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TITOLO TESI

THREE VESSEL OPTICAL COHERENCE TOMOGRAPHY ASSESSMENT OF MACROPHAGES ACCUMULATION IN FIRST NON ST-SEGMENT ACUTE CORONARY SINDROME: DIFFERENCES BETWEEN CULPRIT AND NON CULPRIT CORONARY PLAQUES

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SOMMARIO

ABSTRACT

Background: To investigate in patients with non ST-segment elevation acute coronary syndrome (NSTE-ACS) the prevalence and the features of optical coherence tomography (OCT)-detected macrophages accumulation (MA) in culprit plaques (CP) as compared to non culprit plaques (NCP).

Methods: The study is a post-hoc analysis of a prospective study aimed at evaluating the relationship between aortic inflammation as assessed by ^{18}F-Fluorodeoxyglucose-positron emission tomography and features of coronary plaque vulnerability as assessed by OCT. We enrolled 32 patients with first NSTE-ACS that successfully underwent 3-vessel OCT.

Results: The median age was 65 (54-72) years and 27 patients (84%) were male. CPs were clinically defined. MAs were defined as signal-rich, distinct, or confluent punctuate regions that exceed the intensity of background speckle noise. Their prevalence was 4.2 per patients and MAs were more likely found in CP than NCP (84% vs. 61%, p=0.015). MA had also a higher circumferential extension in CP and the risk for CP associated with macrophages extension was higher (OR = 4.42; 95CI;2.54-9.15, p<0.001) than that associated with the mere presence of MA (OR=3.36; 95%CI;1.30-8.66, p=0.012). CP with thrombus had a lower distance between MA and the luminal surface than CP with no thrombus (0.06 vs. 0.1 mm; p=0.04).

Conclusions: In patients with NSTE-ACS, MAs are more likely present in CP where they disclose also a greater extension compared to those observed in NCP. The distance between MA and the luminal surface is lower in thrombotic CP than that in non thrombotic CP.

Word count = 244

Keywords: non ST-segment elevation acute coronary syndrome; optical coherence tomography; macrophages

INTRODUCTION

The main mechanism leading to acute coronary syndrome (ACS) is represented by coronary athero-thrombosis(1). The lesion substrate prone to thrombosis was termed vulnerable plaque(1). Data from human autopsy studies showed that the main vulnerable plaque phenotype responsible for ACS is represented by the rupture of an atherosclerotic plaque with a large necrotic core, containing free cholesterol crystals and cholesterol esters, a thin fibrous cap and macrophages infiltration and activation(2-4). Frequency domain-optical coherence tomography (FD-OCT) is a recently developed optical imaging invasive technique that provides high-resolution, cross-sectional images of coronary tissue in situ(5). The resolution of FD-OCT (about 10 um) is appropriate for evaluation and measuring of features of plaque vulnerability such as extension of lipid core, the cap thickness, and even the plaque macrophage density(5). In the CLIMA study(6) enrolling 1003 patients undergoing OCT evaluation of proximal left anterior descend artery (LAD), macrophages infiltration not only was associated with an increased risk of clinical event at 1 year follow up [Hazard ratio = 2.7; 95% confidence intervals (95%CI) = 1.2-6.1], but also did it contribute, along with evaluation of minimal lumen area, fibrous cap thickness and lipid arc circumferential extension, to define the phenotype of an OCT-assessed vulnerable plaque associated with the highest risk of cardiac events (HR 7.54, 95%CI 3.1-18.6). However, of the 577 plaques with macrophages accumulation only the 5.2% was associated with the endpoint. Indeed it is known, in keeping with the multifocal nature of atherosclerosis, that macrophages accumulation may be found not only in the culprit plaque but also in the non-culprit plaques(7). We undertook the present three vessel OCT study to describe in patients with first non ST-segment ACS the OCT features associated with the culprit plaques, focusing on macrophages accumulation.

