**CLINICAL STUDY**

**Association of Isoprostanerelated Oxidative Stress with Vulnerability of Culprit Lesions in Diabetic Patients with Acute Coronary Syndrome**

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**Summary**

Urinary excretion of 8-iso-prostaglandin F$_2$α (8-iso-PGF$_2$α), a reliable biomarker for enhanced oxidant stress in vivo, has been described in association with diabetes and coronary heart disease. The aim of this study was to evaluate the relationship between urinary 8-iso-PGF$_2$α levels and the characteristics of coronary culprit lesion in diabetic patients with acute coronary syndrome (ACS). A total of 79 diabetic patients with ACS were included. iMAP intravascular ultrasound (iMAP-IVUS) was performed to evaluate the characteristics of culprit plaques. Fasting urinary 8-iso-PGF$_2$α level was measured and corrected by creatinine clearance. iMAP-IVUS data showed culprit plaques in high urinary 8-iso-PGF$_2$α level patients had a greater percentage of necrotic core and less fibrous components. High urinary 8-iso-PGF$_2$α levels were correlated with increased necrotic plaque components ($r = 0.325$, $P = 0.003$). Meanwhile, the presence of thin-capped fibroatheroma (50.0% versus 11.5%, $P = 0.003$), ruptured plaques (30.8% versus 7.7%, $P = 0.035$), and thrombus (38.5% versus 7.7%, $P = 0.008$) were significantly more frequent in the upper tertile of urinary 8-iso-PGF$_2$α levels than in the low tertile. Multivariate analysis showed high levels of urinary 8-iso-PGF$_2$α (OR 4.240, $P = 0.007$) was independently associated with the presence of vulnerable culprit plaque in diabetic ACS patients. Urinary 8-iso-PGF$_2$α also displayed a significant value in predicting vulnerable plaques in diabetic patients with ACS by constructing the receiver-operating characteristic (ROC) curve (Area under the ROC curve: 0.713, $P = 0.001$). Urinary 8-iso-PGF$_2$α levels are associated with the vulnerability of the coronary culprit lesion in diabetic patients with ACS and may provide additional information for risk assessment in suspected vulnerable patients.

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**Key words:** 8-isoprostane, Vulnerable plaque, Diabetes, Intravascular ultrasound

Acutecoronary syndrome (ACS) is a group of severecardiovascular diseases and the common cause of cardiac death worldwide. Some pathological evidences showed that ACS is likely caused by vulnerable coronary plaques, which is characterized by thin-cap fibroatheroma (TCFA), large lipid-rich necrotic core, and macrophage infiltration. Severe atherosclerotic lesions are frequently observed in diabetic patients. Although diabetes has been clearly established as a risk factor for coronary artery disease (CAD), it still remains elusive on what mechanism is responsible for the accelerated cardiovascular outcomes in diabetic patients. Excess oxidative stress is commonly observed in diabetic patients, which appears to be associated with glucose metabolic disorder and dyslipidemia, and underlies the development of atherosclerotic disease. The oxidative stress should play a key role in the progression of atherosclerotic plaques in diabetic patients. It may be also the potential mechanism for high risk of cardiovascular outcomes and mortality rates in diabetic patients.

The 8-iso-prostaglandin F$_2$α (8-iso-PGF$_2$α) is one of the most reliable in vivo markers of oxidative stress, which has been shown as an independent predictor of the presence and extent of CAD by several studies. However, the relationship between urinary 8-iso-PGF$_2$α and vulnerability of coronary plaque has not been fully evaluated. In the present study, by using iMAP intravascular ultrasound (iMAP-IVUS), we investigated whether any relation exists between urinary 8-iso-PGF$_2$α levels and plaque composition of culprit lesions in diabetic patients with ACS.
Methods

