Arteriosclerosis, Thrombosis, and Vascular Biology

CLINICAL AND POPULATION STUDIES

Healed Plaques in Patients With Stable Angina Pectoris

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OBJECTIVE: Healed plaques, signs of previous plaque destabilization, are frequently found in the coronary arteries. Healed plaques can now be diagnosed in living patients. We investigated the prevalence, angiographic, and optical coherence tomography features of healed plaques in patients with stable angina pectoris.

APPROACH AND RESULTS: Patients with stable angina pectoris who had undergone optical coherence tomography imaging were included. Healed plaques were defined as plaques with one or more signal-rich layers of different optical density. Patients were divided into 2 groups based on layered or nonlayered phenotype at the culprit lesion. Among 163 patients, 87 (53.4%) had layered culprit plaque. Patients with layered culprit plaque had more multivessel disease (62.1% versus 44.7%, P=0.027) and more angiographically complex culprit lesions (64.4% versus 35.5%, P<0.001). Layered culprit plaques had higher prevalence of lipid plaque (83.9% versus 64.5%, P=0.004), macrophage infiltration (58.6% versus 35.5%, P=0.003), calcifications (78.2% versus 63.2%, P=0.035), and thrombus (28.7% versus 14.5%, P=0.029). Lipid index (P=0.001) and percent area stenosis (P=0.015) were greater in the layered group. The number of nonculprit plaques, evaluated using coronary angiograms, tended to be greater in patients with layered culprit plaque (4.2±2.5 versus 3.5±2.1, P=0.053). Nonculprit plaques in patients with layered culprit lesion had higher prevalence of layered pattern (P=0.002) and lipid phenotype (P=0.005). Lipid index (P=0.013) and percent area stenosis (P=0.002) were also greater in this group.

CONCLUSIONS: In patients with stable angina pectoris, healed culprit plaques are common and have more features of vulnerability and advanced atherosclerosis both at culprit and nonculprit lesions.

VISUAL OVERVIEW: An online visual overview is available for this article.

Key Words: atherosclerosis ■ macrophage ■ optical coherence tomography ■ stable angina pectoris ■ thrombosis

Plaque disruptions in the coronary arteries have been found in subjects dying of noncardiovascular causes and multiple plaque ruptures have been described in patients deceased from ischemic heart disease.[1,2] Healed plaques in the coronary arteries have been identified in 61% of patients with sudden coronary death.[3] Histology and in vivo studies reported that episodes of coronary plaque destabilization and luminal thrombosis may occur silently with subsequent thrombus organization and plaque healing.[3–5] These processes may contribute to rapid step-wise progression of atherosclerosis.[4,6,7]

A histology validation study demonstrated that optical coherence tomography (OCT) can identify healed plaques.[8] This study showed that OCT-derived healed plaques, evidenced as plaques with one or more heterogeneous signal-rich layers of different optical density, had high sensitivity and specificity (81% and 98%, respectively) to detect histologically defined healed plaques, suggesting OCT as a useful and unique tool to study healed

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Nonstandard Abbreviations and Acronyms

ACS	acute coronary syndromes
MLA	minimal lumen area
OCT	optical coherence tomography
PCI	percutaneous coronary intervention
SAP	stable angina pectoris

plaques in vivo.[8] In this regard, Otsuka et al[9] reported a more detailed description of OCT appearance of histologically identified healed plaques as a plaque with a characteristic band of high backscattering signal interposed between individual layers, especially after the completion of healing process. The authors suggested that this band may represent the result of a greater optical density of type I collagen as compared with type III, the former substituting the latter at the later stage of the healing.[9] Further confirmation of the role of OCT to detect histologically defined healed plaques has been recently reported by another group.[10] Currently, the biological and clinical significance of healed/layered plaques in the coronary arteries is not completely understood. In autopsy studies, patients with type II diabetes mellitus showed greater number of healed plaque ruptures compared with nondiabetic subjects,[11] and patients with one or more healed ruptures had greater cardiovascular risk profile.[3] In a recent OCT study, layered pattern was found at the culprit site in 29% of patients with acute coronary syndromes (ACS) and it was associated with greater cardiovascular risk factors, systemic inflammation, culprit plaque vulnerability, and multivessel disease.[12] Recently, it has also been reported that culprit plaques with layered phenotype had greater degree of luminal stenosis and more angiographic complexity in a mixed population.[13] Several studies demonstrated different plaque characteristics in the whole coronary tree between patients with ACS and stable angina pectoris (SAP), suggesting different biology between those 2 groups.[14–18]

The aim of the present study was to investigate the prevalence, angiographic, and OCT characteristics of layered plaques in patients with SAP.

Highlights

- Optical coherence tomography can reliably identify healed coronary plaques, signs of previous plaque destabilization, in vivo.
- Healed plaques at the culprit lesion are found in >50% of patients with stable angina pectoris.
- Patients with healed culprit plaques show more multivessel disease and more complex angiographic features, have greater vulnerability and degree of stenosis at the culprit lesion. In addition, nonculprit plaques in those patients tend to be greater in number and also show more features of vulnerability.
- Our study suggests that healed culprit plaques in patients with stable angina pectoris are frequent and are associated with signs of panvascular vulnerability and advanced atherosclerosis.
- Future studies are warranted to understand the clinical significance of healed plaques in patients with stable angina pectoris.

