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CARDIOLOGY



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Pro-inflammatory and pro-thrombotic phenotype of
spontaneously differentiated human monocyte-derived
macrophages in coronary heart disease patients:
implications for plaque morphology and activity

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Introduction



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- Advances in basic science have established a fundamental role for macrophages in mediating all stages of coronary artery disease (CAD) and current emphasis is on a focused shift toward their ability to define the likelihood of coronary plaque growth and vulnerability.
- Recent studies have shown that human monocytes spontaneously differentiated into macrophages (MDMs) display two dominant morphotypes co-existing in the same culture, namely **round MDMs** with functional traits reminiscent of a non-inflammatory and reparative phenotype, and **spindle MDMs** exhibiting a pro-inflammatory profile.



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High-resolution visualization (10 μm) of coronary plaque morphology and its microstructures, including fibrous cap thickness, lipid core, and macrophages accumulation with *in vivo* optical coherence tomography (OCT), is used to obtain relevant information on coronary plaques that can be combined with morpho-phenotypic data of MDM.

Thus ...

providing a unique signature for identification of those atherosclerotic lesions that are most likely to cause a coronary event.



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Aim



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The aim of this study was ...

- To characterize the morphological prevalence and the antigenic and functional profile of the two dominant MDM morpho-phenotypes in CAD patients.
- To investigate whether these *in vitro* findings reflect the *in vivo* morphology features of coronary plaques along with their macrophage content in acute and chronic CAD patients undergoing OCT assessment.



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Materials and methods



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- Study design
- Culture and characterization of MDMs
- OCT image and macrophage analyses



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Study design

The study was carried out at

Centro Cardiologico Monzino, Milan, Italy in collaboration with Institute of Cardiology, Catholic University of the Sacred Heart, Policlinico Gemelli, Rome, Italy and Zagazig University, Egypt.

in a temporal window ranging from October 2016 to October 2017.



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Study population

Ninety consecutive CAD patients undergoing coronary angiography due to stable angina (SA) or acute myocardial infarction (AMI) were enrolled.

In 50 out of the 90 CAD patients, OCT assessment was also performed, aiming at assessing the association between the MDMs morpho-phenotype characterization and coronary plaque morphology.



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Study population

Twenty-five healthy subjects, with neither history of CAD, nor cardiovascular risk factors, nor inflammatory disorders, and specifically not taking any cardiovascular therapy, were recruited as control group.

All the participants provided written informed consent at the time of enrollment



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Study population

Exclusion criteria were:

- previous history of CAD.
- severe chronic heart failure (NYHA class III-IV).
- severe heart valve disease.
- acute and chronic infections.
- liver diseases, neoplasia, evidence of immunologic disorders, use of anti-inflammatory or immunosuppressive drugs, recent (<3 months) surgical procedures or trauma.

In OCT patients, those with poor image quality caused by a large overlying thrombus or residual luminal blood were excluded.



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Study population

The following risk factors were collected:

- family history of early coronary artery disease.
- diabetes mellitus.
- hypercholesterolemia.
- smoking.
- hypertension.
- body mass index (BMI) was also obtained.
- Lab. : full blood count, high-sensitivity C-reactive protein, and peak cardiac Troponin I and creatinine-kinase MB were collected.



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- **Study design**
- **Culture and characterization of MDMs**
- **OCT image and macrophage analyses**



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Culture and characterization of MDMs

MDMs were obtained from a spontaneous differentiation of blood-derived monocytes and characterized using different approaches



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- Study design
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OCT image and macrophage analyses

- OCT image analysis
- OCT macrophages analysis



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OCT image analysis

Frequency domain OCT (FD-OCT) was used.

- In AMI patients, culprit lesion was assessed.
- In SA patients, OCT analysis was performed at the minimal lumen area (MLA) site.

All images were recorded digitally, stored, and analyzed every single frame (0.2 mm) by an OCT core lab (*Institute of Cardiology, Catholic University of the Sacred Heart, Policlinico Gemelli, Rome, Italy*).