Study population: This was a single-center, prospective, and observational study. We included consecutive ACS patients with type 2 diabetes mellitus (T2DM) admitted to the center of cardiology in Beijing An Zhen Hospital between Feb 2016 and Jan 2017. Patients who underwent percutaneous coronary intervention (PCI) with iMAP-IVUS guidance in the culprit vessel were eligible for enrollment. Patients suffering from totally occlusive lesions, restenosis after stenting, previous coronary artery bypass graft surgery, severe heart failure (NYHA functional class III or above), renal failure (creatinine clearance < 30 mL/minute), hepatic insufficiency, infectious disease, more than 80 years old, or have any improper coronary anatomy to perform IVUS were excluded from the study. ACS consisted of ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI), and unstable angina pectoris, which were defined according to the 2013 American College of Cardiology/American Heart Association (ACC/AHA) Guideline for STEMI and 2014 ACC/AHA Guideline for NSTEMI. Diabetes was diagnosed according to the American Diabetes Association criteria or medical history and the use of insulin or glucose-lowering medication. The estimated glomerular filtration rate (eGFR) value was calculated to measure renal function by using the modification of diet in renal disease equation. The study protocol was approved beforehand by the Medical Ethics Committee of Beijing An Zhen Hospital of Capital Medical University, and written informed consent was obtained from all participants. The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association.

Laboratory measurement: Blood samples were taken after overnight fasting for measurement of fasting blood glucose, glycated hemoglobin (HbA1c), creatinine, and lipid levels. The urine sample was collected from each subject after fasting for 12 hours or overnight. Samples were separated immediately after collection by centrifugation at 3000 rpm for 10 minutes and stored at −80°C until analyses. The supernatant was used for the determination of 8-iso-PGF$_\alpha_\alpha$ (Cayman Chemical, Ann Arbor, MI, USA) with a competitive enzyme-linked immunosorbent assay. Urinary creatinine level was measured using a colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA). The urinary 8-iso-PGF$_\alpha_\alpha$ level was corrected by creatinine clearance. Thus, 8-iso-PGF$_\alpha_\alpha$/creatinine ratio was used in the analysis.

Coronary intervention: All patients were performed with coronary angiography by standard Judkins technique. Heparin (5000-10,000 units) was administered intravenously before PCI. A culprit lesion was defined as the lesion related to the clinical event, as identified by both angiography and electrocardiogram findings. IVUS examination was performed before any intervention and after the intracoronary administration of 200 μg nitroglycerine. The IVUS imaging was performed using an IVUS system (iLAB$^\text{TM}$ Ultrasound Imaging System, Boston Scientific, USA) and a 40 MHz intravascular catheter (OptiCross$^\text{TM}$, Boston Scientific, Boston, MA, USA). The imaging catheter was advanced more than 10 mm beyond the lesion, and an automated pullback system was applied to a point greater than 10 mm proximal to the lesion, at a standard pullback speed of 0.5 mm/second. Grayscale IVUS quantitative analysis was performed according to the criteria of the American College of Cardiology Clinical Expert Consensus Document on IVUS. The external elastic membrane (EEM) and lumen cross-sectional areas (CSA) were measured at the lesion minimum lumen site and at the distal and proximal reference segments. Plaque plus media (P&M) CSA = EEM - lumen CSA. Percent plaque burden = P&M CSA / EEM CSA. The coronary artery remodeling was assessed by the remodeling index, which is defined as the ratio of the EEM CSA at the lesion site to the mean reference-segment EEM CSA. The iMAP-IVUS analysis was performed across the entire culprit lesion segment. Coronary plaque components were classified into four categories, fibrotic (green), lipidic (yellow), necrotic (pink), or calcified (blue). Absolute amounts and a percentage of plaque area or volume were reported in the iMAP analysis. Lesions were qualitatively analyzed with QVus (iMap Basic Viewer 2.1.32.0, Medis medical imaging systems, Leiden, the Netherlands). In this study, the vulnerable plaque was defined as the plaque marked by plaque rupture, coronary thrombus, or TCFA. A ruptured plaque contained a cavity that communicated with the lumen with an overlying residual fibrous cap fragment. A fragmented and loosely adherent plaque without a distinct cavity and without a fibrous cap fragment was not considered as a plaque rupture. Thrombus was an intraluminal mass having a layered or lobulated appearance, evidence of blood flow (micro-channels) within the mass, and speckling or scintillation. TCFA was defined as a necrotic core ≥ 10% of plaque area in at least 3 consecutive frames without overlying fibrous tissue in the presence of ≥ 40% plaque burden. IVUS data were analyzed by two independent, experienced interventional cardiologists who were not aware of the patients’ clinical information.

