Biomarkers associated with coronary high-risk plaques

Nakajima, A.; Libby, P.; Mitomo, S.; Yuki, H.; Araki, M.; Seegers, L.M.; ... ; Jang, I.K.

Journal of Thrombosis and Thrombolysis (2022) 54:647–659
https://doi.org/10.1007/s11239-022-02709-2

Biomarkers associated with coronary high-risk plaques

Akihiro Nakajima[1,2] · Peter Libby[3] · Satoru Mitomo[2] · Haruhito Yuki[2] · Makoto Araki[1] · Lena Marie Seegers[1] · Iris McNulty[1] · Hang Lee[4] · Midori Ishibashi[5] · Kazuna Kobayashi[6] · Jouke Dijkstra[7] · Toru Ouchi[2] · Hirokazu Onishi[2] · Hiroto Yabushita[2] · Satoshi Matsuoka[2] · Hiroyoshi Kawamoto[2] · Yusuke Watanabe[2] · Kentaro Tanaka[2] · Shengpu Chou[8] · Tomohiko Sato[2] · Toru Naganuma[2] · Masaaki Okutsu[2] · Satoko Tahara[2] · Naoyuki Kurita[2] · Shotaro Nakamura[2] · David J. Kuter[9] · Sunao Nakamura[2] · Ik-Kyung Jang[1,10]

Accepted: 11 September 2022 / Published online: 7 October 2022

Abstract

Vascular inflammation, lipid metabolism, and thrombogenicity play a key role not only in atherogenesis but also in the development of acute coronary syndromes. Biomarkers associated with coronary high-risk plaques defined according to intravascular imaging have not been systematically studied. A total of 69 patients with coronary artery disease who underwent both optical coherence tomography and intravascular ultrasound imaging, and who provided blood specimens were included. Comprehensive biomarkers for inflammation, lipid, and coagulation were analyzed. Composite models sought biomarker patterns associated with thin-cap fibroatheroma (TCFA) and "high-risk plaques" (TCFA and large plaque burden). Two different composite models were developed for TCFA, based on the finding that high sensitivity C-reactive protein (hsCRP), plasminogen activator inhibitor-1, fibrinogen, IL-6, homocysteine and amyloid A levels were elevated, and high-density lipoprotein cholesterol (HDL) and bile acid levels were decreased in these patients. Both composite models were highly accurate for detecting patients with TCFA (area under curve [AUC]: 0.883 in model-A and 0.875 in model-B, both $p < 0.001$). In addition, creatinine, hsCRP, fibrinogen, tumor necrosis factor-α, IL-6, homocysteine, amyloid A, HDL, prothrombin, and bile acid were useful for detecting patients with "high-risk plaques". Two composite models were highly accurate for detection of patients with "high-risk plaques" (AUC: 0.925 in model-A and 0.947 in model-B, both $p < 0.001$). Biomarkers useful for detection of patients with high-risk coronary plaques defined according to intravascular imaging have been identified. These biomarkers may be useful to risk stratify patients and to develop targeted therapy.
Clinical Trial Registration https://www.umin.ac.jp/ctr/, UMIN000041692.

✉ Sunao Nakamura
boss0606@pluto.plala.or.jp

✉ Ik-Kyung Jang
ijang@mgh.harvard.edu

1 Cardiology Division, Massachusetts General Hospital, Harvard Medical School, 55 Fruit Street, GRB 800, Boston, MA 02114, USA

2 Interventional Cardiology Unit, New Tokyo Hospital, 1271 Wanagaya, Matsudo, Chiba 270-2232, Japan

3 Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

4 Biostatistics Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

5 Department of Clinical Laboratory Medicine, New Tokyo Hospital, Matsudo, Chiba, Japan

6 Clinical Research Center, New Tokyo Hospital, Matsudo, Chiba, Japan

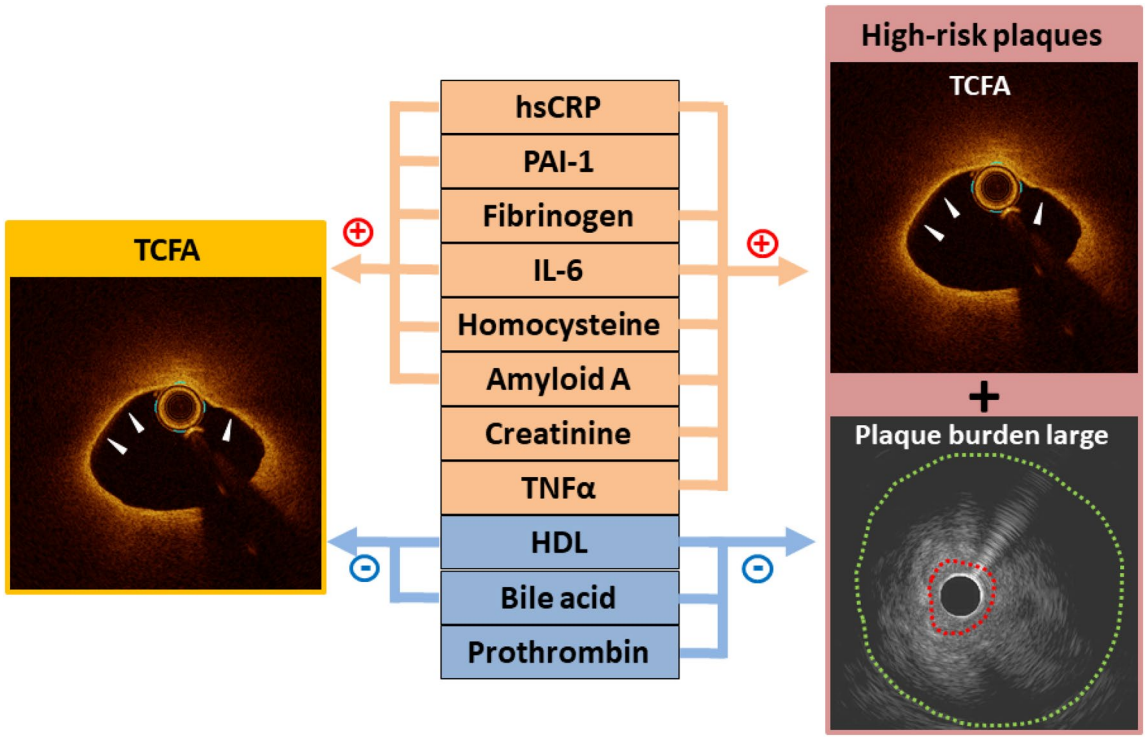
7 Division of Image Processing, Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands

8 Department of Diabetes Internal Medicine, New Tokyo Hospital, Matsudo, Chiba, Japan

9 Hematology Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

10 Division of Cardiology, Kyung Hee University Hospital, Seoul, Korea

Graphical abstract



Biomarkers and high-risk plaqueshsCRP, PAI-1, fibrinogen, IL-6, homocysteine, amyloid A, HDL, and bile acid were useful for detecting patients with TCFA. hsCRP, fibrinogen, IL-6, homocysteine, amyloid A, creatinine, TNFα, HDL, prothrombin, and bile acid were useful for detecting patients with "high-risk plaques" (plaque which has both TCFA and large plaque burden). White arrowhead denotes TCFA. Red and green dashed lines denote lumen area and external elastic membrane area, respectively.

