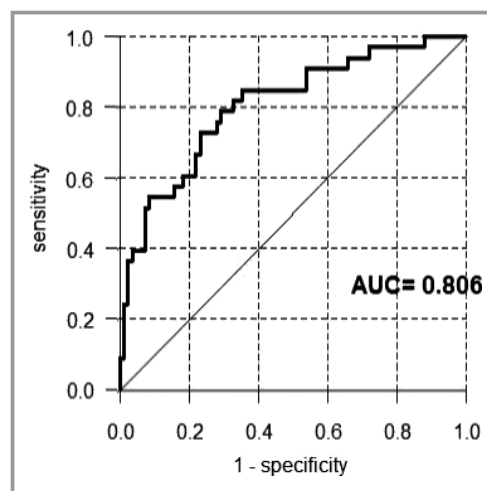


Figure 4. Receiver operating characteristic curve for predictive value of the multivariate regression model on the cumulative MACE occurrence at 1-year follow-up after AMI. The multivariate regression model includes 11-dehydro-thromboxane (TX)₂ on admission, diabetes mellitus, MVD, and baseline LVEF. AMI indicates acute myocardial infarction; AUC, area under the curve; DM, diabetes mellitus; LVEF, left ventricular ejection fraction; MVD, multivessel disease.

by 1 was associated with increased risk of developing MACEs within 1 year after AMI by 58.1%. Prognostic capability of 11-dehydro-TXB₂ and other variables for predicting MACEs is presented on Figure 4 and has an area under the curve related to receiver operating characteristic curve of 0.806. Based on this multivariate model, we developed a mathematical formula for risk prediction, which is presented in Data S1.

Table 3. One-Year Follow-up Cumulative MACE Occurrence in Multivariate Analysis With 11-Dehydro-TXB₂

Variable	OR	95% CI for OR	P Value
Diabetes mellitus	2.476	1.128–5.451	0.024
LVEF during hospitalization, %	0.978	0.95–1.006	0.133
CAD			
1-vessel disease	Reference level		
2-vessel disease	4.671	1.646–14.951	0.006
3-vessel disease	9.141	3.308–29.023	<0.001
Log 11-dehydro-TXB ₂ on admission, pg/mg creatinine	1.581	1.095–2.329	0.017

CAD indicates coronary artery disease; LVEF, left ventricular ejection fraction; MACE, major adverse cardiovascular event; OR, odds ratio; TX, thromboxane. Variables included in the multiple regression model are: age, sex, body mass index, smoking (pack-years), past history of myocardial infarction, diabetes mellitus, hypertension, time from symptoms to percutaneous coronary intervention, multivessel disease, LVEF during hospitalization, maximal troponin level, C-reactive protein, glomerular filtration rate, and 11-dehydro-TXB₂.

When long-term aspirin treatment was included in the regression analysis, it did not influence the results.

Secondary Outcomes Analysis

LVEF and 11-dehydro-TXB₂ level on admission

11-Dehydro-TXB₂ on admission inversely correlated with both measurements of LVEF at initial hospitalization ($R=-0.21$; $P=0.006$) and at 1-year observation ($R=-0.346$; $P<0.001$). Patients with most severely impaired LVEF (<30%) during AMI were found to have the highest levels of 11-dehydro-TXB₂ compared with all others. 11-dehydro-TXB₂ level on admission was also a predictor of LVEF after 1 year; it was higher in patients with impaired LVEF compared with patients with normal LVEF (Table 4).

The 11-dehydro-TXB₂ levels measured at 1 month and 1 year after AMI did not correlate with LVEF.

11-Dehydro-TXB₂ and MACEs at hospital discharge and at 1-month follow-up

Log-transformed 11-dehydro-TXB₂ on admission was predictor of cumulative MACEs for both time points: for discharge MACEs (OR 1.499, 95% CI 1.007–2.253) and for 1-month MACEs (OR 1.57, 95% CI 1.11–2.257). Therefore, the increase in log-transformed baseline 11-dehydro-TXB₂ by 1 increased the risk of MACEs on discharge from hospital by 49.9% and by 57% at 1-month follow-up.