METHODS

Patients

The present study is a post-hoc analysis of a prospective study(8) aimed at evaluating the relationship between aortic inflammation as assessed by ^{18}F-Fluorodeoxyglucose-positron emission tomography (^{18}F-FDG -PET) and features of coronary plaque vulnerability as assessed by OCT**.**

Briefly, we prospectively enrolled consecutive patients with NSTE-ACS referred to the S.Orsola Hospital Catheterization Laboratory and scheduled for PCI for at least 1 coronary obstruction in one of main three epicardial vessels. Patients were recruited from 30th may 2013 to 20th June 2016. They were eligible for the study if all the following criteria were met: age > 18 years, release of written consent, chest pain at rest within 24h plus one of the following: 1) ST-segment deviation ≥0.05 mV in any lead; 2) transient (<20 min) significant ST-segment elevation in two contiguous leads; 3) inverted T waves ≥0.1 mV; 4) positive cardiac biomarkers 5) documentation of coronary artery disease. Exclusion criteria were: ST elevation myocardial infarction (MI), cardiogenic shock, chronic heart failure, diabetes mellitus, left ventricle ejection fraction < 45%, acute life threatening arrhythmia, prior MI, secondary angina, prior PCI or coronary artery bypass grafting, coronary vessels that could not be adequately imaged, left main disease, estimated Modified Diet in Renal Disease glomerular filtration rate < 60 l/min/1.73m^2, acute or chronic infections, dysthyroidism, systemic inflammatory diseases, corticoid treatment, malignancies, alcoholism, mental illness or drug dependence, lack of given informed consent.

For the purpose of the present study we included all patients that successfully underwent 3 vessel FD-OCT. The study was conceived in accordance with the principles of the most recent revision of the Declaration of Helsinki approved by the Ethics Committee of the St. Orsola-Malpighi Hospital (ref: 61/2012/U/Sper).

OCT Protocol and analysis

Coronary angiography was performed either via the trans-radial or the trans-femoral approach with the use of a 6F sheath. The culprit lesion was identified by means of angiography as the most severe stenosis or in the presence of TIMI flow grade < 3 or angiographic signs of endoluminal thrombi and/or plaque rupture, along with ECG ST-segment alterations, and regional wall motion abnormalities on echocardiographic assessment. The angiography images were independently reviewed by two operators (NT, GG), differences were resolved by consensus. There was a 100% accordance in the identification of the culprit lesion. Treatment of non culprit lesions, type of stent, use of intracoronary or intravenous platelet IIb/IIIa inhibitors were left to the operators' discretion. All patients were on aspirin treatment and received a 5000 UI bolus of unfractioned heparin at the time of FD-OCT and PCI. Before or during the procedure all patients received clopidogrel treatment. After PCI each patient received aspirin 75-160 mg indefinitely and clopidogrel 75 mg for 1 year. Standard guidewire will be used for both FD-OCT and angioplasty procedure. OCT was acquired by means of frequency domain C7 XR system (St. Jude Medical, St. Paul, MN, USA) with non-occlusive technique according to a well-standardized methodology. A 20 mm/s pullback speed was applied during automated injection of intracoronary iso-osmolar contrast media. The 3 epicardial vessels were imaged from the distal segment of treated lesions up to the tip of the catheter. More than 1 pullback was allowed but this was discouraged. The coronary images acquired were analyzed off-line by an independent imaging core laboratory (Euroimage Research, Rome, Italy), using validated review stations. (St. Jude Medical, Minnesota, USA). OCT-defined plaque classification was performed according to an international consensus statement and validated criteria(9,10). A lipid plaque was defined as a signal-poor region diffusely bordered by overlying signal-rich bands corresponding to a fibrous cap. A fibrous cap was defined as a signal-rich homogeneous band overlying a lipid core. In presence of lipid plaque the fibrous cap thickness was measured as the mean of three measurements obtained along the fibrous cap. In presence of lipid plaque we also evaluated the presence and extension of macrophages accumulations defined at