Keywords Biomarker · Coronary artery disease · Intravascular ultrasound · Optical coherence tomography · Vulnerable plaque

Abbreviations

ACS	Acute coronary syndrome
EEM	External elastic membrane
FCT	Fibrous cap thickness
HDL	High-density lipoprotein cholesterol
hsCRP	High sensitivity C-reactive protein
IL	Interleukin
IVUS	Intravascular ultrasound
OCT	Optical coherence tomography
PB	Plaque burden
SCFAs	Short-chain fatty acids
TCFA	Thin-cap fibroatheroma
TMAO	Trimethylamine N-oxide
TNFα	Tumor necrosis factor α

Highlights

- Vascular inflammation, lipid metabolism, and thrombogenicity play a key role not only in atherogenesis but also in the development of acute coronary syndromes.
- Blood biomarkers, including hsCRP, PAI-1, fibrinogen, IL-6, homocysteine, amyloid A, HDL-C, and bile acid, are useful for detecting patients with TCFA.
- Creatinine, hsCRP, fibrinogen, TNFα, IL-6, homocysteine, amyloid A, HDL, prothrombin, and bile acid are useful for detecting patients with "high-risk plaques" (both TCFA and large plaque burden).
- Composite models of these biomarkers have higher sensitivity for identifying patients with high-risk coronary plaques.
- Biomarkers may be useful for risk stratification for patients with coronary artery disease and may help guide targeted therapy.

Introduction

Cardiovascular disease is a leading cause of death worldwide. Patients with sudden cardiac death frequently harbor plaques with high-risk features such as thin-cap fibroatheroma (TCFA) and large necrotic core [1–3]. Such patients also often have a large plaque burden [1, 4]. Intravascular imaging modalities, such as optical coherence tomography (OCT) and intravascular ultrasound (IVUS) can identify such features [5–7]. Basic and population based studies have revealed that inflammation, lipid metabolism, thrombogenicity, and possibly gut microbial metabolites are associated with atherosclerosis and cardiovascular events [8]. Biomarkers of these pathways may serve to stratify patients for future risk of adverse cardiovascular events. [9, 10]. However, most studies tested a limited number of biomarkers, and the association between biomarkers and specific plaque characteristics has not been systematically studied. The current study aimed to discover specific blood biomarkers associated with key coronary plaque characteristics using both OCT and IVUS.

Methods

Study population

Patients with coronary artery disease who underwent both OCT and IVUS imaging prior to intervention were prospectively enrolled (UMIN000041692) at New Tokyo Hospital in Japan from October 2020 until April 2021. In the biomarker study, 69 patients whose blood specimens were successfully collected within 12 h prior to the procedure were included (Supplemental Figure S1). Detailed descriptions of the study population and definitions are provided in the Supplemental Materials. The study protocol was approved by the institutional ethics committees at New Tokyo Hospital and Massachusetts General Hospital. Written informed consent was provided by all participants.

Coronary angiography analysis

Methods for coronary angiographic analysis are described in the Supplemental Materials.

OCT and IVUS image acquisition and analysis

OCT imaging was performed using the frequency-domain OPTIS imaging system (Abbott, Minnesota). IVUS imaging was performed using iLab (Boston Scientific, Massachusetts) or VISICUBE (Terumo, Tokyo). Aspiration thrombectomy was allowed before intravascular imaging in patients with TIMI flow grade < 2 and/or occlusive thrombus. All OCT and IVUS images were submitted to the Massachusetts General Hospital core laboratory. OCT and IVUS image analysis was performed using offline review workstations (Ilumien Optis, St. Jude Medical) (QCU-CMS-RESEARCH version 4.69, Leiden University Medical Center, Leiden, The Netherlands) by investigators who were blinded to the clinical, angiographic, and laboratory data. On OCT, lipid was defined as a low-signal region with diffuse border [7]. The degree of lipid arc was measured at 1-mm intervals. Lipid length was measured on the longitudinal view, and lipid index was obtained as the product of mean lipid arc and lipid length [11]. Lipid-rich plaque was defined as a plaque with a maximal lipid arc greater than 90° [12]. In lipid plaques, fibrous cap thickness (FCT) was measured 3 times at the thinnest part and the average value was calculated. TCFA was defined as a plaque with a maximal lipid arc greater than 90° and FCT ≤ 65 μm [13, 14]. Additional OCT analysis was performed according to the previously established criteria as described in the Supplemental Materials [7]. Good intraobserver and interobserver agreement was noted in the identification of TCFA (κ, 0.911 and 0.905, respectively). In IVUS analysis, cross-sectional area of the external elastic membrane (EEM) and lumen area were measured at 1-mm intervals. Plaque burden (PB) was analyzed as: (EEM area at minimal lumen area site—minimal lumen area)/EEM area at minimal lumen area site, using IVUS [15]. Greater PB was defined as plaques which have PB ≥ median PB value (80.26%). Previous studies reported that TCFA and large plaque burden were strongly associated with cardiac events [1, 3]. Thus, we defined a "high-risk plaques" as a plaque which has both TCFA and large PB.

Blood biomarker analysis

The blood samples for biomarker analysis were collected from patients within 12 h prior to the procedure. Details of biomarker analyses are described in the Supplemental Materials.

Statistical analysis

Categorical data are presented as counts and percentages, and are compared using the chi-squared test or Fisher exact test, as appropriate. Continuous data are presented as mean ± standard deviation or median (25th − 75th percentile), as appropriate, depending on the normality of distribution. Between-group differences in continuous variables were compared using the Student *t*-test or Mann–Whitney *U* test, as appropriate. Receiver-operating characteristic curve analyses were performed to determine the best cut-off values of biomarkers for discriminating TCFA and

"high-risk plaques" as well as the sensitivities and specificities. The discriminative composite models were built on multivariable logistic regression including biomarkers which were significantly different between patients with and without specific feature (TCFA and "high-risk plaques"). Specifically, high sensitivity C-reactive protein (hsCRP), plasminogen activator inhibitor-1 (PAI-1), fibrinogen, interleukin-6 (IL-6), homocysteine, amyloid A, high-density lipoprotein cholesterol (HDL), and bile acid were different between patients with TCFA versus without TCFA. Thus, these biomarkers were included in the models for TCFA. Creatinine, hsCRP, fibrinogen, tumor necrosis factor α (TNFα), IL-6, homocysteine, amyloid A, HDL, prothrombin, and bile acid were significantly different between patients with "high-risk plaque" versus without "high-risk plaque". Thus, we included these biomarkers in the composite models for "high-risk plaque. In each analysis, two versions of composite models were built because IL-6 and amyloid A had multicollinearity (variance inflation factor > 10). Analyses were performed with SPSS (version 25 for Windows; SPSS, Inc., Chicago Illinois).

Results

Patient characteristics, angiographic findings, and OCT/IVUS findings

Patient characteristics are shown in Table 1. Among 69 patients, 50 patients (72.5%) were male and the majority of patients presented with stable angina pectoris (87.0%). Patients who had TCFA at the culprit lesion more frequently presented with ACS, and were less frequently on antiplatelet therapy, compared to those without TCFA. In the angiographic analysis, patients with TCFA had greater diameter stenosis than those without TCFA (Supplemental Table S1). OCT and IVUS findings are shown in Table 2. The majority of patients without TCFA had lipid-rich plaque (83%) or macrophages (77%). Patients with TCFA had larger lipid and plaque burden, compared to those without TCFA.

Differences in biomarkers between patients with and without TCFA

Certain biomarkers differed significantly between patients with and without TCFA (Fig. 1, Supplemental Table S2 contains additional biomarker data). Patients with TCFA had significantly higher values of hsCRP, PAI-1, fibrinogen, IL-6, homocysteine, and amyloid A, and significantly lower values of HDL and bile acid, compared to those without TCFA.

ROC curve for detecting patients with TCFA

The ROC curves for distinguishing the patients with TCFA from those without are shown in Fig. 2A–C. ROC curves and cut-off values of biomarkers associated with the presence of TCFA are shown in Fig. 2A and include hsCRP, PAI-1, fibrinogen, IL-6, homocysteine, and amyloid A, whereas biomarkers associated with the absence of TCFA, which include HDL and bile acid, are shown in Fig. 2B. We developed two versions of composite models (model-A: hsCRP, PAI-1, fibrinogen, IL-6, homocysteine, HDL-C, and bile acid; model-B: hsCRP, PAI-1, fibrinogen, homocysteine, amyloid A, HDL-C, and bile acid) because of the multicollinearity of IL-6 and amyloid A (variance inflation factor > 10). The area under the curve (AUC) for distinguishing patients with TCFA from those without was 0.883 (95% confidence interval [95%CI], 0.800–0.966) in model-A and 0.875 (95%CI, 0.789–0.961) in model-B (Fig. 2C). Figure 2D and E show the relationships between the number of predictors and the prevalence of TCFA. The number of predictors were calculated as: the sum of the number of unfavorable biomarkers beyond cut-off value and the number of favorable biomarkers less than cut-off value. The prevalence of TCFA increased as the number of predictors increased.