Table 4. Association of Urinary 11-Dehydro-TXB₂ on Admission With Baseline LVEF During Hospitalization and at 1-Year Follow-up

Systolic LV Function	n	Log 11-Dehydro-TXB ₂ on Admission, pg/mg Creatinine				P-Value	Post-hoc*
		Median	Range	Q1	Q3		
LVEF during hospitalization, %							
Normal (≥55)	55	7.32	5.54–9.45	6.62	7.82	0.002	a
Mildly impaired (45–54)	45	7.1	4.35–9.66	6.71	7.67		a
Moderately impaired (30–44)	57	7.49	5.67–10.74	6.92	8.13		ab
Severely impaired (<30)	23	7.73	5.2–10.26	7.49	8.95		b
LVEF after 12 mo, %							
Normal (≥55)	81	7.15	4.35–9.3	6.42	7.59	<0.001	a
Mildly impaired (45–54)	35	7.49	6.25–9.44	7.1	7.88		b
Moderately impaired (30–44)	21	7.73	5.67–10.13	7.27	8.68		b
Severely impaired (<30)	3	8.76	7.59–9.31	8.17	9.03		b

EF indicates ejection fraction; LV, left ventricular; Q1, quartile; Q3, quartile 3; TX, thromboxane.
*Results of post-hoc analysis. Groups identified with the same letter did not differ significantly.

Discussion

In the present study we evaluated the association of systemic 11-dehydro-TXB₂ production with clinical outcomes of patients with AMI. There are 2 main findings of the study. First, a higher urinary level of 11-dehydro-TXB₂ on admission because of AMI significantly increases the probability of cumulative MACE occurrence at 1-year follow-up. Second, the same biomarker predicts LVEF performance post AMI.

The first observation is in line with the previous studies by Eikelboom et al in which 11-dehydro-TXB₂ was found to be a good predictor of MACEs in a high cardiovascular risk population treated with aspirin.^{9,10} The risk of MACE occurrence in these studies, when 2 extreme quartiles of 11-dehydro-TXB₂ were compared, was between 1.66 and 1.8 times higher in the upper quartile. In the present study, univariate analysis revealed 3.66 times (95% CI 1.47–9.13) higher MACE risk in patients within the highest 11-dehydro-TXB₂ quartile, compared with those in the lowest quartile. Importantly, when these data were reevaluated by using a multivariate analysis, the confounding factors, such as demographic features, treatment options, or main laboratory findings, did not influence performance of urinary 11-dehydro-TXB₂ as a predictor of MACEs in AMI patients. Actually, in the multivariate model only 3 other variables contributed to 11-dehydro-TXB₂ predictive properties: LVEF on admission, presence of diabetes mellitus, and multi-vessel disease. This model proved to have a good prediction capability with an area under the curve of 0.806 and OR for 11-dehydro-TXB₂ of 1.581 (95% CI 1.095–2.329), results similar to those presented by Eikelboom et al.^{9,10}

There are several causes responsible for increased TX production in AMI. First, during the acute phase of AMI, more platelets are released and overactivated.^{1,19} Newly formed

platelets beside their constitutive cyclooxygenase (COX)-1 expression, retain COX-2 activity contributing to additional TX synthesis.^{1,2,11,20,21} Further, intense thrombotic and concomitant acute inflammatory processes present in AMI induce TX synthesis from sources other than platelets, mostly inflammatory (monocytes and macrophages) and endothelial cells.²² These cells produce TX mainly via the COX-2 pathway and stimulate platelets despite their inhibition by aspirin.²³ Aspirin in small doses blocks irreversibly COX-1–dependent TX production; however, inhibition of COX-2 pathway requires much higher doses of aspirin.²

These mechanisms may contribute to elevated TX synthesis and may overcome the aspirin suppression of COX-1 pathway during AMI.²⁴ It also explains why in patients taking aspirin on a long-term basis, 11-dehydro-TXB₂ production is still maintained.