Statistical analysis: All statistical analyses were performed using SPSS for Windows 20.0 (SPSS Inc, Chicago, IL, USA). Data are presented as frequencies and percentages for categorical variables, median for abnormally distributed parameters, and mean ± standard deviation for continuously distributed variables unless otherwise indicated. Differences between two groups were assessed by using the Chi-square, Mann-Whitney rank analysis, and t-tests. Correlation between continuous variables was determined by Pearson correlation coefficients. Patients were categorized according to the tertiles of urinary 8-iso-PGF$_\alpha_\alpha$ levels. Univariate and multivariate logistic regression analyses were performed to identify independent predictors of the vulnerability of the culprit plaque. The predictive value of urinary 8-iso-PGF$_\alpha_\alpha$ for the presence of vulnerable plaque in culprit lesion was calculated by constructing receiver-operating characteristic (ROC) curves. A value of $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics: A total of 86 diabetic patients with ACS who met the inclusion and exclusion criteria...
and underwent iMAP-IVUS were included in this study. Seven patients were excluded because of poor quality IVUS images (2 patients), no IVUS image before PCI (2 patients), or insufficient urinary 8-iso-PGF$_{2\alpha}$ data for analysis (3 patients). As a result, 79 patients were enrolled in the final analysis, who were grouped according to tertiles of the urinary 8-iso-PGF$_{2\alpha}$ level. The mean age of the high tertile group was 62 ± 11 years, and of the intermediate and low tertile groups were 58 ± 12 and 59 ± 8 years, respectively. The majority of patients in these three groups were male. No significant differences were observed between the three tertile groups in terms of body mass index, current smoking, blood pressure, left ventricular ejection fraction, eGFR, the proportion of STEMI and NSTEMI, high-sensitivity C reactive protein (hs-CRP), blood lipid levels, and usage of hypoglycemic agents. Patients of the high tertile group had higher admission blood glucose (9.84 ± 2.70 versus 8.57 ± 3.08 versus 7.81 ± 2.35 mmol/L, P = 0.030) and HbA1c (7.6 ± 1.8 versus 7.1 ± 2.4 versus 6.4 ± 1.0%, P = 0.046) levels, and a trend toward a longer duration of diabetes (4.9 ± 4.9 versus 3.4 ± 5.7 versus 1.8 ± 2.5 years, P = 0.059) compared with patients of the intermediate and low tertile groups. The baseline characteristics are summarized in Table I.

