

Factors Associated with Non-calcified Plaques in Patients with Coronary Artery Calcium Score of Zero

Factores asociados a placa blanda en pacientes con score de calcio igual a cero

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ABSTRACT

Background: The coronary artery calcium score is used for risk stratification in asymptomatic patients. Although coronary artery disease can occur in the absence of coronary artery calcifications, no conditions associated with the presence of soft non-calcified plaques have been described in this scenario, beyond the presence of symptoms.

Objectives: The aim of this study was to determine the associations between non-calcified plaques and independent variables in patients with coronary artery calcium score of zero.

Methods: Consecutive patients with coronary artery score of zero Agatston units who also underwent computed tomography coronary angiography were included in the study. Univariate logistic regression analysis was used to find associations. (15) Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LH+) and negative likelihood ratio (LH-) were calculated.

Results: Among a total of 93 patients, 10% (n = 9) presented non-calcified plaque. A positive exercise stress test was associated with plaques of any degree of severity (OR 6.5; 95% CI, 1.3-33, p = 0.02). This association persisted for non-severe plaques when the positive exercise stress test was combined with a negative myocardial perfusion SPECT or stress echocardiography for ischemia (OR, 12.4; 95% CI 1.5-101, p = 0.02). Sensitivity and specificity of ST-segment depression for non-calcified plaque of any degree of severity was 44.4% and 86%, respectively, with NPV of 94%, PPV of 25%, LR+ of 3.11 and LR- of 0.65.

Conclusions: ST-segment depression could be associated with non-calcified plaques in patients without coronary artery calcifications, even with normal exercise stress myocardial perfusion or wall motion (non-obstructive disease).

Key words: Computed Tomography Angiography / methods - Vascular Calcification / diagnostic imaging - Plaque, Atherosclerotic / diagnostic imaging - Risk Assessment

RESUMEN

Introducción: El score de calcio es una prueba utilizada en la estratificación de riesgo de pacientes asintomáticos. Aunque la enfermedad coronaria puede producirse en ausencia de calcificaciones, no se han descrito afecciones asociadas a la presencia de placa blanda en este contexto, más allá de la presencia de síntomas.

Objetivos: Determinar asociaciones entre la presencia de placa blanda y variables independientes en pacientes con un score de calcio de cero.

Material y métodos: Se incluyeron pacientes consecutivos con un score de calcio de 0 unidades Agatston que se hubieran realizado, además, una angiotomografía coronaria. Se determinaron asociaciones a partir de análisis univariado. Se calculó la sensibilidad, especificidad, VPN, VPP, +LR y -LR.

Resultados: Se incluyeron en el estudio 93 pacientes. El 10% (n = 9) presentaron placa blanda. La ergometría positiva se asoció a placas de cualquier gravedad (OR 6,5; IC del 95%: 1,3-33, p = 0,02). Esta asociación persistió para placas no graves cuando se combinó la ergometría positiva con perfusión miocárdica SPECT o ecocardiograma estrés negativos para isquemia (OR 12,4 IC 95% 1,5-101, p = 0,02). La sensibilidad y la especificidad del infradesnivel del ST para placa blanda de cualquier nivel de gravedad fue del 44,4% y del 86%, respectivamente. El VPN fue del 94% y el VPP del 25%, LR+ fue de 3,11 y LR- fue de 0,65.

Conclusiones: El infradesnivel del ST se asociaría a la presencia de placa blanda en pacientes sin calcificaciones coronarias, incluso en contexto de perfusión miocárdica o motilidad parietal en esfuerzo normales (enfermedad no obstructiva).

Palabras clave: Angiografía por Tomografía Computarizada/métodos - Calcificación Vascular /diagnóstico por imagen - Placa Aterosclerótica/ diagnóstico por imagen - Medición de Riesgo

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Abbreviations

HR	Heart rate	CAC	Coronary artery calcium
LR-	Negative likelihood ratio	AU	Agatston units
LR +	Positive likelihood ratio	NPV	Negative predictive value
EST	Exercise stress test	PPV	Positive predictive value

INTRODUCTION

Assessment of coronary artery calcium (CAC) by non-enhanced computed tomography scan is supported by clinical practice guidelines for risk stratification of asymptomatic patients. (1)

The evidence supporting this recommendation is based on the better performance of CAC to predict coronary events compared with traditional risk factors expressed in the Framingham risk score. In intermediate risk subjects, CAC net reclassification has been reported to improve prediction of events in 31% to 55% of patients. (2-4)

