

Features of atherosclerosis in patients with angina and no obstructive coronary artery disease

Dario Pellegrini^{1,2}, MD; Regina Konst¹, MD; Stijn van den Oord¹, MD, PhD; Aukelien Dimitriu-Leen¹, MD, PhD; Jan-Quinten Mol¹, MD; Tijn Jansen¹, MD; Angela Maas¹, MD, PhD; Helmut Gehlmann¹, MD; Robert-Jan van Geuns¹, MD, PhD; Suzette Elias-Smale¹, MD, PhD; Niels van Royen¹, MD, PhD; Peter Damman^{1*}, MD, PhD

1. Radboud University Medical Center, Department of Cardiology, Nijmegen, the Netherlands; 2. Aziende Socio Sanitarie Territoriale Papa Giovanni XXIII, Bergamo, Italy

This paper also includes supplementary data published online at: <https://eurointervention.pcronline.com/doi/10.4244/EIJ-D-21-00875>

KEYWORDS

- optical coherence tomography
- other techniques
- stable angina

Abstract

Background: An association between atherosclerosis and coronary vasospasm has previously been suggested. However, to date, no conclusive data on the whole spectrum of these disorders have been published.

Aims: This study aimed to define specific morphological features of atherosclerosis in patients with angina and no obstructive coronary artery disease (ANOCA) due to coronary vasospasm.

Methods: From February 2019 to January 2020, we enrolled 75 patients referred to our laboratory for a coronary function test (CFT) due to ANOCA and suspected coronary vasomotor dysfunction. The CFT consisted of an acetylcholine test and a physiology assessment with hyperaemic indexes using adenosine. Patients were divided into two groups according to the presence or absence of coronary vasospasm triggered by acetylcholine (ACH+ and ACH-, respectively). In addition, optical coherence tomography (OCT) was performed to assess the lipid index (LI), a surrogate for lipid area, and the prevalence of markers of plaque vulnerability.

Results: ACH+ patients had a higher LI than ACH- patients (LI: 819.85 [460.95-2489.03] vs 269.95 [243.50-878.05], respectively, $p=0.03$), and a higher prevalence of vulnerable plaques (66% vs 38%, $p=0.04$). Moreover, ACH+ patients showed a higher prevalence of neovascularisation compared to ACH- subjects (37% vs 6%, $p=0.02$) and a trend towards a higher prevalence of all individual markers, in particular thin-cap fibroatheroma (20% vs 0%, $p=0.06$). No differences were detected between patterns of coronary vasospasm.

Conclusions: The presence of coronary vasospasm, regardless of its phenotype, is associated with higher lipid burden, plaque vulnerability and neovascularisation.

*Corresponding author: Department of Cardiology, Radboud University Medical Center, Postbus 9101, 6500 HB, Nijmegen, the Netherlands. E-mail: peter.damman@radboudumc.nl

Abbreviations

ACH	acetylcholine
ANOCA	angina and no obstructive coronary artery disease
CAD	coronary artery disease
CFR	coronary flow reserve
CFT	coronary function test
FFR	fractional flow reserve
IMR	index of microvascular resistance
LI	lipid index
OCT	optical coherence tomography
RFR	resting full-cycle ratio
TCFA	thin-cap fibroatheroma

Introduction

Despite the scientific advancements in the diagnosis and management of coronary artery disease (CAD), progress is limited in the field of angina with non-obstructive CAD (ANOCA), an often neglected condition with a high prevalence¹ and a significant impact on prognosis and quality of life²⁻⁴.

Among possible causes of ANOCA, coronary vasomotor disorders are prominent and are associated with an increased risk of adverse cardiovascular events⁵⁻⁹. Coronary vasospasm represents one of the most important coronary vasomotor disorders. It is a condition mainly related to endothelium-dependent vascular dysfunction, and it can affect both the epicardial vessels (epicardial spasm) or the microcirculation (microvascular vasospasm).

Despite being traditionally considered a merely functional disorder, epicardial vasospasm has been associated with a variable degree of plaque burden and of markers of plaque vulnerability, suggesting an association between atherosclerosis and coronary function disorder¹⁰⁻¹³. Optical coherence tomography (OCT) has emerged as the preferred method to assess atherosclerotic morphological characteristics, due to its high spatial resolution¹³⁻¹⁴. However, significant variability has been reported in study results, and to date no study has performed a comprehensive analysis of all the different markers of plaque vulnerability in the different types of coronary vasospasm. A better understanding of the underlying anatomic and physiologic features could potentially allow improved risk stratification and tailored treatments.

The aim of the current study was to assess the atherosclerotic burden and morphological characteristics of coronary vasospasm using OCT in ANOCA.

Methods

STUDY DESIGN AND POPULATION

This single-centre observational study was conducted at the Radboud University Medical Center (Radboudumc) in Nijmegen, the Netherlands, a large tertiary referral centre specialised in patients with persistent angina in the absence of obstructive CAD. Between February 2019 and January 2020, we enrolled all patients undergoing an invasive coronary function test (CFT) for suspected coronary vasomotor dysfunction and we performed OCT to correlate functional and anatomical findings.

In all patients, obstructive CAD had been ruled out before CFT by previous anatomical imaging (coronary angiography or coronary computerised tomography angiography) or non-invasive ischaemia detection. Absence of significant coronary lesions was confirmed by coronary angiography at the beginning of the CFT. The extent and severity of CAD burden upon angiography was quantified with the Gensini score, which was the scoring system of choice due to its ability to stratify the degree of stenosis¹⁵. Exclusion criteria for the OCT study were obstructive CAD, previous coronary revascularisation and severe kidney disease (eGFR <30 mL/min). The study was approved by the local institutional ethical review board of the Radboudumc and complies with the Declaration of Helsinki. All patients gave written informed consent to the study and data collection.

STUDY PROCEDURES

A detailed description of the CFT is reported in the **Supplementary Appendix 1**. Per protocol, all tests focused on the left anterior descending artery (LAD), in accordance with available recommendations¹⁶, due to its prognostic relevance, the large territory of distribution and logistical considerations (procedural time, radiation dose and amount of dye). Briefly, after confirming the absence of significant CAD by angiography, we performed a provocative test with increasing doses of acetylcholine (10, 20, 100, 200 µg) in the left coronary artery to detect signs and symptoms of spasm. After the injection of nitrates, a pressure wire was placed in the distal LAD (PressureWire XTM; Abbott) and measurements of resting full-cycle ratio (RFR), fractional flow reserve (FFR), index of microvascular resistance (IMR) and coronary flow reserve (CFR) were performed using dedicated software (CoroFlow software; Coroventis).

