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The association between in-stent neoatherosclerosis and native coronary artery disease progression: a long-term angiographic and optical coherence tomography cohort study

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Aims The purpose of the present study was to investigate the relationship between in-stent neoatherosclerosis (NA) and native atherosclerosis progression of untreated coronary segments.

Methods and results In-stent NA was assessed by optical coherence tomography (OCT) among patients included in the SIRTAX-LATE OCT study 5 years after drug-eluting stent (DES) (sirolimus-eluting and paclitaxel-eluting stents) implantation. Neoatherosclerosis was defined as the presence of fibroatheroma or fibrocalcific plaque within the neointima of stented segments with a longitudinal extension >1.0 mm. Atherosclerosis progression in untreated native coronary segments was evaluated by serial quantitative coronary angiography (QCA). The change in minimal lumen diameter (MLD) was serially assessed within matched segments at baseline and 5-year angiographic follow-up. The key clinical endpoint was non-target lesion (non-TL) revascularization throughout 5 years. A total of 88 patients with 88 lesions were available for OCT analysis 5 years after DES implantation. In-stent NA was observed in 16% of lesions with the majority of plaques being fibroatheromas (11.4%) followed by fibrocalcific plaques (5.7%). A total of 704 non-TL segments were serially evaluated by QCA. Between baseline and 5-year follow-up, the reduction in MLD was significantly more pronounced in patients with NA (−0.25 mm, 95% CI −0.36 to −0.17 mm) when compared with patients without NA (−0.13 mm, 95% CI −0.17 to −0.10 mm, $P = 0.002$). Similarly, non-TL revascularization was more frequent in patients with NA (78.6%) when compared with patients without NA (44.6%, $P = 0.028$) throughout 5 years.

Conclusions In-stent NA is more common among patients with angiographic and clinical evidence of native atherosclerosis progression suggesting similar pathophysiological mechanisms.
SIRTAX trial is registered at http://www.clinicaltrials.gov/ct2/show/NCT00617084.

Keywords Drug-eluting stent • Sirolimus-eluting stent • Paclitaxel-eluting stent • Neoatherosclerosis • Target lesion revascularization • Long-term outcomes

Clinical perspective

The significant association between in-stent neoatherosclerosis (NA) and progression of native coronary atherosclerosis suggests similar pathophysiological mechanisms. Therapeutic strategies known to attenuate atherosclerosis progression—such as high-dose statin therapy—may be also effective to suppress the development or progression of NA. In addition, the impact of NA on cardiovascular events has not been sufficiently investigated and requires confirmation in adequately powered observational intra-coronary imaging studies.

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Introduction

Drug-eluting stents (DES) reduce the risk of repeat revascularization compared with bare metal stents, but late stent failure may still occur due to restenosis or stent thrombosis. In-stent neoatherosclerosis (NA)—a novel disease entity—is characterized by the development of atherosclerotic changes in the nascent neointimal tissue within previously implanted stents. Although there is no large–scale prospective study assessing the impact of NA on late stent failure and associated clinical outcomes, NA has been identified as the culprit for delayed in-stent-restenosis or stent thrombosis in intra-coronary imaging studies and case reports.[1–3] Accordingly, NA may represent an accelerated and possibly more unstable manifestation of atherosclerosis.[1,4] While histological analyses were performed for the documentation of NA in human *ex vivo* pathology studies, optical coherence tomography (OCT) is able to accurately characterize the *in vivo* vascular response after stent implantation including the development of in-stent NA.[1,3]

Despite the potential clinical impact of NA during the long-term course following DES implantation, little is known about the pathophysiological mechanisms underlying the development of NA. Based on histological similarities between NA and native atherosclerosis, we hypothesized that patients with progression of atherosclerosis in native coronary segments would be at increased risk for the development of NA within stented segments.[4] We therefore investigated the type and frequency of in-stent NA as assessed by OCT and native atherosclerosis progression in the entire untreated coronary artery tree assessed by quantitative coronary angiography (QCA) among patients included in the SIRTAX-LATE OCT cohort study 5 years after DES implantation.[5]

Methods

Patient population

The design and results of the SIRTAX and SIRTAX-LATE study (Sirolimus-Eluting Stent Compared With Paclitaxel-Eluting Stent for Coronary Revascularisation) have been previously reported.[6,7] For the purpose of the present study, we analysed all patients included in the SIRTAX-LATE OCT study. Among 145 patients who underwent angiographic follow-up 5 years after DES implantation between December 2008 and July 2009, 88 patients with 88 lesions were included in the OCT study.[5] A detailed patient flow is shown in *Figure 1*. The study complied with the Declaration of Helsinki regarding investigation in humans and was approved by the institutional ethics committees at Bern University Hospital, Switzerland. All patients provided written informed consent.

Optical coherence tomography imaging and analysis

Optical coherence tomography was performed with a time domain M2 system (Lightlab Imaging, Westford, MA, USA) using a pullback speed of 2 mm/s and the non-occlusive flushing technique. After the diagnostic angiography and administration of 5000 IU unfractionated heparin, the ImageWire (Lightlab Imaging) was carefully advanced distal to the study lesion. Following administration of 200 μg of intra-coronary nitroglycerin, the target vessel was flushed via the guiding catheter with nonionic, isomolar contrast liquid using a power injector with flush rates between 3 and 5 mL/s. Optical coherence tomography pullbacks were assessed offline using a proprietary software (Lightlab Imaging). Stented segments were analysed for strut coverage, apposition, and protrusion at frames with 1-mm interval by two independent analysts blinded (L.R. and S.B.) for stent type. For in-stent NA assessment, frames were analysed at 0.125 mm intervals by two independent investigators (L.R. and M.T.). Frames were considered not analysable when more than one-quarter of the circumference was not visible due to insufficient flush or out of zoom. Definitions used for stent strut analyses were previously reported.[5]

Neointima was defined as the tissue between the luminal border and the endoluminal border of the struts. Neoatherosclerosis lesion was defined as the presence of a fibroatheroma or fibrocalcific plaques within the neointima of a stented segment with a longitudinal extension of $\geq$1 mm. A gap of at least 0.5 mm was used to define the boundary between two NA lesions.