MATERIALS AND METHODS

The data that support the findings of the present study are available from the first corresponding author upon reasonable request. In the present study, the words healed and layered have been used interchangeably, as a layered pattern on OCT has been shown to represent a healed phenotype on histology.[8–10]

Study Population

Patients with diagnosis of SAP who had undergone intracoronary OCT imaging performed during cardiac catheterization were selected from the Massachusetts General Hospital OCT Registry (URL: http://www.clinicaltrials.gov. Unique identifier: NCT01110538), an international multicenter registry involving 20 institutions in 6 different countries, between August 2010 and May 2014 (please see the Major Resources Table in the Data Supplement). SAP was defined as chest pain on exertion without changes in frequency, intensity, and duration of symptoms in the previous 4 weeks and/or positive stress test. The culprit lesion was identified by the site investigators who performed the procedure, and the data was included in the data collection form. Subsequently, the data were reviewed and compared with coronary angiogram by the readers at the core lab at Massachusetts General Hospital, to confirm the culprit lesion. The culprit lesion was defined as the tightest lesion on coronary angiogram or as the site of percutaneous coronary intervention (PCI), if PCI was performed. If a multivessel PCI was performed during the same procedure, the culprit plaque was considered the one with the highest degree of stenosis. In any case of discordance between collection form data and the interpretation of the readers at the core lab, or in those cases in which it was not possible to establish the culprit lesion with certainty, the culprit lesion was considered uncertain, and the case was excluded from further analysis (Figure 1). Culprit plaques treated with balloon predilatation or stenting before OCT imaging were excluded. Distal embolization was assessed by interventional cardiologists who performed the procedure at each study site by coronary angiography (evaluated as no-flow or slow-flow phenomenon distal to PCI site), and the data was entered to the data collection form. According to this evaluation, no patient included in the final population of our study reported distal embolization following PCI. We defined nonculprit plaques as any plaque, excluding the culprit lesion, with diameter stenosis ≥30% on coronary angiogram using a quantitative coronary angiography analysis program. A minimum gap of 5 mm was required to consider 2 lesions as separate. Plaques with a distance <5 mm to the PCI site[19] or continuous to an implanted stent were excluded. The number of nonculprit plaques was counted using coronary angiogram. The decision to perform OCT imaging and the vessels to study by OCT examination was left to the cardiologist who was performing the procedure at each site. The data was collected in a data collection form and images sent to the core lab at Massachusetts General Hospital.

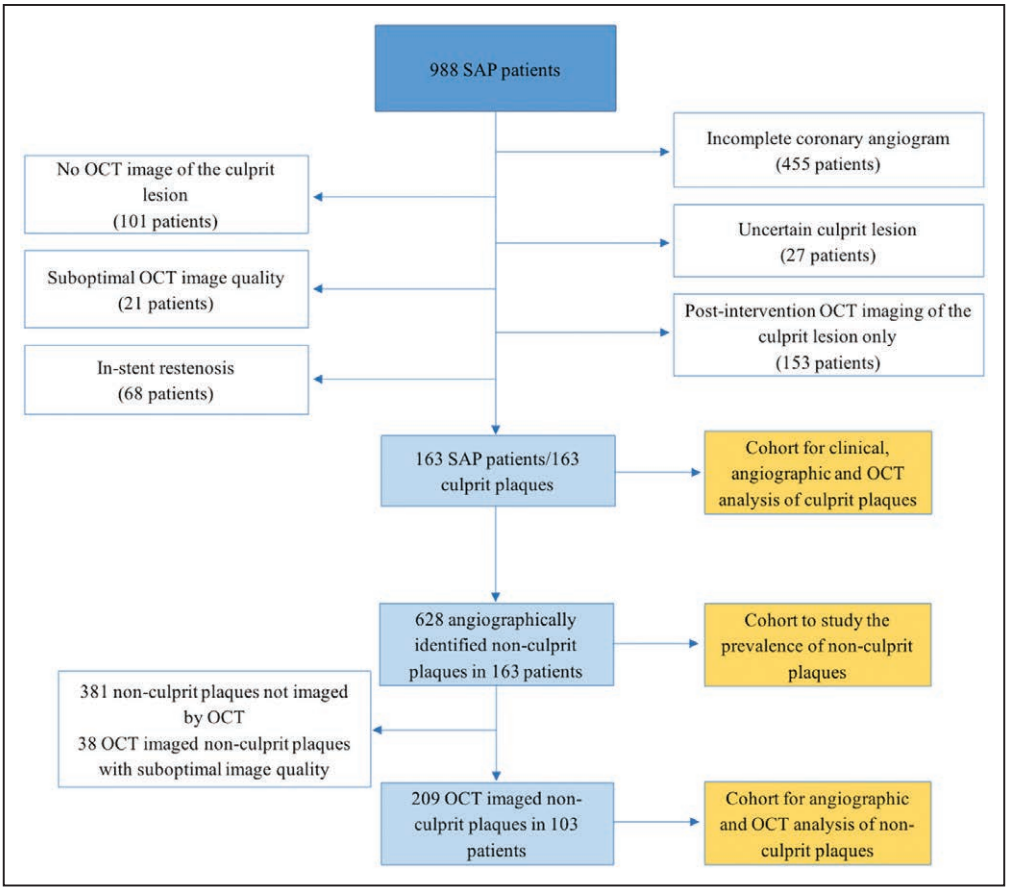

Figure 1. Flowchart of study.
From 988 patients presenting with stable angina pectoris (SAP), 163 patients were selected for this study. In 163 patients, 628 nonculprit plaques were identified by coronary angiogram, which were used to study the prevalence of nonculprit plaques. A total of 209 optical coherence tomography (OCT) imaged nonculprit plaques in 103 patients were included in OCT analysis.

OCT images and coronary angiogram were matched using landmarks such as side branches, stent edges, and calcifications.

Among 279 patients with SAP who had preintervention OCT image of the culprit lesion and complete coronary angiogram, 21 patients were excluded for suboptimal OCT image quality, 27 for uncertain culprit lesion, and 68 for in-stent restenosis. Therefore, our final population included 163 patients with SAP with 163 culprit plaques (Figure 1). In 163 patients, 628 nonculprit plaques were identified on angiogram (201 in the culprit vessel and 427 in nonculprit vessels) and were used to study the prevalence of nonculprit plaques. Of 628 nonculprit plaques, 381 were not imaged by OCT and 38 were excluded for suboptimal image quality; therefore, 209 OCT imaged nonculprit plaques in 103 patients were available for angiographic and OCT analysis. In our study, 79 patients (48.5%) had 1 vessel imaged, 44 patients (27.0%) 2 vessels imaged, and 40 (24.5%) 3 vessels imaged. No differences were found in the number of vessels studied by OCT between patients with or without layered plaque at the culprit site, except that the right coronary artery was more frequently imaged by OCT in patients with layered culprit plaque (Table I in the Data Supplement). Clinical data including demographics, cardiovascular risk factors, history of cardiovascular diseases, laboratory data, and medications were collected at each institution.