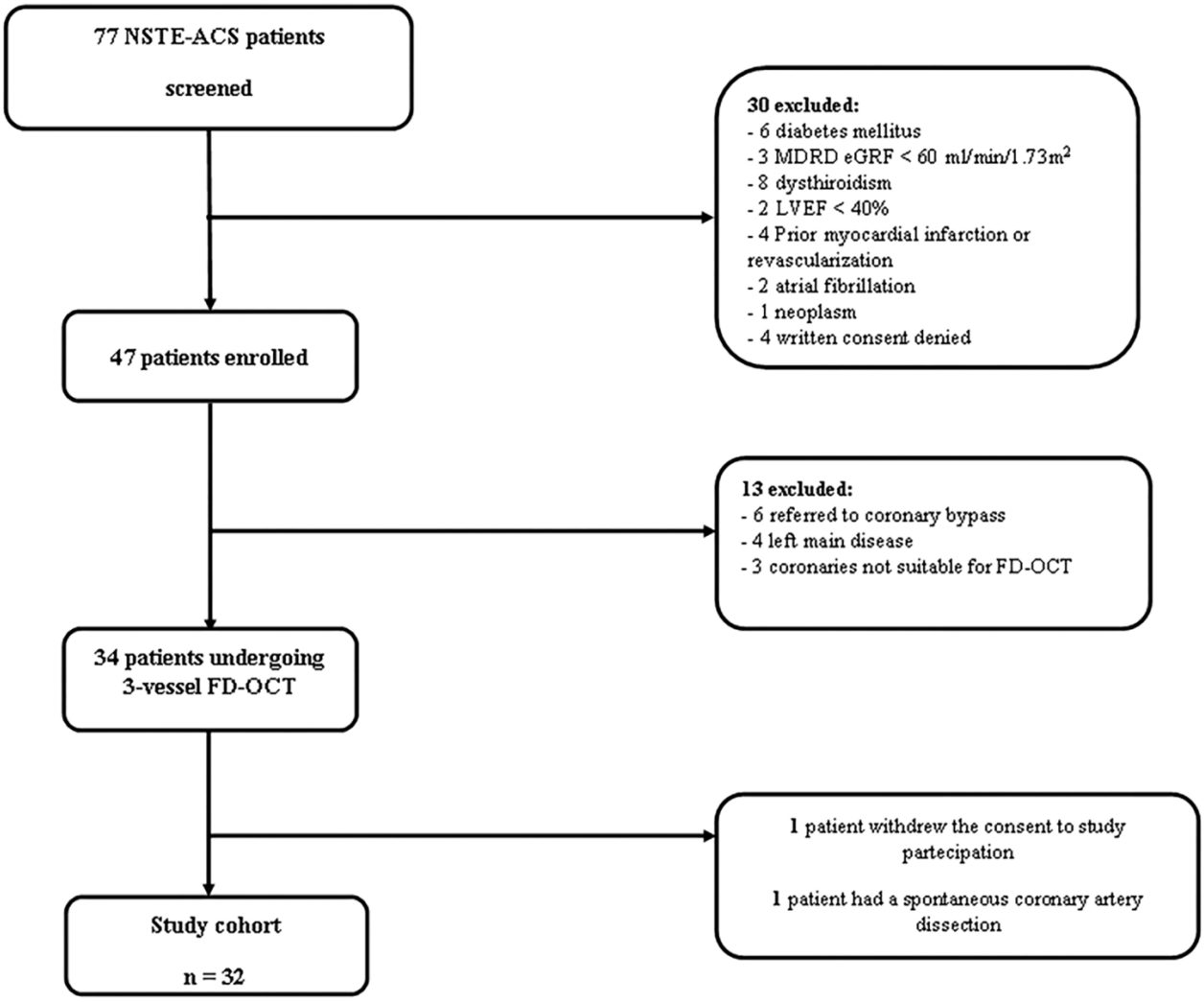
visual estimation as signal-rich, distinct, or confluent punctuate regions that exceed the intensity of background speckle noise(9). The inter-observer variability of the Corelab, tested in a 782 plaques with macrophages accomulation from CLIMA registry, was as follows: R values were 0.97 for the circumferential arc extension, 0.95 for the minimum distance and 0.98 for the mean distance. Culprit lesions were further divided on the basis of presence/absence of OCT-detected thrombus defined as an intraluminal irregular mass adherent or protruding from the plaque.

Statistical analysis

Continuous variables were presented as median (25th-75th). Categorical data were presented as numbers (percentage). For comparisons between groups, the Fisher chi-square test and Whitney's test were used, as appropriate. The association between OCT findings and the culprit plaque was evaluated by means of univariable random effect logistic regression adjusted for correlated observations in the same patient. Continuous variables were modelled also as categorical variables using cut-off obtained by receiver operating curves .