Differences in biomarkers for patients with and without "high-risk plaques"

Out of 69 patients, 14 had "high-risk plaques" as defined above. We identified the blood biomarkers that can distinguish patients with or without "high-risk plaques" (differences in each biomarker value between patients with and without "high-risk plaques" are shown in Supplemental Table S3). ROC curves and cut-off values are shown in Fig. 3A–C. ROC curves showed that creatinine, hsCRP, fibrinogen, TNFα, IL-6, homocysteine, and amyloid A were useful for predicting the presence of "high-risk plaques" (unfavorable biomarkers) (Fig. 3A). In contrast, HDL, prothrombin, and bile acid were useful for predicting the absence of "high-risk plaques" (favorable biomarkers) (Fig. 3B). Like the analyses for TCFA, we made two composite models (model-A: creatinine, hsCRP, fibrinogen, TNFα, IL-6, homocysteine, HDL, prothrombin, and bile acid; model-B: creatinine, hsCRP, fibrinogen, TNFα, homocysteine, amyloid A, HDL, prothrombin, and bile acid) because of multicollinearity of IL-6 and amyloid. The AUC for distinguishing patients with "high-risk plaques" from those without was 0.925 (0.850–1.000) in model-A and 0.947 (0.893–1.000) in model-B (Fig. 3C). Figure 3D and E show the relationship between the number of predictors

Table 1 Patient characteristics

	All (n = 69)	Patients with TCFA (n = 22)	Patients without TCFA (n = 47)	p value
Age, y	70.7 ± 11.1	71.1 ± 11.7	70.5 ± 10.9	0.691
BMI	25.2 ± 3.6	25.9 ± 3.1	24.8 ± 3.7	0.246
Male, n (%)	50 (72.5)	18 (81.8)	32 (68.1)	0.243
Clinical presentation				**0.002**
Stable angina pectoris, n (%)	60 (87.0)	15 (68.2)	45 (95.7)	
STEMI, n (%)	3 (4.3)	3 (13.6)	0 (0.0)	
NSTE-ACS, n (%)	6 (8.7)	4 (18.2)	2 (4.3)	
Prior MI, n (%)	7 (10.1)	3 (13.6)	4 (8.5)	0.394
Prior PCI, n (%)	23 (33.3)	6 (27.3)	17 (36.2)	0.465
Prior CABG, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	–
Hypertension, n (%)	56 (81.2)	16 (72.7)	40 (85.1)	0.184
Dyslipidemia, n (%)	59 (85.5)	18 (81.8)	41 (87.2)	0.398
Diabetes mellitus, n (%)	26 (37.7)	8 (36.4)	18 (38.3)	0.877
Renal Insufficiency, n (%)	19 (27.5)	9 (40.9)	10 (21.3)	0.089
Family history of CAD, n (%)	2 (2.9)	0 (0.0)	2 (4.3)	0.461
Smoking				0.458
Current smoker, n (%)	13 (18.8)	6 (27.3)	7 (14.9)	
Past smoker, n (%)	30 (43.5)	9 (40.9)	21 (44.7)	
Never smoker, n (%)	26 (37.7)	7 (31.8)	19 (40.4)	
LVEF, %	63.9 (60.7–66.1)	63.9 (54.4–65.8)	63.9 (60.9–67.8)	0.223
Medication at admission				
Aspirin, n (%)	47 (68.1)	9 (40.9)	38 (80.9)	**0.001**
P2Y12 inhibitor, n (%)	42 (60.9)	9 (40.9)	33 (70.2)	**0.020**
Warfarin, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	–
DOAC, n (%)	9 (13.0)	2 (9.1)	7 (14.9)	0.402
Statin, n (%)	48 (69.6)	12 (54.5)	36 (76.6)	0.064
PCSK9 inhibitor, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	–
β blocker, n (%)	15 (21.7)	4 (18.2)	11 (23.4)	0.439
ACEI/ARB, n (%)	34 (49.3)	11 (50.0)	23 (48.9)	0.934
Culprit vessel				0.073
LAD, n (%)	48 (69.6)	14 (63.6)	34 (72.3)	
LCX, n (%)	9 (13.0)	1 (4.5)	8 (17.0)	
RCA, n (%)	12 (17.4)	7 (31.8)	5 (10.6)	

Statistically significant ($p < 0.05$) are given in bold

Values are mean ± SD, n (%), or median (interquartile range)

ACEI/ARB angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker, *BMI* body mass index, *CABG* coronary artery bypass graft, *CAD* coronary artery disease, *DOAC* direct oral anticoagulant, *LAD* left anterior descending artery, *LCX* left circumflex artery, *LVEF* left ventricular ejection fraction, *MI* myocardial infarction, *NSTE-ACS* non-ST-segment elevation acute coronary syndrome, *PCI* percutaneous coronary intervention,*RCA* right coronary artery, *STEMI* ST-segment elevation myocardial infarction

and the prevalence of "high-risk plaques". The prevalence of "high-risk plaques" increased as the number of predictors increased.

Discussion

This study identified blood biomarkers useful for distinguishing patients with TCFA and "high-risk plaques" from those without. To have a more robust method to predict high-risk plaques, composite models were devised. Furthermore, we identified the optimal cut-off of each biomarker and

Table 2 OCT and IVUS analysis

	All (n = 69)	Patients with TCFA (n = 22)	Patients without TCFA (n = 47)	p-value
Qualitative OCT analysis				
Lipid-rich plaque, n (%)	61 (88.4)	22 (100.0)	39 (83.0)	**0.040**
TCFA, n (%)	22 (31.9)	22 (100.0)	0 (0.0)	–
Macrophage, n (%)	58 (84.1)	22 (100.0)	36 (76.6)	**0.013**
Microvessels, n (%)	33 (47.8)	14 (63.6)	19 (40.4)	0.072
Cholesterol crystal, n (%)	21 (30.4)	9 (40.9)	12 (25.5)	0.196
Calcification, n (%)	52 (75.4)	16 (72.7)	36 (76.6)	0.728
Layered plaque, n (%)	48 (69.6)	16 (72.7)	32 (68.1)	0.696
Culprit etiology in ACS cases (n = 9)				**0.028**
Plaque rupture, n (%)	7 (77.8)	7 (100.0)	0 (0.0)	
Plaque erosion, n (%)	2 (22.2)	0 (0.0)	2 (100.0)	
Quantitative OCT and IVUS analysis				
Minimal flow area, mm^2	1.19 (0.88–1.84)	1.27 (1.02–1.68)	1.14 (0.79–1.93)	0.444
Reference lumen area, mm^2	6.67 (4.72–8.78)	7.26 (6.72–10.29)	6.00 (4.59–8.10)	**0.009**
Area stenosis, %	78.2 ± 10.8	80.6 ± 8.9	77.1 ± 11.5	0.209
Lipid analysis				
Thinnest FCT, μm	87 (60–131)	54 (43–60)	107 (87–150)	**< 0.001**
Max lipid arc, degree	246 (148–360)	360 (269–360)	181 (137–264)	**< 0.001**
Mean lipid arc, degree	157 ± 56	206 ± 39	133 ± 47	**< 0.001**
Lipid length, mm	10.1 (6.1–12.8)	12.3 (9.6–20.7)	8.2 (5.2–11.5)	**0.001**
Lipid index	1419.0 (829.8–2298.9)	2491.7 (1859.4–4113.2)	1080.0 (499.8–1828.5)	**< 0.001**
Plaque burden by IVUS, %	80.3 (73.3–86.5)	82.7 (76.6–89.8)	79.9 (71.5–83.0)	**0.039**

Statistically significant ($p < 0.05$) are given in bold

Values are n (%), median (interquartile range), or mean ± SD.