In the primary analysis, we aimed to compare clinical data, and angiographic and OCT characteristics of culprit plaques between patients with or without layered culprit plaque. In the secondary analysis, we studied the number and the angiographic and OCT characteristics of nonculprit plaques in patients with or without layered plaque at the culprit site. In the tertiary analysis, layered and nonlayered plaques at nonculprit sites, irrespective of culprit lesion phenotype, were compared. The study protocol complies with the Declaration of Helsinki. The Massachusetts General Hospital OCT Registry was approved by the institutional review board at each participating site, and all patients provided written informed consent before enrollment.

Coronary Angiogram Analysis

Quantitative coronary angiography analysis software (CASS 5.10.1, Pie Medical Imaging BV, Maastricht, the Netherlands) was used to analyze coronary lesions. The parameters included lesion length, minimal lumen diameter, reference diameter, and percent diameter stenosis. American Heart Association/American College of Cardiology classification was used to evaluate lesion complexity,[20] and type B2 and C lesions were considered complex.

OCT Analysis

OCT procedures were performed using a frequency-domain or a time-domain OCT system. OCT images were de-identified and submitted to Massachusetts General Hospital Cardiology Laboratory for Integrative Physiology and Imaging, where they

were analyzed by one investigator who was blinded to patients' data using an offline review workstation.

A frame-by-frame analysis was conducted to evaluate OCT qualitative and quantitative characteristics along the entire plaque, as previously described.[21] OCT visualization of all imaged plaques (culprit and nonculprit plaques) was performed before PCI. We defined layered plaques as those plaques presenting with one or more signal-rich layers of different optical density and a clear demarcation from underlying components in at least 3 consecutive frames along the entire plaque (Figure 2).[8] Plaques were dichotomized into fibrous or lipid plaques, as previously performed.[8,12] Fibrous plaques were defined by the presence of high backscattering and homogeneous signal-rich regions; lipid plaques were identified as signal-poor regions with diffuse borders.[22] Fibrous cap thickness and lipid burden plaque parameters including lipid arc, lipid length, and lipid index were measured and calculated in lipid plaques. Lipid arc was identified at 1 mm intervals, and lipid length was measured using the longitudinal view; lipid index was calculated by multiplication of lipid length and lipid arc mean. Fibrous cap thickness was calculated by the average of 3 different measurements performed at the thinnest part of fibrous cap covering a lipid core. We defined thin cap fibroatheroma a plaque with a lipid arc wider than 90° and with the thinnest part of the fibrous cap measuring <65 μm.[23] Macrophage infiltration in coronary plaques was identified by the presence of superficial highly backscattering focal granular regions.[24] Microvessels were identified as signal-poor vesicular or tubular structure delineated in at least 3 contiguous frames.[24] Calcifications were identified as an area with low backscattering signal and a sharp border inside a plaque.[24] Spotty calcium was defined as the presence of lesions with maximal arc <90° and length <4 mm.[25] Large calcifications were defined as lesions with maximal arc ≥90° or length ≥of 4 mm.[25] Cholesterol crystals were identified as thin and linear regions of high signal intensity with high backscattering within a plaque.[24] Thrombus was defined as an irregular mass attached to the luminal surface or floating into the lumen with diameter >250 μm.[23] Plaque length was measured on the longitudinal view. For each plaque, reference lumen area, minimal lumen area (MLA), and percent area stenosis were measured. Reference lumen area was defined as the mean of the largest lumen area proximal and distal to the stenosis within 10 mm from the edge of the plaque; MLA identified the smallest lumen area found within the length of the entire lesion. Percent area stenosis was calculated using the formula ([mean reference lumen area−MLA]/mean reference lumen area×100). Fifty randomly selected plaques were analyzed again at least 2 weeks after the first reading. A second investigator analyzed 50 randomly selected plaques. Intra- and inter-observer coefficient κ indices about layered phenotype, lipid pattern, and macrophage infiltration features were evaluated. Intraobserver and inter-observer coefficient κ indexes were 0.84 and 0.76 for layered phenotype, 0.94