A p value < 0.05 in the two-tailed tests was considered significant. All analyses were performed with STATA 14.0 software (STATA Corporation, College Station, Tex).

Figure 1 - Study flow chart



RESULTS

Figure 1 shows the study flow chart. Among 77 patients screened 47 were initially enrolled. Of these 13 were excluded after angiography and 1 withdrew the consent to study participation before undergoing FD-OCT and 1 had a spontaneous coronary artery dissection. Therefore, the study population comprised 32 NSTE-ACS.

Baseline characteristics are shown in Supplemental Table 1 and Table 1. The median age of the study population was 65 (54-72) years and 27 patients (84%) were male. Ischemic ST changes on admission ECG were observed in 23 cases (70%). Multivessel disease was present in 16 (50%) patients and multivessel treatment was performed in 13 (41%) cases.

Supplemental Table 1. Baseline characteristics of included patients

Variable	**All patients**
No. of patients	**n = 32**
Age, years, median (25^{th}-75^{th})	65 (54-72)
Male gender - no. (%)	27 (84)
Risk factors	
Hypercholesterolemia - no. (%)	20 (63)
Hypertension - no. (%)	20 (63)
Smokers - no. (%)	26 (81)
Family-history of CAD - no. (%)	8 (25)
Presenting characteristics	
Systolic BP, mmHg, median (25^{th}-75^{th})	158 (145-168)
Heart rate, pulse/min, mean ± SD	75 (64-87)
ECG changes - no. (%)	23 (70)
Hb, g/dl, median (25^{th}-75^{th})	14.7 (14.1-15.6)
WBC,*10^3/mm^3 median (25^{th}-75^{th})	8425 (6655-11775)
Creatinine, mg/dl, median (25^{th}-75^{th})	0.93 (0.80-1.02)
CRP, ng/l median (25^{th}-75^{th})	0.45 (0.25-0.63)
LVEF, % median (25^{th}-75^{th})	57 (50-61)
Procedure	
Multivessel disease - no. (%)	16 (50)
Multivessel treatment	13 (41)
DES - no. (%)	31 (97)

Table 1. Characteristic of lipid plaques at the culprit site compared with those at the non culprit site

Variable	**Culprit site**	**No culprit site**	**P value**
No. of patients	**n = 32**	**n = 173**	
Minimum lumen area, *mm* median (25^{th}-75^{th})	1.31 (0.93-1.77)	4.86 (2.8-7.02)	<0.0001
Max lipid pool arc, *degree*s median (25^{th}-75^{th})	352 (238-360)	149 (101-218)	<0.0001
Lipid pool length, *mm* median (25^{th}-75^{th})	15.2 (8.8-22.9)	8.2 (3.8-17.0)	0.0014
Fibrous cap thickness, *μm* median (25^{th}-75^{th})	72 (55-85)	103 (83-140)	<0.0001
Presence of macrophages accumulation, *n* (%)	27 (84)	106 (61)	0.015
----Macrophages arc, *degree*s median (25^{th}-75^{th})	181 (115-360)	114 (83-170)	<0.0013
----Macrophages distance from lumen, *mm* median (25^{th}-75^{th})	0.07 (0.05-0.11)	0.08 (0.06-0.13)	0.26
Presence of cholesterol crystals, *n* (%)	9 (28)	35 (20)	0.35