Fibroatheroma (FA) were characterized as a signal-poor region displaying a high attenuation (to differentiate from layered neointima) with diffuse borders and a lateral extension of at least one quadrant.[8] Thin-cap fibroatheroma (TCFA) were defined as FA with a fibrous cap $\leq$65 μm and thick-cap fibroatheroma (ThCFA) with a fibrous cap $>$65 μm. Fibrocalcific plaques were defined as signal-poor region with low attenuation and clear borderlines extending over one quadrant. Whenever the calcific pool was located both inside and behind the stent, we disregarded the presence of NA. Signal rich bands, suggestive of macrophage accumulations, were defined as lines or dots with strong signal attenuation producing a shadow with a sharply delineated lateral border.

Additional characteristics which potentially reflect neoatherosclerotic changes were investigated and their definition are provided in Supplementary material online, Data. To determine the intra- and inter-observer reproducibility of NA assessment, 20 OCT pullbacks were randomly chosen and analysed by two assessors at two time points (2 months apart) and then Cohen's Kappa was calculated.

Quantitative coronary angiography analysis

After administration of intra-coronary nitroglycerin, standard biplane angiographic images were obtained so that each coronary segment was recorded in at least two orthogonal views. All angiographies were analysed by the angiographic core laboratory at Bern University Hospital. Assessors were blinded to the OCT analysis and clinical outcomes. Methods for the serial assessment of the target lesions (TLs) were previously reported.[7] All three major untreated epicardial vessels including all side branches with a reference vessel diameter (RVD) of $>$1.5 mm in diameter were assessed by QCA at baseline and at follow-up using similar projections whenever possible. For this purpose, segments were divided in subsegments according to the modified American Heart Association/American College of Cardiology (AHA/ACC) classification using the QCA—CMS software version 7.3 (Medis Medical Imaging Systems, Leiden, the Netherlands) (*Figure 2*).[9,10] Minimal lumen diameter (MLD), RVD, segment length, and diameter stenosis ([1 – MLD/RVD] × 100) were assessed. In case a segment was revascularized prior to the 5-year follow-up examination, the latest available angiography prior to revascularization was used for analysis. The change of all variables was derived for each segment as outcome (follow-up) – outcome (baseline). The angiographic endpoint was mean change in MLD.

Clinical follow-up

An independent clinical events committee adjudicated all data on case report forms. All adverse events were assessed in hospital, at 1, 6, and 9 months, and on an annual basis up to 5 years. The clinical endpoint of this study was the occurrence of any non-TL revascularization within the 5-year angiographic follow-up window. Non-TL revascularization was defined as any revascularization except for TLR. Definitions of secondary clinical endpoints are provided in the Supplementary material

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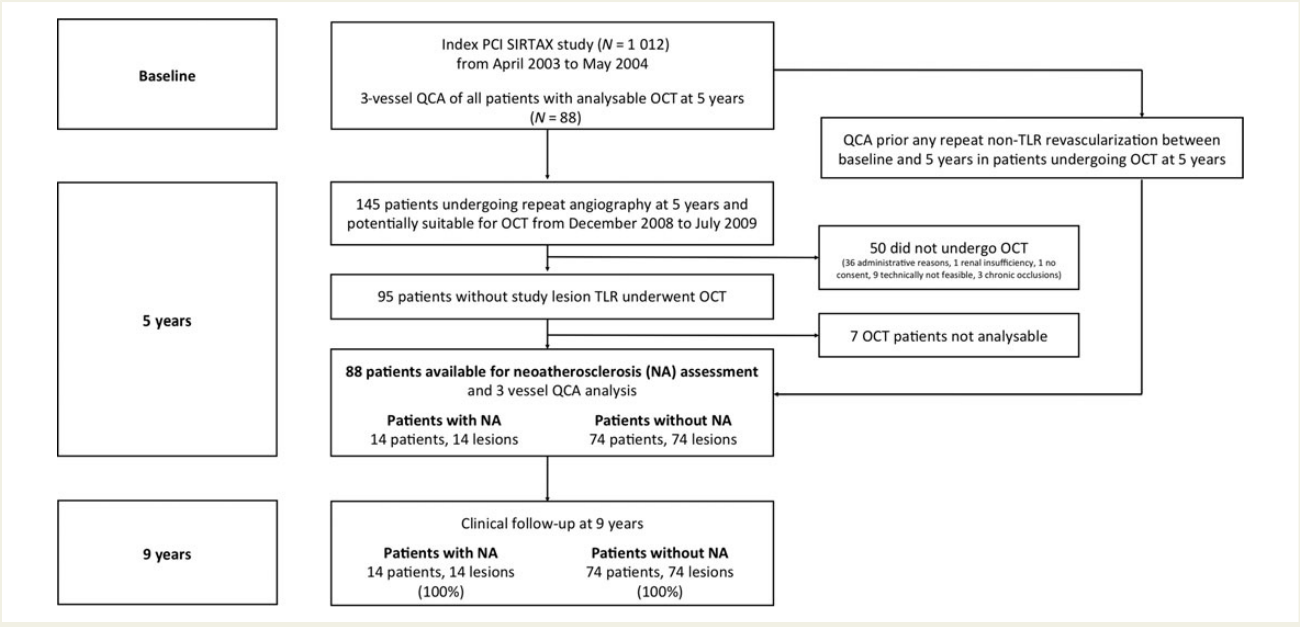

Figure 1 Study flow.

online, Data. To further investigate the clinical impact of NA findings, we extended the clinical follow-up to 9 years from index procedure (until July 2013).

Statistical analysis

Comparison of baseline characteristics, medication, lipid profiles, and stents were performed with Wilcoxon rank-sum or Fisher's exact tests.

Stratification: Patients were stratified in two groups according to the presence of at least one NA plaque detected by OCT at 5-year follow-up in the TL (based on criterion that an NA plaque is $\geq$1.0 mm).