Urinary excretion of 11-dehydro-TXB₂ is assumed to reflect systemic TX biosynthesis most accurately²⁵ and, in contrast to serum TXB₂, can also detect the in vivo “extraplatelet” TX production.

In a recent study by Frelinger et al, the authors proved in a prospective study that patients presenting for diagnostic catheterization with elevated levels of serum TXB₂ had higher incidence of MACEs despite aspirin treatment in a 2-year observation period.²⁶ It confirms the findings of the present study and highlights the importance of residual TX production by ASA suppressed platelets.

Extraplatelet pathways are described as responsible for ≈30% of 11-dehydro-TXB₂ production²⁷ and significantly increase in inflammatory and prothrombotic processes—which are present during AMI. Our findings support this hypothesis, as increased production of 11-dehydro-TXB₂ during AMI correlated with inflammatory parameters: high-sensitivity CRP and

increased white blood cells. Further, 11-dehydro-TXB₂ substantially decreased at both 1-month and 1-year follow-up compared with the baseline measurements in the acute phase, which cannot be solely explained by the long-term aspirin treatment after AMI. This is in parallel with the recent study by Cangemi et al, where the authors described TXB₂ elevation in patients with pneumonia and subsequent AMI.²⁸

Results of the present study demonstrated that patients with higher levels of 11-dehydro-TXB₂ have decreased LVEF during AMI and increased levels of baseline 11-dehydro-TXB₂ predicted decreased LVEF at 1-year follow-up. This relationship between elevated TX biosynthesis and reversible myocardial dysfunction may suggest impairment of microcirculation and may play a pivotal role in the pathogenesis of MI. Moreover, 11-dehydro-TXB₂ on admission correlated with maximal values of biomarkers of myocardial damage, such as creatine kinase and hs-TnT, which confirmed the results of Valles et al—that TXA₂ was found to correlate with the myonecrosis in nonresponding to aspirin-treated patients.²⁹ Also, in a study by Nicolli et al, TXB₂ production correlated with the severity and extent of atherosclerotic changes in coronary vessels despite aspirin treatment.³⁰

In our study, we also found that 11-dehydro-TXB₂ levels were higher in STEMI patients compared with NSTEMI patients, which can be explained by more intense inflammatory response and a crucial role of myonecrosis in pathogenesis of STEMI compared with NSTEMI.³¹

Complementary to our findings, it has been described by Nicolli et al that TXA₂ measurement could be an independent predictor of no-reflow syndrome following coronary intervention, which probably contributes to the short-term complications after AMI and corresponds with our study findings.³² This could explain why patients with higher levels of 11-dehydro-TXB₂ had a higher incidence of cumulative MACEs in a short-term follow-up after AMI (at hospital discharge and 1 month). However, these findings should be treated with caution, as the number of cumulative MACEs was relatively small at this time period.

To our best knowledge, no previous studies have examined 11-dehydro-TXB₂ in AMI and its associations with future MACEs and left ventricular performance. The strengths of the present study include very low dropout of patients (3%) during follow-up and strict inclusion criteria that made the studied group homogeneous. We evaluated only patients assumed to have AMI of type I who underwent PCI, whereas symptoms lasted not longer than 24 hours. In this cohort of 180 patients, a relatively large proportion of patients had cumulative MACEs at 1-year follow-up (60 patients), which enabled us to draw conclusions on the 11-dehydro-TXB₂ predictive value as a biomarker. We used the most sensitive quantitative assay of combined high-performance liquid chromatography–tandem mass spectrometry. This is of great importance, as

several previous studies described problems with reliability with the monoclonal antibody ELISA, which detects not only 11-dehydro-TXB₂ but also an additional metabolite like 11-dehydro-2,3-dinor-TXB₂.⁴

