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"CLINICAL, ANGIOGRAPHIC AND OPTICAL COHERENCE TOMOGRAPHY PREDICTORS OF CORONARY PLAQUE HEALING"

Relatore
Chiar.mo Prof. Italo Porto
Correlatore
Prof. Rocco Vergallo

Candidato
Nicolò Maria Ispano

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Alla mia mamma,

perché in tutto ciò che sono,
e in tutto ciò che sarò,

ci sarà sempre qualcosa di te.

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1 INTRODUCTION:

1.1 ATHEROSCLEROSIS: EPIDEMIOLOGY, RISK FACTORS, A GENERAL OVERVIEW

Atherosclerosis remains the major cause of death in both developed and developing countries, manifesting as multiple clinical events such as acute myocardial infarction and ischemic stroke(1,2). The nature of the disease is systemic, predominantly affecting medium and large-sized arteries(3). Epidemiologically, cardiovascular diseases cause 17,9 million deaths every year, representing 32% of all.

Over decades the epidemiological profile of patients with acute coronary syndromes has significantly changed. Historically, the "typical" patient with myocardial infarction was described as a middle-aged white man with hypertension, hypercholesterolaemia and smoking habits. However, implementation of effective antihypertensive therapies, lipid-lowering agents (statins) and the reduction of smoking prevalence have substantially modified the traditional cardiovascular risk. As a result, coronary artery disease now affects an increasing number of elderly patients, due to population ageing, and an increasing portion of women(2).

In recent years, the global epidemic of obesity has emerged as a major driver of atherosclerotic cardiovascular disease, closely associated with type 2 diabetes, hypertension and metabolic syndrome, specifically in South American countries and Asia. Here the widespread of these factors increase the prevalence of atherosclerosis.

In parallel with these changes, the predominant pattern of dyslipidaemia has evolved. Whereas elevated low-density lipoprotein cholesterol (LDL) was historically the primary lipid abnormality, contemporary patients (receiving LDL-lowering therapy, such as statins or PCSK9 inhibitors) commonly exhibit atherogenic dyslipidaemia characterized by elevated triglyceride-rich lipoproteins (TGRL) and reduced high-density lipoprotein cholesterol (HDL).

This lipid phenotype is linked to insulin resistance and the metabolic syndrome, clusters of conditions that include increased waist circumference, hypertriglyceridemia, low HDL, elevated blood pressure, and impaired fasting glucose. The growing prevalence of metabolic syndrome has contributed to a growing global burden of ischaemic heart disease, increasing from approximately 100 million cases in 1990 to over 180 million cases in 2019(2).

In this evolving landscape, the development of atherosclerosis is driven by multiple cardiovascular risk factor, stemming from the complex interaction between traditional and non-traditional risk factors(2). As previously described, among traditional risk factors, elevated levels of low-density lipoprotein (LDL) cholesterol, arterial hypertension, cigarette smoking, and diabetes mellitus play established causal roles, promoting endothelial dysfunction and lipid accumulation(3,4). However, the risk profile is continuously changing. Recent evidence has downplayed the protective role of HDL cholesterol, emphasizing instead the causal role of triglyceride-rich lipoproteins(2). In addition, second line non-traditional factors are emerging as critical drivers: sleep disturbance, physical inactivity, air pollution, environmental stress, and alterations in the gut microbiome are now recognized as significant contributors to disease development(2).

In this scenery, understanding the evolving cardiovascular risk profile may provide important information into mechanism underlying of plaque healing.

In this context, chronic low-grade inflammation has emerged as a key driver of atherosclerosis, contributing not only to plaque initiation and progression but also to plaque destabilization and thrombotic complications. Recent evidence has highlighted the role of residual inflammatory risk, often assessed by high-sensitivity C-reactive protein (hsCRP), as an independent predictor of cardiovascular events even in patients receiving optimal lipid-lowering therapy (5).

1.1.1 PATHOPHYSIOLOGY OF THE PLAQUE: CELLULAR, MOLECULAR AND IMMUNOLOGICAL MECHANISMS

A normal artery has three layers: intima, tunica media and adventitia. Under normal conditions the intima monolayer does not accumulate leukocytes. The Endothelium plays an important role between blood circulation and tissues transducing changes in blood to layers, concerning wall shear stress and changes in the concentration of metabolic factors (such as coagulant and anti-coagulants, vasodilators or vasoconstrictors and pro or anti-inflammatory molecules). The main forces acting on the wall are tensile stress induced by blood pressure and wall shear stress. Wall shear stress changes can affect the morphology and functions of endothelium; especially higher tension is linked to be at higher risk for the development of atherosclerosis and migration of mononuclear cells. According to this information it is known that atherosclerotic lesions mainly occur in branch points and bifurcations characterised by flow separation and low wall shear stress.