Optical coherence tomography: Frame-level OCT outcomes were analysed with linear mixed models with patient as random intercept and lesion-level OCT outcomes with linear models.

Quantitative coronary angiography: Quantitative coronary angiography outcomes were recorded for several segments per patient at baseline and at angiographic follow-up. The absolute change from baseline to follow-up was computed for each segment. Patient-level outcomes and their changes were then derived by taking the arithmetic mean over several segments. To compare the strata, medians taken over the patients are reported with 95% CIs from non-parametric bootstrap with *P*-values from Wilcoxon rank-sum tests.

Clinical events: The occurrence of revascularization events up to and including the 5-year angiographic follow-up was compared between the strata. Analyses were based on the first event per patient. Crude percentages are reported, odds ratios and *P*-values are from logistic regression models. Statistical analyses were done with the computing environment R (The R Foundation for Statistical Computing) and with Stata (StataCorp, College Station, TX, USA).

Results

Frequency, type, and distribution of neoatherosclerosis

A total of 97% (11 772/12 124 frames) of all OCT frames were suitable for analysis. Neoatherosclerosis formation was observed in 14 (15.9%) of 88 lesions with the majority of plaques fulfilling the diagnostic criteria of fibroatheromas ($N = 9$, 10.2%) and less frequently fibrocalcific plaques ($N = 4$, 4.5%) and both plaque types in one case ($N = 1$, 1.2%) (*Table 1*, *Figure 3*). Multiple NA lesions in the same stent were observed in four lesions (4.5%). The intra- and inter-observer reproducibility (Cohen's Kappa) were 0.886 and 0.857, respectively. The most frequently observed findings potentially related to NA were signal rich bands, which were observed in 31.8% of stents. Other findings potentially related to NA were infrequent (microvessels: 2.3%, surface erosion: 3.4%).

Neoatherosclerosis was more common among lesions treated with PES (25.5%) compared with sirolimus-eluting stent (SES; 4.9%; $P = 0.009$) and differences between stent types applied to both the frequency of fibrocalcific plaques (SES 0 vs. PES 10.6%, $P = 0.058$) as well as fibroatheromas (SES 4.9 vs. PES 17.0%, $P = 0.10$). Similarly, signal rich bands were more frequent among lesions treated with PES than SES (46.8 vs. 14.6%, $P = 0.001$).

Baseline characteristics of patients with and without neoatherosclerosis

Baseline clinical, angiographic, and procedural characteristics of patients with and without NA are summarized in *Table 2*. No significant differences were recorded with the exception of type of implanted stent as mentioned above. We assessed the adherence to cardiovascular medications including acetylsalicylic acid, β-blocker, angiotensin converting enzyme (ACE) inhibitor/angiotensin receptor (AR) blocker, statin, or their combination throughout 5 years. There was no difference in the intake of any of these medications (or combinations of 1, 2, or 3 medications) between patients with compared with those without NA throughout 5 years (Supplementary material online, *Table S1*). In addition, no difference in lipid levels at baseline or at 5 years was noted and no difference in the reduction of low-density lipoprotein-cholesterol

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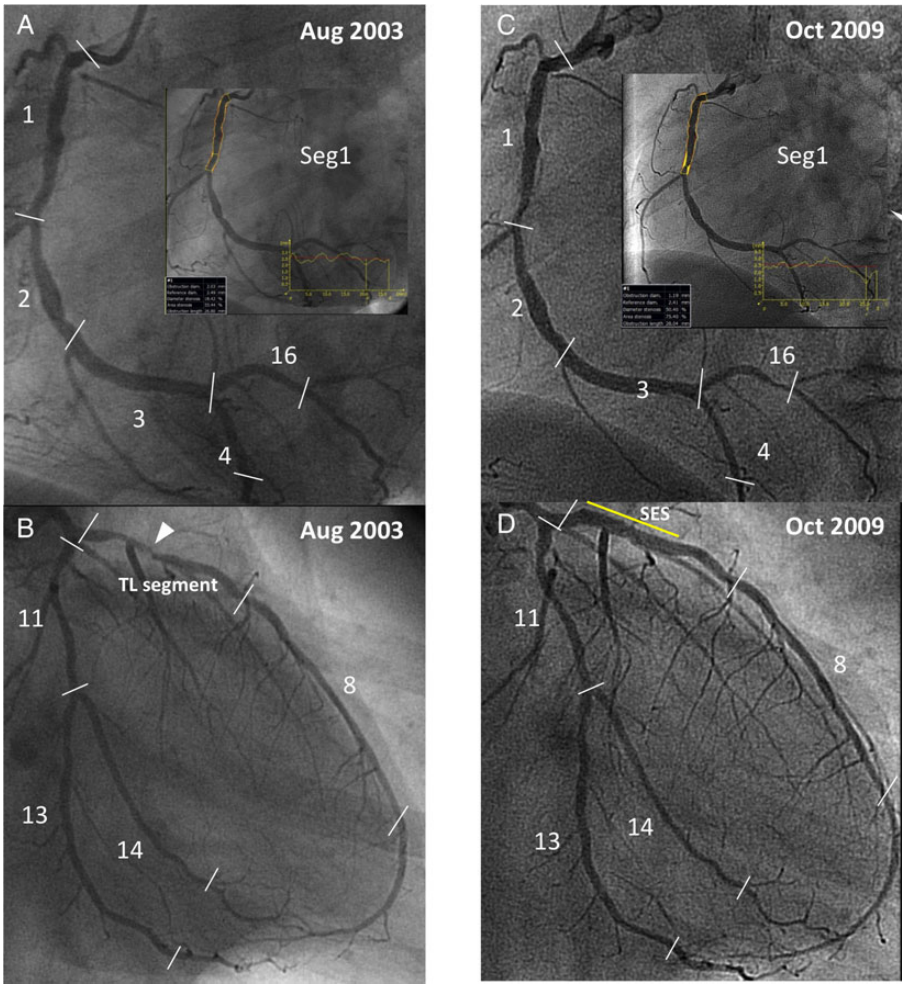

Figure 2 Serial quantitative coronary angiography analysis. This figure shows the serial quantitative coronary angiography analysis within matched regions of all untreated coronary artery segments at baseline (*A* and *B*) and at 5-year follow-up (*C* and *D*). Untreated coronary artery segments were classified according to the modified AHA/ACC classification. The treated lesion is shown in the proximal left anterior descending (LAD).