Limitations of the Study

This study is a single-center study with a limited number of patients. The study group was sufficient to evaluate the cumulative MACE outcomes but not large enough to evaluate each different MACE complication separately; thus, they had to be evaluated cumulatively. Being aware of this necessity when developing the study protocol, we designed the LTIMI study to evaluate cumulative MACEs as a primary outcome. Nevertheless, a future multicenter study is warranted to verify the outcomes in a larger cohort of patients with AMI. Another limitation of the study is that some patients were taking long-term aspirin treatment before study enrollment. This bias imposed by long-term treatment with aspirin was, however, balanced by the fact that all patients received aspirin immediately when suspected of having AMI and at least 30 minutes before sample collection for further analysis. After oral intake of uncoated aspirin, it reaches its concentration peak in blood at ≈30 minutes. This approach represents more of a “real-life scenario” and, importantly, long-term aspirin treatment did not influence the statistical models of 11-dehydro-TXB₂ performance as a biomarker. Finally, several other factors not evaluated in this study could influence 11-dehydro-TXB₂ measurements such as a patient’s genetic variability of TX production or TX receptor synthesis and turnover. This study also did not aim to assess the interindividual resistance to aspirin or other antiplatelet drugs, which may also influence the TX production and MACE incidence.

In conclusion, 11-dehydro-TXB₂ measured in AMI patients is an independent predictor of major cardiovascular outcomes that occur during the first year after the incidence and may provide prognostic information on left ventricular performance. These findings shed additional light on the pathological role of TXA₂ in AMI complications.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Supplemental Methods

MACE Definitions

1- Recurrent MI - clinical signs and symptoms of ischemia that are distinct from the presenting ischemic event and meeting at least 1 of the following criteria:

1. Within 48 h after PCI:

A. In patients with the baseline biomarkers values elevated and stable or falling, a rise of troponin values $\geq 20\%$. In addition, symptoms suggestive of myocardial ischemia or new ischemic electrocardiographic changes or angiographic findings consistent with a procedural complication or imaging demonstration of new loss of viable myocardium.

B. Patients with new, significant Q waves in at least 2 contiguous leads of an ECG that were not present with the presenting ischemic event.

C. Stent thrombosis associated with MI detected by coronary angiography or autopsy in the setting of myocardial ischemia and with a rise and/or fall of cardiac biomarker values with at least 1 value above the 99th percentile URL.

2. Within 48 h after CABG: A CABG-related MI defined by elevation of cardiac biomarker values 10 times the 99th percentile URL in patients with normal baseline troponin values (99th percentile URL) plus either new pathological Q waves or new LBBB or angiographically documented new graft or new native coronary artery occlusion or imaging evidence of new loss of viable myocardium or new regional wall motion abnormality. Patients with cardiac biomarkers above the ULN before CABG with the increase in biomarkers $\geq 20\%$ above the most recent value associated with symptoms/signs of myocardial ischemia were coded as reinfarction.

3. Spontaneous (before or without subsequent revascularization, > 48 h after PCI, and/or after CABG): new, significant Q waves in at least 2 contiguous leads of an ECG that were not present with the presenting ischemic event and/or an increase in CK-MB or troponin above the 99th percentile ULN, which is at least $\geq 20\%$ above the most recent value in patients whose most recent cardiac markers drawn before reinfarction were normal [1].

2 - Cardiogenic shock - occurring after primary PCI, sustained, lasting > 30 min. episode of decrease in blood pressure (hypotension) with SBP <80 to 90 mmHg and/or CI <2.2L/min/m², associated with peripheral hypoperfusion, manifested clinically by alteration in mental status, cooling of the extremities and decreased urine output, determined to be secondary to cardiac dysfunction and/or by requirement for parenteral inotropic or vasopressor agents or mechanical support (e.g., IABP, extracorporeal circulation, VADs) to maintain blood pressure and cardiac index above those specified levels [1].