OCT PROCEDURE ANALYSIS

Detailed OCT protocol and definitions are reported in the **Supplementary Appendix 2**. After CFT, one high-definition OCT run (54 mm, 20 mm/sec) of the LAD was performed (proximal marker in the LAD ostium) with a frequency-domain OCT catheter (Dragonfly Optis; St. Jude Medical). Offline analysis of each OCT pullback was performed by two independent expert reviewers, blinded to the results of the CFT. Disagreements were resolved by a third reviewer. In-depth analysis of cross-sectional images was performed in one frame per millimetre of longitudinal length. Plaque analysis followed international consensus¹⁷.

The main variables of interest were the lipid index (LI) (**Figure 1**), and the presence of vulnerable plaques, defined as plaques presenting at least one marker of vulnerability (presence of a fibroatheroma, macrophage infiltration, neovascularisation or plaque erosion) (**Figure 2**), both individually and as a composite endpoint. The LI, a surrogate for lipid pool area, was defined as the mean lipid arc (**Figure 1A**) multiplied by lipid-core longitudinal length¹⁸. It was introduced to provide an index of atherosclerotic burden, as light in OCT cannot penetrate lipid-rich plaques due to significant attenuation, and a thorough assessment of plaque burden such as in intravascular imaging

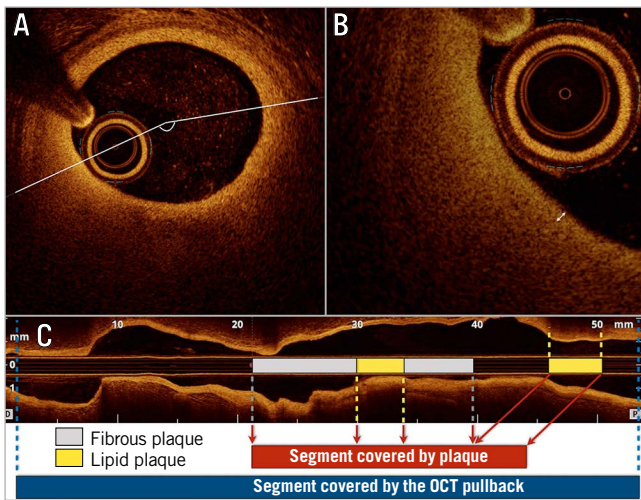


Figure 1. Representation of lipid pool analysis. A) Lipid angle, B) thickness of the fibrous cap and C) longitudinal extension. After frame-by-frame analysis, the total amount of plaque along the scanned segment was computed. Then, in the total segment covered by plaque, the extension of each plaque component was calculated. OCT: optical coherence tomography

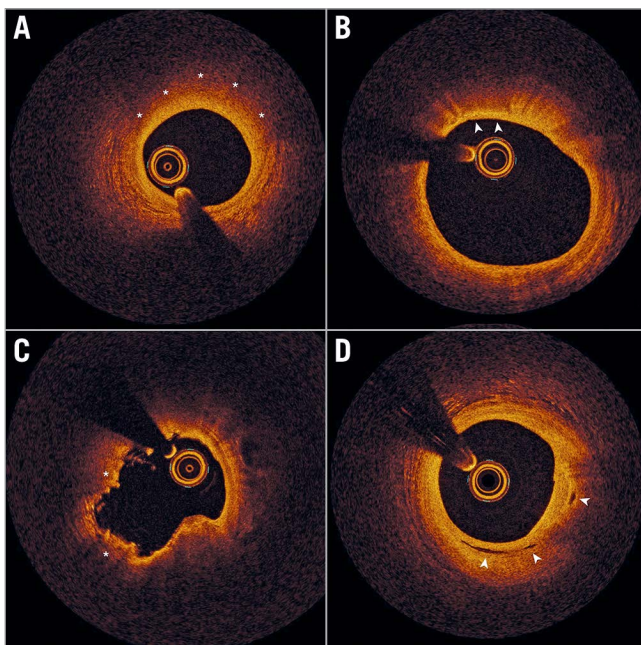


Figure 2. Examples of plaque vulnerability markers detected with optical coherence tomography (OCT). A) Fibroatheroma (asterisks), shown as a lipid plaque with an angle $>90^\circ$. The bright fibrous cap at the top of the plaque, when $\leq 65 \mu\text{m}$, defines the presence of a thin-cap fibroatheroma. B) Macrophage infiltration (arrowheads), shown as bright spots with significant attenuation, in the context of a lipid plaque. C) Erosion (asterisks), shown as irregular endothelium with overlying thrombus. D) Neovascularisation (arrowheads), shown as small vascular structure in the context of the plaque, without connections with the lumen.

would not be possible. Fibroatheroma was defined as a lipid plaque with a maximum lipid arc $>90^\circ$. Thin-cap fibroatheroma

(TCFA) was defined as a fibroatheroma with a cap thickness $\leq 65 \mu\text{m}$ (Figure 1B). The individual morphological atherosclerosis characteristics were analysed both on a patient-level (number of patients with at least one OCT frame showing the variable of interest) and on a frame-level (extension of those variables in terms of number of millimetres indexed per total length of plaque segments) (Figure 1C).

DEFINITIONS

We defined the presence or absence of coronary vasospasm (either epicardial or microvascular spasm) according to an intracoronary acetylcholine (ACH) test result (ACH+ vs ACH-), in line with current guidelines^{16,19}. Epicardial spasm was defined as a focal or diffuse epicardial coronary diameter reduction $\geq 90\%$ in response to ACH, compared to the relaxed state after the intracoronary nitroglycerine infusion, with reproduction of both recognisable symptoms and ischaemic electrocardiogram (ECG) changes. Microvascular spasm was defined as the reproduction of recognisable symptoms and ischaemic ECG changes, in the absence of $\geq 90\%$ epicardial diameter reduction during ACH infusion. Ischaemic ECG changes were defined as transient ST-segment elevation or depression of $\geq 0.1 \text{ mV}$, or ischaemic T-wave changes in at least two contiguous leads. Impaired microvascular dilatation was defined by a CFR < 2.0 , an IMR ≥ 25 , or a combination of both.