In particular, offline analysis was performed by two expert and independent investigators, who were blinded to clinical and laboratory values.



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OCT image analysis

OCT analysis was targeted on :

- plaque characterization (calcified, fibrous, or lipid plaques).
- presence of plaque rupture (PR).
- measurement of fibrous cap thickness.
- presence of intracoronary thrombus and intra-plaque microchannels.

OCT macrophages analysis

Macrophage infiltration (MØI) in the analyzed lesions was assessed by OCT ...

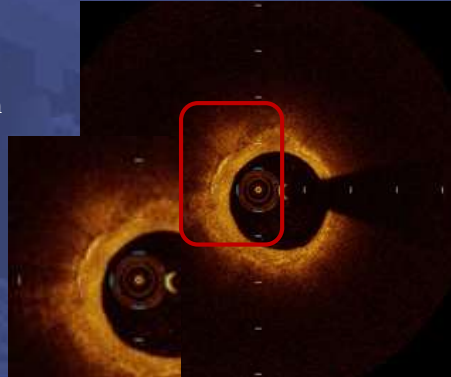
- Qualitative analysis
- Quantitative analysis

OCT macrophages analysis**Qualitative analysis**

- on raw OCT data
- within a $300 \times 125 \mu\text{m}^2$ (lateral x axial) region of interest (ROI)
- according to International Working Group for Intravascular Optical Coherence Tomography (IWG-IVOCT) Consensus standards

In particular, macrophages have been visualized by OCT imaging as :

signal-rich, distinct, or confluent punctate regions that exceed the intensity of background speckle noise and generate a backward shadowing.



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OCT macrophages analysis**Quantitative analysis**

obtained by :

measuring the normalized standard deviation (NSD) known to have a high degree of positive correlation with histological measurements of macrophage content.

by using :

a dedicated software provided by S. Jude medical.

$$\text{NSD}(x,y) = [\sigma(x,y)125 \mu\text{m}^2 / (\text{Smax} - \text{Smin})] \times 100.$$

Where NSD (x,y) is the normalized standard deviation of the OCT signal at pixel location (x,y), Smax is the maximum OCT image value, and Smin is the minimum OCT image value



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Results



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- **Clinical features**
- **MDMs characterization**
- **MDMs and OCT plaque features**



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Variables	SA (n=41)	AMI (n=49)	P value
Age (years)	68±9	62±12	0.02
Male sex, n (%)	33 (81)	38 (77)	0.73 [#]
Current smoking, n (%)	26 (63)	30 (61)	0.83
Diabetes mellitus, n (%)	11 (27)	23 (47)	0.05
Dyslipidemia, n (%)	21 (51)	26 (49)	0.86
Hypertension, n (%)	24 (58)	27 (55)	0.74
Family history of CAD, n (%)	19 (46)	27 (55)	0.41
BMI (kg/m ²)	28.6 ± 3.1	29.2 ± 4.1	0.45
LVEF (%)	55 ± 7	47 ± 6	0.45


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Variables	SA (n=41)	AMI (n=49)	P value
Laboratory data			
WBC ($\times 10^9/L$)	8.77 ± 3.04	9.21 ± 3.60	0.53
RBC ($\times 10^{12}/L$)	4.62 ± 0.70	5.44 ± 3.59	0.15
Platelets ($\times 10^9/L$)	220 ± 74	227 ± 74	0.67
hs-CRP (mg/L)	2.10 (1.40-3.20)	13.20 (5.00-26.00)	0.0001*
Peak TnI ($\mu g/dL$)	0.001 (0.001-0.002)	12.30 (1.40-34.60)	0.0001*
Peak CK-MB ($\mu g/dL$)	2.10 (1.60-3.42)	85.40 (13.20-213.00)	0.0001*


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Variables	SA (n=41)	AMI (n=49)	P value
Angiographic data			
Culprit or treated vessel, n(%)			
LAD	16 (39)	33 (67)	
LCX	7 (17)	5 (10)	0.03 [#]
RCA	18 (44)	11 (23)	
Multivessel disease, n (%)	28 (68)	23 (47)	0.04