Disruption of the mechanism involved in vascular homeostasis permits vasoconstriction and lipid infiltration. Oxidative stress, glycation, platelet activation are considered the first steps

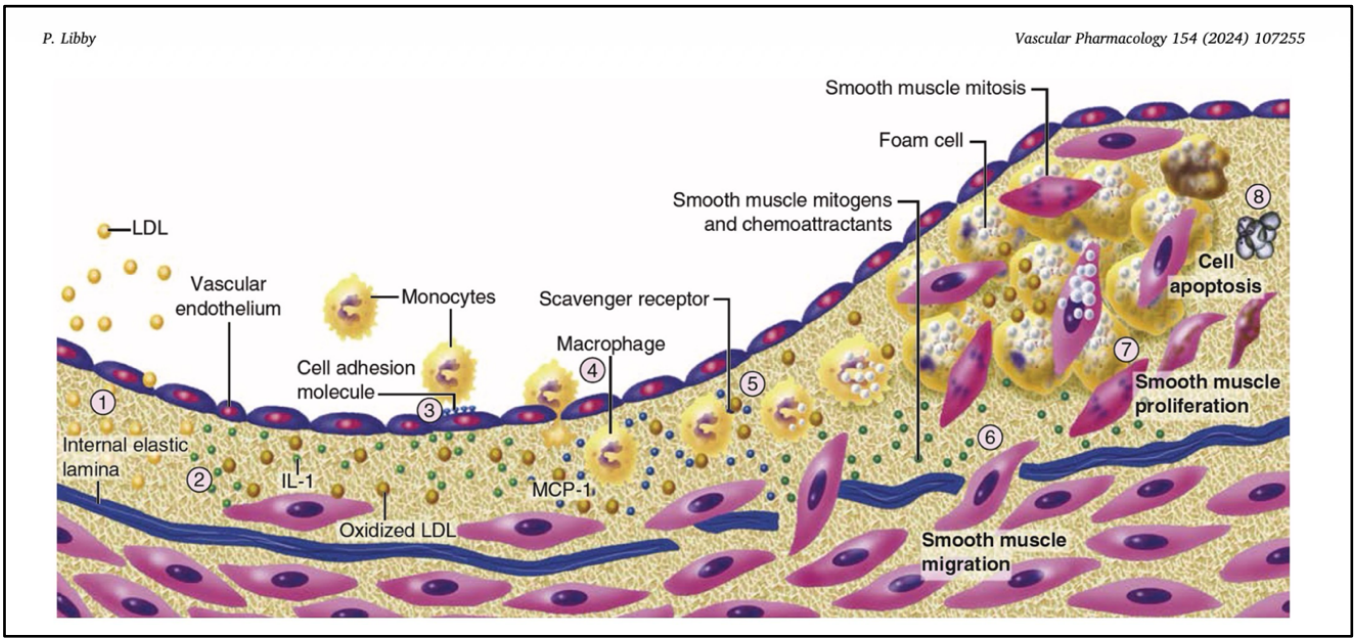


Figure 1 Evolution of atherosclerotic plaque

Adapted from Libby P. Inflammation and the pathogenesis of atherosclerosis. Vascular Pharmacology. 2024

of atheromatous plaque formation and fatty streak deposition. Oxidized LDL are key inflammatory component containing oxidised lipids and products from their degradation that contributes to the progression of the disease. The process is characterised by loss of antioxidants carried by LDL, followed by degradation of polyunsaturated fatty acids leading

to the formation of short-chain aldehydes. At the same time oxLDL modifies apoB-100 causing lack of affinity toward LDL-receptors and increasing affinity for the scavenger receptors. These factors induce endothelium to express PDGFs, that enhance VSMC migration, eNOS or PECAM-1, which contribute to plaque development. Moreover, reduction of these mediators is strongly related to lack of homeostatic factors.

Other molecules express by the endothelium are VCAM-1, ICAM-1 and MCP-1, receptors promoting rolling, adherence and interaction of monocytes.

Briefly, monocyte capture and rolling are mainly mediated by P-selectin. Interaction with ICAM and VCAM permit monocity to remain attached. In addition, monocytes are activated by endothelial chemokines (CXCL1, CXCL2, CXCL4 and CCL5) increasing adhesiveness that consent transmigration of these cells into the intima.