(LDL-C) over 5 years (NA: −25.9 vs. no-NA: −9.1 mg/dL, $P = 0.62$) or change in high-density lipoprotein-cholesterol (HDL-C) over 5 years (NA −1.4 vs. no-NA −2.6 mg/dL, $P = 0.85$) was observed.

Optical coherence tomography analysis

Optical coherence tomography findings at 5 years in lesions with and without NA are summarized in Supplementary material online, *Table S2*. Lesions with NA showed a thicker neointima (0.15 vs. 0.11 mm, $P = 0.001$), neointimal area (1.27 vs. 0.96 mm^2, $P = 0.003$), and percent volume obstruction (19.6 vs. 13.0%, $P = 0.001$). Less protruding stent struts were found in lesions with (0.06%) vs. without NA (0.40%, $P = 0.023$).

Quantitative coronary angiography analysis

A total of 704 untreated, native coronary artery segments at baseline were matched with the corresponding segments at 5 years follow-up, allowing the assessment of longitudinal changes over time (*Table 3*). A reduction in MLD was observed in both groups (with and without NA). The reduction in MLD of untreated, native coronary artery segments between baseline and 5-year follow-up was more pronounced in lesions of patients with NA (−0.25 mm, 95% CI −0.36 to −0.17) compared with lesions of patients without NA (−0.13 mm, 95% CI −0.17 to −0.10, $P = 0.002$) (*Figure 4*). Similarly, the change of % diameter stenosis was higher in lesions of patients with NA (6.0%, 95% CI 5.3–11.1) compared with those without NA (4.3%, 95% CI 2.5–6.2, $P = 0.048$). Two representative examples of patients with NA lesion formation and native atherosclerosis progression are provided in *Figure 5*. We performed four sensitivity analyses: In the first, we applied a more strict definition of NA lesions requiring a longitudinal extension of 1.5 mm. With this criteria, the difference in mean change in MLD remained unchanged (−0.32 vs. −0.13 mm, $P = 0.0005$) (Supplementary material online, *Table S3*). In the second sensitivity analysis, we only included patients treated with PES and found similar MLD changes (−0.27 vs. −0.11 mm, $P = 0.004$) (Supplementary material online,

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Table 1 Neoatherosclerosis-related findings of lesions undergoing optical coherence tomography analysis

	Overall (*N* = 88)	SES (*N* = 41)	PES (*N* = 47)	*P*-value
Plaque type				
NA (lesions with at least one plaque)	14 (15.9)	2 (4.9)	12 (25.5)	0.009
Fibrocalcific plaque	5 (5.7)	0 (0)	5 (10.6)	0.058
FA	10 (11.4)	2 (4.9)	8 (17.0)	0.10
Thick-cap FA	8 (9.1)	1 (2.4)	7 (14.9)	0.06
Thin-cap CFA	3 (4.3)	1 (2.4)	2 (4.3)	1.00
Incidence of multiple fibrocalcific plaques	1 (1.1)	0 (0)	1 (2.1)	1.00
Incidence of multiple thick-cap FA	1 (1.1)	0 (0)	1 (2.1)	1.00
Incidence of multiple thin-cap FA	0 (0)	0 (0)	0 (0)	
Multiple neoatherosclerotic plaque	4 (4.5)	0 (0)	4 (8.5)	0.12
Non-plaque-related neoatherosclerotic findings				
Signal rich band	28 (31.8)	6 (14.6)	22 (46.8)	0.001
Microvessel	2 (2.3)	0 (0)	2 (4.3)	0.50
Intimal tear	1 (1.1)	0 (0)	1 (2.1)	1.00
Intra-luminal thrombus	10 (11.4)	4 (9.8)	6 (12.8)	0.75
Erosion	3 (3.4)	3 (7.3)	0 (0)	0.10
Plaque rupture	0 (0)	0 (0)	0 (0)	
Other findings				
Peri-strut low-signal intensity layer	15 (17.1)	2 (4.9)	13 (27.7)	0.005
Lesions with any potential NA findings	36 (40.9)	10 (24.4)	26 (55.3)	0.005

Values are the number of patients (%), one stented lesion per patient underwent OCT analysis. *P*-values from Fisher's exact test. Plaque defined as at least eight consecutive frames (≥1 mm) with fibrocalcific, thick- or thin-cap fibroatheroma. Non-plaque findings defined as at least three consecutive frames with the same finding.