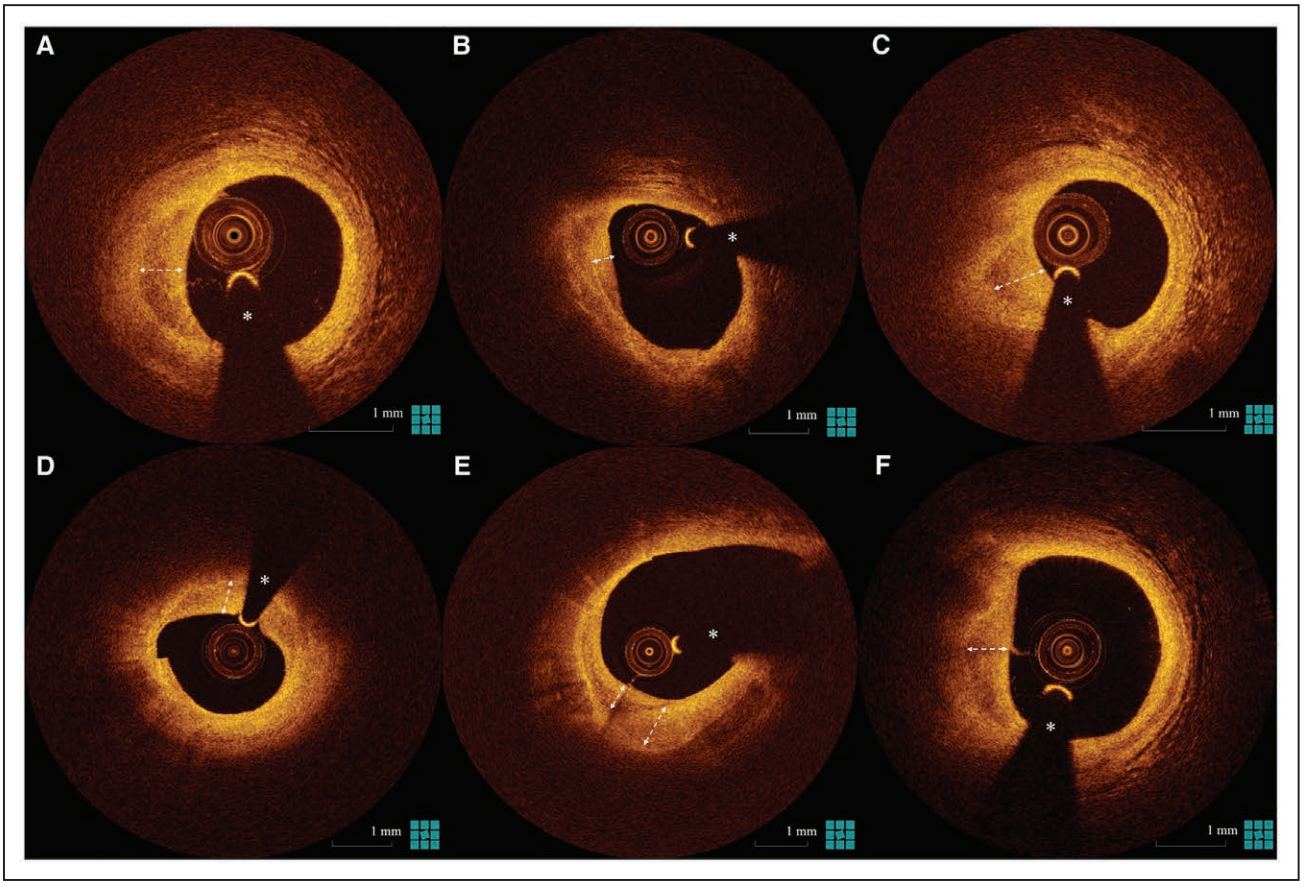

Figure 2. Layered plaque phenotypes.
Examples of layered plaques visualized by optical coherence tomography. Layered plaque is defined as a plaque with one or more signal-rich layers of different optical density and a clear demarcation from underlying components. Double dotted arrows denotes layers of different optical density; and asterisk, guidewire artifact.

and 0.86 for lipid plaques, and 0.84 and 0.78 for macrophage infiltration, respectively.

Statistical Analysis

In the primary and secondary analysis, clinical data, angiographic and OCT features of culprit and nonculprit plaques, and the number of nonculprit plaques, were compared between patients with or without layered plaque at the culprit lesion; in the tertiary analysis, layered versus nonlayered nonculprit plaques were compared, irrespective of culprit lesion phenotype. Categorical variables were expressed as counts (percentage) and were compared using the χ^2 test or Fisher exact test, as appropriate. Mean±SD was used to summarize continuous variables with a normal distribution, while median and percentiles (25th–75th percentiles) were used for those that were not normally distributed. Student *t* test was used to compare normally distributed continuous variables, while the Mann–Whitney *U* test was used for not normally distributed variables. In angiographic and OCT analysis of nonculprit plaques, comparisons between different groups were performed using a generalized estimating equations approach to take into account potential clustering of multiple plaques in a single patient. A 2-sided *P* value of <0.05 was considered statistically significant. All analyses were performed using the SPSS version 23.0 (SPSS, Inc, Chicago, IL).

RESULTS

Among 163 patients, 87 had layered culprit plaque (53.4%). Mean age was 63.5±10.2 years and about 3 quarters were males. Demographics, cardiovascular risk factors, history of cardiovascular diseases, laboratory data, and medications were similar between patients with or without layered plaque at the culprit lesion (Table 1).

Angiographic Findings

Patients with layered plaque at the culprit lesion had higher prevalence of multivessel disease (62.1% versus

Table 1. Comparison of Clinical Characteristics Between Patients With SAP With or Without Layered Culprit Plaque Phenotype

	Overall Study Population, 163 (100%)	Layered Culprit Plaque, 87 (53.4%)	Nonlayered Culprit Plaque, 76 (46.6%)	*P* Value
Demographic data				
Age, y	63.5±10.2	63.0±10.2	64.2±10.2	0.451
Male gender	128 (78.5)	68 (78.2)	60 (78.9)	0.903
BMI, kg/m^2	25.4±4.2	25.5±4.6	25.2±3.7	0.640
Cardiovascular risk factors				
Hypertension	111 (68.9)	59 (69.4)	52 (68.4)	0.892
Hyperlipidemia	111 (70.7)	62 (74.7)	49 (66.2)	0.244
Current smoking	29 (18.6)	16 (19.0)	13 (18.1)	0.874
Diabetes mellitus	44 (27.2)	21 (24.4)	23 (30.3)	0.404
Familial history of cardiovascular diseases	20 (12.7)	10 (12.0)	10 (13.3)	0.808
History of cardiovascular diseases				
Previous MI	34 (21.9)	20 (24.4)	14 (19.2)	0.434
Previous PCI	65 (39.9)	34 (39.1)	31 (40.8)	0.824
Previous CABG	0 (0.0)	0 (0.0)	0 (0.0)	…
Laboratory data				
Total cholesterol, mg/dL	156.5 (143.0–194.0)	166.5 (147.3–195.5)	155.0 (140.8–194.0)	0.112
LDL-C, mg/dL	89.0 (77.0–116.0)	92.5 (77.0–116.0)	78.0 (77.0–116.0)	0.209
HDL-C, mg/dL	47.6±15.6	49.0±16.8	46.0±13.9	0.258
Triglycerides, mg/dL	102.0 (89.0–177.0)	104.0 (89.0–177.0)	99.0 (89.0–177.0)	0.727
Creatinine clearance, mL/min per 1.73 m2	85.2±26.4	84.5±26.1	86.1±26.8	0.712
Medications				
Aspirin	140 (85.9)	76 (87.4)	64 (84.2)	0.565
Clopidogrel	83 (50.9)	44 (50.6)	39 (51.3)	0.925
Statins	120 (73.6)	68 (78.2)	52 (68.4)	0.159
β-blockers	86 (52.8)	46 (52.9)	40 (52.6)	0.975
ACE-inhibitors/ARBs	87 (53.4)	51 (58.6)	36 (47.4)	0.151