Once in the intima, monocytes differentiate into pro-inflammatory, M1, and anti-inflammatory, M2, phenotype. It is known that macrophage plasticity is important for a successful response with M1 predominating in disease progression and M2 in regression.

M1 macrophages are activated in response to inflammatory cytokines, chemokines, NO and ROS, which promote recruitment of other macrophages and inflammatory progression.

Moreover, in the inflammatory environment of the arterial intima, low-density lipoproteins (LDL) undergo several modifications, including oxidation facilitating their uptake by macrophages through scavenger receptors such as CD36, SR-A and LOX-1, which are not downregulated, leading to progressive accumulation. Once internalized, oxidized LDL particles are degraded in lysosomes and cholesterol is stored in the cytoplasm as lipid droplets.

Under physiological conditions, cholesterol can be removed from macrophages through efflux pathways mediated by transporters such as ABCA1 and ABCG1, which transfer cholesterol to high-density lipoproteins (HDL).

However, in the pro-inflammatory environment of atherosclerotic lesions these mechanisms become impaired, promoting lipid accumulation and the transformation of macrophages into foam cells, a key feature of early atherosclerotic plaque development. This process maintains endothelial activation, monocyte recruitment and foam cell formation leading to the growth of atherosclerotic lesions. Moreover, this process leads to the formation of cholesterol crystals both inside and outside the cells, bringing to activation of pro-inflammatory

pathways such as NLRP3 inflammasome and activation of caspase-1. These mechanisms represent key drivers of plaque progression and instability.

Another protagonist of this process are vascular smooth muscle cells, located in the intima. These cells can internalise oxLDL in a non-regulated pathway that is mediated by scavenger receptor like SR-A, CD36, LOX-1. Their contribution to foam cell population is significant, at least 50% of foam cells in human coronary arteries are derived by smooth muscle cells and monocyte, underling their important role in this process.

In addition to this process, smooth muscle cells migrate from media to intima where they produce extra cellular matrix and promote accumulation of these products in growing plaque.

During these changes, atheroma plaque leads a transition from fatty streak to plaque with necrotic core, characterised by cell-free and lipid rich areas. In this process, to stabilise the plaque, the necrotic core is covered by a developing fibrous cap, reducing the likelihood of regression.

Specifically, fibrous cap is a sub-endothelial barrier that is made to serve as a structural support to avoid the exposure of material from necrotic core acting as prothrombotic trigger for thrombus development.

When an acute event occurs, foam cells or ECs activate the healing process by producing epidermal growth factor, PDGF, TGF-beta, VEGF. Healing process may stabilize plaque or lead to consecutive acute event and relapsing of unsuccessful healing process.

Generally, during plaque healing vascular smooth muscle cells migrates from tunica media to the intima increasing production of collagen, elastin and proteoglycans. These components, produced by VSMC and ECM generate a fibrous cap that surround the lesion and prevent rupture.

Fibrous cap characteristics such as thickness and matrix composition are important determinants of plaque stability.

During the development of atherosclerotic lesion, necrotic core increases its size because of macrophage death and loss of efferocytosis, a process that consist in the removing of apoptotic cells.

In addition, cholesterol crystals derived from dying cells accumulate in the lesion amplifying this inflammatory phenomenon.

The progressive enlargement of the necrotic core and the release of proteolytic enzymes, such as matrix metalloproteinases, contribute to the thinning of fibrous cap and increase plaque vulnerability. Importantly, the same process also influences plaque healing dynamics.

Another option for atheroma plaque is calcification process, advanced hallmark atherosclerosis, characteristic of inflammatory regions with local reduction of collagen fibres. The process is driven by releasing of matrix vesicles and synthetic VSMC death. The accumulation starts with calcium orthophosphate, covered by amorphous calcium phosphate. This process is made by VSMC and pericytes that differentiate into osteoblast-like phenotypes, acquiring the capacity to deposit calcium and generate mineralized matrix, like bone. These deposits grow from microcalcifications to larger that extend from the core to the surrounding matrix. The amount and size of calcium deposits do not correlate with vulnerability, which is rather linked with other features like calcification type, environment, location. (1,2,6,7)

These mechanisms provide the biological basis for plaque healing and highlight the importance of identifying clinical and imaging predictors of this process.