STATISTICAL ANALYSIS

Continuous variables are presented as mean \pm standard deviation or as median (interquartile range [IQR]), as specified. After testing for normal distribution, comparisons were performed using the unpaired t-test, and the Mann-Whitney U test or Kruskal-Wallis test, as appropriate. Categorical variables are presented as counts and percentages (%). Comparisons were performed using Pearson's χ^2 or Fisher's exact test. A two-sided p-value < 0.05 was considered statistically significant. All statistical analyses were performed with JMP v.14.0 (SAS Institute).

Results

BASELINE CHARACTERISTICS AND FLOW PARAMETERS

From February 2019 to January 2020, 83 patients underwent a CFT at our institution. Of these, 75 patients met the eligibility criteria and were enrolled in the study.

Baseline characteristics are reported in Table 1. The study population was composed almost entirely of women (93%) with a mean age of 55 years and a moderate cardiovascular risk profile. As per protocol, significant CAD had been ruled out either by previous coronary angiography (50 subjects, 67% of the population), coronary computerised tomography angiography (37 subjects, 49%) or non-invasive ischaemia detection test (50 subjects, 67%). Before CFT, coronary angiography confirmed the absence of significant stenoses. No cases of FFR or RFR below the ischaemic threshold were recorded.

Overall, there were no fatal or serious non-fatal complications related to the performance of CFT or OCT.

Table 1. Baseline characteristics of the study population.

Variable	Total	ACH+	ACH-	p-value
Demographics				
Age (years)	55.17±7.28	55.51±7.67	53.94±5.67	0.41
Female sex	70 (93%)	55 (93%)	15 (94%)	1.00
History of smoking	38 (51%)	32 (54%)	6 (38%)	0.27
Current smoker	5 (6%)	4 (7%)	1 (6%)	1.00
Familiar history	47 (63%)	39 (66%)	8 (50%)	0.26
Hypertension	38 (51%)	30 (51%)	8 (50%)	1.00
Hypercholesterolaemia	34 (45%)	27 (46%)	7 (44%)	1.00
Diabetes mellitus	6 (8%)	5 (8%)	1 (6%)	1.00
Peripheral artery disease	3 (4%)	3 (5%)	0	1.00
Thyroid disorder	14 (19%)	10 (17%)	4 (25%)	0.48
Rheumatic disease	23 (31%)	19 (32%)	4 (25%)	0.76
Self-reported migraine	37 (49%)	27 (46%)	10 (63%)	0.27
Pregnancy-related hypertension	17 (24%)	11 (19%)	4 (25%)	0.73
Pre-eclampsia	8 (11%)	3 (5%)	3 (12%)	0.14
Angiographic and physiologic indexes				
Gensini score	0.0 [0.0-2.5]	1.0 [0.0-3.5]	0.0 [0.0-1.1]	0.04
RFR	0.93 [0.92-0.94]	0.93 [0.92-0.95]	0.93 [0.91-0.94]	0.65
FFR	0.89 [0.86-0.92]	0.89 [0.84-0.93]	0.88 [0.87-0.92]	0.43
CFR	3.40 [2.50-4.60]	3.30 [2.50-4.53]	3.53 [2.87-4.90]	0.51
IMR	18.10 [14.00-26.00]	19.00 [14.00-27.15]	18.00 [16.10-21.00]	0.51

ACH: acetylcholine; CFR: coronary flow reserve; FFR: fractional flow reserve; IMR: index of microvascular resistance; RFR: resting full-cycle ratio

An abnormal reaction to acetylcholine (ACH+) occurred in 59 patients (79%), with 28 patients having epicardial spasm and 31 patients having microvascular spasm. Baseline clinical characteristics were similar between ACH+ and ACH- patients (**Table 1**). At angiography, coronary artery disease burden was very low, and ACH+ patients had a significant, yet clinically non-relevant, increase compared to ACH- patients (Gensini score 1 and 0, respectively, $p=0.04$). Of the patients with vasospasm, 23 (39%) had coexistent impaired microvascular dilatation as measured by adenosine, compared to three subjects in the ACH- group (19%). Twelve individuals (16%) had a negative CFT.

Three patients did not undergo an adenosine test due to drug contraindications (severe asthma) and could not perform a complete physiology evaluation (only resting indexes were measured). Resting and hyperaemic flow indexes were within the normal range, and no differences were detected between the groups (**Table 1**).

OCT ANALYSES

ACH+ patients had a significantly higher LI than ACH- patients (819.85 vs 269.95, respectively, $p=0.03$) (**Table 2, Central illustration**), due to a significant increase in mean lipid angle (87.66° vs 64.14°, respectively, $p<0.01$). Furthermore, total plaque

Table 2. Comparison of optical coherence tomography (OCT) findings in patients with and without an abnormal reaction to acetylcholine (ACH+/ACH-).

	ACH+ (n=59)	ACH- (n=16)	p-value
Main outcome			
Lipid index	819.85 [460.95-2489.03]	269.95 [243.50-878.05]	0.03
Vulnerable plaque (% subjects)	39 (66%)	6 (38%)	0.04
Marker prevalence (% of subjects)			
Fibroatheroma	29 (49%)	4 (25%)	0.10
Thin-cap fibroatheroma	12 (20%)	0	0.06
Macrophage infiltration	28 (47%)	5 (31%)	0.27
Neovascularisation	22 (37%)	1 (6%)	0.02
Erosion	9 (15%)	0	0.19
Analysis of plaque extension (% length)			
Total plaque extension (%)	38.10 [8.00-76.09]	10.88 [0.98-45.74]	0.06
Lipid-rich (%)	48.51 [16.62-81.03]	15.25 [0.00-75.00]	0.31
Fibroatheroma (%)	12.70 [0.00-38.40]	0.00 [0.00-3.41]	0.07
Calcification (%)	0.00 [0.00-22.39]	0.00 [0.00-0.00]	0.11
Macrophages (%)	5.07 [0.00-28.96]	0.00 [0.00-24.16]	0.41
Neovessels (%)	0.00 [0.00-9.82]	0.00 [0.00-0.00]	0.11
Analysis of lipid pools			
Mean lipid arc (degrees)	87.66 [71.77-99.46]	64.14 [60.03-77.12]	<0.01
Max lipid arc (degrees)	133.80 [88.03-180.95]	90.25 [66.40-120.38]	0.03
Cap thickness (µm)	214.44 [188.33-255.52]	256.67 [182.50-386.57]	0.25
Lipid length (mm)	10.00 [4.50-27.50]	4.00 [4.00-10.75]	0.10