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- Clinical features
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- 
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***in vitro* MDM characterization**

Healthy subjects:
ratio spindle/round MDMs 1:1




apoptotic bodies

TG-2

TG-2

TG-2

Jurkat apoptotic T-cell

Normal Thrombin Generation

Normal Efferocytosis

***in vivo* plaque characterization**

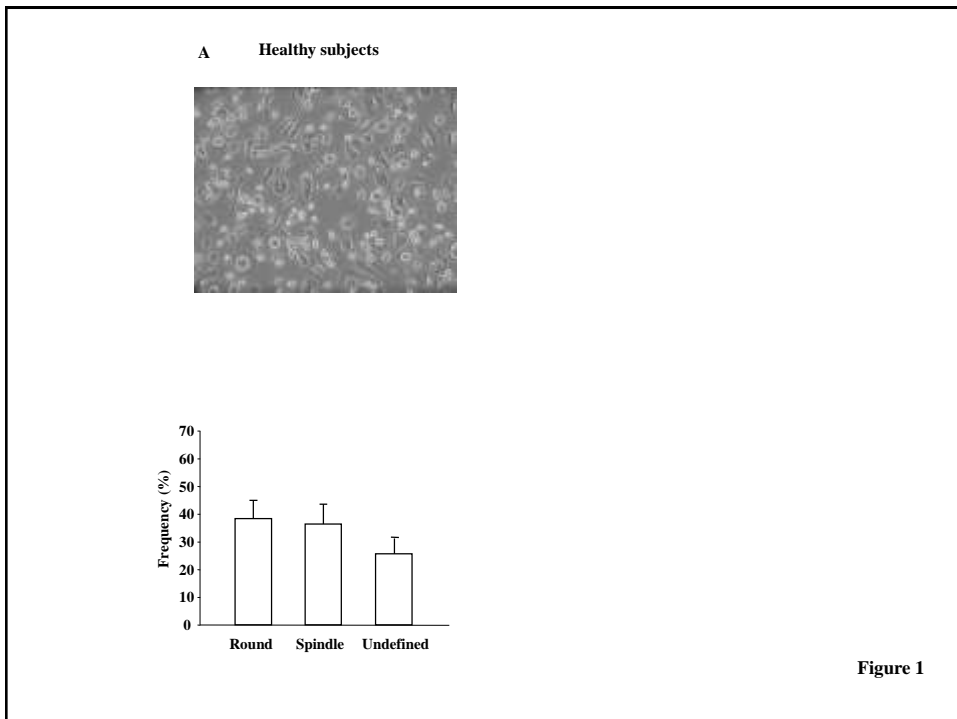
Healthy subject

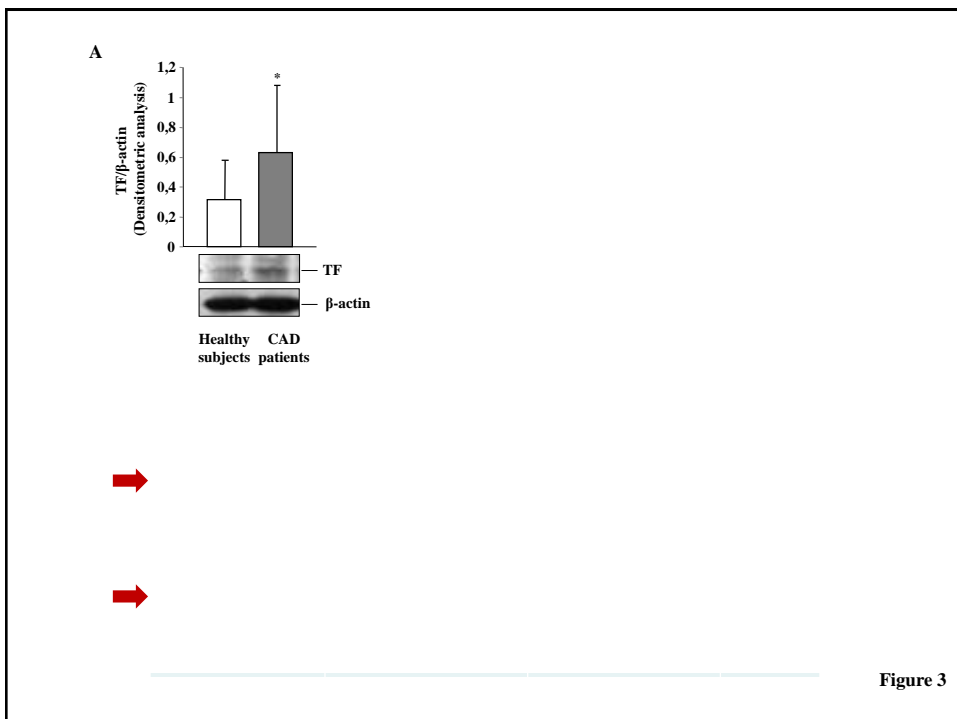
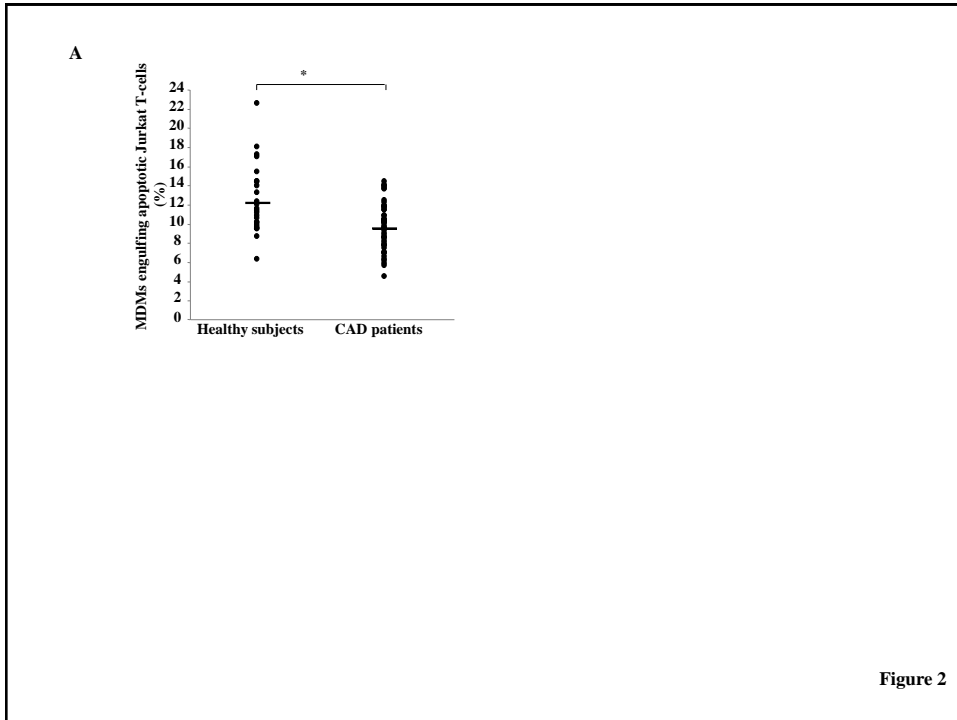