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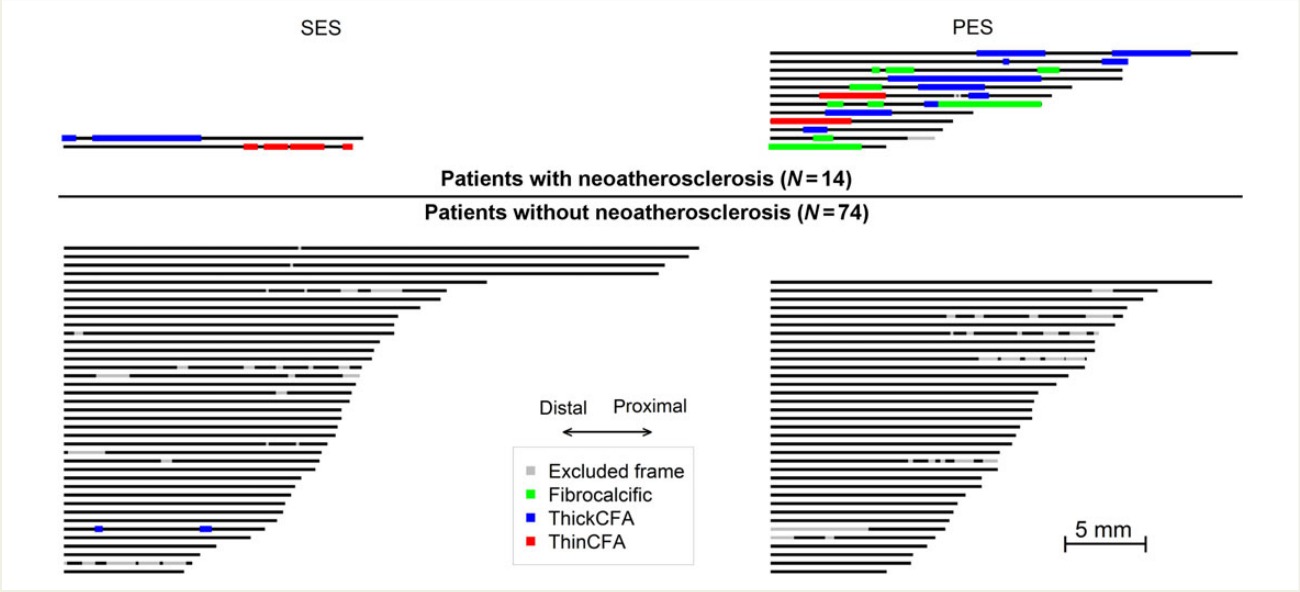

Figure 3 Longitudinal neoatherosclerosis lesion map. The stented vessel regions are represented by black lines with indication of the location of neoatherosclerosis plaque types (colored) and non-analysable frames (grey). The longitudinal resolution is 0.125 mm. Stents with neoatherosclerosis plaques (i.e. ≥1 mm longitudinal extension) are shown in the upper part of the figure and stents without in the lower part.

Table S4). Third, we applied sensitivity analysis assessing the change in MLD in non-target vessels vs. non-stented target vessels and found consistent results[non-target vessels (−0.26 vs. −0.13 mm, $P = 0.003$), non-stented segments in target vessel (−0.30 vs. −0.09 mm, $P = 0.02$)] (Supplementary material online, *Table S5*). Fourth, we compared the change in MLD between ACS and

Table 2 Baseline clinical, procedural, stented lesion, and angiographic characteristics

	Patients with NA (*N* = 14)	Patients without NA (*N* = 74)	*P*-value
Age (years)	56 (49.0–71.5)	60 (53.0–64.0)	0.89
Male	10 (71.4)	60 (81.1)	0.47
BMI (kg/m^2)	27.4 (24.9–28.9)	27.8 (24.7–30.8)	0.75
Cardiac risk factors			
Diabetes mellitus	3 (23.1)	13 (18.8)	0.71
Hyperlipidaemia	8 (61.5)	42 (60.9)	0.47
Hypertension	10 (76.9)	41 (59.4)	0.35
Current smoker	6 (46.2)	29 (42.0)	1.00
Previous PCI	4 (28.6)	15 (20.3)	0.49
Previous MI	3 (21.4)	22 (29.7)	0.75
Left ventricular ejection fraction (%)	62.5 (52.5–65.0)	60 (50.0–65.0)	0.97
Clinical presentation			
Stable CAD	6 (42.9)	30 (40.5)	1.00
Acute coronary syndrome	8 (57.1)	44 (59.5)	1.00
Unstable angina	0	5 (6.7)	1.00
NSTEMI	6 (42.9)	14 (18.9)	0.08
STEMI	2 (14.3)	25 (33.8)	0.21
TL coronary artery			
LAD	7 (50.0)	35 (47.3)	1.00
LCX	3 (21.4)	17 (23.0)	1.00
LMCA	0 (0.0)	2 (2.7)	1.00
RCA	4 (28.6)	20 (27.0)	1.00
Pre-procedure angiographic measurements			
MLD (mm)	0.35 (0.09–0.54)	0.41 (0.16–0.76)	0.61
RVD (mm)	2.91 (2.48–3.00)	2.86 (2.54–3.14)	0.78
Diameter stenosis (%)	86.5 (82.0–96.8)	86.0 (75.0–95.0)	0.59
SYNTAX score	13.3 (6.5–15.5)	11 (7.0–17.0)	0.78
Procedures			
Lesion length (mm)	14.5 (10.0–20.0)	15.0 (10.0–20.0)	0.98
Total stent length (mm)	19.0 (13.0–20.8)	18.0 (13.0–23.0)	0.95
SES implantation	2 (14.3)	39 (52.7)	0.009
PES implantation	12 (85.7)	35 (47.3)	0.009
Post-procedure angiographic measurements			
MLD in segment (mm)	2.73 (2.62–3.20)	2.63 (2.28–3.00)	0.77
MLD in stent (mm)	2.68 (2.48–2.93)	2.64 (2.38–3.00)	0.88
RVD in segment (mm)	2.83 (2.77–3.17)	2.94 (2.50–3.24)	0.93
RVD in stent (mm)	2.94 (2.53–3.04)	2.85 (2.54–3.06)	0.99
Diameter stenosis (%)			
In-segment	4.0 (4.0–6.0)	7.0 (4.0–12.0)	0.38
In-stent	6.0 (1.8–7.0)	7.0 (3.0–9.0)	0.28

Values shown are median (lower to upper quartile) or number (%). *P*-values from Wilcoxon rank-sum test or Fisher's exact test.

stable patients, again with similar results [ACS (−0.27 vs. −0.11 mm, $P = 0.021$), stable CAD (−0.25 vs. −0.14, $P = 0.044$)]) (Supplementary material online, *Table S6*).

No significant difference in terms of the angiographic SYNTAX score at index procedure was observed (NA: 13.3 vs. no-NA: 11.0, $P = 0.78$).