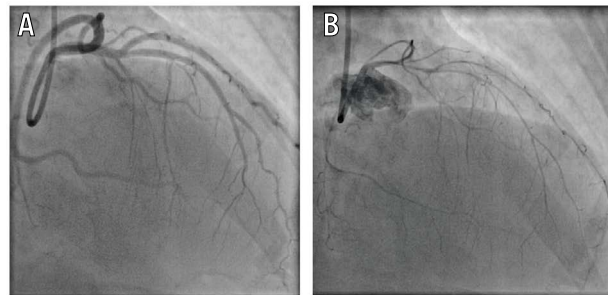
extension tended to be higher; as in ACH+ patients, 38.10% of all frames were covered by plaque, as compared with 10.88% in ACH- patients ($p=0.06$). In the ACH+ group, the prevalence of vulnerable plaque markers was significantly higher than in the ACH- group (66% vs 38%, $p=0.04$), led by a higher, yet non-significant, rate of TCFA (20% vs 0%, $p=0.06$) and a significant increase in neovascularisation (37% vs 6%, $p=0.02$). No differences were recorded in the prevalence and extension of the other markers of plaque vulnerability. No correlations were identified between the variables of interest and baseline characteristics or flow parameters. In particular, exploratory analyses found no correlations between atherosclerosis and CFR/IMR values.

A comparison was performed between patients with microvascular spasm and epicardial spasm (**Table 3**), also showing no major differences in terms of LI (668.00 [310.10-2348.18] vs 885.40 [461.45-2582.13], respectively, $p=0.51$) and in terms of markers of plaque vulnerability (overall rate of 64% and 68% in patients with epicardial and microvascular spasm, respectively, $p=0.78$).

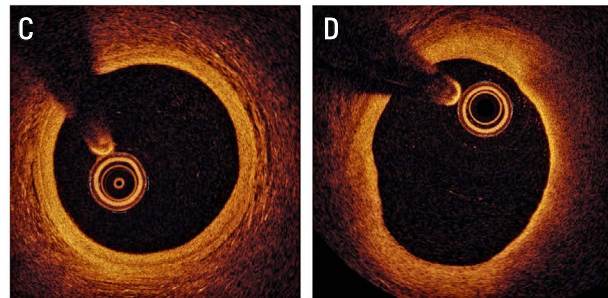
Finally, in an exploratory analysis of the subgroup of patients with epicardial spasm, LI was compared between subjects with focal and diffuse epicardial spasm, yielding non-significant differences (982.25 vs 885.40, respectively, $p=0.52$).

CENTRAL ILLUSTRATION Graphical representation of the study.**75 ANOCA patients****Acetylcholine test**

79%: vasospasm (ACH+)
21%: negative test (ACH-)

**Optical coherence tomography**

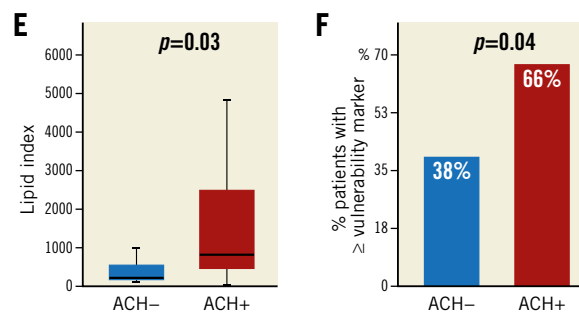
Lipid index
Plaque vulnerability markers*



*Vulnerability markers: thin-cap fibroatheroma, macrophage infiltration, neovascularisation, plaque erosion

OCT findings in vasospasm

Higher lipid index
Higher plaque vulnerability



Upper row: result of the acetylcholine test, showing either an angiographically normal artery (A) or a diffuse epicardial vasospasm (B). Middle row: optical coherence tomography assessment was performed, to detect normal segments (C) or coronary plaques, and to evaluate the lipid index and the presence of markers of plaque vulnerability. D) an example of a thin-cap fibroatheroma. Lower row: study results. Vessels prone to vasospasm were associated with a higher lipid index (E) and a higher prevalence of vulnerable plaques (F). ACH: acetylcholine; ANOCA: angina with no obstructive coronary artery disease; OCT: optical coherence tomography

Discussion

In this study of patients with ANOCA undergoing CFT and OCT we obtained three major findings. First, patients with vasospasm elicited by acetylcholine (either epicardial or microvascular spasm) had more advanced atherosclerosis compared to patients without vasospasm. Second, patients with coronary vasospasm had more characteristics of plaque vulnerability compared to patients without vasospasm. Third, there were no differences in terms of disease extension and morphology between patients with epicardial and microvascular spasm.

ASSOCIATIONS BETWEEN VASOMOTOR DYSFUNCTION AND ATHEROSCLEROSIS

Patients with coronary vasospasm showed more advanced atherosclerosis compared to those without vasospasm. Although

isolated reports have addressed similar hypotheses in specific phenotypes of vasomotor disorders (mainly vasospastic angina), to our knowledge this is the first study to analyse the full spectrum of vasomotor dysfunction. Despite conflicting reports in the last decades, with earlier studies suggesting that spasm may be associated with mild atherosclerosis^{12,20}, our data support recent OCT and near-infrared spectroscopy (NIRS) studies^{10,11} demonstrating that epicardial spasm may be associated with more advanced atherosclerosis, even in the absence of obstructive CAD. In our study, in vessels prone to vasospasm, atherosclerotic plaques were both more extensive (38% of the assessed segment was covered by plaque, compared to 11% in ACH- vessels, $p=0.06$), and more prone to rupture (66% of ACH+ patients had at least one marker of plaque vulnerability, compared to 38% of ACH- group, $p=0.04$).

Table 3. Comparison of optical coherence tomography (OCT) findings in patients with epicardial vasospasm and microvascular spasm.