FD-OCT findings

Figure 2 shows the distribution of overall lipid plaques and lipid plaques containing macrophages, according to the vessel imaged. They were quite represented in sites other than the culprit one with a rate of 6.4 and 4.2 per patient, respectively. However, lipid plaques at the culprit site disclosed a different phenotype. As expected they had a smaller minimal luminal area, a higher extension of lipid component and a thinner fibrous cap (Table 1). They also were more likely to have macrophages infiltration. Noteworthy, macrophages infiltrations had a higher circumferential extension at the culprit sites than those in the non culprit site, whereas there were no differences in terms of distance to the lumen. Table 2 shows the association between OCT findings and the culprit plaque, adjusted for 32 patients. The extension of macrophages accumulation was associated with the culprit plaque both as continuous and categorical variable. In particular the risk associated with macrophages extension $\geq$ 132° (OR = 4.42; 95CI;2.54-9.15, p<0.001) was higher than that associated with the mere presence of macrophages accumulation (OR=3.36; 95%CI;1.30-8.66, p=0.012). Of 32 culprit plaques, the presence of thrombus was observed in 17 plaques. Table 3 shows that culprit plaques with thrombus had a thinner fibrous cap and a lower distance between macrophages accumulation and the luminal surface than culprit lesions with no thrombus, whereas, although statistically non significant, culprit plaques with no thrombus had a greater macrophages circumferential extension than culprit plaques with thrombus (median degrees: 237° vs. 140°; p=0.16). Macrophages in non culprit plaque, thrombotic and non thrombotic culprit plaque are shown in figure 3.

Figure 2. Distribution of lipid plaques and macrophages accumulation according to the vessel explored