1.2 VULNERABLE PLAQUE: A NEW LANDSCAPE BETWEEN PLAQUE RUPTURE, EROSION AND CALCIFIED PLAQUE

Nowadays, attention to ‘’vulnerable plaque’’ has increased in parallel with the changing landscape of atherosclerosis and mechanisms of thrombotic complications. In recent decades, attention has focused on plaque instability, particularly in the context of improved cardiovascular therapies.

Over years, plaque rupture has become less frequent with an increasing portion of ACS attributed to endothelial erosion with thrombosis accounted for approximately one-fifth to one-third of ACS cases. Moreover, factors like lipid-lowering drugs and people’s aging translate into a moderate increase of eruptive calcified nodule causing ACS. In this landscape also clinical presentation has modified. STEMI prevalence decreased while NSTEMI has become more prevalent. Historically, the term ‘’vulnerable plaque’’ referred to plaque with increased risk for ruptures; now all major substrates that have been identified (plaque rupture, plaque erosion and eruptive calcified nodule) as ‘’ high-risk plaque ‘’ are part of this group.

In this context it's important to identify biological and morphological characteristics (such as plaque burden, lipid-rich core, thin fibrous cap, protective and pro-thrombotic mechanism) that can stratify the risk of a plaque to cause ACS or cardiac death.

The major criteria that identify plaque as ''high risk plaque'' are thin fibrous cap, large lipid core or necrotic core, a large burden, active inflammation with macrophage and phenomenon of endothelial damage/denudation.

As minor criteria of high-risk plaque the literature describes about superficial protruding calcified nodule, intra-plaque haemorrhage, cholesterol crystals, absence of healing plaque, disturbed local hemodynamic and location in proximal or mid coronary artery (8).

TABLE 1 Major and Minor Criteria for the Definition of High-Risk Plaque

Major criteria
Thin fibrous cap
Large lipid/necrotic core
Large plaque burden
Active plaque inflammation (macrophage)[a]
Endothelial damage/denudation[a]
Minor criteria
Superficial protruding calcified nodule
Intraplaque hemorrhage[a]
Neovascularization
Cholesterol crystals
Positive remodeling
Absence of healed plaque
Disturbed local hemodynamics (eg, shear stress, shear stress gradient)[a]
Location in a proximal or mid coronary artery (large area at risk)[b]

[a]Reliable noninvasive or invasive imaging modalities for their detection are not yet available. [b]This criterion increases the likelihood that a plaque thrombosis will manifest clinically but has not been shown to be an independent risk factor for plaque instability or thrombosis.

Figure 2 Major and Minor Criteria for the Definition of High-Risk Plaque

Vergallo R, Park SJ, Stone GW, et al. Vulnerable or High-Risk Plaque: A JACC: Cardiovascular Imaging Position Statement. JACC Cardiovasc Imaging. 2025

Historically, the profile of vulnerable and high-risk plaque is represented by TCFA plaque, characterised by thin fibrous cap (< 65 μm) and lipid-necrotic core containing smooth muscle cells, microcalcifications and accumulation of activated macrophages. During last 3 decades scientists thought that TCFA was the main cause of plaque rupture, shown by autopsy studies of patients who died from ACS. The major limitation of these studies was the inability to assess silent TCFAs in the same patients. During years, studies using virtual imaging has shown that other presentation of plaque, such as erosion or eruptive calcified nodule, could cause ACS. Moreover, PROSPECT study shows that only 5% of TCFA in