**Table I.** Baseline Characteristics

| Variables                              | Tertiles of Urinary 8-iso-PGF$_{2\alpha}$ (pmol/mmolCr) | P-value |
|----------------------------------------|---------------------------------------------------------|---------|
|                                        | Low (≤ 87) | Intermediate (88-127) | High (≥ 128) |     |
| n                                      | 26 | 27 | 26 |     |
| Age (years)                            | 59 ± 8 | 58 ± 12 | 62 ± 11 | 0.322 |
| Males                                  | 17 (65.4) | 19 (70.4) | 19 (73.1) | 0.829 |
| Prior MI                               | 2 (7.7) | 4 (14.8) | 3 (11.5) | 0.717 |
| Current smoking                        | 9 (34.6) | 15 (55.6) | 14 (53.8) | 0.242 |
| Hypertension                           | 17 (65.4) | 16 (59.3) | 18 (69.2) | 0.746 |
| Clinical presentation                  |                      |                      |         |     |
| Unstable angina                        | 23 (88.5) | 21 (77.8) | 18 (69.2) | 0.239 |
| NSTEMI                                 | 0 (0) | 2 (7.4) | 2 (7.7) | 0.355 |
| STEMI                                  | 3 (11.5) | 4 (14.8) | 6 (23.1) | 0.512 |
| Duration of diabetes (months)          | 1.8 ± 2.5 | 3.4 ± 5.7 | 4.9 ± 4.9 | 0.059 |
| BMI (kg/m$^2$)                         | 24.8 ± 2.7 | 25.6 ± 3.5 | 26.6 ± 3.2 | 0.135 |
| LVEF (%)                               | 60.7 ± 7.4 | 57.0 ± 7.9 | 57.6 ± 8.2 | 0.182 |
| eGFR (mL/minute/1.73m$^2$)             | 90.8 ± 23.8 | 83.4 ± 21.6 | 84.7 ± 21.5 | 0.441 |
| SBP (mmHg)                             | 128 ± 12 | 130 ± 14 | 131 ± 13 | 0.575 |
| DBP (mmHg)                             | 75 ± 9 | 77 ± 11 | 76 ± 11 | 0.764 |
| TG (mmol/L)                            | 1.95 (1.00, 2.42) | 1.92 (1.11, 2.50) | 2.05 (0.98, 3.17) | 0.661 |
| TC (mmol/L)                            | 4.91 (4.27, 5.62) | 4.98 (4.09, 5.40) | 5.24 (4.41, 6.05) | 0.447 |
| HDL-C (mmol/L)                         | 1.01 (0.83, 1.14) | 1.04 (0.89, 1.19) | 1.12 (0.95, 1.31) | 0.134 |
| LDL-C (mmol/L)                         | 2.84 (2.26, 3.52) | 2.92 (2.05, 3.51) | 3.28 (2.35, 3.95) | 0.419 |
| WBC (10$^3$/L)                         | 7.4 ± 1.2 | 7.9 ± 2.0 | 8.1 ± 1.7 | 0.248 |
| hs-CRP (mg/dL)                         | 1.19 (0.84, 2.77) | 1.75 (0.65, 3.72) | 1.87 (0.91, 4.76) | 0.144 |
| Urinary 8-iso-PGF$_{2\alpha}$ (pmol/mmolCr) | 52.0 (38.3, 63.0) | 101.3 (95.0, 111.3) | 189.1 (141.9, 244.7) | < 0.001 |
| FBG (mmol/L)                           | 7.81 ± 2.35 | 8.57 ± 3.08 | 9.84 ± 2.70 | 0.030 |
| HbA1c (%)                              | 6.4 ± 1.0 | 7.1 ± 2.4 | 7.6 ± 1.8 | 0.046 |
| Hypoglycemic agents                    |                      |                      |         |     |
| Insulin secretagogues                  | 2 (7.7) | 7 (25.9) | 6 (23.1) | 0.194 |
| Metformin                              | 11 (42.3) | 11 (40.7) | 11 (42.3) | 0.991 |
| Glucosidase inhibitors                 | 16 (61.5) | 13 (48.1) | 11 (42.3) | 0.363 |
| Insulin                                | 7 (26.9) | 4 (14.8) | 7 (26.9) | 0.477 |

Data are given as numbers (percentage) for categorical variables and mean (± standard deviation) or median (interquartile range) for continuous variables. 8-iso-PGF$_{2\alpha}$ indicates 8-iso-prostaglandin F$_{2\alpha}$; STEMI, ST-elevated myocardial infarction; NSTEMI, non ST-elevated myocardial infarction; BMI, body mass index; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoproteins; LDL-C, low-density lipoproteins; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell; FBG, fasting blood glucose; and HbA1c, glycated hemoglobin.

**Angiographic results:** There were no significant differences in culprit lesion location, TIMI flow grade, and the percent of diameter stenosis between the three groups. However, the three-vessel disease was observed more frequently in the high tertile group than in the intermediate and low tertile groups (38.5% versus 14.8% versus 11.5%, P = 0.036). Angiographic findings are summarized in Table II.