ACS acute coronary syndrome, *FCT* fibrous cap thickness, *IVUS* intravascular ultrasound, *OCT* optical coherence tomography, *TCFA* thin-cap fibroatheroma

demonstrated the relationship between the number of predictors and the presence of TCFA and "high-risk plaques".

This is a pilot study demonstrating potential for the utilization of blood biomarkers to identify patients with high-risk coronary plaque features defined according to intravascular imaging. Biomarkers may provide insights into a link between underlying pathological process and high-risk coronary plaque phenotype. Although the number of the patient was limited (especially patients who presented with ACS), if the findings are replicated in larger studies, this simple approach will help not only to risk stratify but also to guide targeted therapy for patients with coronary artery disease.

Inflammation and coronary artery disease

Inflammation contributes to atherosclerotic plaque formation and adverse cardiac events [8, 16, 17]. This study demonstrated that blood inflammatory biomarkers (such as hsCRP, IL-6, fibrinogen, and amyloid A) may help to detect patients with coronary plaques with higher risk features on intravascular imaging. This result is consistent with previous studies which showed inflammatory biomarkers have significant associations with coronary artery disease burden and cardiovascular events [18–20]. Atherosclerotic lesion formation generally follows accumulation and modification of plasma-derived lipoproteins and their uptake by macrophages (foam cell formation) [21]. Cell death and deficient clearance of dead cells (efferocytosis) promotes formation of the plaque's necrotic core. Within the plaque, inflammatory activation of macrophages, mast cells, and T cells provokes the release of pro-inflammatory cytokines which inhibit interstitial collagen synthesis and proteases which digest fibrous cap components [17]. Thus, inflammation contributes to TCFA formation. Atherosclerosis also involves endothelial dysfunction. Pro-inflammatory cytokines such as IL-1 and TNFα promote the interaction between circulating leukocytes and the endothelium through induction of leukocyte adhesion molecules (such as vascular cell adhesion molecule 1) [22]. Downstream of IL-1 and TNF, IL-6 exacerbates the progression of atherosclerosis through activation of Janus kinase 1 and signal transducer and activator of transcription 1 and 3 [22]. IL-6

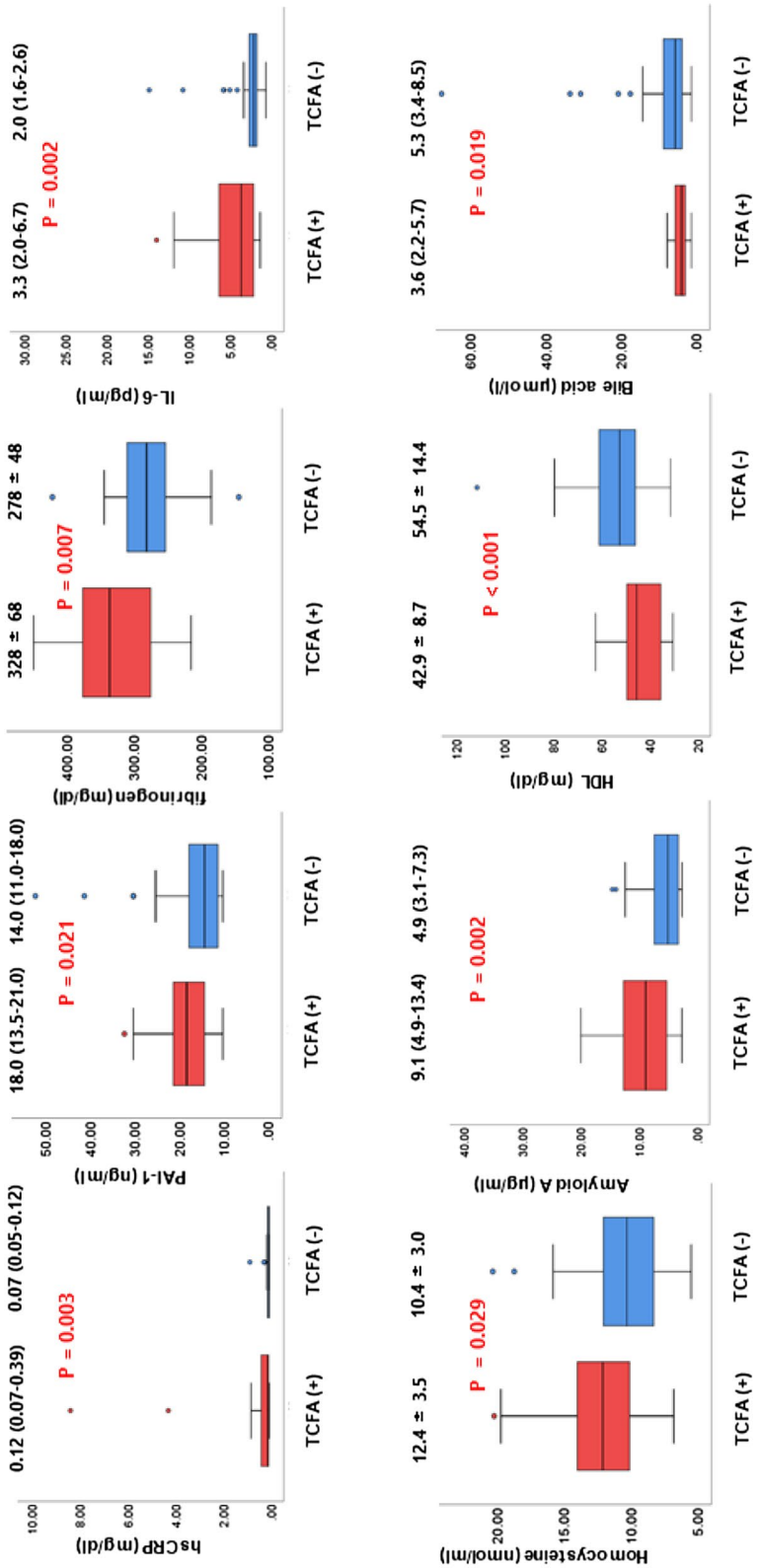
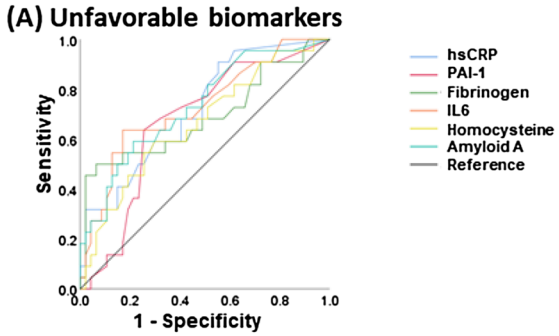
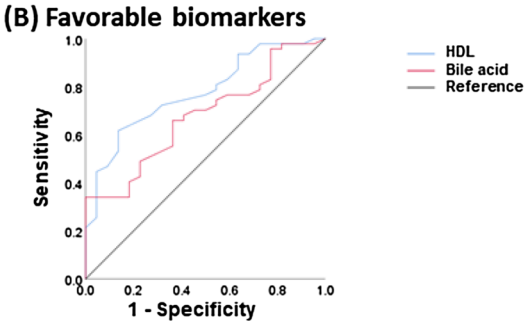