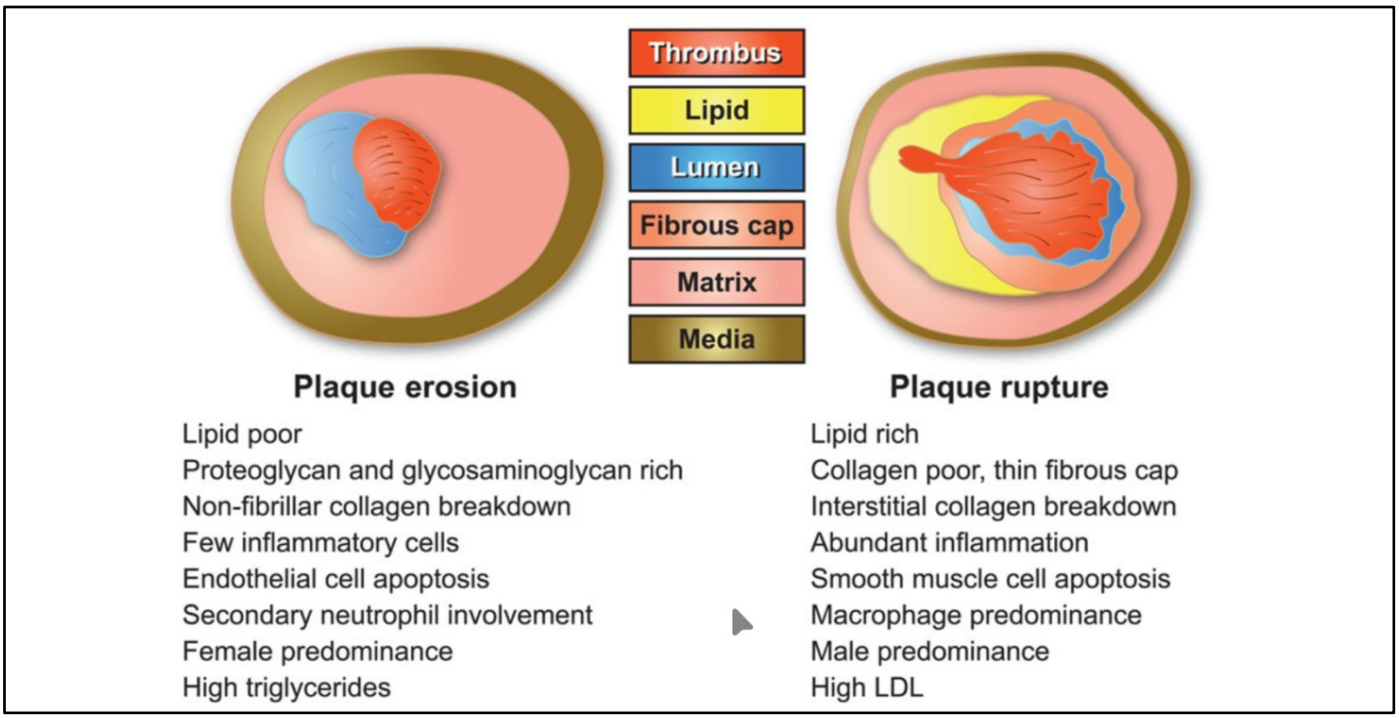


Figure 3 Characteristics and Differentiation between phenotype of plaque

Libby P, Pasterkamp G. Requiem for the 'vulnerable plaque'. European Heart Journal. 2015

high-risk patients' plaque, defined by virtual histology, led coronary events during 3.4 years of follow up period while the incidence of events in low-risk patients is lower (8). In line with these findings, pathological and clinical evidence has shown that plaque rupture and thrombus formation frequently occur in the absence of clinical symptoms, suggesting that additional systemic and local factors (including local and blood thrombogenic factors) are required for the development of acute coronary syndromes (9). These findings have challenged the traditional concept of vulnerable plaque as a single lesion entity.

As previously discussed, for these reasons the concept of vulnerable plaque as TCFA is now in doubt thanks to other histological presentations of ACS. Furthermore, PROSPECT studies show that overall atherosclerotic burden is more important than angiographic stenosis in

predicting future coronary events. This concept is now changing what scientists considered over years (8,10,11).

Moreover, PROSPECT II confirmed the limited predictive value of angiographic stenosis for ACS, highlighting that the combination of large plaque burden and lipid-rich composition is major determinant of plaque vulnerability (12).

These findings suggest that plaque vulnerability is not only a local phenomenon but reflects a systemic disease involving both plaque characteristics and patient-related factors. Consistently, contemporary evidence suggests that acute coronary syndromes represent a heterogeneous condition driven by multiple pathophysiological mechanisms, including plaque rupture, erosion, and functional alterations, rather than a single unifying process (13).

In this context, a future vision of the vulnerable plaque is shifting from a single lesion vision to a pancoronary assessment of atherosclerosis integrating clinical parameters and scale with non-invasive and invasive techniques like CT and invasive coronary study. This view is going to lead to a patient-centred treatment as a systemic disease and a more comprehensive, patient-centred approach integrating clinical, angiographic and imaging data to better identify high-risk plaques (14,15).