**Grayscale and iMAP IVUS analysis:** Grayscale and iMAP-IVUS findings in the culprit lesions are shown in Table III. Lumen area and plaque burden were not significantly different in the patients of the three groups. No significant differences were observed in culprit lesion volume, length, and remodeling index between those tertiles.
### Table II. Angiographic Characteristics

| Variables          | Tertiles of Urinary 8-iso-PGF$_{2\alpha}$ (pmol/mmolCr) |   | P-value |
|--------------------|-------------------------------------------------|---|---------|
|                   | Low (≤ 87) | Intermediate (88-127) | High (≥ 128) |   |
| Culprit lesion     |   |   |   |   |
| LM                 | 1 (3.8) | 2 (7.4) | 2 (7.7) | 0.817 |
| LAD                | 14 (53.8) | 15 (55.6) | 14 (53.8) | 0.990 |
| LCX                | 5 (19.2) | 1 (3.7) | 3 (11.5) | 0.206 |
| RCA                | 6 (23.1) | 9 (33.3) | 7 (26.9) | 0.701 |
| Diseased vessels   |   |   |   |   |
| 1                  | 15 (57.7) | 13 (48.1) | 9 (34.6) | 0.246 |
| 2                  | 8 (30.8) | 10 (37.0) | 7 (26.9) | 0.726 |
| 3                  | 3 (11.5) | 4 (14.8) | 10 (38.5) | 0.036 |
| TIMI flow grade    |   |   |   |   |
| 0                  | 2 (7.7) | 4 (14.8) | 4 (15.4) | 0.648 |
| 1                  | 0 (0) | 4 (14.8) | 3 (11.5) | 0.139 |
| 2                  | 5 (19.2) | 3 (11.1) | 6 (23.1) | 0.506 |
| 3                  | 19 (73.1) | 16 (59.3) | 13 (50.0) | 0.230 |
| Percent diameter stenosis (%) | 79.4 ± 13.6 | 81.9 ± 11.4 | 82.8 ± 9.0 | 0.556 |

Data are given as number (percentage) for categorical variables and mean (± standard deviation) for continuous variables. 8-iso-PGF$_{2\alpha}$ indicates 8-iso-prostaglandin F$_{2\alpha}$; LM, left main coronary artery; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; RCA, right coronary artery; and TIMI, thrombolysis in myocardial infarction.