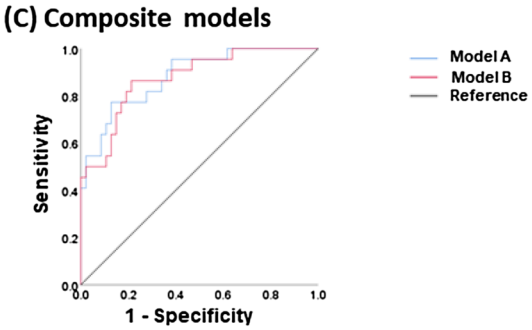
Fig. 1 Difference in biomarkers between patients with and without TCFA



	AUC (95% CI)	Cut-off	Sensitivity	Specificity	P value
hsCRP	0.720 (0.594 – 0.845)	0.0635	0.909	0.447	0.003
PAI1	0.673 (0.540 – 0.807)	17.5	0.636	0.745	0.021
Fibrinogen	0.688 (0.539 – 0.836)	333.5	0.500	0.936	0.012
IL-6	0.730 (0.600 – 0.860)	2.75	0.636	0.830	0.002
Homocysteine	0.663 (0.525 – 0.802)	11.550	0.591	0.702	0.030
Amyloid A	0.733 (0.605 – 0.860)	7.7	0.591	0.787	0.002

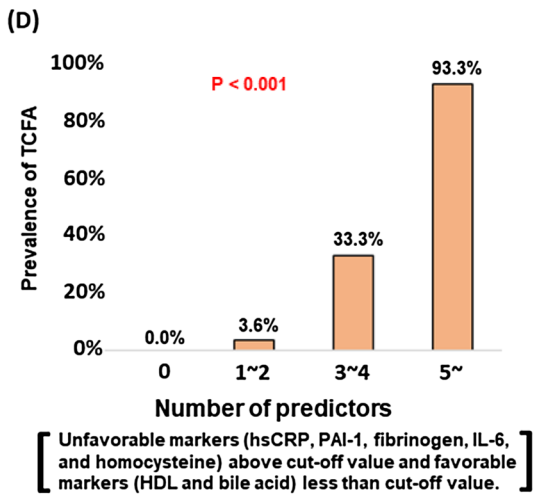


	AUC (95% CI)	Cut-off	Sensitivity	Specificity	P value
HDL	0.776 (0.664 – 0.888)	50.5	0.617	0.864	< 0.001
Bile acid	0.676 (0.547 – 0.805)	4.05	0.660	0.636	0.019

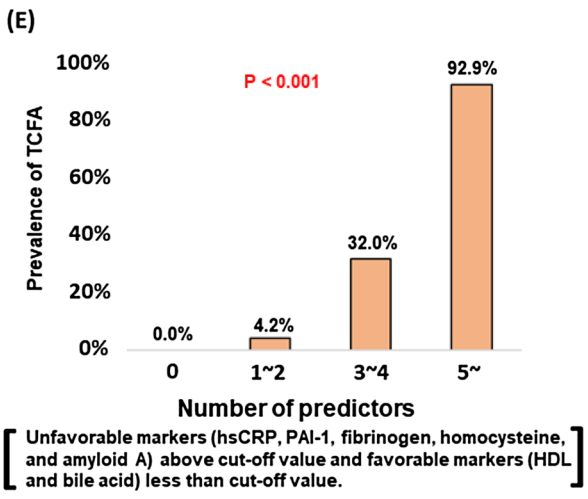


	AUC (95% CI)	P value
Model A	0.883 (0.800 – 0.966)	< 0.001
Model B	0.875 (0.789 – 0.961)	< 0.001

Model A: hsCRP, PAI-1, Fibrinogen, IL-6, Homocysteine, HDL, Bile acid
Model B: hsCRP, PAI-1, Fibrinogen, Homocysteine, Amyloid A, HDL, Bile acid



[Unfavorable markers (hsCRP, PAI-1, fibrinogen, IL-6, and homocysteine) above cut-off value and favorable markers (HDL and bile acid) less than cut-off value.]



[Unfavorable markers (hsCRP, PAI-1, fibrinogen, homocysteine, and amyloid A) above cut-off value and favorable markers (HDL and bile acid) less than cut-off value.]

also unleashed the acute phase response in hepatocytes. Fibrinogen, an acute phase reactant induced by IL-6, not only reflects inflammation but also furnishes fibrin for thrombus formation and increases plasma viscosity [23]. Amyloid A is a highly sensitive acute phase reactant and known to associate with atherosclerosis [24]. In addition to these mechanistic reports, CANTOS demonstrated the effectiveness of biomarker guided anti-inflammatory therapy by showing that allocation of a monoclonal antibody targeting IL-1β based on hsCRP reduces major adverse

◂**Fig. 2** ROC curves and cut-off values for distinguishing between the patients with and without TCFA and the relationship between the number of predictors and the prevalence of TCFA. **A** shows the ROC curves and best cut-off values of biomarkers useful for detecting the patients with TCFA (unfavorable biomarkers). **B** shows the ROC curves and best cut-off values of biomarkers useful for detecting the patients without TCFA (favorable biomarkers). **C** shows the ROC curves of composite models (model-A: hsCRP, PAI-1, fibrinogen, IL-6, homocysteine, HDL, and bile acid, model-B: hsCRP, PAI-1, fibrinogen, homocysteine, amyloid A, HDL, and bile acid). We made 2 versions of the model because IL-6 and amyloid A had multicollinearity (variance inflation factor > 10). Both models were highly accurate for distinguishing between patients with and without TCFA. **D** and **E** show the relationship between number of predictors and the prevalence of TCFA. The number of predictors was calculated as: the number of unfavorable biomarkers (hsCRP, PAI-1, fibrinogen, IL-6, and homocysteine in **D**, hsCRP, PAI-1, fibrinogen, homocysteine, and amyloid A in **A**]) above each cut-off value and favorable markers (HDL and bile acid) less than each cut-off value. In both panels, the prevalence of TCFA increased as the number of predictors increased.

cardiovascular events [25]. This result illustrated the utility of inflammatory biomarkers in guiding optimal anti-inflammatory therapy.

Thrombogenic and lipid factors, and coronary artery disease

Prothrombotic factors also associate with coronary artery disease and ACS. The acute phase reactant PAI-1 can promote thrombosis and atherosclerosis [26]. PAI-1 inhibitors tissue-type and urokinase-type plasminogen activator, key components of the endogenous fibrinolysis. In addition to hepatocyte production, endothelial cell and smooth muscle cell activation or injury can increase local vascular PAI-1 synthesis. By suppressing the fibrinolytic system, PAI-1 facilitates clot stability [26]. Previous studies reported the increase of PAI-1 in atherosclerosis, coronary artery disease, obesity, and insulin resistance [26]. Homocysteine may also contribute to coronary artery disease. Homocysteine is associated with an increase in vascular oxidative burden through an increase in superoxide radical production and alteration of intracellular antioxidant enzymes [27]. As a result, homocysteine can lead to endothelial dysfunction, a prothrombotic state, and atherosclerosis. In addition, the pro-oxidative state caused by homocysteine can activate inflammatory mediators such as that governed by the transcription factor nuclear factor kappa B, which can exacerbate atherosclerosis [27]. In our current study, HDL was a protective factor against TCFA and "high-risk plaques". HDL concentrations correlate inversely with atherosclerotic events. HDL can mediate reverse cholesterol transport; the process of removing excess cholesterol from arterial wall's macrophages to the liver, bile, and feces [28]. In addition, HDL has antioxidant components, which might protect against atherosclerosis. HDL inhibits the expression of adhesion molecules and migration of monocytes into the subendothelial space [28]. Furthermore, HDL also enhances nitric oxide synthesis, increasing the production of nitric oxide, which protects against inflammation activation in the endothelium [28]. On the other hand, our current study showed prothrombin was a protective biomarker for "high-risk plaques". However, prothrombin is associated with the coagulation cascade and it can have an unfavorable effect on atherosclerosis from a theoretical perspective (it can promote mural thrombus after minor endothelial disruption, which results in rapid plaque progression following thrombus organization). The role of prothrombin on atherosclerosis remains incompletely understood and other factors such as liver disease and alcohol consumption can affect prothrombin [29, 30]. Thus, further mechanistic and validation studies are required.