Although its systematic indication as a screening method in asymptomatic patients has not demonstrated a reduction of hard outcomes in randomized studies, a recent systematic review has shown that CAC increases the likelihood of initiation or continuation of pharmacological therapy for the prevention of cardiovascular disease. (5)

However, testing for calcium scoring in patients with symptoms suggestive of coronary artery disease is considered rarely adequate in the Multimodality Appropriate Use Criteria in Cardiovascular Imaging American guidelines. (6) This is due to the prevalence of soft (non-calcified) plaques in this group of patients. In a cohort of symptomatic patients with CAC score of zero, Villines et al. reported that 16.5% had non-calcified plaques and 3.5% presented significant plaques. (7)

Although the limitation of calcium scoring in symptomatic patients is well established, there are no studies evaluating the presence of other conditions associated with the presence of non-calcified plaques in patients without coronary artery calcifications. Identifying these factors would help in the detection of populations at higher cardiovascular risk and in the future design of studies to determine the usefulness of calcium scoring to predict hard outcomes.

The aim of this study was to determine the associations between non-calcified plaques and independent clinical variables or those evidenced by functional tests in a population of patients without known coronary artery disease and with CAC score of zero Agatston units (AU).

METHODS

Study population

This single-center, retrospective study analyzed the database-incorporated registries of a consecutive cohort of patients evaluated in our center from September 2017 to October 2018. The inclusion criteria were:

1. Age >18 years

2. Previous exercise stress test (EST) with a readable baseline electrocardiogram.
3. Absence of coronary artery calcifications after performing CAC scoring and a computed tomography coronary angiography (CTCA) according to the indication of the attending physician.

Tests that motivated the attending physician to require CAC scoring and CTCA due to the presence of symptoms, and functional tests with abnormal results or functional mismatch, were included in the analysis. A previous graded EST with ST-segment depression >2 mm 0.06 s after the J point was considered positive and an EST with ST-segment depression <2 mm was considered abnormal but not positive. Functional mismatch was defined as a positive EST with no evidence of ischemia in exercise stress myocardial perfusion SPECT or stress echocardiography. A functional test with evidence of ischemia was considered positive and stress imaging tests without signs of ischemia but with a report of "probable breast attenuation" or "probable diaphragmatic attenuation" were considered abnormal but not positive. Patients referred by the attending physician for CAC score measurement and gated CTCA to evaluate aortic diameters or pulmonary vein diameters before atrial fibrillation ablation were also included. In the interview before undergoing the CT scan, all patients presented their complementary tests and the clinical record was taken. The EST previously required by the attending physicians had been indicated to rule out coronary artery disease (even in patients undergoing CT scan to evaluate aortic diameters before atrial fibrillation ablation). These stress tests had been required due to the presence of symptoms or to write a medical fitness certificate.

Patients with known coronary artery disease, CAC score >0 AU, cardiomyopathies with baseline electrocardiographic abnormalities that could affect the predictive value of the previous EST, and patients with motion artifacts in the CT scan due to breathing or arrhythmias that did not allow visualization of at least one coronary artery segment were excluded from the analysis.

Computed tomography coronary angiography image acquisition and calcium scoring

Computed tomography coronary angiography images were acquired using a 64-detector row computed tomography scanner (Ingenuity Core, Phillips, the Netherlands) with slice thickness of 0.9 mm, rotation time 0.4s, pitch 0.55, tube voltage adjusted for patient's BMI (120 kV if BMI ≥30 kg/m² and 100 kV if BMI <30 kg/m²) and tube current between 700 and 1000 mA. The images were acquired using ECG gating and modulated prospective or retrospective protocol according to heart rate (HR) or presence of arrhythmias during acquisition. Beta-blockers were indicated at the time of acquisition if HR was >65 bpm and sublingual nitrites were administered 2 to 3 min before acquisition in the absence of contraindications. Intravenous injection of 90-125 mL iodinated contrast (Optiray®, Ioversol 320 mg/mL, Mallinckrodt, St. Louis, USA) was administered at a flow rate of 4 to

6 mL/s using the bolus tracking technique to synchronize CT acquisition with the first passage of contrast material through the coronary arteries.

A prospective ECG-gated protocol was used to acquire images for calcium scoring, with rotation time of 0.4 s, tube voltage of 120 kV, tube current of 55 mA and slice thickness of 3 mm.