Values shown are n (%), mean±SD, or median (25th and 75th percentile). Percentages were indicated as percentages of valid data considering that a small proportion of clinical data were missing (not >5%). ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; CABG, coronary artery bypass grafting; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; PCI, percutaneous coronary intervention; and SAP, stable angina pectoris.

Table 2. Angiographic Characteristics of Culprit Plaques in Patients With Stable Angina Pectoris Based on Layered or Nonlayered Culprit Plaque Phenotype

	Layered Culprit Plaque, 87 (53.4%)	Nonlayered Culprit Plaque, 76 (46.6%)	*P* Value
Culprit vessel			0.527
LAD	50 (57.5)	50 (65.8)	
RCA	20 (23.0)	13 (17.1)	
LCx	17 (19.5)	13 (17.1)	
Multivessel disease	54 (62.1)	34 (44.7)	0.027
B2/C lesion	56 (64.4)	27 (35.5)	<0.001
Lesion length, mm	12.2±5.4	11.0±4.5	0.134
MLD, mm	1.4±0.5	1.5±0.5	0.234
RD, mm	3.2±0.9	3.1±0.9	0.587
DS, %	56.0±13.0	52.4±11.8	0.062

Values shown are n (%) and mean±SD. DS indicates diameter stenosis; LAD, left anterior descending artery; LCx, left circumflex artery; MLD, minimal lumen diameter; RCA, right coronary artery; and RD, reference diameter.

44.7%, *P*=0.027) and more complex lesions at the culprit site (64.4% versus 35.5%, *P*<0.001; Table 2).

OCT Characteristics of Culprit Lesion (Primary Analysis)

Layered culprit plaques had a higher prevalence of lipid plaque (83.9% versus 64.5%, *P*=0.004), macrophage infiltration (58.6% versus 35.5%, *P*=0.003), calcifications (78.2% versus 63.2%, *P*=0.035), and thrombus (28.7% versus 14.5%, *P*=0.029) than nonlayered ones (Figure 3). Layered culprit plaques also had greater lipid burden and higher percent area stenosis (Table 3).

OCT Characteristics of Nonculprit Plaques (Secondary Analysis)

The number of nonculprit plaques evaluated by coronary angiogram in the total population (163 patients) tended to be greater in patients with layered culprit plaque (4.2±2.5 versus 3.5±2.1, *P*=0.053). Among 209 nonculprit plaques imaged by OCT in 103 patients, 123 plaques were in patients with layered culprit plaque and 86 in patients with nonlayered culprit plaque (Table 4). No statistically significant differences were found in clinical characteristics between patients with (57 patients) and without (46 patients) layered culprit plaque in this subpopulation (103 patients; Table II in the Data Supplement). Nonculprit plaques in patients with layered culprit plaque had higher prevalence of layered pattern (56.1% versus 33.7%, *P*=0.002) and lipid plaque (86.2% versus 67.4%, *P*=0.005). In this group, lipid burden and percent area stenosis were also greater than those without layered culprit plaque (Table 4). Nonculprit plaques in patients with layered culprit plaque showed greater percent diameter stenosis (40.1±7.9%

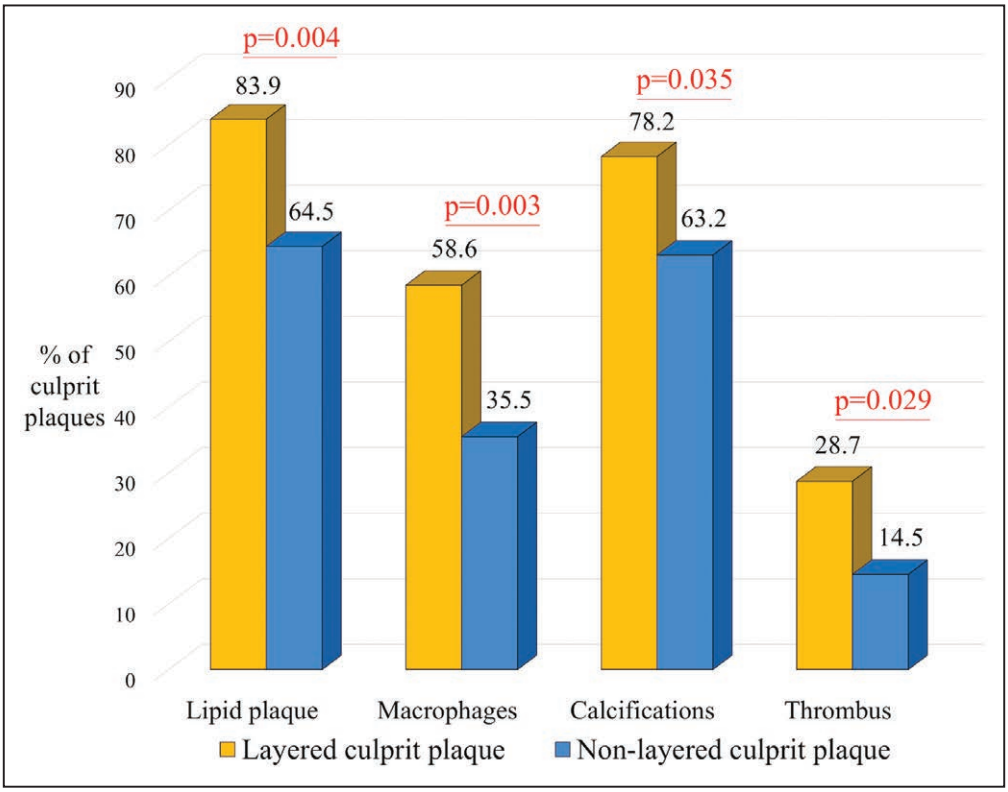

Figure 3. Comparison of plaque characteristics between layered and nonlayered culprit plaques.
Layered culprit plaques showed higher prevalence of lipid plaque, macrophage infiltration, calcifications, and thrombus compared with nonlayered ones.