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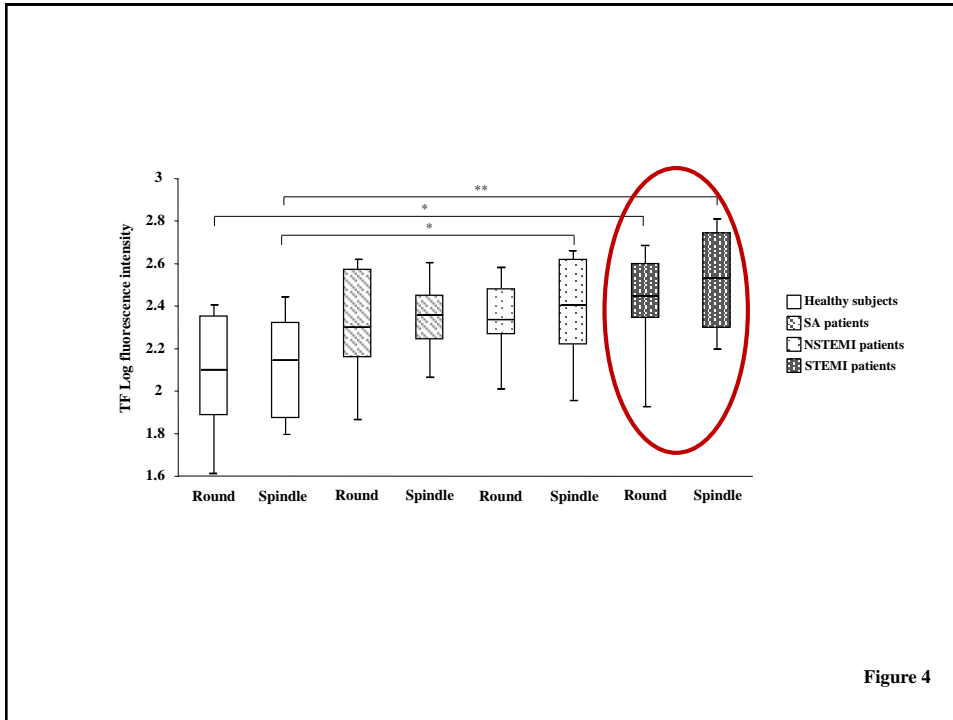