Analysis of computed tomography coronary angiography images and calcium scoring

The coronary artery segments were analyzed using three-dimensional curved multiplanar reconstructions and maximum intensity projections. Initially, the images were reconstructed at end-diastole (75% of the RR interval) and image quality was evaluated in each coronary segment. If the quality was insufficient, the subsequent reconstructions were obtained at 70% and 80% of the RR interval (prospective protocol) and additionally at 30% and 40% in case of a modulated retrospective protocol. Two independent observers reported the presence of plaques according to the Society of Cardiovascular Computed Tomography grading scale for stenosis severity (8) (0% no visible stenosis, 1% to 24% minimal stenosis, 25% to 49% mild stenosis, 50% to 69% moderate stenosis, 70% to 99% severe stenosis and 100% occlusion). Segments with at least a visible stenosis of 25% were manually quantified using commercially available software (Philips IntelliSpace Portal 8.0) and the results of both observers were averaged. Any visual and quantitative interobserver variability >50% was resolved by a third observer. The quantification of coronary calcium was performed using Philips IntelliSpace Portal 8.0, including all pixels with a density >130 HU. A calcification was defined as a lesion with a density >130 HU and area > 2 adjacent pixels. The CAC score was calculated according to the Agatston method. (9)

Definition of non-calcified plaque

Non-calcified plaque was defined as a lesion that produces at least a minimal stenosis and does not present any focus of calcification. This definition obtained from the CTCA was considered as endpoint to compare results. As all patients with at least one focus of coronary calcification (Agatston score > 0 AU) were excluded, all the plaques found in our cohort met the definition.

Statistical analysis

Univariate logistic regression analysis was used to find associations. Non-calcified plaques of any degree of severity were considered as dependent variables in the first analysis, while those non-severe non-calcified plaques (producing stenosis <70%) were the dependent variables in the second analysis. The following variables were considered to search for association: age >50 years; male sex; hypertension; dyslipidemia; current or former smoker; presence of symptoms (chest pain or dyspnea as reasons to order the test); positive EST with ST-segment depression of at least 2 mm at 0.06 s from the J point; functional mismatch with positive EST and absence of ischemia on stress myocardial perfusion SPECT or stress echocardiography. Two by two tables were used to assess the diagnostic accuracy of any associated variable, and sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-) were calculated. Patients with at least one non-calcified plaque as defined in the previous section were considered to have the disease (with true positive or false negative results of the predictor analyzed). Continuous

variables with normal distribution were expressed as mean and were compared using Student's t test while continuous variables with non-Gaussian distribution were expressed as median and compared using the Mann-Whitney test. Categorical variables were expressed as proportions and were compared using Fisher's test. A p value <0.05 was considered statistically significant. All calculations were performed using Epi-Info version 7.2.2.2 software package.

Ethical considerations

As this was an observational study, we only performed the tests requested by the attending physicians and analyzed the data obtained without any additional intervention.

At our institution, patients sign an informed consent form in which they accept the possibility that data linked to the results of their tests may be used anonymously for scientific purposes.

The study protocol was approved in 2017 by TCBA Ethics Committee.

RESULTS

Ninety-three patients with CAC score = 0 AU were included in the study. In 28% of cases, computed tomography scans were ordered for other reasons and not for suspected coronary artery disease. Seven percent of the patients presented symptoms and 26% had at least a positive functional test that was the indication for the CT scan (Table 1).

Patients with plaques were older and presented more positive EST than those without plaques (Table 2). Non-calcified plaques were present in 10% of the patients (n = 9) and 2% of the patients had severe stenosis (n = 2, Tables 3 and 4).

On univariate analyses, positive EST was associated with plaques of any degree of severity (OR 6.5

Table 1. Demographic patient characteristics (n = 93)

Age (years ± SD)	45 ± 15
Male sex, (%)	50 (54)
HT (%)	50 (54)
DLP (%)	11 (12)
DBT (%)	2 (2)
SH (%)	9 (10)
ASCVD risk score (median ± SD)	11 ± 7
Symptoms (%)	6 (7)
Positive EST (%)	16 (17)
Myocardial perfusion SPECT/stress echocardiography +* (%)	8 (9)
Functional mismatch (%)	6 (7)
Aortic disease (%)	13 (14)
Pulmonary veins (%)	13 (14)
Radiation received (mSv ± SD)	8 ± 3

* Myocardial perfusion SPECT/stress echocardiography + refers to patients with stress myocardial perfusion SPECT or stress echocardiography with evidence of myocardial ischemia. HT: Hypertension. DLP: Dyslipidemia. DBT: Diabetes. SH: Smoking habits. ASCVD: Atherosclerotic cardiovascular disease. EST: Exercise stress test. SPECT: Single-photon emission computed tomography.