Table 3. OCT Characteristics of Culprit Plaques in Patients With Stable Angina Pectoris Based on Layered or Nonlayered Culprit Plaque Phenotype

	Layered Culprit Plaque, 87 (53.4%)	Nonlayered Culprit Plaque, 76 (46.6%)	*P* Value
Plaque type			0.004
Fibrous plaque*	14 (16.1)	27 (35.5)	
Lipid plaque	73 (83.9)	49 (64.5)	
Fibrous cap thickness, μm	111.9±51.5	124.2±52.9	0.203
Lipid arc mean, °	189.9±30.4	179.0±35.4	0.072
Lipid length, mm	13.0±8.0	9.1±5.1	0.001
Lipid index, mm°	2503.0±1669.4	1648.2±1105.8	0.001
TCFA	21 (24.1)	12 (15.8)	0.186
Macrophages	51 (58.6)	27 (35.5)	0.003
Microvessels	54 (62.1)	38 (50.0)	0.121
Cholesterol crystals	38 (43.7)	23 (30.3)	0.077
Calcifications	68 (78.2)	48 (63.2)	0.035
Spotty calcium	43 (49.4)	27 (35.5)	0.074
Large calcifications	54 (62.1)	31 (40.8)	0.007
Thrombus	25 (28.7)	11 (14.5)	0.029
Plaque length, mm	22.5±10.6	16.3±6.8	<0.001
MLA, mm^2	1.8±1.2	2.0±1.1	0.182
RA, mm^2	8.2±3.1	7.7±3.2	0.328
AS, %	77.5±11.4	73.1±11.1	0.015

Values shown are n (%), mean±SD. AS indicates area stenosis; MLA, minimal lumen area; OCT, optical coherence tomography; RA, reference lumen area; and TCFA, thin cap fibroatheroma.

*Calcifications were present in 33 of 41 (80.5%) fibrous culprit plaques: 12/14 (85.7%) fibrous layered culprit plaques and 21/27 (77.8%) fibrous nonlayered culprit plaques.

Table 4. OCT Characteristics of Nonculprit Plaques in Patients With or Without Layered Plaque at the Culprit Site

	Patients With Layered Culprit Plaque, 123 Nonculprit Plaques	Patients With Nonlayered Culprit Plaque, 86 Nonculprit Plaques	*P* Value
Layered phenotype	69 (56.1)	29 (33.7)	0.002
Plaque type			0.005
Fibrous plaque	17 (13.8)	28 (32.6)	
Lipid plaque	106 (86.2)	58 (67.4)	
Fibrous cap thickness, μm	129.0±57.8	128.8±56.4	0.989
Lipid arc mean, °	180.7±43.4	166.1±43.1	0.081
Lipid length, mm	10.8±5.5	9.2±5.6	0.086
Lipid index, mm °	2048.8±1318.6	1567.1±1039.6	0.013
TCFA	17 (13.8)	10 (11.6)	0.670
Macrophages	63 (51.2)	38 (44.2)	0.351
Microvessels	56 (45.5)	51 (59.3)	0.050
Cholesterol crystals	36 (29.3)	14 (16.3)	0.090
Calcifications	89 (72.4)	58 (67.4)	0.491
Spotty calcium	48 (39.0)	36 (41.9)	0.725
Large calcifications	65 (52.8)	36 (41.9)	0.157
Thrombus	7 (5.7)	3 (3.5)	0.442
Plaque length, mm	17.0±8.0	15.4±8.5	0.222
MLA, mm^2	3.1±1.5	3.6±1.7	0.073
RA, mm^2	8.3±3.0	8.8±3.9	0.401
AS, %	62.1±10.4	58.4±7.4	0.002

Values shown are n (%), mean±SD. AS indicates area stenosis; MLA, minimal lumen area; OCT, optical coherence tomography; RA, reference lumen area; and TCFA, thin cap fibroatheroma.

versus 37.4±6.8%, *P*=0.007) than those in patients without layered culprit plaque on angiogram (Table III in the Data Supplement).

Layered Versus Nonlayered Plaques at the Nonculprit Lesions, Irrespective of Culprit Lesion Phenotype (Tertiary Analysis)

Among 209 OCT imaged nonculprit plaques, 98 (46.9%) had layered phenotype. These layered nonculprit plaques had a higher prevalence of lipid plaque (91.8% versus 66.7%, *P*<0.001), macrophage infiltration (61.2% versus 36.9%, *P*<0.001), cholesterol crystals (40.8% versus 9.0%, *P*<0.001), calcifications (81.6% versus 60.4%, *P*=0.003), and spotty calcium (49.0 % versus 32.4%, *P*=0.006) than nonlayered ones (Figure I in the Data Supplement). Layered nonculprit plaques also had significantly smaller MLA (3.1±1.4 versus 3.6±1.8 mm^2, *P*=0.039) on OCT analysis (Figure I in the Data Supplement) and greater percent diameter stenosis (40.2±8.0% versus 37.9±6.9%, *P*=0.021) on quantitative coronary angiogram analysis, compared with nonlayered ones (Table IV in the Data Supplement).

DISCUSSION

In the present study we demonstrated that (1) >50% of culprit lesions in patients with SAP had layered pattern; (2) patients with layered culprit plaques had more multivessel disease and complex culprit lesions on angiograms; (3) patients with layered culprit plaques had panvascular vulnerability and greater plaque burden; (4) layered plaques at nonculprit sites, irrespective of culprit lesion phenotype, showed greater vulnerability and plaque burden.