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Table 3 Quantitative coronary angiography analysis of non-target lesion segments

	Patients with NA median (95% CI)	Patients without NA median (95% CI)	*P*-value
Number of patients	*N* = 14	*N* = 73[a]	
Number of segment	*N* = 120	*N* = 584	
Number of segments per patient	8.6	8.0	
Segment localization[b]			0.31
LAD	38 (32%)	194 (33%)	0.82
LCX	34 (28%)	197 (34%)	0.30
RCA	48 (40%)	193 (33%)	0.18
RVD (mm)			
BL	2.32 (2.23–2.67)	2.36 (2.28–2.47)	0.94
FUP	2.38 (2.12–2.5)	2.38 (2.28–2.44)	0.50
Mean segment length per pat. (mm)			
BL	30.76 (29.53–33.21)	30.94 (29.57–31.98)	0.68
FUP	31.37 (29.5–32.66)	31.34 (29.48–32.5)	0.99
Total segment length per pat. (mm)			
BL	278 (249–302)	256 (237–274)	0.15
FUP	280 (251–293)	255 (236–281)	0.34
Minimal Lumen diameter (mm)			
BL	1.9 (1.66–2.16)	1.9 (1.84–2.01)	0.62
FUP	1.54 (1.41–1.88)	1.78 (1.72–1.88)	0.072
Change[c] in MLD (FUP-BL)	−0.25 (−0.36 to −0.17)	−0.13 (−0.17 to −0.10)	0.002
Diameter stenosis (%)			
BL	20.94 (19.15–22.55)	20.03 (18.45–21.92)	0.32
FUP	27.67 (25.21–35.9)	23.61 (21.49–28.79)	0.063
Change[c] in %DS (FUP-BL)	6.03 (5.28–11.06)	4.31 (2.47–6.24)	0.048

Values are medians over several patients (95% CIs from non-parametric bootstrap). *P*-values from Wilcoxon rank-sum test. Patient-level outcomes derived as the mean from several segments.
QCA, quantitative coronary angiogram; FUP, follow-up; BL, baseline.
[a]In one patient allocated in the non-NA group, the baseline angiography was not available and thus, this patient had to be excluded from the serial QCA analysis.
[b]Reported as count (%), *P*-values from Pearson χ^2-test.
[c]Change was derived at the level of segments.

Clinical events

Clinical events throughout 5 years are summarized in *Table 4*. Non-TL revascularizations occurred more frequently in patient with NA (78.6%) when compared with patients without NA [44.6%, odds ratio = 4.56 (95% CI 1.17–17.69), $P = 0.028$]. Similarly, non-TVR (71.4 vs. 43.2%, OR = 3.28 (0.94–11.42), $P = 0.06$) was more frequently observed in the NA lesion group. We further assessed clinical events over additional 4 years after OCT and did not record any differences between patients with vs. without NA (Supplementary material online, *Table S7*).

Discussion

This cohort study of patients with coronary artery disease previously treated with DES allowed to correlate the process of in-stent NA with native atherosclerosis progression, due to its design including serial angiographic surveillance, annual clinical follow-up, and intra-coronary imaging using OCT at 5 years.

Association between neoatherosclerosis and native coronary atherosclerosis progression

The principal finding of this cohort study is a significant association between in-stent NA and the progression of native coronary atherosclerosis. The more pronounced reduction in MLD of NA compared with non-NA patients was of similar extent in the target and non-target vessel and in ACS vs. stable CAD patients, attesting to the robustness of the results. The *P*-value of 0.002 obtained from a robust rank-based method for the comparison of MLD change between patients with and without NA indicates strong evidence against the null hypothesis of no difference, even when considering stringent criteria. In the presence of an initially conservative treatment approach in non-target segments, the ability of MLDs to

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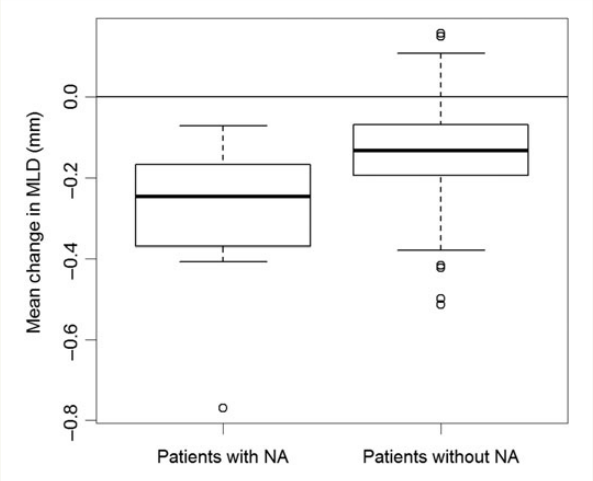

Figure 4 Quantitative angiographic analysis. Box-plot representation of the per-patient mean angiographic change in minimal lumen diameter (minimal lumen diameter follow-up − minimal lumen diameter baseline) from untreated coronary artery segments that were serially assessed and matched. Analysis is stratified according to presence ($N = 14$ patients) or absence ($N = 74$ patients) of neoatherosclerosis plaques in the stented vessel that underwent optical coherence tomography analysis. Lower and upper box edges are the quartiles and thick line is the median. A horizontal reference line at change = 0 is drawn.

change over time appears limited. The observed delta of 0.12 mm correspond to 0.8 pooled SD units, indicating a large biological signal. Of note, the change in MLD did result in significant differences in non-TL revascularizations.

The mechanism underlying in-stent NA are poorly understood and it is believed to be a multifactorial process. It has been suggested that NA occurs in the context of incompetently regenerated endothelium, which results in an excessive uptake of circulating lipids and leucodiapedesis leading to an accelerated atherosclerosis formation within the neointima.[11] Of note, in a recent *ex vivo* histological analysis NA was observed at a similar frequency following implantation of new generation everolimus-eluting stents compared with early generation DES despite evidence of other signs of improved arterial healing.[12] These findings suggest that small alterations of the endothelium within the neointima may be sufficient to determine an accelerated in-stent NA formation. As NA is less frequent and occurs later in BMS compared with DES, the anti-proliferative agent released from DES may be suspected as a causative factor. Our findings indicate that NA is more likely to develop in patients with a progressive native atherosclerosis phenotype during long-term follow-up. Therefore, pathogenetic factors contributing to the progression of native atherosclerosis appear to be similar to those involved in NA formation. Of note, coronary artery disease complexity as assessed by the angiographic SYNTAX score at baseline was comparable in patients with and without evidence of in-stent NA, suggesting that the observed association between NA and native atherosclerosis progression is independent from the initial disease severity. It could be assumed that patients with unfavourable plaque phenotypes show a more aggressive NA formation and that the atherosclerotic progression at non-target sites detected throughout 5 years are related to the more aggressive disease already present at baseline. In this context, the lack of a baseline OCT is a limitation; however, we did not observe any association between NA formation and clinical indication (ACS vs. stable).