3 - Cardiac arrest with successful resuscitation - sudden loss of mechanical heart function occurring after effective PCI procedure, characterized by the absence of the patient's response to stimuli, palpable pulse and/or apnea in the mechanism of ventricular fibrillation, pulseless ventricular tachycardia, asystole or pulseless electrical activity, requiring cardiopulmonary resuscitation.

4 – Urgent PCI – urgent need for coronary angioplasty for presumed culprit lesion(s) suggested by increasing signs and symptoms of angina, documented ischemia in resting ECG recordings or induced by exercise stress test or angiographic evidence of new or worse $\geq 70\%$ lesion and/or thrombus in an epicardial coronary artery that is believed to be responsible for the myocardial ischemic symptoms/signs [2].

5 - Urgent CABG – urgent need for coronary artery bypass grafting surgery in the case of documented ischemia and multi-vessel coronary artery disease evidence on coronary angiography requiring surgical revascularization as the only modality of treatment.

6 – Heart failure event/pulmonary edema – episode of acute heart failure presenting clinically as pulmonary edema caused by a sudden deterioration of left ventricular function determined on the basis of signs and symptoms, objective diagnostic tests, requiring initiation or intensification of pharmacotherapy specifically for acute HF, including intravenous diuretic or vasoactive agents (e.g., inotrope, vasopressor, or vasodilator) or mechanical or surgical intervention [3].

7 - Stroke/TIA – documented sudden episode of focal neurological dysfunction of CNS lasting < 24 hours (Transient Ischemic Attack) or \geq 24 hours or until death (stroke) attributable to ischemic etiology (TIA, ischemic stroke) or hemorrhagic etiology (intracerebral hemorrhage), based on pathological, imaging, or other objective evidence of cerebral, spinal cord, or retinal focal ischemic or hemorrhagic injury in a defined vascular distribution and other etiologies excluded [4].

8 - Cardiovascular death – a death due to immediate consequences of MI occurring within 30 days of MI and invasive procedure (heart failure, stroke, complications of invasive procedure, cardiovascular haemorrhage) and distant >30 days after MI (acute MI, heart failure, stroke, sudden cardiac death, death due to other cardiovascular causes) [2].

Thromboxane measurements (detailed)

Aliquoted urine samples were stored not longer than 6 months. After thawing on ice, creatinine concentration was measured using Vitros 350 Chemistry System (Ortho Clinical Diagnostics, Neckargemuend, Germany). Aliquot of urine (0.5 mL) was added deuterated and chemically identical internal standard (9S,15S-dihydroxy-11-oxo-thromboxa-5Z, 13E-dien-1-oic acid (11-dehydro-TXB2-d4, 1 ng; Cayman Chemical Co., Ann Arbor, MI, USA). Next, the sample was acidified using acetic acid to pH 3.5 and extracted twice with equal volume of methyl-tert-butyl ether:methanol (8:2 v/v). Following extraction, the organic phase was

pooled and evaporated at 37°C under nitrogen. Before analysis, extracted sample was dissolved in methanol (50 microL). High performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) measurements were done using HPLC equipped with autosampler (Shimadzu Sil-2-AC, Shimadzu Scientific Instruments, Inc. Columbia, MD, USA) and mass spectrometer (Qtrap 4000, Applied Biosystems, Foster City, CA, USA) with electrospray ion source and operating in multiple reaction monitoring negative ionization mode (MRM). Ten microL of the sample extract was injected on reverse phase column (Zorbax Eclipse XDB C-18, Agilent Technologies, Inc. Santa Clara, CA, USA) stabilized thermally at 37°C and eluted using a linear gradient of acetonitrile/water/acetic acid/isopropanol as published previously (ref.17). Urinary content of 11-dehydro-TXB2 was calculated using a stable isotope dilution method from area under peaks of 11-dehydro-TXB2 (monitored 367-171 m/z ions pair) to the area under the peak of internal deuterated standard (monitored 371-165 m/z ions pair). Results were expressed in picograms per mg creatinine.