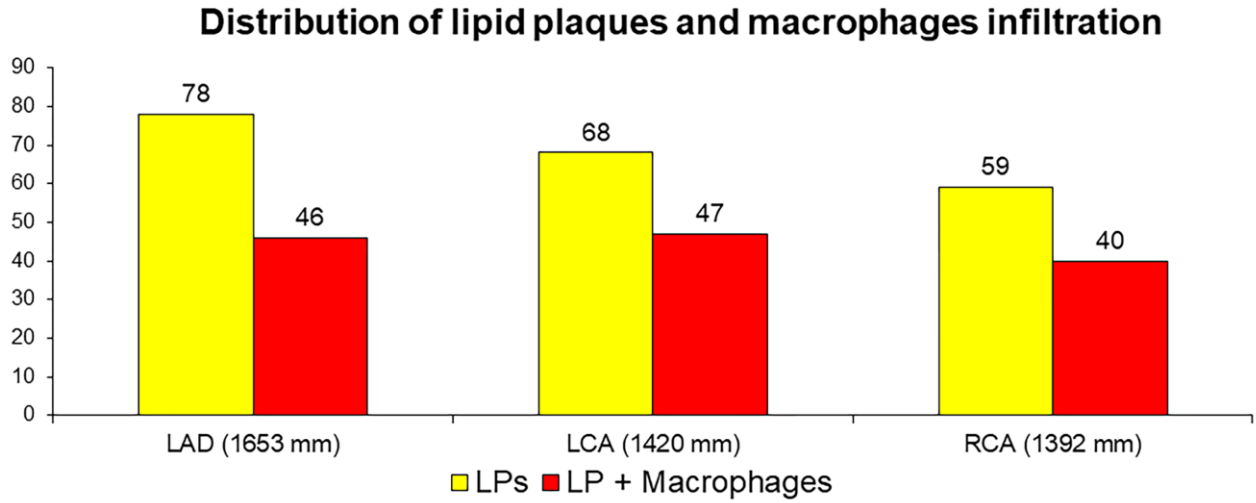


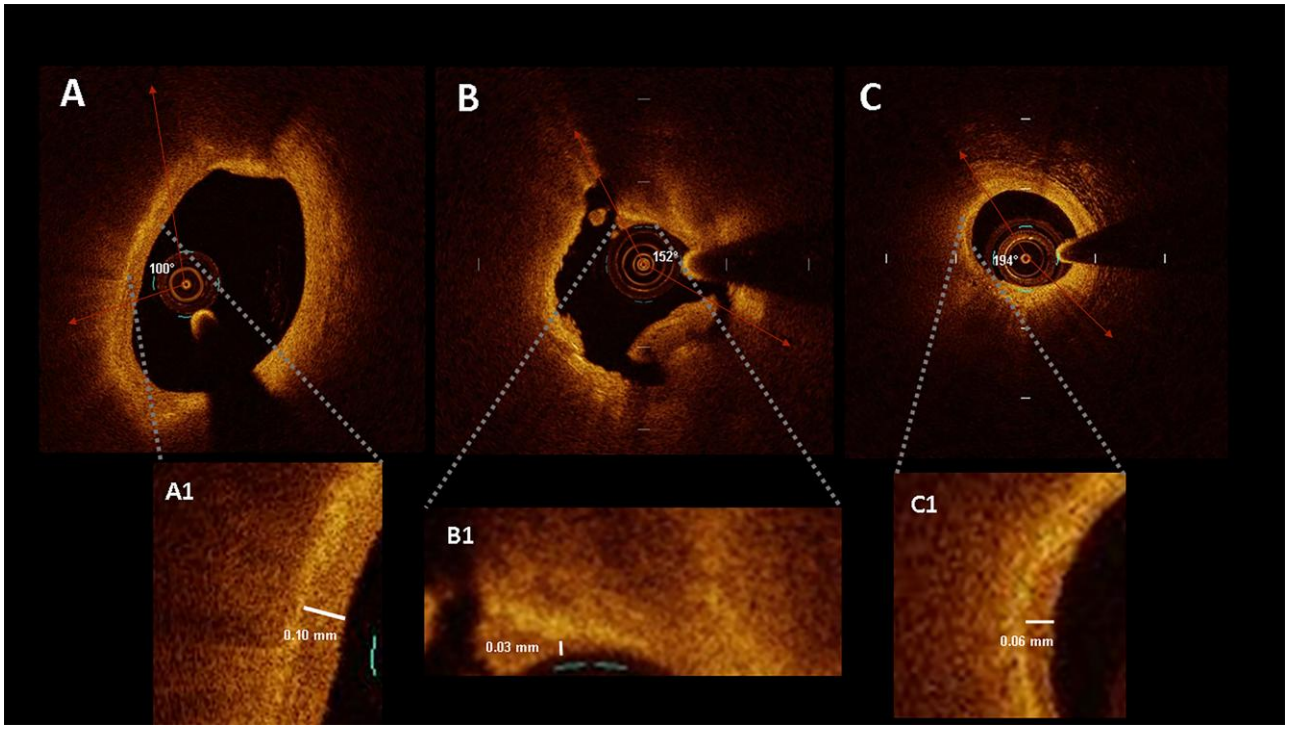
Table 2. Univariable association between OCT findings and culprit lesion. Random effect logistical regression with standard error adjusted for 32 patients

Variable	**Univariable analysis**	
No. of patients	**OR (95%CI)**	**P value**
minimal lumen area, mm^2	0.21 (0.062-0.683)	0.01
Lipid pool length, *mm*	1.04 (1.02-1.07)	<0.001
Max lipid pool arc, *degree*s	1.01 (1.008-1.02)	<0.001
*Lipid pool length ≥ 12 mm	2.74 (1.48-5.05)	0.001
*Max Lipid Arc ≥ 215°	11.87 (4.54-31.06)	<0.001
Presence of macrophages	3.36 (1.30-8.66)	0.012
Macrophages arc, *degree*s	1.008 (1.005-1.01)	<0.0001
*Max Macrophage Arc ≥ 132°	4.42 (2.54-9.15)	<0.0001
Fibrous cap thickness, *μm*	0.97 (0.95-0.99)	0.03
‖Fibrous cap thicknes < 75 *μm*	7.73 (3.73-16.01)	<0.001

Table 3. Culprit lesion characteristics according to the presence or absence of OCT detected thrombus

Variable **No. of patients**	**Thrombus** **n = 17**	**No Thrombus** **n = 15**	**P value**
Minimum lumen area, *mm* median (25^{th}-75^{th})	1.31 (0.95-1.59)	1.4 (0.7-2.0)	0.85
Max lipid pool arc, *degree*s median (25^{th}-75^{th})	360 (245-360)	313(167-360)	0.16
Lipid pool length, *mm* median (25^{th}-75^{th})	18.8 (11.8-34.4)	13.2 (8.4-21.2)	0.16
Fibrous cap thickness, *µm* median (25^{th}-75^{th})	60 (50-70)	80 (73-106)	0.001
Presence of macrophages accumulation, *n* (%)	15 (88)	12 (80)	0.437
----Macrophages arc, *degree*s median (25^{th}-75^{th})	140 (100-247)	237 (159-360)	0.16
----Macrophages distance from lumen, *mm* median (25^{th}-75^{th})	0.06 (0.03-0.08)	0.1 (0.06-0.14)	0.04
Presence of cholesterol crystals, *n* (%)	7 (41)	2 (13)	0.087