### Table III. Intravascular Ultrasound Analysis

| Variables          | Tertiles of Urinary 8-iso-PGF$_{2\alpha}$ (pmol/mmolCr) |   | P-value |
|--------------------|-------------------------------------------------|---|---------|
|                   | Low (≤ 87) | Intermediate (88-127) | High (≥ 128) |   |
|                   |   |   |   |   |
| Proximal reference |   |   |   |   |
| EEM CSA (mm$^2$)   | 18.8 ± 5.7 | 19.5 ± 3.5 | 20.5 ± 6.1 | 0.494 |
| Lumen CSA (mm$^2$) | 12.0 ± 4.5 | 12.8 ± 3.7 | 13.2 ± 5.1 | 0.616 |
| P&M CSA (mm$^2$)   | 6.8 ± 4.0 | 6.7 ± 3.1 | 7.3 ± 3.3 | 0.784 |
| Plaque burden (%)  | 35.0 ± 16.0 | 34.5 ± 14.2 | 36.0 ± 14.4 | 0.931 |
| Minimum lumen site |   |   |   |   |
| EEM CSA (mm$^2$)   | 17.4 ± 6.4 | 16.9 ± 4.1 | 18.1 ± 6.3 | 0.713 |
| Lumen CSA (mm$^2$) | 3.5 ± 1.0 | 3.4 ± 0.9 | 3.2 ± 1.3 | 0.688 |
| P&M CSA (mm$^2$)   | 13.9 ± 6.2 | 13.4 ± 4.0 | 14.9 ± 5.3 | 0.595 |
| Plaque burden (%)  | 77.5 ± 9.6 | 78.8 ± 7.3 | 81.4 ± 5.8 | 0.188 |
| Distal reference   |   |   |   |   |
| EEM CSA (mm$^2$)   | 17.2 ± 9.1 | 15.9 ± 4.6 | 16.2 ± 8.1 | 0.809 |
| Lumen CSA (mm$^2$) | 12.2 ± 5.9 | 10.9 ± 4.1 | 11.3 ± 7.1 | 0.694 |
| P&M CSA (mm$^2$)   | 4.9 ± 4.0 | 5.0 ± 3.9 | 4.9 ± 1.9 | 0.991 |
| Plaque burden (%)  | 26.2 ± 11.7 | 30.9 ± 17.3 | 32.2 ± 9.5 | 0.236 |
| Culprit lesion length (mm) | 18.3 ± 6.9 | 19.8 ± 6.8 | 20.0 ± 13.3 | 0.760 |
| Culprit lesion volume (mm$^3$) | 174.2 ± 91.9 | 180.6 ± 69.9 | 191.3 ± 124.3 | 0.815 |
| Remodeling index   | 1.01 ± 0.21 | 1.03 ± 0.28 | 1.13 ± 0.36 | 0.270 |
| Absolute volumetric culprit lesion components |   |   |   |   |
| Fibrotic (mm$^3$)  | 114.6 ± 53.8 | 118.4 ± 44.3 | 100.6 ± 53.5 | 0.415 |
| Lipidic (mm$^3$)   | 16.4 ± 11.3 | 16.1 ± 9.1 | 19.7 ± 15.4 | 0.508 |
| Necrotic (mm$^3$)  | 40.9 ± 33.9 | 42.6 ± 22.7 | 67.5 ± 64.5 | 0.056 |
| Calcified (mm$^3$) | 2.2 ± 3.2 | 3.4 ± 3.0 | 3.5 ± 4.5 | 0.349 |
| Relative volumetric culprit lesion components |   |   |   |   |
| Fibrotic (%)       | 66.7 ± 10.2 | 66.2 ± 7.2 | 58.2 ± 14.7 | 0.011 |
| Lipidic (%)        | 9.4 ± 2.6 | 8.4 ± 2.1 | 10.0 ± 3.9 | 0.157 |
| Necrotic (%)       | 22.8 ± 8.8 | 23.4 ± 6.5 | 29.8 ± 12.7 | 0.018 |
| Calcified (%)      | 1.1 ± 0.9 | 2.0 ± 1.3 | 2.0 ± 1.9 | 0.048 |

Data are given as number (percentage) for categorical variables and mean (± standard deviation) for continuous variables. 8-iso-PGF$_{2\alpha}$ indicates 8-iso-prostaglandin F$_{2\alpha}$; EEM, external elastic membrane; CSA, lumen cross-sectional areas; and P&M, Plaque plus media.
Correlations between urinary 8-iso-prostaglandin F₃α (8-iso-PGF₃α) levels and the percentage of plaque components in the culprit lesion identified by iMAP intravascular ultrasound (iMAP-US).

and low tertiles (67.5 ± 64.5 versus 42.6 ± 22.7 versus 40.9 ± 33.9 mm³, \( P = 0.056 \)). There were greater necrotic (29.8 ± 12.7% versus 23.4 ± 6.5% versus 22.8 ± 8.8%, \( P = 0.018 \)) and less fibrous components (58.2 ± 14.7% versus 66.2 ± 7.2% versus 66.7 ± 10.2%, \( P = 0.011 \)) in the high than in the intermediate and low tertiles. Urinary 8-iso-PGF₃α values were positively correlated with percent necrotic volumes (\( r = 0.325, P = 0.003 \)) and negatively with percent fibrous volumes (\( r = -0.288, P = 0.010 \)) (Figure 1). The presence of TCFA (50.0% versus 11.5%, \( P = 0.003 \)), ruptured plaque (30.8% versus 7.7%, \( P = 0.035 \)), and thrombus (38.5% versus 7.7%, \( P = 0.008 \)) were more common in the high tertile than in the low tertile (Figure 2).