Gut microbial metabolites and coronary artery disease

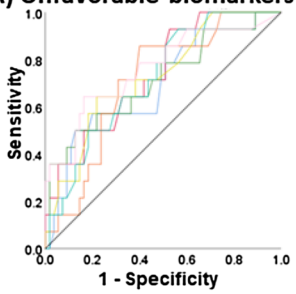
Recent accumulating evidence suggests that gut microbiota and their metabolites may influence atherosclerosis and adverse coronary events. Previous large studies have reported the unfavorable effect of trimethylamine oxide (TMAO) [31, 32]. In contrast, certain short chain fatty acids (SCFAs) may exert cardioprotective functions [33]. This study did not show clear relationships between vulnerable plaque features, and TMAO and SCFAs (Supplemental Tables S2 and S3). This study lacked healthy controls because of the invasive nature of the intravascular imaging.

Our study demonstrated that bile acid was associated with favorable features (related to the absence of TCFA and "high-risk plaques"). Bile acids have been reported to be associated with lipid metabolism, glucose/insulin metabolism, and inflammation [33]. Although the effects of bile acids on atherosclerosis have not been fully understood, they may modulate the gut microbial microflora and modulate immune responses and have an impact on host physiology through farnesoid X receptor, liver X receptor, pregnane X receptor, and G-protein-coupled receptors [33]. Bile acids comprise several species which have different concentrations. However, our current study analyzed only total bile acid. Thus, defining the effects of specific bile acids on plaque characteristics will require further study.

Clinical implication

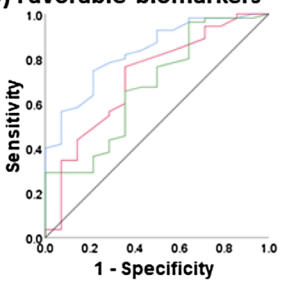
Our current study demonstrated the possibility of risk stratification for high-risk plaques in patients with coronary artery disease using a combination of blood biomarkers. Based on biomarkers, a targeted therapy for inflammation, lipid, coagulation, or endothelial dysfunction may be chosen. In addition, although large validation studies are required, the composite models of biomarkers may help identify patients

(A) Unfavorable biomarkers



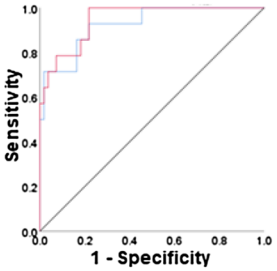
	AUC (95% CI)	Cut-off	Sensitivity	Specificity	P value
Creatinine	0.694 (0.536 – 0.852)	1.065	0.500	0.873	0.026
hsCRP	0.738 (0.602 – 0.873)	0.071	0.929	0.473	0.006
Fibrinogen	0.712 (0.550 – 0.875)	333.5	0.500	0.873	0.015
TNFα	0.708 (0.571 – 0.845)	0.710	0.857	0.600	0.017
IL-6	0.729 (0.587 – 0.871)	2.950	0.643	0.782	0.009
Homocysteine	0.719 (0.585 – 0.853)	9.550	0.929	0.436	0.012
Amyloid A	0.762 (0.609 – 0.914)	9.050	0.643	0.836	0.003

(B) Favorable biomarkers



	AUC (95% CI)	Cut-off	Sensitivity	Specificity	P value
HDL	0.831 (0.721 – 0.942)	46.5	0.745	0.786	< 0.001
Prothrombin	0.713 (0.554 – 0.872)	77.0	0.764	0.643	0.014
Bile acid	0.678 (0.517 – 0.839)	1.75	0.946	0.357	0.041

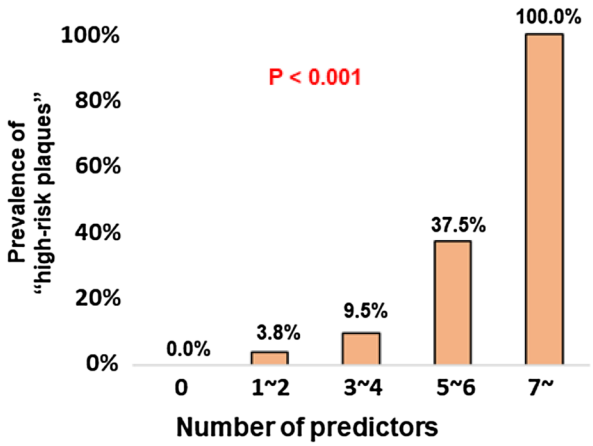
(C) Composite models



	AUC (95% CI)	P value
Model A	0.925 (0.850 – 1.000)	< 0.001
Model B	0.947 (0.893 – 1.000)	< 0.001

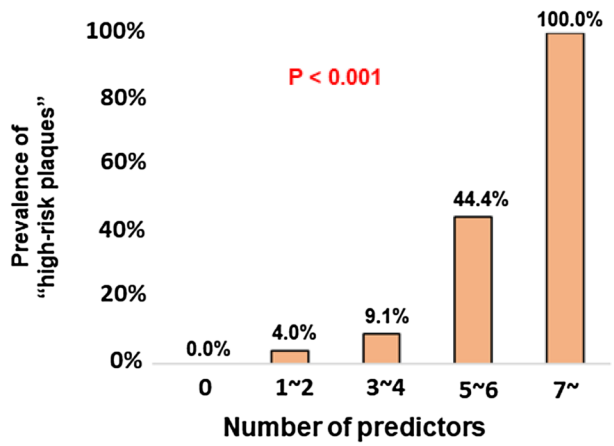
Model A: Creatinine, hsCRP, Fibrinogen, TNFα, IL6, Homocysteine, HDL, Factor 2, Bile acid
Model B: Creatinine, hsCRP, Fibrinogen, TNFα, Homocysteine, Amyloid A, HDL, Factor 2, Bile acid

(D)



[Unfavorable markers (creatinine, hsCRP, fibrinogen, TNFα, IL-6, and homocysteine) above cut-off value and favorable markers (HDL, prothrombin, and bile acid) less than cut-off value.]

(E)



[Unfavorable markers (creatinine, hsCRP, fibrinogen, TNFα, homocysteine, and amyloid A) above cut-off value and favorable markers (HDL, prothrombin, and bile acid) less than cut-off value.]

with high-risk plaques in the coronary arteries without invasive examinations, such as intra-coronary imaging.

Limitations

This study has several limitations. First, the current study was a proof-of-concept study, therefore the number of patients was small. In addition, the majority of the patients

◂**Fig. 3** ROC curves and cut-off values for distinguishing between the patients with and without "high-risk plaques" and the relationship between the number of predictors and the prevalence of "high-risk plaques". **A** shows the ROC curves and best cut-off values of biomarkers useful for detecting the patients with "high-risk plaques" (unfavorable biomarkers). **B** shows the ROC curves and best cut-off values of biomarkers useful for detecting the patients without "high-risk plaques" (favorable biomarkers). **C** shows the ROC curves of composite models (model-A: creatinine, hsCRP, fibrinogen, TNFα, IL-6, homocysteine, HDL, prothrombin, and bile acid, model-B: creatinine, hsCRP, fibrinogen, TNFα, homocysteine, amyloid A, HDL, prothrombin, and bile acid). We made 2 versions of the model because IL-6 and amyloid A had multicollinearity (variance inflation factor > 10). Both models were highly accurate for distinguishing between patients with and without "high-risk plaques". **D** and **E** show the relationship between number of predictors and the prevalence of "high-risk plaques". The number of predictors was calculated as: the number of unfavorable biomarkers (creatinine, hsCRP, fibrinogen, TNFα, IL-6, and homocysteine in **A**, creatinine, hsCRP, fibrinogen, TNFα, homocysteine, and amyloid A in **B**) above each cut-off value and favorable biomarkers (HDL, prothrombin, and bile acid) less than each cut-off value. In both panels, the prevalence of "high-risk plaques" increased as the number of predictors increased.