	Epicardial spasm (n=28)	Microvascular spasm (n=31)	p-value
Main outcome			
Lipid index	885.40 [461.45-2584.13]	668.00 [310.10-2348.18]	0.51
Vulnerable plaque (% subjects)	18 (64%)	21 (68%)	0.78
Marker prevalence (% of subjects)			
Fibroatheroma	15 (54%)	14 (45%)	0.61
Thin-cap fibroatheroma	7 (25%)	5 (16%)	0.52
Macrophage infiltration	13 (46%)	15 (48%)	1.00
Neovascularisation	13 (46%)	9 (29%)	0.19
Erosion	2 (7%)	7 (23%)	0.15
Analysis of plaque extension (% length)			
Total plaque extension (%)	41.67 [8.62-77.33]	21.21 [8.00-76.09]	0.58
Lipid-rich (%)	46.14 [2.63-80.94]	48.52 [29.17-81.43]	0.51
Fibroatheroma (%)	15.15 [0.00-45.31]	6.29 [0.00-31.87]	0.40
Calcification (%)	2.99 [0.00-21.60]	0.00 [0.00-22.90]	0.58
Macrophages (%)	4.76 [0.00-38.33]	5.07 [0.00-23.74]	0.74
Neovessels (%)	2.68 [0.00-12.00]	0.00 [0.00-5.04]	0.16
Analysis of lipid pools			
Mean lipid arc (degrees)	92.16 [74.43-111.37]	83.34 [69.15-97.00]	0.28
Max lipid arc (degrees)	141.55 [108.30-164.28]	114.55 [87.63-198.33]	0.74
Cap thickness (µm)	224.84 [181.54-257.60]	214.44 [187.67-238.69]	0.99
Lipid length (mm)	10.00 [4.00-26.00]	12.00 [4.75-29.25]	0.73

Considering individual markers, the prevalence of neovascularisation was increased in arteries with vasospasm, in agreement with previous experience^{21,22}, and a consistent trend towards a higher rate was identified in all other variables^{21,23}, particularly in TCFA (20% vs 0%, p=0.06).

We found no correlations between physiology indexes and markers of plaque vulnerability. This is in contrast with the study by Usui and colleagues²⁴, which reported a positive correlation between IMR and markers of plaque severity, regardless of other indexes. However, this study was performed in the setting of angiographically moderate-severe lesions, and the generalisability of these results in the setting of ANOCA is questionable and needs additional evidence.

Macrophage infiltration, which has been suggested as a risk factor for plaque progression and rupture^{21,25}, was similar between those with and without vasospasm. We considered macrophages in the setting of lipid-rich plaques, as suggested by consensus documents, due to lack of validation studies in non-lipidic plaques¹⁷.

However, exploratory analyses that also considered bright spots outside lipid-rich plaques led to similar results. Additional data are needed to draw additional conclusions on the role of this marker of plaque inflammation.

Another interesting finding is related to intraplaque neovascularisation. This marker was detected in 37% of ACH+ patients, with a higher prevalence compared to ACH- patients (6%, p=0.02). A possible association between neovessels and spasm has been questioned, as the dysfunctional endothelium of these vessels may trigger the paradoxical reaction to acetylcholine, through an impaired nitric oxide pathway²¹. We were able to reproduce this finding. However, it is noteworthy that the study by Choi and colleagues considered a combination of intraplaque neovessels and vasa vasorum. We decided to include only intraplaque vessels in the analysis. These develop in reaction to the growth of the lipid pool, triggered by ischaemia and inflammation, and may be a better expression of such a deranged physiology. The role of vasa vasorum in our study is, therefore, not known.

Our results seem to support the theory that coronary vasospasm is not only a functional disorder, but is associated with more advanced atherosclerotic disease. These data may also explain the higher rate of ischaemic adverse events reported by recent studies on this population⁵⁻⁸. Indeed, the prognostic value of high-risk plaque characterisation by OCT was confirmed by the recent CLIMA study²⁶, which found cap thickness <75 µm, lipid arc >180° and macrophage infiltration to be associated with a higher risk of adverse cardiovascular events at one year. Interestingly, low cap thickness resulted in the single strongest risk factor. In our study, despite the use of an even stricter cut-off (<65 µm), this marker was found in 20% of subjects with coronary vasospasm.

If confirmed in a larger series, these findings may have an impact on the management and treatment of patients with coronary vasospasm, who usually show a low to moderate cardiovascular risk profile and receive drug therapy only to control symptoms (mainly calcium channel blockers and nitrates), with no significant benefit in terms of prognosis. There is still widespread belief that coronary vasomotor dysfunction is a relatively benign condition, and this evaluation bias (i.e., the false belief that, if there is no angiographic stenosis, there is no significant atherosclerosis) may also lead to an underestimation of future events and, therefore, to a decreased level of awareness and insufficient clinical monitoring over time. CFT proved to be effective at better defining the vasomotor dysfunction phenotype and optimising drug therapy²⁷. The addition of anatomic information might provide additional prognostic value, which would in turn set the goal of treatment on harder outcomes, beyond simple symptom control. Current consensus documents focus on lifestyle factors and behavioural interventions, comparable to patients with CAD. With regard to risk factor management, ACE inhibitors and statins are recommended⁴. With regard to aspirin and anti-inflammatory therapy directed at atherosclerosis, no clear recommendations are available. We hypothesise that this medication might be beneficial in vasospastic ANOCA patients.

RELATIONSHIPS BETWEEN MICROVASCULAR AND EPICARDIAL SPASM

The definition of microvascular spasm is based on the absence of visible severe epicardial spasm in the setting of typical chest pain and ECG changes triggered by acetylcholine²⁸. Currently, the Coronary Vasomotor Disorders International Study Group (COVADIS) lists microvascular spasm in the group of conditions called microvascular angina, along with impaired microvascular dilatation. The latter is considered an endothelium-independent vascular dysfunction and includes impaired vasodilatory capacity and increased microvascular resistance (marked by an abnormal CFR and/or IMR values⁴). Although all these conditions may cause disturbed functioning of the microcirculation, in our study, microvascular spasm shared significant similarities with epicardial spasm, particularly in terms of plaque distribution and composition. Understanding the mutual relationships between these conditions, either in terms of temporal evolution or spatial distribution, would provide interesting insights. Indeed, microvasculature may present the same features of dysfunctional endothelium and inflammation that were found in the epicardial arteries. Pro-inflammatory mediators produced in the larger vessels may also affect the downstream vasculature. The current diagnostic criteria define epicardial vasospasm as severe dynamic stenosis upon angiography, so all those abnormal reactions to acetylcholine which do not fulfil this strict criterion fall into the group of microvascular spasm, regardless of the degree of epicardial stenosis, if any. As a consequence, the concomitant existence of epicardial and microvascular vasospasm is not considered; so it is not possible to understand whether overlapping syndromes may exist, the evolution of these patterns over time, and whether specific triggers may elicit different spastic reactions in the same individual.