**Independent predictors of culprit plaque vulnerability:** The presence of TCFA, plaque rupture, and thrombus were used as markers for plaque vulnerability on iMAP-US. We performed multivariate analysis to determine independent predictors of vulnerable plaque in culprit lesions. The following variables were tested (all with \( P < 0.2 \) in univariate analysis): age (≥ 65 years), current smoking, high HbA₁c (> 7%), low-density lipoprotein cholesterol (≥ 3.40 mmol/L), hs-CRP (> 3 mg/L) and urinary 8-iso-PGF₃α (upper tertile, ≥ 128 pmol/mmol Cr). Urinary 8-iso-PGF₃α (OR 4.240, 95% CI 1.493-12.037, \( P = 0.007 \)) and hs-CRP (OR 3.985, 95% CI 1.177-13.490, \( P = 0.026 \)) were the independent predictors of the vulnerable culprit plaque in diabetic ACS patients (Table IV). We constructed a ROC curve for predicting vulnerable plaques by urinary 8-iso-PGF₃α and blood hs-CRP levels. The area under the ROC curve for urinary 8-iso-PGF₃α and blood hs-CRP were 0.713 (95% CI 0.600-0.827, \( P = 0.001 \)) and 0.594 (95% CI 0.467-0.721, \( P = 0.151 \)), respectively (Figure 3). Urinary 8-iso-PGF₃α displayed a significant value in predicting vulnerable plaques in diabetic patients with ACS.

**Discussion**

It is now accepted that vulnerable plaques are an important characteristic of the “vulnerable patient”, who is proposed for the identification of subjects with a high likelihood of developing cardiac events in the near future. However, available screening and diagnostic methods are insufficient to identify the victims before the event occurs. The search for a noninvasive approach to detecting
Figure 2. The incidence of culprit lesion thin-cap fibroatheroma (TCFA). (A) plaque rupture. (B) and thrombus (C) in relation to tertiles of urinary 8-iso-prostaglandin F2α (8-iso-PGF2α) level.

the vulnerable plaques and vulnerable patients was encouraged to perform. In our present study, the principal result shows that urinary 8-iso-PGF2α, as a stable biomarker of oxidative stress, might be an important surrogate marker of vulnerable coronary culprit lesions in diabetic patients with ACS.

Plaque rupture and subsequent thrombosis are the most important mechanisms leading to ACS and sudden coronary death. Diabetic patients had more plaque ruptures and thrombus than non-diabetic patients in AMI, which may be associated with the greater rates of cardiovascular events in diabetic patients. Plaque composition may play a role in the plaque disruption and thrombosis. The pathological study showed necrotic core size played a greater role in the progression of atherosclerosis in diabetic subjects in sudden coronary death. TCFA is an important precursor of plaque rupture and thrombosis, which can account for a majority of acute coronary ischemic events. In the present study, there was a greater percentage of necrotic components in the high urinary 8-iso-PGF2α group than in the intermediate and low tertile groups, and increasing urinary 8-iso-PGF2α level was found to be associated with a higher percentage of necrotic volumes of culprit lesions. Elevated levels of markers of systemic oxidative stress, such as urinary 8-iso-PGF2α were reported to play a key role in the established risk factors of diabetic cardiovascular complications. The study of Schwedhelm, et al. showed urinary 8-iso-PGF2α was a sensitive and independent risk marker of coronary heart disease. The present study showed patients with a high tertile of urinary 8-iso-PGF2α had more three-vessel disease than in intermediate and low tertiles, which was in agreement with the study of Basarici, et al. who found urinary 8-iso-PGF2α levels reflected the extent and severity of CAD. Szułdrzyński, et al. reported that plasma levels of 8-iso-PGF2α are significantly higher in patients with ACS compared with stable CAD. In a pathological study by Nishibe, et al. 8-iso-PGF2α was found enriched in coronary plaque specimens especially from vulnerable patients, suggesting a crucial role of free radicals in the formation of vulnerable plaques. These findings suggest that 8-isoprostanes-related oxidative stress may be involved in the progression and destabilization of atherosclerotic plaques. In the present study, iMAP-IVUS data showed that there were clear associations between urinary 8-iso-PGF2α levels and plaque characteristics (TCFA, plaque rupture, and thrombus). TCFA, plaque rupture, and thrombus were observed more frequently in diabetic ACS patients with high urinary levels of 8-iso-PGF2α. An elevated urinary 8-iso-PGF2α level was an independent predictor of the vulnerability of culprit plaques in those patients.