Figure 4

- Clinical features
- MDMs characterization
- MDMs and OCT plaque features

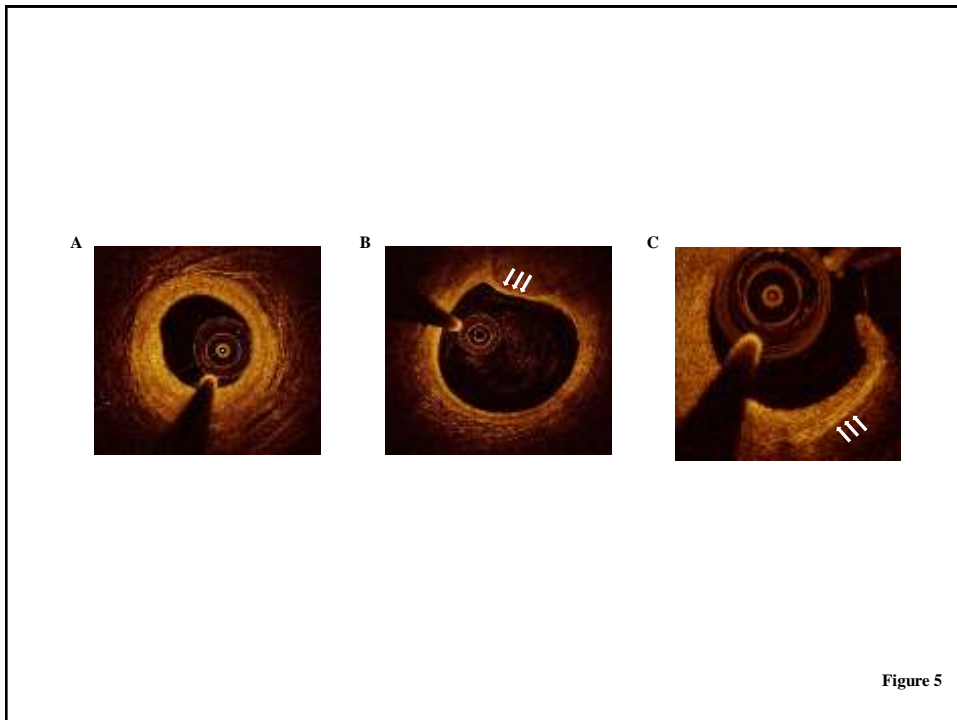



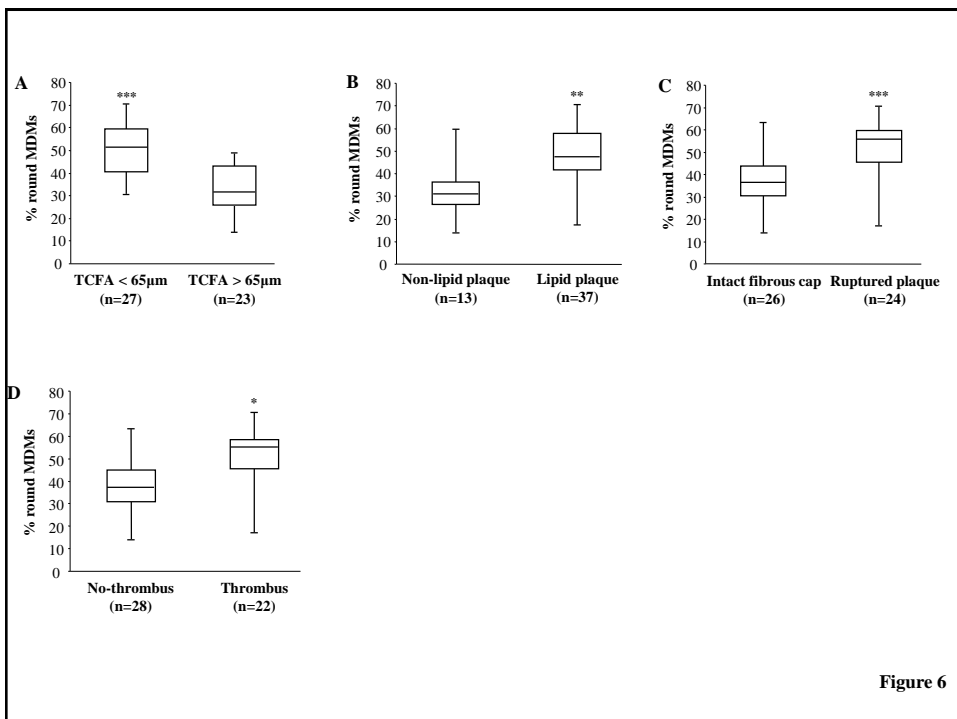
Figure 5

Variables	SA (n=26)	AMI (n=24)	P value
Lipid plaque, n (%)	9 (35)	20 (84)	0.0006
Fibrous plaque, n (%)	9 (35)	2 (8)	0.04 [#]
Calcific plaque, n (%)	7 (27)	2 (8)	0.14 [#]
Plaque rupture, n (%)	2 (8)	15 (62)	0.02
MLA, mm ²	1.59 (0.71-3.85)	1.54 (0.70-3.75)	0.99
TCFA, n (%)	9 (35)	19 (79)	0.001
Thrombus, n (%)	2 (8)	25 (96)	0.0001 [#]
White, n (%)	1 (4)	18 (75)	0.0001 [#]
Red, n (%)	1 (4)	7 (29)	0.02 [#]
Lipid quadrants, n	2.1±1.0	3.1±1.6	0.0001
Lipid arc degree °	135 (87-265)	269 (161-280)	0.0001
Presence of microchannels, n (%)	4 (15)	15 (63)	0.0006 [#]

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Variables	SA (n=26)	AMI (n=24)	P value
Macrophage infiltration detection, n (%)	7 (27)	18 (75)	0.002
Macrophage NSD	3.45±1.01	6.19±1.95	0.001