Supplemental Tables

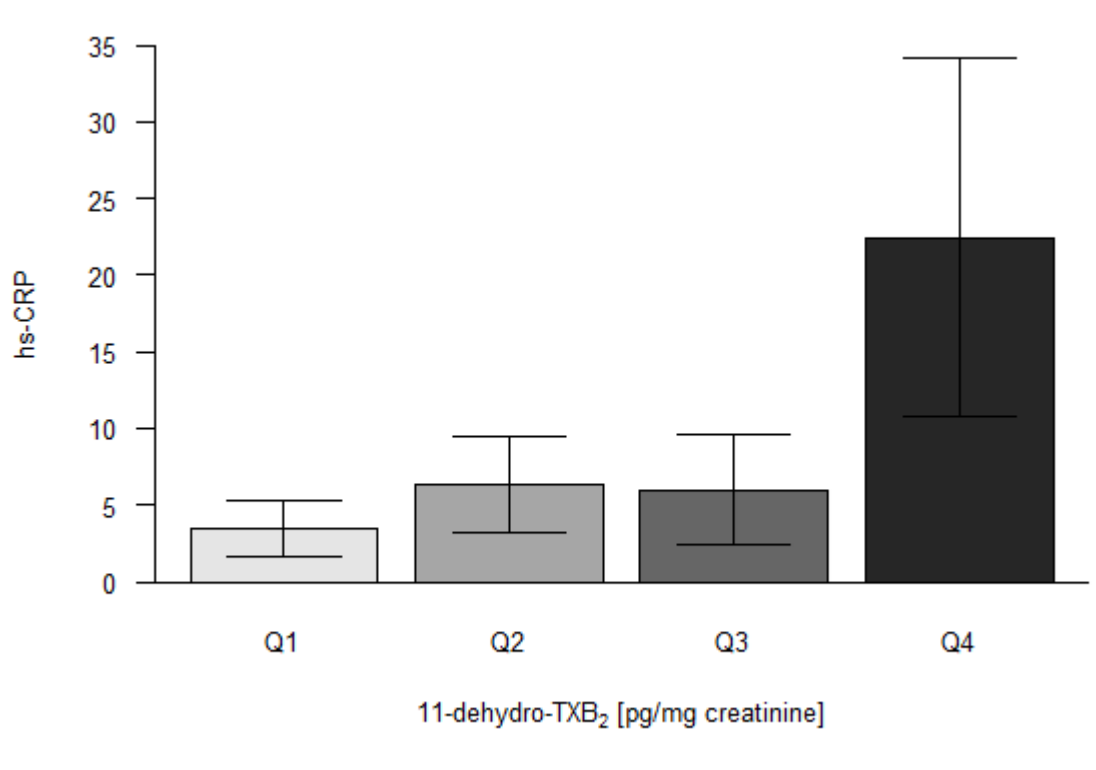
Table S1. The incidence of MACE and cumulative MACE during hospitalization, at 1-month and 1-year follow-up.

Major adverse cardiovascular events (MACE)	During hospitalization n (%)	After 1 month n (%)	After 1 year n (%)
1- Subsequent MI	0 (0%)	10 (5.8%)	12 (7.1%)
2- Cardiogenic shock	6 (3.3%)	2 (1.2%)	4 (2.4%)
3- Cardiac arrest with successful resuscitation	9 (5.0%)	2 (1.2%)	7 (4.1%)
4- Urgent PCI	4 (2.2%)	9 (5.2%)	10 (5.9%)
5- Urgent CABG	7 (3.9%)	2 (1.2%)	3 (1.8%)
6- Heart failure event/pulmonary edema	12 (6.7%)	3 (1.7%)	1 (0.6%)
5- Stroke/TIA	1 (0.6%)	2 (1.2%)	2 (1.2%)
6- Cardiovascular death	4 (2.2%)	1 (0.6%)	9 (5.3%)
Cumulative MACE	27* (15.0%)	42* (24.3%)	60* (35.5%)