enrolled in this study presented with stable angina. Hence, the intravascular imaging findings which suggestive of high-risk plaques in the current study are not fully representative for all vulnerable/high-risk plaques and the results of this study should be limited to those patients with stable coronary artery disease. The biomarkers associated with vulnerable plaque features may differ depending on clinical presentation: ACS or stable angina pectoris. Larger studies are warranted to confirm this preliminary finding. Second, only patients with coronary artery disease were enrolled. Therefore, there is no healthy control data in our current study. Third, although we investigated more than 40 blood biomarkers in our current study, the panels tested may not include some potentially informative biomarkers including for example non-coding RNAs. A large study with comprehensive biomarker assays to confirm the findings of this pilot study is warranted. Fourth, multiple other factors (not only plaque characteristics) could have influenced blood biomarker levels. Fifth, comparison of the biomarker patterns developed here to correlate with morphologic characteristics of plaques with other risk stratification algorithms and prediction of events will require further study. Finally, all patients were enrolled in Japan. There is a possibility that the results could differ in a cohort with different ethnic backgrounds.

Conclusion

This study identified patterns of blood biomarkers that associate with high-risk plaque features defined according to intravascular imaging. Such patterns of biomarkers may aid risk assessment and help plan targeted therapeutic strategies.

Patients with TCFA had significantly higher values of hsCRP, PAI-1, fibrinogen, IL-6, homocysteine, and amyloid A and lower values of HDL and bile acid, compared to patients without TCFA.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11239-022-02709-2.

Acknowledgements The authors thank Asuka Beppu, RN and Tomoka Furuya, RN (Clinical Research Center, New Tokyo Hospital) for data collection and management work; Masaomi Kono, MT (Department of Clinical Laboratory Medicine, New Tokyo Hospital) for specimen handling; Hiroaki Obata, Miyuki Tanaka, and Taeko Shiino (Intravascular imaging team, New Tokyo Hospital) and Akira Muzuma, RT (Department of radiology, New Tokyo Hospital) for image acquisition and transfer; and all other staff at New Tokyo Hospital for their support of this study.

Author contributions AN: Conceptualization, methodology, investigation, formal analysis, writing—original draft. PL: writing—review and editing, supervision. SM: investigation, methodology. HY: investigation, methodology. MA: investigation, methodology. LMS: investigation, methodology. IM: investigation, writing—review and editing. HL: methodology, investigation, formal analysis, writing—review and editing. MI: investigation, methodology. KK: investigation, methodology. JD: methodology, supervision. TO: investigation. HO: investigation. HY: investigation. SM: investigation. HK: investigation. YW: investigation. KT: investigation. SC: investigation. TS: Investigation. TN: investigation. MO: investigation. ST: investigation. NK: investigation. SN: investigation. DJK: writing—review and editing, supervision. SN: conceptualization, methodology, investigation, resources, supervision. IKJ: conceptualization, methodology, investigation, resources, formal analysis, writing—review and editing, supervision, project administration.

Funding Dr Jang's research has been supported by Mrs. Gillian Gray through the Allan Gray Fellowship Fund in Cardiology and Mr. and Mrs. Michael and Kathryn Park. They had no role in the design or conduct of this research.

Declarations

Conflict of interest Dr. Jang has received educational grant support from Abbott Vascular. All other authors have no relationships relevant to the contents of this paper to disclose.

Informed consent Written informed consent was provided by all participants.

References

1. Stone GW, Maehara A, Lansky AJ, de Bruyne B, Cristea E, Mintz GS, Mehran R, McPherson J, Farhat N, Marso SP, Parise H, Templin B, White R, Zhang Z, Serruys PW (2011) A prospective