Although the current study was not designed to explore mechanisms of 8-iso-PGF2α in vulnerable plaques of diabetic ACS patients, some important evidences have been provided by previous studies in understanding the implications of increased production of 8-iso-PGF2α in diabetic cardiovascular disease. Urinary 8-iso-PGF2α has been proved to be the most reliable marker to assess lipid peroxidation, which is a key mechanism for the development of atherosclerotic plaques in diabetes.
low-density lipoprotein accelerates the proliferation of macrophages, the transformation of foam cells, and the formation of a necrotic core, thus to make the plaque vulnerable. The study by Yura, et al. showed that 8-iso-

PGF$\alpha$ per se could stimulate smooth muscle cell proliferation, endothelin-1 mRNA, and protein expression in a bovine aortic endothelial cell. The endothelin-1 present may then cause migration of monocytes, cell proliferation,
and movement. These macrophages then take lipids and transform them into foam cells that cause a higher macrophage-to-smooth muscle cell ratio and a lipid-filled necrotic core. On the other hand, 8-iso-PGF$_{2\alpha}$ could cause persistent platelet activation and platelet shape change, enhance platelet adhesion, and attenuate the antithrombotic and antiaggregatory effects of nitric oxide. Increasing concentrations of 8-iso-PGF$_{2\alpha}$ resulted in irreversible platelet aggregation in a dose-dependent manner in the presence of subthreshold concentrations of collagen, ADP, arachidonic acid, and analogs of prostaglandin H$_2$. The amplification of platelet functions (activation, adhesion, and aggregation) may lead to coronary thrombosis in diabetic patients with high urinary 8-iso-PGF$_{2\alpha}$ level, providing a mechanistic link and interpretation of the association between oxidative stress and instability of diabetic patients.

**Study limitations:** Several study limitations should be considered in the interpretation of the results. Firstly, the sample size was relatively small, so that it may have influenced the results and the statistical analyses. Secondly, the nature of this study does not allow us to provide a mechanism for the effect of 8-iso-PGF$_{2\alpha}$ on the characteristics of coronary culprit lesion in diabetic patients with ACS. Thirdly, the assessment of urinary 8-iso-PGF$_{2\alpha}$ level was made at a single point in time, so the study cannot account for previous exposure to oxidative stress. Finally, this is an observational study performed at a single national center. The observational nature of analysis means that we cannot infer causality in the associations we have demonstrated.

**Conclusion**

The current study is the first to use iMAP-IVUS to examine the characteristics of coronary plaque vulnerability associated with the production of urinary 8-iso-PGF$_{2\alpha}$ in diabetic patients with ACS. The urinary 8-iso-PGF$_{2\alpha}$ might be an important surrogate marker for risk assessment in vulnerable coronary culprit lesions and suspected vulnerable patients. These findings also support the hypothesis that isoprostanes-related oxidative stress is involved in the atherosclerotic vulnerable plaque process, suggesting therapies aimed at reducing oxidative stress would benefit diabetic patients at risk of developing cardiac events.

**Disclosures**

**Conflicts of interest:** The authors declare that they have no competing interests.

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