Unfortunately, no comparison with available evidence is possible, as studies focusing on microvascular spasm are lacking, and these results should be considered as hypothesis-generating. Moreover, there is still a lack of evidence in subjects with abnormal CFR/IMR values. Additional data may provide insights on morphological differences and similarities between these conditions, with the potential for further refinements of current classifications and risk stratification.

Limitations

This is a single-centre study, on a small sample of individuals. A larger population may allow additional comparisons, as well as a higher statistical power, especially for the analysis of markers of plaque vulnerability and for additional comparisons with non-vasospastic microvascular dysfunction. The study population included mostly women; although a higher prevalence in the female sex is expected in the field of vasomotor disorders, the generalisability to male sex may be limited.

Finally, we followed standard assessment protocols for the acquisition and interpretation of OCT images, and relied on multiple, independent assessments to limit variability. Nevertheless, a certain degree of inter- and intra-operator variability in the measurements must be taken into account.

Conclusions

Coronary vasospasm triggered by an abnormal reaction to acetylcholine is associated with a higher degree of atherosclerosis, a higher prevalence of vulnerable plaques and a higher rate of neovascularisation. Different patterns of vasospasm (focal, diffuse, or microvascular) are not associated with differences in intracoronary findings.

Impact on daily practice

Coronary vasospasm, regardless of its pattern, is associated with more advanced atherosclerotic disease, both in terms of plaque extension and of plaque vulnerability, compared with non-spastic vessels. Thus, patients with coronary vasospasm may have a higher risk of adverse cardiovascular events compared to the general population. This may allow identification of high-risk groups and improve long-term prognosis through the early adoption of strict management of cardiovascular risk factors and close follow-up.

Conflict of interest statement

P. Damman has received consultancy and lecture fees from Philips and Abbott. R.E. Konst, N. van Royen and S.E. Elias-Smale have received a research grant from Abbott. The other authors have no conflicts of interest to declare.

References

- Patel MR, Peterson ED, Dai D, Brennan JM, Redberg RF, Anderson HV, Brindis RG, Douglas PS. Low diagnostic yield of elective coronary angiography. *N Engl J Med*. 2010;362:886-95.
- Jespersen L, Hvelplund A, Abildstrøm SZ, Pedersen F, Galatius S, Madsen JK, Jørgensen E, Kelbæk H, Prescott E. Stable angina pectoris with no obstructive coronary artery disease is associated with increased risks of major adverse cardiovascular events. *Eur Heart J*. 2012;33:734-44.
- Asbury EA, Creed F, Collins P. Distinct psychosocial differences between women with coronary heart disease and cardiac syndrome X. *Eur Heart J*. 2004;25:1695-701.
- Kunadian V, Chieffo A, Camici PG, Berry C, Escaned J, Maas AHEM, Prescott E, Karam N, Appelman Y, Fraccaro C, Buchanan GL, Manzo-Silberman S, Al-Lamee R, Regar E, Lansky A, Abbott JD, Badimon L, Duncker DJ, Mehran R, Capodanno D, Baumbach A. An EAPCI Expert Consensus Document on Ischaemia with Non-Obstructive Coronary Arteries in Collaboration with European Society of Cardiology Working Group on Coronary Pathophysiology & Microcirculation Endorsed by Coronary Vasomotor Disorders International Study Group. *EuroIntervention*. 2021;16:1049-69.
- AlBadri A, Bairey Merz CN, Johnson BD, Wei J, Mehta PK, Cook-Wiens G, Reis SE, Kelsey SF, Bittner V, Sopko G, Shaw LJ, Pepine CJ, Ahmed B. Impact of Abnormal Coronary Reactivity on Long-Term Clinical Outcomes in Women. *J Am Coll Cardiol*. 2019;73:684-93.
- Suda A, Takahashi J, Hao K, Kikuchi Y, Shindo T, Ikeda S, Sato K, Sugisawa J, Matsumoto Y, Miyata S, Sakata Y, Shimokawa H. Coronary Functional Abnormalities in Patients With Angina and Nonobstructive Coronary Artery Disease. *J Am Coll Cardiol*. 2019;74:2350-60.
- Taqeti VR, Shaw LJ, Cook NR, Murthy VL, Shah NR, Foster CR, Hainer J, Blankstein R, Dorbala S, Di Carli MF. Excess Cardiovascular Risk in Women Relative to Men Referred for Coronary Angiography Is Associated With Severely Impaired Coronary Flow Reserve, Not Obstructive Disease. *Circulation*. 2017;135:566-77.
- Taqeti VR, Shaw LJ, Cook NR, Murthy VL, Shah NR, Foster CR, Hainer J, Blankstein R, Dorbala S, Di Carli MF. Coronary microvascular dysfunction and future risk of heart failure with preserved ejection fraction. *Eur Heart J*. 2018;39:840-9.
- Hung MJ, Cheng CW, Yang NI, Hung MY, Cherng WJ. Coronary vasospasm-induced acute coronary syndrome complicated by life-threatening cardiac arrhythmias

in patients without hemodynamically significant coronary artery disease. *Int J Cardiol.* 2007;117:37-44.

10. Choi BJ, Prasad A, Gulati R, Best PJ, Lennon RJ, Barsness GW, Lerman LO, Lerman A. Coronary endothelial dysfunction in patients with early coronary artery disease is associated with the increase in intravascular lipid core plaque. *Eur Heart J.* 2013;34:2047-54.

11. Lavi S, Bae JH, Rihal CS, Prasad A, Barsness GW, Lennon RJ, Holmes DR Jr, Lerman A. Segmental coronary endothelial dysfunction in patients with minimal atherosclerosis is associated with necrotic core plaques. *Heart.* 2009;95:1525-30.