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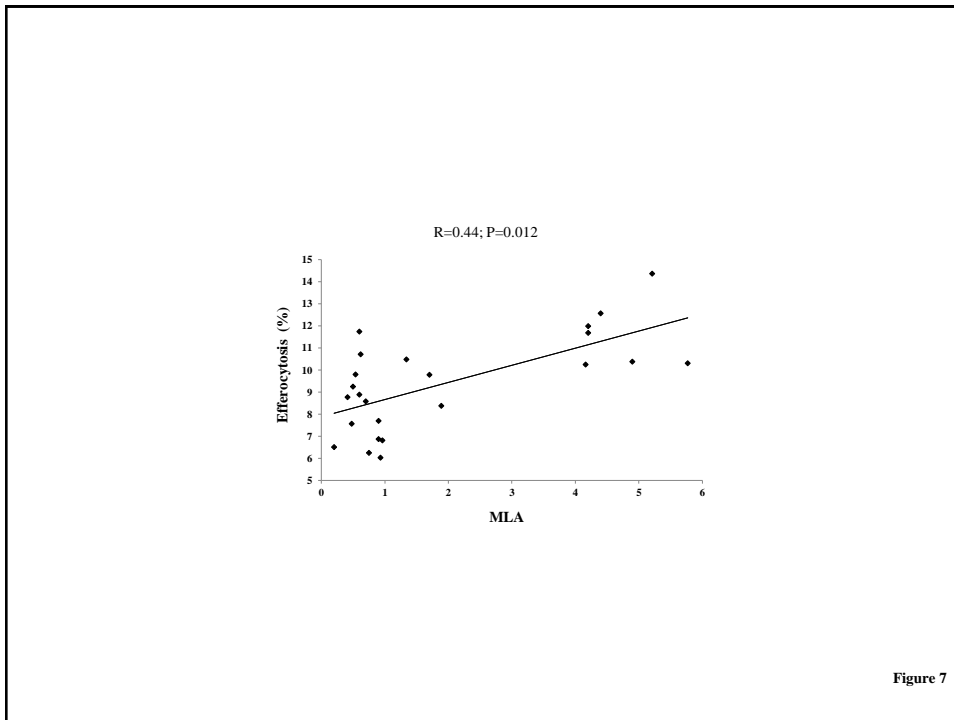


Figure 7

To summarize

In CAD patients :

higher prevalence of round MDMs, with a lower MDM efferocytic capacity, and an enhanced propensity to thrombus formation, as compared to subjects with no history of CAD.

This is the first study that assesses the relationship between the MDM morpho-phenotype profile and coronary stenosis dimension and vulnerability using OCT in CAD patients.

So ... the propensity of monocytes to differentiate into round MDMs in CAD patients is associated with the presence of a vulnerable plaque, prone to rupture, and with PR.

To summarize

The greater is the prevalence of round MDMs, the higher is the intra-plaque macrophage content.

Finally, the MDM efferocytic ability is directly linked to OCT coronary plaque dimension.



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Clinical implications



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- The determination of MDM subsets might have a role in predicting plaque composition and might contribute to the differentiation between stable and unstable plaques.
- Moreover, the development of therapies that switch off macrophage cytotoxicity and plaque growth and destabilizing functions would prove a powerful new approach to curtail macrophage-mediated injury and limit plaque progression and enhance plaque stability.



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- Of interest, our findings if confirmed in larger study populations, may pave the way to novel therapies, able to manipulate MDM morpho-phenotype, that should be tested, at least, in high-risk individuals, aiming at reducing first or recurrent cardiovascular events.



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Limitations



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- The small sample size of our population
- Our experimental model may have several limitations, such as the lack of cell turnover, the absence of tissue-specific matrix proteins, and a “tailored” lifespan, which are all critical in shaping tissue macrophage behavior.
- Finally, although OCT has shown good correlation in validation studies, intracoronary imaging findings are a suboptimal reflection of true histology, especially for macrophage infiltration.



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Conclusion



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- MDMs obtained from CAD patients show a morpho-phenotypic differences with a prevalence of round MDMs displaying pro-inflammatory and pro-thrombotic properties.
- Revealing the functional characteristics of individual macrophage phenotypes may lead to a better understanding of their contribution to coronary atherosclerosis, and the potential of pharmacological modulation of phenotypes may, thus, provide novel diagnostic, therapeutic, and, more importantly, preventative regimens for CAD.



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