It could be hypothesized that therapeutic strategies known to attenuate atherosclerosis progression—such as high-dose statin therapy—may be also effective to suppress the development or progression of NA. We investigated whether patients with in-stent NA were less adherent to evidence based cardiovascular medications including statins, but did not observe any differences between groups. Similarly, the reduction in LDL-C and the increase in HDL-C, both known to be associated with atherosclerosis progression, were not different between patients with compared with those without NA lesions. Based on the relatively small sample size and of this cohort, we consider these observations the latter results as indefinite.

Frequency of neoatherosclerosis

The reported frequency of in-stent NA substantially differs from previous studies. This is explained at least in part by the variety of NA definitions applied and the substantial differences in patient selection. In a human pathology study, Nakazawa and colleagues reported a frequency of 31% in 209 DES lesions at a mean of 1.2 years after stent implantation by defining NA as the presence of either peri-strut foamy macrophage clusters, fibroatheromas, thin-cap fibroatheromas, or plaque ruptures with thrombosis.[4] More recently, the same group of investigators observed a similar frequency of NA among patients treated with everolimus-eluting stents (29%), SES (35%), and PES (19%) 30 days to 3 years after stent implantation.[12] The overall frequency of any NA-related findings in our OCT investigation (40.9%) was comparable with these two pathology studies with the exception that NA was more frequently observed after PES implantation—which could be explained by a substantially different patient selection and a longer follow-up time. *In vivo* studies using OCT to describe the frequency of in-stent NA are scarce. Yonetsu and colleagues defined NA as the presence of lipid-laden neointima and reported a frequency of 75% at 4 years of follow-up.[13] We found a lower frequency, even when considering a wider definition under the inclusion of any potential in-stent NA-related findings. A possible explanation for this discrepancy is the higher proportion of symptomatic patients in the study by Yonetsu and colleagues. Another explanation is the risk of overestimation of NA using OCT, as suggested by Nakano and colleagues. In this study, a conservative definition of NA was applied requiring a longitudinal extension of at least 1.0 mm in length under the exclusion of potential macrophage accumulation and fibrin deposition by identifying signal rich bands and peri-strut low-signal intensity areas.[14]

Predictors of neoatherosclerosis lesion formation

With the exception of device type, we have not observed any differences in baseline clinical, procedural, or angiographic variables between patients with NA when compared with those without NA at 5 years. In a histology study, younger age, longer implant duration,

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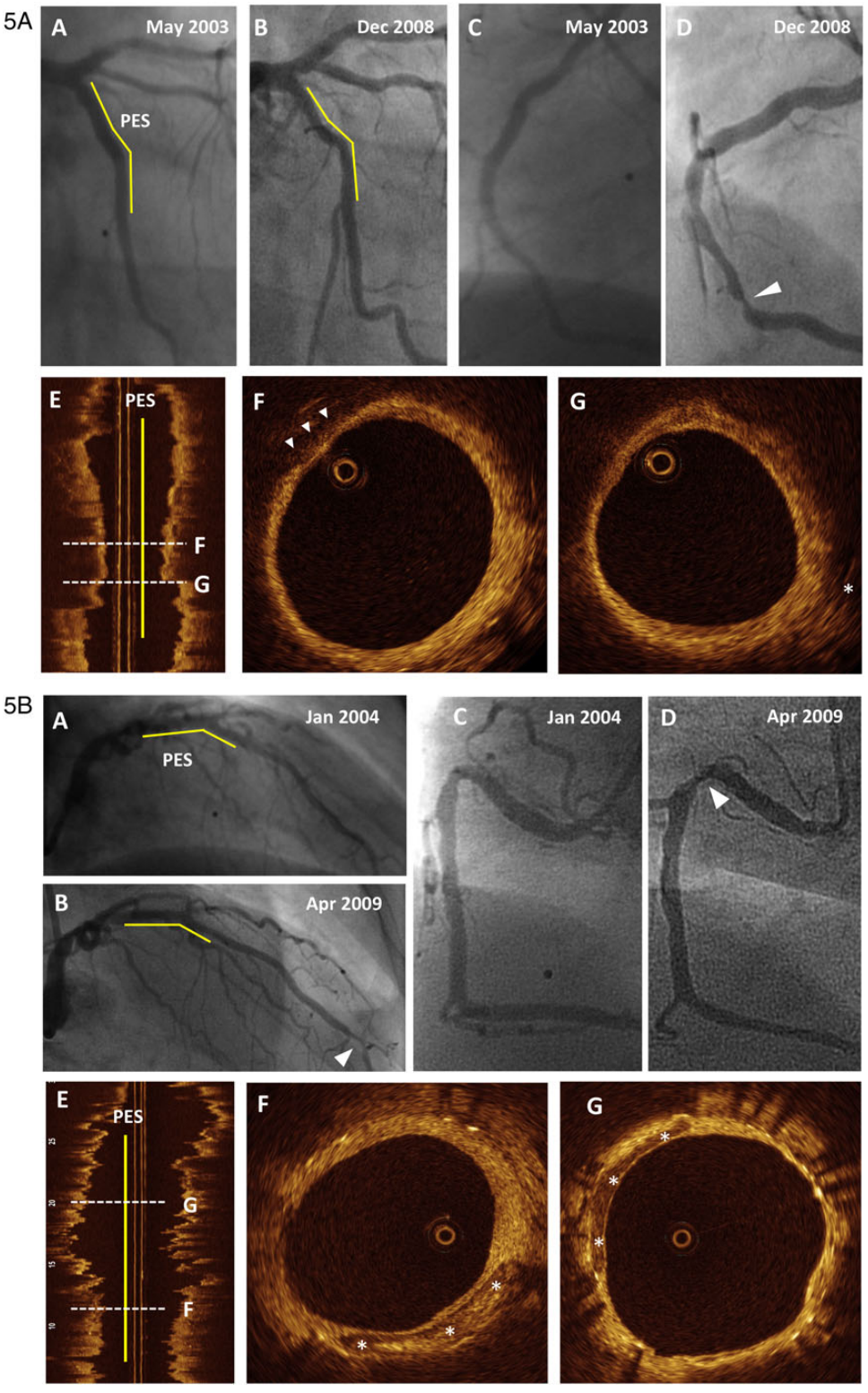