Layered Culprit Plaques in Patients With SAP

Plaques following subclinical destabilization and nonocclusive thrombosis undergo complex healing processes with organization of residual thrombus, smooth muscle cell proliferation, and proteoglycan and collagen type III deposition (with subsequent conversion to collagen type I).[9] In autopsy studies, signs of previous plaque destabilization have been found in 53% of subjects with sudden coronary death, who had no evidence of previous myocardial infarction.[3] Mann and Davies[4] also reported

that >70% of plaques with diameter stenosis ≥51%, had a healed pattern, suggesting the potential contribution of plaque healing to progression of coronary atherosclerosis. Recently, it became possible to study this phenomenon in vivo. A histology validation study demonstrated that OCT can reliably detect healed plaques in vivo.[8] On OCT, healed plaques show heterogeneous signal-rich layers of different optical density with a typical high backscattering signal band interposed between individual layers.[8,9] Fracassi et al[12] reported that 29% of patients with ACS had layered phenotype at the culprit lesion and the layered plaques had more features of vulnerability. In the present study, we investigated this phenomenon in patients with SAP and found that 53% of culprit lesions had layered phenotype, a prevalence almost twice as high as that in ACS patients.[12] Vergallo et al[26] also reported more healed plaques in nonculprit segments in patients with long-standing stable angina and single episode of myocardial infarction, while their prevalence was lower in patients with recurrent ACS. These studies together suggest that episodes of silent plaque destabilizations are more frequent in SAP than in ACS. We also demonstrated that patients with SAP with layered plaque at the culprit site had higher prevalence of multivessel disease and more complex culprit lesions on angiogram. Moreover, plaques with layered phenotype at the culprit site showed more signs of vulnerability (more lipid plaque, greater lipid burden, and more macrophage infiltration), higher prevalence of thrombus and greater plaque burden (longer plaques and greater percent area stenosis) than nonlayered ones. The present findings are in line with the previous studies showing that layered culprit plaque had greater macrophage infiltration in stable patients[8] and greater vulnerability in patients with ACS.[12] Thrombus was found in one of 5 patients in our study. Previous studies reported a similar prevalence in patients with SAP.[27,28] In detail, Mann et al[28] found thrombus in 22% of the culprit lesions in patients with SAP. Our results also showed a higher prevalence of calcifications in layered culprit plaques as compared with nonlayered ones. Previous histopathology studies reported that the prevalence of healed plaque ruptures was higher in segments with diffuse calcifications, and that healed ruptures had the greatest mean area of calcification.[29,30] To explain these results, Burke et al[29] suggested that plaque disruption, via hemorrhage and cellular breakdown, may contribute to coronary calcification. We hypothesize that a greater lipid burden and macrophage infiltration increase the risk of plaque destabilization. After plaque disruption, a complex healing process with collagen type III and then type I deposition may occur. At the same time, cellular breakdown and local hemorrhage may favor processes of calcium formation with local coronary calcification. However, necrotic core and local inflammation may persist after the healing process,[9] increasing the risk of new episodes of destabilization. This hypothesis would explain the different OCT features (lipid, macrophages, calcifications) found in layered plaques. Future studies will provide definitive answers on these mechanisms. Finally, the finding that healed plaque had greater degree of stenosis is also consistent with the previous reports.[3–5,12,13] This finding would support the hypothesis about the contribution of plaque destabilization and subsequent healing to coronary plaque progression.

During the process of repetitive plaque destabilization and healing, a balance between local/systemic thrombogenicity and endogenous protective antithrombotic mechanism determines the fate of a plaque disruption. When local thrombogenicity is less strong or endogenous antithrombotic mechanism is more robust, the disruption will result in nonocclusive thrombus formation, which may contribute to the progression of the plaque. When the plaque reaches a threshold, the patient may develop SAP secondary to supply demand imbalance. On the contrary, in patients with ACS, thrombogenicity may dominate and plaque disruption results in occlusive thrombus formation. The notion that local/systemic thrombogenicity is an important determinant of outcome of plaque disruption is supported by the fact that features of plaque vulnerability are prevalent in layered plaques, irrespective of clinical presentations (ACS or SAP).[12]

Nonculprit Plaques in Patients With SAP With or Without Layered Culprit Plaque

In the present study, we also studied angiographic and OCT features of nonculprit plaques of patients with layered culprit plaque, to investigate atherosclerosis pattern in the whole coronary tree in these patients. Our study showed that, in patients with SAP, those with layered plaque at the culprit site tended to have a greater number of nonculprit plaques and had higher prevalence of multivessel disease than those with nonlayered culprit plaque: the pattern similar to that in patients with ACS.[12] We also showed that nonculprit plaques in patients with layered culprit plaque had more layered phenotype, more lipid plaque, greater lipid index and greater degree of stenosis, compared with those in patients without layered culprit plaque. These results indicated that the presence of layered phenotype at the culprit lesion in patients with SAP represents a higher level of panvascular vulnerability and greater plaque burden through previous repetitive plaque destabilization (Figure 4).