1.2.1 PLAQUE RUPTURE

Plaque rupture is the most frequent phenotype of myocardial infarction, accounting from 40% to 60% of ACS. The mechanism underlying plaque rupture consists of a structural

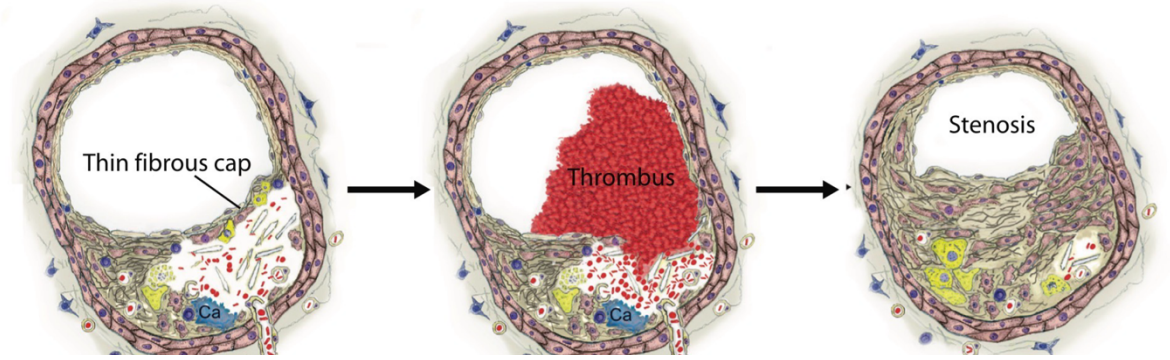


Figure 4 Plaque rupture and healing. Reproduced from Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of Plaque Formation and Rupture. Circ Res. 2014;114:1852–1866.

defect in the fibrous cap, exposing highly thrombogenic core to the blood, as confirmed by the presence of plaque material within the thrombus. As shown in studies by Virmani, plaque rupture occurs where fibrous cap is thinnest and infiltrated by foam cells, in particular the weakest spot is often the cap margin or shoulder region. Autopsy studies shows that TCFAs with a fibrous cap thickness <65 μm are those who have the majority risk of rupture. Histologically, plaque rupture occurs typically in area with reduced smooth muscle cells content. In this contest, foam cells produce proteolytic enzymes (like plasminogen activators, cathepsins, metalloproteinases) leading to extracellular matrix degradation and finally plaque rupture (11,16–18). In line with this, specific immune imbalances between pro-inflammatory CD4+ CD28 T-cells and regulatory T cells have been associated with fibrous cap thinning and plaque rupture as assessed by OCT (19).

Plaque rupture is commonly associated with ST-elevation myocardial infarction (STEMI) and prior intraplaque haemorrhage, which is more frequent in rupture plaques and contributes to expansion of the necrotic core and increasing plaque vulnerability. Moreover, studies show that the prevalence of red thrombus is higher in plaque rupture than plaque erosion or eruptive calcified nodule.

For decades, studies suggested that plaque rupture is a phenomenon that involve thin cap fibroatheroma (TCFA). Observations of TCFA prevalence in this patient, support the concept that plaques share similar characteristic, except for the integrity of the fibrous cap. Pathological studies have shown that the prevalence of TCFA in non-culprit vessels is higher

in patient with plaque rupture than in those with plaque erosion, while reported rates ranging from 70% to 30%. Scientist have proposed criteria for TCFA associated with a higher risk of acute clinical events linked to 3 main key factors including large plaque burden, luminal obstruction and fibrous cap thickness < 52 μm. In addition, studies between TCFA, culprit-rupture-plaque and no-culprit-rupture-plaque have shown that fibrous cap thickness is lower in ruptured plaque than TCFA confirming that fibrous cap thickness is a key determinant of plaque instability. Furthermore, culprit rupture plaque is more likely to produce symptoms due to higher plaque burden and lumen narrowing (compared to asymptomatic phenotype of minor lesion). This demonstrates the presence of asymptomatic ruptured plaque and highlights the role of luminal stenosis in determining clinical presentation.

In conclusion, relevant factors are lipid core size, plaque volume, focal haemodynamic factors and fewer macrophage activity. Although plaque rupture can occur spontaneously, ACS are often triggered by emotional or physical stressors that act as precipitating factors in susceptible individuals. These triggers increase the relative risk of ACS, thanks to sympathetic activation and enhancement of thrombogenicity, however their impact on absolute risk remain marginal (11,16–18).

1.2.2 EROSION-PRONE PLAQUE

Plaque erosion is the second most common substrate of acute coronary syndrome (ACS). Unlike plaque rupture, this phenotype is characterized by a different pathophysiological mechanism. Plaque erosion leads to thrombus formation adjacent to endothelium damage of denudation involving plaque without sign of disruptions or critical obstructions. This phenotype is more common in younger individuals, women, diabetic patients and the elderly. The anatomopathological substrate of this process is different, involving plaques rich in smooth muscle cells with a preserved fibrous cap, with abundant extracellular matrix (proteoglycan and glycosaminoglycans) lacking a lipid or necrotic core. These structural features contribute to a different thrombotic pathway compared to rupture-prone plaques. This type of plaque expresses hyaluronidase 2 which activates TLR-2 and is rich in neutrophils which generate extracellular traps that promote procoagulant activity, trap platelets and fibrin and stimulate endothelial cell stress and apoptosis. Moreover, thrombus in erosion plaque is particularly rich in platelets and levels of myeloperoxidase are higher.