Characteristics	Non-calcified plaque		p
	YES (n = 9)	NO (n = 84)	
Age (years ± SD)	55 ± 7	45 ± 16	0.02
Male sex, (%)	4 (44)	46 (55)	0.7
DBT (%)	0	2 (2.4)	1
HT (%)	2 (22)	7 (8.3)	0.2
SH (%)	2 (22)	7 (8.3)	0.2
DLP (%)	1 (11)	10 (11.9)	1
Symptoms (%)	1 (11)	5 (6)	0.46
Positive EST (%)	4 (44)	12 (14.3)	0.04
Functional mismatch* (%)	2 (22)	4 (4.8)	0.1

*Functional mismatch refers to a positive EST with no evidence of ischemia in exercise stress myocardial perfusion SPECT or exercise stress echocardiography. DBT: Diabetes. HT: Hypertension. SH: Smoking habits. DLP: Dyslipidemia. EST: Exercise stress test.

Table 2. Demographic characteristics according to the presence or absence of non-calcified plaque.

Patient	LMCA	LAD	Diagonal	LCx	RCA
1	no	yes (proximal)	no	yes (proximal)	no
2	no	yes (proximal)	no	yes (proximal)	no
3	no	yes (mid-third)	no	yes (mid-third)	no
4	no	no	no	no	no
5	no	yes (proximal)	no	yes (proximal)	no
6	no	yes (proximal)	no	yes (proximal)	no
7	no	yes (prox. and mid-third)	no	yes (prox. and mid-third)	no
8	yes	yes (proximal)	no	yes (proximal)	yes (mid-third)
9	yes	yes (proximal)	yes	yes (proximal)	no

LMCA: Left main coronary artery. LAD: Left anterior descending coronary artery. LCX: Left circumflex coronary artery. RCA: Right coronary artery.

Table 3. Characteristics of coronary artery anatomy of the 9 patients with non-calcified plaque

Patient	Number of vessels with severe plaque	Number of vessels with non-severe plaque	Number of non-calcified plaques	Severe plaque
1	0	2	2	25-49%
2	0	1	1	25-49%
3	1	0	1	70-99%
4	0	1	1	25-49%
5	0	1	1	25-49%
6	0	1	1	25-49%
7	1	1	2	70-99%
8	0	3	3	50-69%
9	0	2	2	25-49%

Table 4. Characteristics of coronary artery anatomy of the 9 patients with non-calcified plaque

95% CI 1.3-33, p = 0.02) and with non-severe plaques (OR 14.9 95% CI 2-106, p = 0.007) (Tables 5 and 6).

Functional mismatch with positive EST and absence of ischemia by stress gated myocardial perfusion SPECT or stress echocardiography was associated with non-severe plaques (OR 12.4; 95% CI, 1.5-101; p = 0.02) (Table 6).

Exercise stress test sensitivity and specificity for non-calcified plaque of any degree of severity was 44.4% (95% CI, 13.7%-78.8%) and 86% (95% CI, 76.38%-92.39%), respectively, with NPV of 94% (95% CI, 88.86%-96.30%), PPV of 25% (95% CI, 11.95%-

45.02%); LR+ of 3.11 (95% CI, 1.27-7.64) and LR- of 0.65 (95% CI, 0.36-1.17) (Tables 7 and 8).

DISCUSSION

In this cohort of patients with CAC score of zero, the prevalence of non-calcified plaque was 10% and a positive EST was associated with plaques of any degree of severity. The association of a positive EST with non-severe plaques remained when stress gated myocardial perfusion SPECT or stress echocardiography were negative for ischemia. The specificity and LR+ of ST-segment depression for the diagnosis of non-calcified

Table 5. Univariate analysis using non-calcified plaque of any degree of severity as dependent variable

Variable	OR	95% CI	p
Age > 50 years	1.84	0.14-12.63	0.77
Male sex	0.66	0.16-2.63	0.55
HT	3.14	0.54-18.12	0.19
DLP	0.92	0.10-8.19	0.94
SH	2.50	0.24-31.80	0.43
Positive EST (%)	4.80	1.12-25.15	0.43
Functional mismatch	5.71	0.88-36.89	0.06
Symptoms	1.97	0.20-19.05	0.55

HT: Hypertension. DLP: Dyslipidemia. SH: Smoking habits. EST: Exercise stress test.