Figure 5 (*5A*) Association between neoatherosclerosis lesion formation and atherosclerosis progression in untreated coronary artery segments. This figure shows a representative example of a patient with a TCFA within the neointima (*F* and *G*) displaying a strong attenuation, which prevents the visualization of the stent struts behind the lipid pool/necrotic core. Arrowheads indicate the localization of the minimal cap thickness. In the distal part of the right coronary artery (RCA), a non-significant stenosis at baseline (*C*) progressed over 5 years to a significant stenosis (*D*). The longitudinal view is presented in (*E*). (*5B*) Association between neoatherosclerosis lesion formation (fibrocalcific plaque) and atherosclerosis progression in untreated coronary artery segments. This figure shows a representative example of a patient with a fibrocalcific plaque within the neointima (*F* and *G*) displaying a signal-poor region with low attenuation and sharp borders (asterisk). In the distal LAD and proximal RCA, a non-significant stenosis at baseline (*A* and *C*) progressed over 5 years to a significant stenosis (*B* and *D*). The longitudinal view is presented in (*E*).

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Table 4 Clinical events up to 5 years angiographic follow-up

	Patients with NA (*N* = 14)	Patients without NA (*N* = 74)	Odds ratio (95% CI)	*P*-value
Non-TVR	10 (71.4%)	32 (43.2%)	3.28 (0.94–11.42)	0.062
Non-TLR[a]	11 (78.6%)	33 (44.6%)	4.56 (1.17–17.69)	0.028
Non-TLR TVR	1 (7.1%)	5 (6.8%)	–	1.000
Any revascularization[b]	11 (78.6%)	37 (50.0%)	3.67 (0.95–14.22)	0.060
Any MI	0 (0%)	2 (2.70%)	–	1.000

No. of events (%) are reported. Median follow-up time was 1933 days (IQR: 1847–2012). Odds ratios and *P*-values from logistic regression model. For each patient, clinical events were included until and including the day of 5 years imaging follow-up. If <5 events, *P*-values from Fisher's exact test.
[a]Non-TVR or TVR excluding TLR.
[b]Non-TVR or TVR including TLR.

and SES and PES usage were identified as independent predictors of NA formation.[4] Moreover, in an *in vivo* OCT study Yonetsu and colleagues identified time from stent implantation, active smoking, chronic kidney disease, and use of ACE-AR-II as independent predictors of NA.[15] Our study results may assist in understanding why active smoking and chronic kidney disease emerged as predictors for NA formation, both established risk factors for native atherosclerosis.

Clinical impact of neoatherosclerosis

The impact of NA on clinical outcomes has not been prospectively investigated at this point in time. Observational studies and case reports, however, suggest an association between NA lesions and late stent failures.[1,3] We observed no significant differences in target lesion related outcomes between patients with and without NA during additional 4 years of follow-up. However, in view of the selection of TLR-free patients throughout 5 years and the relatively small number of patients with NA in our study, further prospective investigations are required for definitive conclusions on the clinical impact of NA.

Study limitations

Our study needs to be interpreted in light of some limitations. First, only selected patients free from TL-related events were considered eligible for angiographic and OCT long-term evaluation. Thus, the generalizability of our findings may be limited. Second, the sample size was relatively small. This limits secondary analyses focusing on predictors of NA as well as the evaluation of the clinical impact of NA. However, the findings related to our primary hypothesis are statistically robust and mechanistically plausible. Third, we investigated the occurrence of NA in early generation DES that are no longer used in clinical practice. It remains to be shown if our findings apply to new generation DES, although new generation everolimus-eluting stents have been reported to have a similar propensity to develop NA as early generation DES in a recent *ex vivo* histological analysis. Although OCT has been validated against histology for the assessment of atherosclerotic plaque composition and phenotype, we are unaware of a dedicated validation study for the diagnosis of in-stent NA. Whilst the assessment of calcifications is not expected to be the cause of misinterpretations, the differentiation between in-stent fibroatheroma and macrophage accumulations, artefacts (e.g. tangential drop out), fibrin accumulations surrounding stent struts, hypersensitivity reaction (T-lymphozyte and eosinophil infiltration), granulation tissue or the penetration of necrotic core from the original plaque appears more challenging and might be the source of misdiagnosis and a reason for NA overreporting.[16] Serial QCA was performed one single projection, which may results in an underestimation of angiographic lesion progression. The use of similar projections for serial QCA analyses, however, prevented an overestimation of the observed angiographic lesion progression.

Conclusions

The formation of in-stent NA is closely associated with progression of native coronary atherosclerosis, suggesting similarities in the pathophysiologic mechanisms of these two entities. These findings may have important clinical implications for the development and implementation of strategies to prevent NA among patients undergoing PCI.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Authors' contributions

L.R. and S.W. conceived the study. M.T., S.B. and L.R. did the OCT analysis. M.T. and T.Z. did the angiographic analysis. S.Z. and P.J. performed the statistical analysis and interpreted the findings in collaboration with L.R., S.W., M.T., and all other authors. M.T. and L.R. wrote the first draft of the manuscript, which was critically revised for important intellectual content by S.W. and all authors.

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