Recent evidence suggests that clonal haematopoiesis driven by JAK2 V617F mutation could also contribute to plaque erosion. This mutation stimulates neutrophil activation and enhances inflammatory activity, leading to adhesion, migration and release of proteolytic enzymes such as MMP-9. This process can result in endothelial damaged layer, denudation and thrombus formation may occur.

These biological differences are reflected in imaging findings. Studies comparing OCT findings in plaque erosion and plaque rupture show that patient with ACS caused by plaque erosion have less stenosis, thicker fibrous cap, shorter lipid arc and shorter lipid length.

From a clinical perspective, these differences translate into distinct patient characteristics and presentations. Contemporary studies using imaging techniques such as OCT show that the prevalence of plaque erosion is not only increasing (due to statin therapy, reduction in smoking habits, decrease of passive smoking and higher prevalence of factor such as hypertriglyceridemia, metabolic syndrome, diabetes and older people) but also evidence

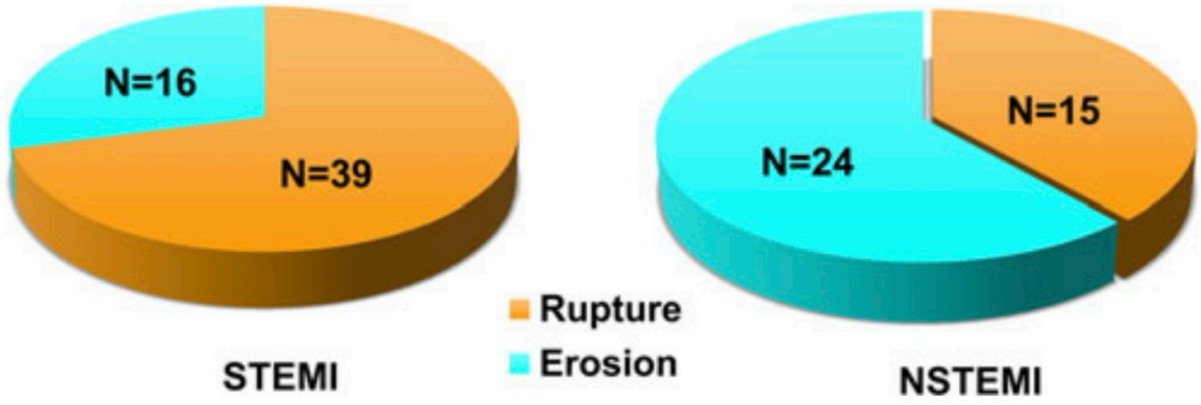


Figure 5 Association between plaque morphology and clinical presentation of acute coronary syndromes. Adapted from Libby and Pasterkamp, Eur Heart J. 2015.

provides that plaque erosion is more frequently associated with NSTEMI than STEMI (7,8,20–24). Moreover, clinical studies show that patients with plaque erosion present lower total cholesterol, triglycerides, low-density lipoprotein and C-reactive protein (CRP) than patient with plaque rupture. These laboratory differences may be related to a lower pancoronary inflammation and lower lipid accumulation (20).

Moreover, these findings have important therapeutic implications. Scientists have shown that patients with plaque erosion presenting with STEMI and without significant stenosis can be treated with medical therapies with outcomes to those patients treated with traditional stenting.

Moreover, studies based on coronary imaging, like EROSION, show that patients with ACS due to OCT-confirmed plaque erosion, TIMI 3 and residual stenosis <70% can be treated

with medical therapy (based on DAPT and thrombus-aspiration) without stent implantation. Alternative approach with drug-coated balloons could represent a potential future strategy. Future perspectives using clinical or laboratory findings (currently unknown) approaches integrating imaging techniques such as CTA to exclude significant stenosis may lead to medical treatment for these patients (7,8,20–24).

Altogether, these features highlight the need for a tailored approach in the management of erosion plaques.