natural-history study of coronary atherosclerosis. N Engl J Med 364(3):226–235. doi:https://doi.org/10.1056/NEJMoa1002358
2. Xing L, Higuma T, Wang Z, Aguirre AD, Mizuno K, Takano M, Dauerman HL, Park SJ, Jang Y, Kim CJ, Kim SJ, Choi SY, Itoh T, Uemura S, Lowe H, Walters DL, Barlis P, Lee S, Lerman A, Toma C, Tan JWC, Yamamoto E, Bryniarski K, Dai J, Zanchin T, Zhang S, Yu B, Lee H, Fujimoto J, Fuster V, Jang IK (2017) Clinical significance of lipid-rich plaque detected by optical coherence tomography: a 4-year follow-up study. J Am Coll Cardiol 69(20):2502–2513. doi:https://doi.org/10.1016/j.jacc.2017.03.556
3. Kedhi E, Berta B, Roleder T, Hermanides RS, Fabris E, AJJ IJ, Kauer F, Alfonso F, von Birgelen C, Escaned J, Camaro C, Kennedy MW, Pereira B, Magro M, Nef H, Reith S, Al Nooryani A, Rivero F, Malinowski K, De Luca G, Garcia Garcia H, Granada JF, Wojakowski W (2021) Thin-cap fibroatheroma predicts clinical events in diabeticpatients with normal fractional flow reserve: the COMBINE OCT-FFR trial. Eur Heart J 42(45):4671–4679. doi:https://doi.org/10.1093/eurheartj/ehab433
4. Arbab-Zadeh A, Fuster V (2015) The myth of the "vulnerable plaque": transitioning from a focus on individual lesions to atherosclerotic disease burden for coronary artery disease risk assessment. J Am Coll Cardiol 65(8):846–855. doi:https://doi.org/10.1016/j.jacc.2014.11.041
5. Aguirre AD, Arbab-Zadeh A, Soeda T, Fuster V, Jang IK (2021) Optical coherence tomography of plaque vulnerability and rupture: JACC focus seminar part 1/3. J Am Coll Cardiol 78(12):1257–1265. doi:https://doi.org/10.1016/j.jacc.2021.06.050
6. Mintz GS, Nissen SE, Anderson WD, Bailey SR, Erbel R, Fitzgerald PJ, Pinto FJ, Rosenfield K, Siegel RJ, Tuzcu EM, Yock PG (2001) American college of cardiology clinical expert consensus document on standards for acquisition, measurement and reporting of intravascular ultrasound studies (IVUS). A report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents. J Am Coll Cardiol 37(5):1478–1492. doi:https://doi.org/10.1016/s0735-1097(01)01175-5
7. Araki M, Park SJ, Dauerman HL, Uemura S, Kim JS, Di Mario C, Johnson TW, Guagliumi G, Kastrati A, Joner M, Holm NR, Alfonso F, Wijns W, Adriaenssens T, Nef H, Rioufol G, Amabile N, Souteyrand G, Meneveau N, Gerbaud E, Opolski MP, Gonzalo N, Tearney GJ, Bouma B, Aguirre AD, Mintz GS, Stone GW, Bourantas CV, Räber L, Gili S, Mizuno K, Kimura S, Shinke T, Hong MK, Jang Y, Cho JM, Yan BP, Porto I, Niccoli G, Montone RA, Thondapu V, Papafaklis MI, Michalis LK, Reynold H, Saw J, Libby P, Weisz G, Iannaccone M, Gori T, Toutouzas K, Yonetsu T, Minami Y, Takano M, Raffel OC, Kurihara O, Soeda T, Sugiyama T, Kim HO, Lee T, Higuma T, Nakajima A, Yamamoto E, Bryniarski KL, Di Vito L, Vergallo R, Fracassi F, Russo M, Seegers LM, McNulty I, Park S, Feldman M, Escaned J, Prati F, Arbustini E, Pinto FJ, Waksman R, Garcia-Garcia HM, Maehara A, Ali Z, Finn AV, Virmani R, Kini AS, Daemen J, Kume T, Hibi K, Tanaka A, Akasaka T, Kubo T, Yasuda S, Croce K, Granada JF, Lerman A, Prasad A, Regar E, Saito Y, Sankardas MA, Subban V, Weissman NJ, Chen Y, Yu B, Nicholls SJ, Barlis P, West NEJ, Arbab-Zadeh A, Ye JC, Dijkstra J, Lee H, Narula J, Crea F, Nakamura S, Kakuta T, Fujimoto J, Fuster V Jang IK (2022) Aassessment of coronary atherosclerosis and intervention. Nat Rev Cardiol
8. Libby P (2021) The changing landscape of atherosclerosis. Nature 592(7855):524–533. doi:https://doi.org/10.1038/s41586-021-03392-8
9. Drakopoulou M, Toutouzas K, Stefanadi E, Tsiamis E, Tousoulis D, Stefanadis C (2009) Association of inflammatory markers with angiographic severity and extent of coronary artery disease. Atherosclerosis 206(2):335–339. doi:https://doi.org/10.1016/j.atherosclerosis.2009.01.041
10. Lawler PR, Bhatt DL, Godoy LC, Lüscher TF, Bonow RO, Verma S, Ridker PM (2020) Targeting cardiovascular inflammation: next steps in clinical translation. Eur Heart J. doi:https://doi.org/10.1093/eurheartj/ehaa099
11. Vergallo R, Uemura S, Soeda T, Minami Y, Cho JM, Ong DS, Aguirre AD, Gao L, Biasucci LM, Crea F, Yu B, Lee H, Kim CJ, Jang IK (2016) Prevalence and predictors of multiple coronary plaque ruptures: in vivo 3-vessel optical coherence tomography imaging study. Arterioscler Thromb Vasc Biol 36(11):2229–2238. doi:https://doi.org/10.1161/ATVBAHA.116.307891
12. Yamamoto E, Yonetsu T, Kakuta T, Soeda T, Saito Y, Yan BP, Kurihara O, Takano M, Niccoli G, Higuma T, Kimura S, Minami Y, Ako J, Adriaenssens T, Boeder NF, Nef HM, Fracassi F, Sugiyama T, Lee H, Crea F, Kimura T, Fujimoto JG, Fuster V, Jang IK (2019) Clinical and laboratory predictors for plaque erosion in patients with acute coronary syndromes. J Am Heart Assoc 8(21):e012322. doi:https://doi.org/10.1161/jaha.119.012322
13. Kato K, Yonetsu T, Jia H, Abtahian F, Vergallo R, Hu S, Tian J, Kim SJ, Lee H, McNulty I, Lee S, Uemura S, Jang Y, Park SJ, Mizuno K, Yu B, Jang IK (2013) Nonculprit coronary plaque characteristics of chronic kidney disease. Circ Cardiovasc Imaging 6(3):448–456. doi:https://doi.org/10.1161/CIRCIMAGING.112.000165
14. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM (2000) Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 20(5):1262–1275. doi:https://doi.org/10.1161/01.atv.20.5.1262
15. Cho YK, Hwang J, Lee CH, Kim IC, Park HS, Yoon HJ, Kim H, Han SW, Hur SH, Kim KB, Kim JY, Doh JH, Shin ES, Koo BK, Nam CW (2020) Influence of anatomical and clinical characteristics on long-term prognosis of FFR-guided deferred coronary lesions. JACC Cardiovasc Interv 13(16):1907–1916. doi:https://doi.org/10.1016/j.jcin.2020.05.040
16. Ross R (1999) Atherosclerosis—an inflammatory disease. N Engl J Med 340(2):115–126. doi:https://doi.org/10.1056/nejm199901143400207
17. Hansson GK, Libby P, Tabas I (2015) Inflammation and plaque vulnerability. J Intern Med 278(5):483–493. doi:https://doi.org/10.1111/joim.12406
18. Lombardo A, Biasucci LM, Lanza GA, Coli S, Silvestri P, Cianflone D, Liuzzo G, Burzotta F, Crea F, Maseri A (2004) Inflammation as a possible link between coronary and carotid plaque instability. Circulation 109(25):3158–3163. doi:https://doi.org/10.1161/01.cir.0000130786.28008.56
19. Ridker PM, Hennekens CH, Buring JE, Rifai N (2000) C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342(12):836–843. doi:https://doi.org/10.1056/nejm200003233421202
20. Espinola-Klein C, Rupprecht HJ, Bickel C, Lackner K, Schnabel R, Munzel T, Blankenberg S (2007) Inflammation, atherosclerotic burden and cardiovascular prognosis. Atherosclerosis 195(2):e126–134. doi:https://doi.org/10.1016/j.atherosclerosis.2007.01.021
21. Bäck M, Yurdagul A Jr, Tabas I, Öörni K, Kovanen PT (2019) Inflammation and its resolution in atherosclerosis: mediators and therapeutic opportunities. Nat Rev Cardiol 16(7):389–406. doi:https://doi.org/10.1038/s41569-019-0169-2
22. Tousoulis D, Oikonomou E, Economou EK, Crea F, Kaski JC (2016) Inflammatory cytokines in atherosclerosis: current therapeutic approaches. Eur Heart J 37(22):1723–1732. doi:https://doi.org/10.1093/eurheartj/ehv759
23. Kampoli AM, Tousoulis D, Antoniades C, Siasos G, Stefanadis C (2009) Biomarkers of premature atherosclerosis. Trends Mol

Med 15(7):323–332. doi:https://doi.org/10.1016/j.molmed.2009.06.001
24. Webb NR (2021) High-density lipoproteins and serum amyloid A (SAA). Curr Atheroscler Rep 23(2):7. doi:https://doi.org/10.1007/s11883-020-00901-4
25. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P, Glynn RJ (2017) Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 377(12):1119–1131. doi:https://doi.org/10.1056/NEJMoa1707914
26. Yamamoto K, Takeshita K, Kojima T, Takamatsu J, Saito H (2005) Aging and plasminogen activator inhibitor-1 (PAI-1) regulation: implication in the pathogenesis of thrombotic disorders in the elderly. Cardiovasc Res 66(2):276–285. doi:https://doi.org/10.1016/j.cardiores.2004.11.013
27. Antoniades C, Antonopoulos AS, Tousoulis D, Marinou K, Stefanadis C (2009) Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials. Eur Heart J 30(1):6–15. doi:https://doi.org/10.1093/eurheartj/ehn515
28. Natarajan P, Ray KK, Cannon CP (2010) High-density lipoprotein and coronary heart disease: current and future therapies. J Am Coll Cardiol 55(13):1283–1299. doi:https://doi.org/10.1016/j.jacc.2010.01.008
29. Violi F, Ferro D, Basili S, Quintarelli C, Saliola M, Alessandri C, Cordova C, Balsano F (1992) Hyperfibrinolysis increases the risk of gastrointestinal hemorrhage in patients with advanced cirrhosis. Hepatology 15(4):672–676. doi:https://doi.org/10.1002/hep.1840150420
30. González-Reimers E, Galindo-Martín L, Santolaria-Fernández F, Sánchez-Pérez MJ, Alvisa-Negrín J, García-Valdecasas-Campelo E, González-Pérez JM, Martín-González MC (2008) Prognostic value of serum selenium levels in alcoholics. Biol Trace Elem Res 125(1):22–29. doi:https://doi.org/10.1007/s12011-008-8152-5
31. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL (2013) Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 368(17):1575–1584. doi:https://doi.org/10.1056/NEJMoa1109400
32. Tang WH, Wang Z, Fan Y, Levison B, Hazen JE, Donahue LM, Wu Y, Hazen SL (2014) Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: refining the gut hypothesis. J Am Coll Cardiol 64(18):1908–1914. doi:https://doi.org/10.1016/j.jacc.2014.02.617
33. Witkowski M, Weeks TL, Hazen SL (2020) Gut Microbiota and cardiovascular disease. Circ Res 127(4):553–570. doi:https://doi.org/10.1161/CIRCRESAHA.120.316242