Layered Nonculprit Plaques, Irrespective of Culprit Lesion Morphology

Finally, we studied layered nonculprit plaques, irrespective of culprit lesion phenotype. Layered plaques in nonculprit sites were prevalent (46.9%) and had more signs of vulnerability (more lipid plaque, more macrophage infiltration, and more cholesterol crystals) than

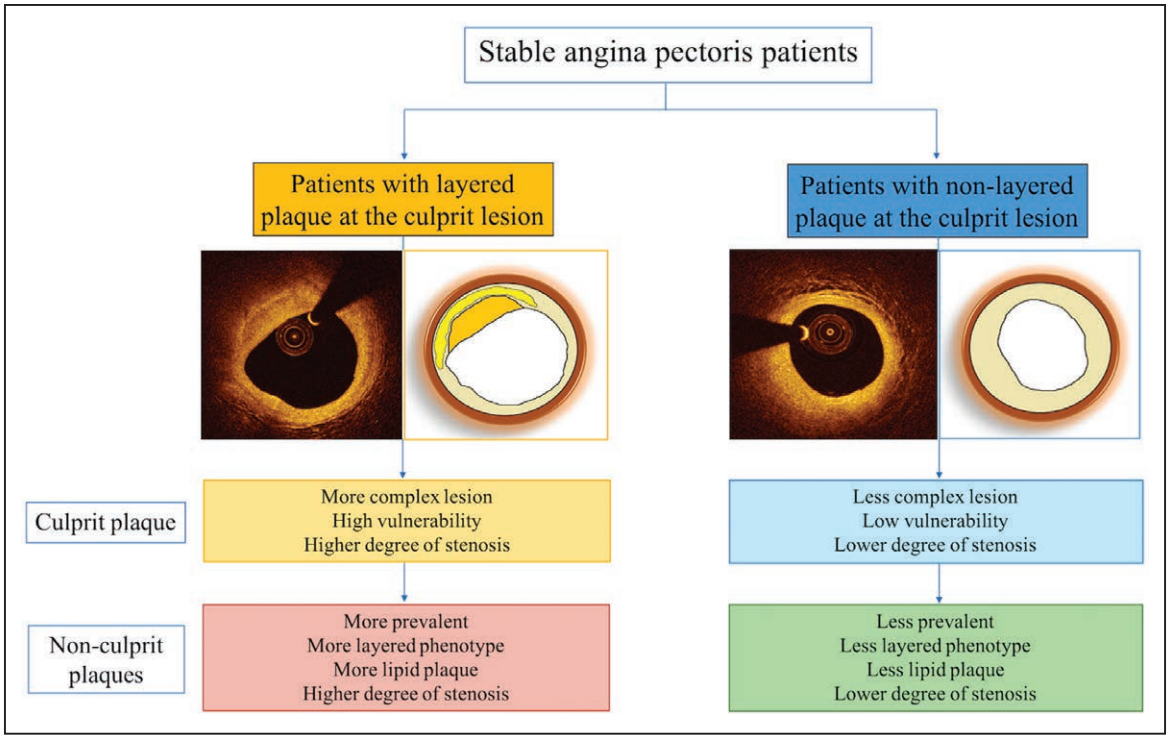

Figure 4. Patients with stable angina pectoris with or without layered culprit plaque.
Optical coherence tomography (OCT) images with corresponding illustrations of a layered culprit plaque (**left**) and a nonlayered culprit plaque (**right**). Patients with layered culprit plaques have higher prevalence of complex lesions on angiogram, greater vulnerability, and higher degree of stenosis at the culprit lesions. Patients with layered culprit plaque also had a trend toward greater number of nonculprit plaques and the nonculprit plaques had more layered pattern, lipid plaque and had greater degree of stenosis than those in patients with nonlayered culprit plaque. These data suggest that patients with layered culprit plaque have higher degree of panvascular vulnerability and more advanced coronary atherosclerosis both at the culprit and nonculprit lesions.

nonlayered ones. Layered nonculprit plaques also had greater plaque burden. In an autopsy study, Burke et al[3] demonstrated a positive correlation between the number of healed plaque rupture sites and the degree of stenosis in 142 subjects with sudden coronary death. A recent study by Vergallo et al[26] demonstrated that healed coronary plaques in nonculprit segments were more frequently lipid-rich (66.7%) and segments with healed plaques had smaller MLA than those without healed pattern. The result of the present study is consistent with these studies that layered plaques are associated with greater vulnerability and greater atherosclerosis burden.

Limitations

Several limitations should be noted. First, not all patients underwent 3-vessel OCT imaging. However, in real-world clinical practice, OCT imaging of all 3 vessels is not always feasible. Nevertheless, no differences were found in the number of vessels imaged by OCT between patients with or without layered culprit plaque. Second, the study represents a post hoc analysis of data collected in the Massachusetts General Hospital OCT Registry, a large international multicenter registry. Moreover, a large number of patients had to be excluded. Thus, a selection bias cannot be excluded. Therefore, the data should be interpreted with caution. Large-scale prospective studies will provide definitive answers. Third, co-registration of angiogram and OCT was not done. However, the corresponding sites were carefully identified on angiogram and on OCT images using landmarks such as side branches, stents, or calcifications. Fourth, as reported in the histology validation study,[8] despite the high specificity of OCT-defined healed plaques to detect histologically defined healed plaques, sensitivity was slightly lower. Therefore, care should be taken in the interpretation of the present results. Moreover, in the presence of lipid plaques, OCT showed lower concordance with histology. Consequently, layered plaques might have been underestimated. Fifth, although OCT criteria for detection of healed plaques have been validated against histology, the data was derived from a single-center study with a relatively small sample.[8] Moreover, it is sometimes difficult to differentiate a layered phenotype from other plaque components, such as lipid, calcifications, macrophages, or residual thrombus. However, intra- and inter-observer agreement on layered pattern was good in our study. Future studies will help to improve OCT accuracy for detection of healed plaques. Sixth, unfortunately, pro/antithrombotic biomarker data were not available. Biomarker data would have helped us to better understand the mechanism. Seventh, complete clinical and imaging

follow-up to investigate the natural history of plaques with different phenotype was unavailable. Follow-up studies will provide definitive answers about long-term prognosis of patients with layered plaques.

Conclusions

In patients with SAP, layered plaque was identified in >50% of patients at the culprit lesion: a prevalence that is twice as high as that in patients with ACS. Layered plaques either at the culprit lesion or at the nonculprit lesion, were associated with signs of panvascular vulnerability and advanced atherosclerosis, irrespective of clinical presentations (SAP or ACS). In SAP, systemic endogenous antithrombotic mechanisms may be dominant, relative to local/systemic thrombogenicity, that plaque disruption results in nonocclusive thrombosis, leading to silent progression of the plaque, rather than development of ACS.

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