12. Tanaka A, Shimada K, Tearney GJ, Kitabata H, Taguchi H, Fukuda S, Kashiwagi M, Kubo T, Takarada S, Hirata K, Mizukoshi M, Yoshikawa J, Bouma BE, Akasaka T. Conformational change in coronary artery structure assessed by optical coherence tomography in patients with vasospastic angina. *J Am Coll Cardiol.* 2011;58:1608-13.

13. Shin ES, Ann SH, Singh GB, Lim KH, Yoon HJ, Hur SH, Her AY, Koo BK, Akasaka T. OCT-Defined Morphological Characteristics of Coronary Artery Spasm Sites in Vasospastic Angina. *JACC Cardiovasc Imaging.* 2015;8:1059-67.

14. Kitano D, Takayama T, Sudo M, Kogo T, Kojima K, Akutsu N, Nishida T, Haruta H, Fukamachi D, Kawano T, Kanai T, Hiro T, Saito S, Hirayama A. Angioscopic differences of coronary intima between diffuse and focal coronary vasospasm: Comparison of optical coherence tomography findings. *J Cardiol.* 2018;72:200-7.

15. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol.* 1983;51:606.

16. Beltrame JF, Crea F, Kaski JC, Ogawa H, Ong P, Sechtem U, Shimokawa H, Bairey Merz CN; Coronary Vasomotion Disorders International Study Group (COVADIS). International standardization of diagnostic criteria for vasospastic angina. *Eur Heart J.* 2017;38:2565-8.

17. Tearney GJ, Regar E, Akasaka T, Adriaenssens T, Barlis P, Bezerra HG, Bouma B, Bruining N, Cho JM, Chowdhary S, Costa MA, de Silva R, Dijkstra J, Di Mario C, Dudek D, Falk E, Feldman MD, Fitzgerald P, Garcia-Garcia HM, Gonzalo N, Granada JF, Guagliumi G, Holm NR, Honda Y, Ikeno F, Kawasaki M, Kochman J, Koltowski L, Kubo T, Kume T, Kyono H, Lam CC, Lamouche G, Lee DP, Leon MB, Maehara A, Manfrini O, Mintz GS, Mizuno K, Morel MA, Nadkarni S, Okura H, Otake H, Pietrasik A, Prati F, Räber L, Radu MD, Rieber J, Riga M, Rollins A, Rosenberg M, Sirbu V, Serruys PW, Shimada K, Shinke T, Shite J, Siegel E, Sonoda S, Suter M, Takarada S, Tanaka A, Terashima M, Thim T, Uemura S, Ughi GJ, van Beusekom HM, van der Steen AF, van Es GA, van Soest G, Virmani R, Waxman S, Weissman NJ, Weisz G; International Working Group for Intravascular Optical Coherence Tomography (IWG-IVOC). Consensus standards for acquisition, measurement, and reporting of intravascular optical coherence tomography studies: a report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation. *J Am Coll Cardiol.* 2012;59:1058-72.

18. Kato K, Yonetsu T, Kim SJ, Xing L, Lee H, McNulty I, Yeh RW, Sakhuja R, Zhang S, Uemura S, Yu B, Mizuno K, Jang IK. Comparison of nonculprit coronary plaque characteristics between patients with and without diabetes: a 3-vessel optical coherence tomography study. *JACC Cardiovasc Interv.* 2012;5:1150-8.

19. Ong P, Camici PG, Beltrame JF, Crea F, Shimokawa H, Sechtem U, Kaski JC, Bairey Merz CN; Coronary Vasomotion Disorders International Study Group (COVADIS). International standardization of diagnostic criteria for microvascular angina. *Int J Cardiol.* 2018;250:16-20.

20. Yamagishi M, Miyatake K, Tamai J, Nakatani S, Koyama J, Nissen SE. Intravascular ultrasound detection of atherosclerosis at the site of focal vasospasm in angiographically normal or minimally narrowed coronary segments. *J Am Coll Cardiol.* 1994;23:352-7.

21. Choi BJ, Matsuo Y, Aoki T, Kwon TG, Prasad A, Gulati R, Lennon RJ, Lerman LO, Lerman A. Coronary endothelial dysfunction is associated with inflammation and vasa vasorum proliferation in patients with early atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2014;34:2473-7.

22. Park KH, Sun T, Diez-Delgado F, Liu Z, Yang SW, Lennon RJ, Herrmann J, Gulati R, Rodriguez-Portel M, Lerman LO, Lerman A. Association between coronary microvascular function and the vasa vasorum in patients with early coronary artery disease. *Atherosclerosis.* 2016;253:144-9.

23. Shin ES, Her AY, Ann SH, Balbir Singh G, Cho H, Jung EC, Shim EB, Koo BK, Akasaka T. Thrombus and Plaque Erosion Characterized by Optical Coherence Tomography in Patients With Vasospastic Angina. *Rev Esp Cardiol (Engl Ed).* 2017;70:459-66.

24. Usui E, Yonetsu T, Kanaji Y, Hoshino M, Yamaguchi M, Hada M, Fukuda T, Sumino Y, Ohya H, Hamaya R, Kanno Y, Yuki H, Murai T, Lee T, Hirao K, Kakuta T. Optical Coherence Tomography-Defined Plaque Vulnerability in Relation to Functional Stenosis Severity and Microvascular Dysfunction. *JACC Cardiovasc Interv.* 2018;11:2058-68.

25. Libby P. Inflammation in atherosclerosis. *Nature.* 2002;420:868-74.

26. Prati F, Romagnoli E, Gatto L, La Manna A, Burzotta F, Ozaki Y, Marco V, Boi A, Fineschi M, Fabbiochi F, Taglieri N, Niccoli G, Trani C, Versaci F, Calligaris G, Ruscica G, Di Giorgio A, Vergallo R, Albertucci M, Biondi-Zoccai G, Tamburino C, Crea F, Alfonso F, Arbustini E. Relationship between coronary plaque morphology of the left anterior descending artery and 12 months clinical outcome: the CLIMA study. *Eur Heart J.* 2020;41:383-91.

27. Ford TJ, Stanley B, Good R, Rocchiccioli P, McEntegart M, Watkins S, Eteiba H, Shaukat A, Lindsay M, Robertson K, Hood S, McGeoch R, McDade R, Yii E, Sidik N, McCartney P, Corcoran D, Collison D, Rush C, McConnachie A, Touyz RM, Oldroyd KG, Berry C. Stratified Medical Therapy Using Invasive Coronary Function Testing in Angina: The CorMicA Trial. *J Am Coll Cardiol.* 2018;72:2841-55.