Table 6. Univariate analysis using non-severe non-calcified plaque as dependent variable

Variable	OR	95% CI	p
Age > 50 years	1.18	0.19-17.91	0.58
Male sex	1.15	0.24-5.49	0.85
HT	1.62	0.17-15.25	0.07
DLP	1.27	0.13-11.63	0.83
SH	3.42	0.32-35.60	0.30
Positive EST (%)	8.24	1.63-41.47	0.01
Functional mismatch*	8.20	1.19-56.07	0.03
Symptoms	0.27	26.97	0.39

*Functional mismatch refers to patients with positive EST with no evidence of ischemia in exercise stress myocardial perfusion SPECT or exercise stress echocardiography. HT: Hypertension. DLP: Dyslipidemia. SH: Smoking habits. EST: Exercise stress test.

Table 7. Diagnostic accuracy of EST to detect non-calcified plaque

EST	Non-calcified plaque		total
	Present	Absent	
Positive	4	12	16
Negative	5	72	77
Total	9	84	93

EST: Exercise stress test.

Table 8. Sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of exercise stress test to detect non-calcified plaque

Sensitivity	44.4% (13.7-78.8)
Specificity	86% (76.38-92.39)
LR+	3.11 (1.27-7.64)
LR-	0.65 (0.36-1.17)

LR+: Positive likelihood ratio. LR-: Negative likelihood ratio

plaque of any degree of severity were 86% and 3.11%, respectively.

The prevalence of non-calcified plaque in our cohort is consistent with the one described in the CONFIRM registry for patients with symptoms. This could evidence a limitation of the CAC score for risk stratification of asymptomatic patients with abnormal EST. In other words, in asymptomatic patients with CAC score of zero, ST-T abnormalities could be due to a non-calcified plaque. This hypothesis would be supported by the specificity and LR+ of the EST found in this cohort.

The second relevant finding in our cohort is the persistence of the association of a positive EST with non-significant plaques in the setting of normal myocardial perfusion (Figure 1). It is worth remembering that stress myocardial perfusion and stress echocardiography are not the adequate methods for differentiating between absence of plaques and non-obstructive coronary artery disease (mild or moderate plaques). This explains why the absence of ischemia is a common finding in the history of patients with cardiovascular events (10) and the significant improvement in risk stratification when the CAC score is added to myocardial perfusion imaging tests (SPECT/CT systems). (11)

The implications of our findings could be summarized as follows: the electrocardiographic abnormalities during EST associated with the detection of mild to moderate non-calcified plaques in patients without evidence of ischemia or coronary artery calcifications would demonstrate the validity of ST-segment depression as a diagnostic marker. This is consistent with previous publications from our group about impaired ventricular arterial coupling in patients with ST-segment depression and normal myocardial perfusion. (12) Several publications show that the combination of ST-segment depression and normal myocardial perfusion would reflect global subendocardial abnormalities which are underestimated by ischemia provocative tests due to the phenomenon of balanced ischemia. (13-16) Therefore, our interpretation is that the presence of non-significant non-calcified plaques associated with abnormal EST could represent a surrogate of microvascular disease that is not detected by gated myocardial perfusion SPECT due to its global, non-segmented character. (17, 18).

It is important to emphasize that our findings do not contradict the basic principles of the ischemic cascade. Although according to the ischemic cascade, electrocardiographic changes appear later than wall motion or perfusion abnormalities, the phenomenon of balanced ischemia typical of microvascular disease and its subendocardial nature hamper its detection by the technologies used in stress ultrasound and myocardial perfusion SPECT. In other words, although perfusion abnormalities appear before ECG changes, they should be documented by technologies not limited by the phenomenon of balanced ischemia or by lack