Figure 3. OCT images of A) lipid plaque at non culprit site, B) thrombotic culprit lesion, C) non thrombotic culprit lesion. Angles denote the circumpherential extension of macrophages accumulation. In boxes A1-C1 the white lines indicate the minimum distance of macrophages accumulation to the lumen surface Abbreviations: FCT = fibrous cap thickness



DISCUSSION

The main findings of the present study enrolling 32 patients with first NSTE-ACS undergoing PCI and three vessel OCT are as follows: 1) Macrophages accumulation is a multifocal and frequent phenomenon in the coronary tree of ACS patients; 2) Culprit plaques are more likely to have macrophages accumulation that non culprit plaques 3) Macrophage accumulations have a greater circumferential extension in the culprit plaques than in the non culprit plaques; 4) among the culprit lesions those with thrombus have macrophages at a lower distance from the lumen surface compared to those with no thrombus.

Macrophages are inflammatory cells that play a key role in the development and progression of atherosclerosis and ACS. Indeed, plaque rupture and disruption are directly linked to macrophages plaque infiltration and activation enzymes(3, 5). In the recent CLIMA study (6), OCT detected macrophages accumulation contributed to define an OCT-based vulnerable plaque phenotype associated with an increase risk of clinical event at 1 year. However, of the 36 OCT defined vulnerable plaques only 7 were associated with events showing a very low positive predictive value (PPV = 19%). Therefore, a further in vivo definition of plaque vulnerability is highly advisable.

In the present study we compared OCT features of lipid plaques between culprit and non culprit lesions by means of 3-vessel OCT in NSTE-ACS patients. Our analysis adjusted for correlated observations in the same patient confirmed that a smaller minimal luminal area, a higher extension of lipid component and a thinner fibrous cap are features associated with the culprit lesion. Macrophages accumulation was a quite frequent phenomenon with a rate of 4.2 foci per patients. Culprit lesions were more likely to have an OCT evidence of macrophages accumulation that, compared to those observed in non culprit lesion, disclosed also a higher extension. Of note, the risk of culprit lesion was higher when considering the extension of macrophages accumulation than its mere presence. Although there was no differences in terms of macrophages distance to the lumen surface between culprit lesions as a whole and non culprit lesions, our study suggests that culprit lesions with thrombus tend to have a lower distance of macrophages accumulation to the lumen

surface than culprit lesion with no thrombus. Indeed, macrophages secrete matrix metalloproteinase, express tissue factor which may promote both plaque rupture and thrombosis(11). Nonetheless culprit lesion with no thrombus disclosed the highest circumferential extension of macrophages accumulation confirming a key role of macrophages in promoting plaque progression(5).

Taken together, our findings suggest that a thorough evaluation of macrophages extension end their relationship with the luminal surface may contribute to a further in vivo definition of vulnerable plaque in order to ameliorate its positive predictive value with reference to future coronary events.

Study limitations

Due the observational design the study is not immune to source of bias however it represents a pure model of first NSTEMI thanks to the strict inclusion criteria and several exclusion criteria. Although we used a validated methodology we could have overestimated or misclassified the presence of macrophage accumulation since it has been suggested, by studies based on OCT images co-registered with histology(12), that other plaque materials with different index of refraction may be responsible for bright spots in OCT images. On the contrary pools of lipid-rich macrophages may have been missed due to the same index of refraction of a lipid pool. Yet, in culprit plaques with thrombus we might have underestimated the extension of macrophage since thrombus may shadow obscure underlying structures.

Finally, although highly desirable, in the present study we were prevented from characterizing coronary plaques simultaneously with OCT and ^{18}F-FDG -PET due to the known technical difficulties to image the coronary tree with an unselective tracer such as ^{18}F-FDG. Nonetheless, future PET studies with novel markers(13) of inflammation, with excellent macrophage specificity, could be contribute to improve our understanding in interpretating of OCT findings.

CONCLUSION

In patients with NSTE-ACS lipid plaques with macrophages accumulation are frequent and more likely to be associated with culprit lesion. The risk of culprit lesion depends on both its presence and extension. The distance of macrophages to lumen surface is lower in patients with thrombotic culprit lesion.

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