28. Ong P, Athanasiadis A, Borgulya G, Vokshi I, Bastiaenen R, Kubik S, Hill S, Schäufele T, Mahrholdt H, Kaski JC, Sechtem U. Clinical usefulness, angiographic characteristics, and safety evaluation of intracoronary acetylcholine provocation testing among 921 consecutive whit patients with unobstructed coronary arteries. *Circulation.* 2014;129:1723-30.

Supplementary data

Supplementary Appendix 1. Description of study procedures.

Supplementary Appendix 2. OCT analysis.

The supplementary data are published online at:

<https://eurointervention.pconline.com/>

doi/10.4244/EIJ-D-21-00875



Supplementary data

Supplementary Appendix 1. Description of study procedures.

Coronary angiography

Patients were instructed to withhold from taking all vasoactive medication (e.g., calcium channel blockers [CCBs], long-acting nitrates) and methylxanthine-containing substances (including caffeine) for 24-48 hours before the procedure, depending on half-life time. Administration of nitroglycerin was avoided during the procedure if possible. First, a diagnostic CAG was performed to confirm the absence of an obstructive coronary artery disease (CAD), defined as a visual stenosis of more than 50% in combination with a measured resting full-cycle ratio (RFR) >0.89 and/or fractional flow reserve (FFR) >0.80 . If no or non-obstructive CAD was present, the procedure continued with the assessment of the coronary vasoreactivity in the CFT.

Coronary function test

Endothelium-dependent vasoreactivity: spasm provocation

First, endothelium-dependent coronary function was tested with acetylcholine (ACH) provocation testing. This was performed in accordance with the standardised protocol by Ong et al²⁸. Heart rate, blood pressure and a 12-lead ECG were continuously monitored. Incremental doses of 2, 20, 100, and 200 mcg of ACH were manually infused over a period of 1-3 minutes into the left coronary artery (LCA) through a guiding catheter. After each infusion, cine-images were obtained to assess the change in coronary diameter. After the 200 mcg dose or if significant epicardial or microvascular spasm was present, 0.2 mg nitroglycerin was injected into the LCA. In case of persistent coronary spasm or haemodynamically significant atrioventricular conduction disorders, intravenous atropine could be administered. At the discretion of the operator, an uneventful provocation test of the LCA could be followed by infusion of 80 mcg of ACH in the RCA in a similar matter. The ACH provocation test was always concluded with the intracoronary administration of nitroglycerin.

Pressure indexes

Second, a guidewire with distal pressure and temperature sensors (PressureWire X; Abbott Vascular) was used to measure the RFR, a novel non-hyperaemic pressure ratio that evaluates the entire cardiac cycle. With the sensor positioned at the tip of the catheter, the pressure measurement from the wire was equalised with that of the guiding catheter. The sensor was then positioned in the distal LAD coronary artery. A minimum of five steady-state cardiac cycles was used to calculate RFR. The fractional flow reserve (FFR) was measured during assessment of the endothelium-independent vasoreactivity.

Endothelium-independent vasoreactivity: CFR and IMR

Subsequently, using the same guidewire already in place, the endothelium-independent (hyperaemic) vasoreactivity was assessed with the thermodilution method. The aortic pressure (Pa) at the guiding catheter and the distal coronary pressure (Pd) at the tip of the guidewire were recorded simultaneously throughout the measurement. The resting mean transit time (Tmn) was determined by injections of 3-5 mL room temperature saline into the LAD artery, averaging at least three consecutive overlapping measurements. Next, adenosine (typically 140 µg/kg/min) was administered via a large peripheral or central vein to induce steady state maximal hyperaemia - and thereby minimal microvascular resistance – and at least three more injections of room temperature saline were recorded and averaged to determine the hyperaemic Tmn. Measurements of Tmn were only accepted if the variability between the three measurements of Tmn was <20%. A drift check was performed at the end of the procedure. In case of significant drift (more than 2 mmHg), measurements were repeated.

FFR was calculated by the ratio of mean Pd and mean Pa at maximal hyperaemia. Microvascular resistance, measured as the index of microvascular resistance (IMR), was calculated as the Pd at maximal hyperaemia divided by the inverse of the hyperaemic Tmn. The coronary flow reserve (CFR) was determined by dividing the average resting Tmn by the average hyperaemic Tmn. All measurements were automatically analysed by dedicated software (Coroventis Coroflow).

Supplementary Appendix 2. OCT analysis

Analysis of the OCT pullbacks was performed by two independent expert reviewers, blinded to the results of the previous tests. Discordance was solved by a third reviewer. Every OCT run was

reviewed in its full length. In-depth analysis of cross-sectional images was performed in one frame per millimetre of longitudinal length.

Plaque was defined as an intima-media thickness $>500\ \mu\text{m}$. Each plaque was then classified as fibrous or lipid-rich, according to international consensus documents. In particular, lipid was defined as a diffusely bordered, signal-poor region, with significant signal attenuation, and with an overlying signal-rich layer (fibrous cap). In the context of lipid plaque, lipid arc was measured every mm. Fibroatheroma (FA) was defined as a lipid-rich plaque with a lipid arc $>90^\circ$. Lipid index (LI) was defined as the mean lipid arc multiplied by lipid-core longitudinal length. Fibrous cap thickness (FCT) was measured once every mm at its thinnest part (visual selection), and measurement was then repeated three times in the frame with the smallest value and averaged, to compensate intra-observer variability. Thin-cap fibroatheroma (TCFA) was defined as a fibroatheroma with a FCT $\leq 65\ \mu\text{m}$. Calcification was defined as an area with low attenuation and a sharp border, in the context of a plaque. Macrophages were defined as linear or spotty formations with an increased signal compared to the background speckle noise, and with heterogeneous backward shadows. Neovessels were defined as spherical or tubular, signal-void structures, with a diameter of 50 to 300 μm , in the context of a plaque, without any connection to other branches. Thrombus was defined as a floating or protruding mass $>200\ \mu\text{m}$ on multiple adjacent frames and was categorised accordingly as either red (high backscattering and high attenuation), or white (low backscattering and low attenuation). Plaque erosion was identified by the presence of thrombus with or without lumen irregularity overlying an intact fibrous cap on multiple adjacent OCT frames.