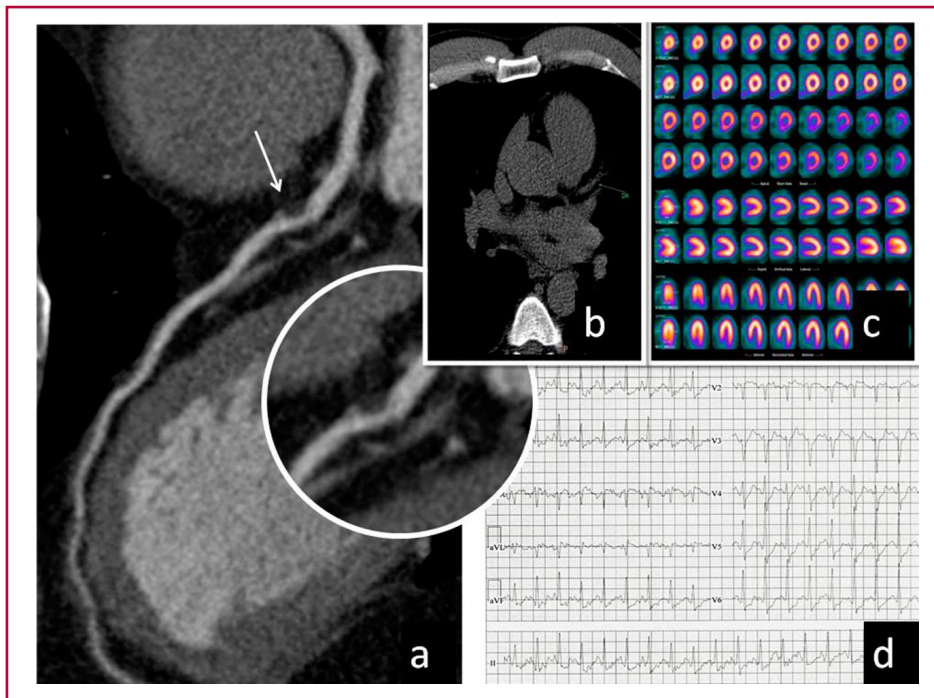


Fig. 1. Images from a 55-year-old high-performance athlete male with a history of dyslipidemia and without known cardiovascular disease with a 40% non-calcified plaque in the proximal segment of the left anterior descending coronary artery (LAD) (a) coronary artery calcium score of zero (b) normal myocardial perfusion (c) and ST-segment abnormalities (d)

of resolution for non-transmural phenomena (quantification of coronary flow by PET myocardial perfusion imaging, [19] D-SPECT [20] or stress cardiac MRI [21] to assess non-transmural ischemia).

Study limitations

As with any retrospective, observational and single-center study, the design has high risk of bias. The sample size is small and has low prevalence of symptoms (7% of our population) due to the inclusion of patients with several indications for CTCA, even not related with the assessment of coronary arteries (e.g., evaluation of the pulmonary veins). Considering the high prevalence of non-calcified plaques in symptomatic patients described in other publications, it will be necessary to perform a study including more patients with symptoms to give consistency to our statements. On the other hand, the low prevalence of severe plaques in our cohort (2%) does not allow us to be certain about the role of EST to predict severe plaques in patients with CAC score of 0 and normal functional imaging tests. Moreover, we cannot talk about the predictive role of EST for non-calcified plaques, since our study is just a proof of concept (association between positive EST and non-calcified plaques). This proof of concept is the first of several steps for the assessment of a cardiovascular risk marker. (22) If studies with a larger number of patients show that, in the absence of coronary calcifications and normal perfusion imaging tests, EST is not a marker of severe plaques (i.e. it only predicts mild and moderate plaques in this context), they would provide a basis for analyzing a strategy of CTCA versus calcium score without CTCA

in a randomized study. This could modify the current recommendation of clinical practice guidelines for performing CTCA in all patients with functional mismatch, leaving the use of contrast and radiation dose characteristic of CTCA only for cases of EST-SPECT mismatch with CAC score >zero.

CONCLUSIONS

Absence of coronary artery calcifications in CT scan does not necessarily mean the absence of plaques, due to the existence of non-calcified lesions within the atherosclerotic process. The presence of coronary artery disease in this setting could be suspected from an abnormal EST. ST-segment depression could be associated with the presence of non-calcified plaques of any degree of severity. In patients without CAC, perfusion and wall motion defects, ST-segment abnormalities could warn us about the presence of non-obstructive disease. This could probably be explained by diffuse microvascular involvement without significant macrovascular impairment expressed as subendocardial injury (an underestimated phenomenon in stress SPECT and stress echocardiography due to its homogeneous and subendocardial character). These hypotheses and their implications should be confirmed by future prospective studies with a larger number of patients and in randomized studies evaluating relevant clinical outcomes.

Conflicts of interest

None declared.

(See authors' conflicts of interest forms on the website/Supplementary material)

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