* Cumulative MACE during hospitalization, at 1-month and 1-year follow-up do not add up because one patient could have more than one adverse cardiovascular event. Cumulative MACE assessment during hospitalization was performed for 180 patients, after 1 month for 173 (4 patients died during hospitalization and 3 patients were lost to follow-up). Cumulative MACE assessment after 1 year was performed for 169 patients (5 patients died and 6 patients were lost to follow-up).

At 1-year follow-up the composite MACE appeared in 60 patients (35.5%) and in this group 14 patients died (7.8%).

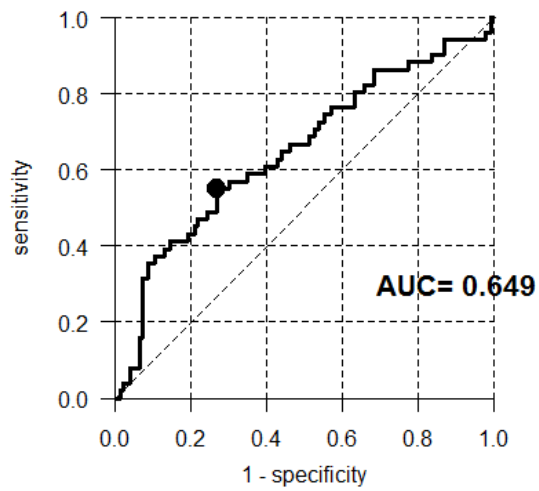
Figure S1. Association of hs-CRP with quartile distribution of log-transformed 11-dehydro-TXB₂



Footnotes: The average (bars) and SD (whiskers) of 11-dehydro-TXB₂ quartile distribution on admission expressed in log-transformed values in association with hs-CRP. Ranges of log 11-dehydro-TXB₂ quartiles are as follows: Q1- <6.85; Q2- 6.85-6.39; Q3- 7.40-8.01; Q4- >8.01 [pg/mg creatinine].

Abbreviations: hs-CRP – high sensitive C-reactive protein

Figure S2. ROC curve for 11-dehydro-TXB₂ on admission as a biomarker of cumulative MACE occurrence one year post-AMI in univariate analysis.



Abbreviations: AUC – area under the curve.

The cut-off point for natural logarithm of 11-dehydro-TXB₂ was assumed 7.7 (pg/mg creatinine), with area under the curve (AUC) of 0.649 (specificity of 73.13% and sensitivity of 54.9%).

Supplementary Data (Data S1)

Mathematical formula based on multivariate analysis to calculate the chance and probability of cumulative MACE occurrence 1 year post-AMI:

$$\text{chance} = \exp (0,978 \cdot \text{LVEF}_{\text{hosp}} + 2,476 \cdot \text{DM} + \text{MVD} + 1,581 \cdot \log 11\text{-dehydro-TXB2}_{\text{hosp}})$$

Abbreviations:

- $\text{LVEF}_{\text{hosp}}$ – LVEF on admission [%]
- DM (diabetes mellitus) = 1 for diabetic patients, 0 for non-diabetic patients
- MVD (multivessel disease) = 0 for patients with 1-vessel disease; MVD = 4.671 for 2-vessel disease and MVD = 9.141 for 3-vessel disease
- $\log\text{-}11\text{-dehydro-TXB}_2_{\text{ hosp}}$ – log of 11-dehydro-TXB₂ on admission [pg/mg creatinine]

Footnote: *probability = chance / (1 + chance)*

Supplemental References

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