1.2.3 ERUPTIVE CALCIFIED NODULE

Eruptive calcified nodules account for approximately 10% of acute coronary syndromes (ACS), more frequently in patients with chronic kidney disease, diabetes, hypertension and

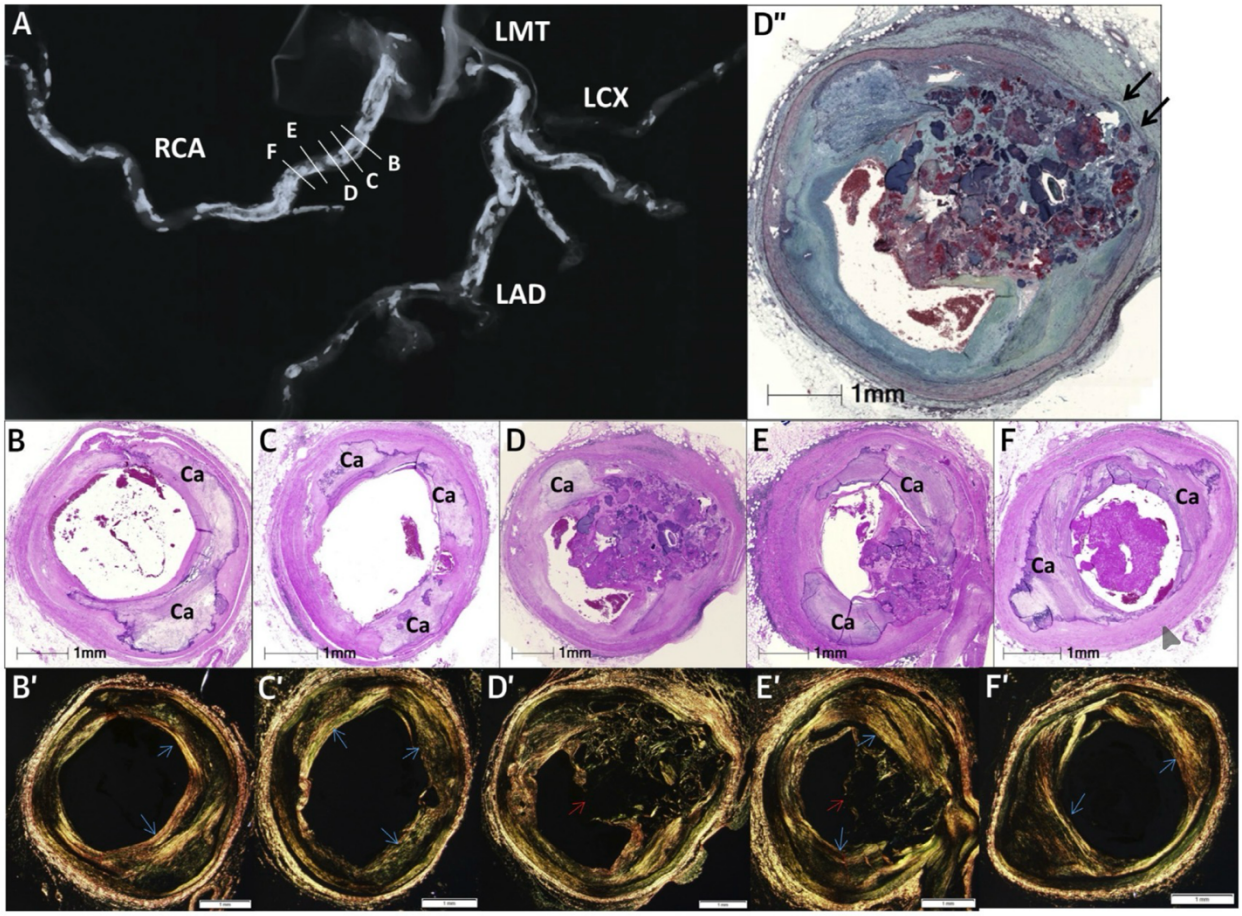


Figure 6 Plaque classification algorithm based on optical coherence tomography (OCT). Culprit lesions were classified as plaque rupture, OCT-defined erosion, OCT-defined calcified nodule, or other causes according to fibrous cap integrity, thrombus presence, and

chronically elevated levels of creatinine. These lesions are characterised by protruding nodular calcifications, fibrous cap disruption, overlying thrombus and endothelial loss. The underlying pathophysiology differs from plaque rupture and plaque erosion as it is primarily related to fragmentation of calcified nodules rather than the breakdown of collagen-rich structures. In this setting, disruption is mainly driven by hemodynamic factors that cause fragmentation of calcified deposits with core protrusion into the lumen and disruption of fibrous cap.

The spectrum of vascular calcifications is highly heterogeneous; calcification composed of collagen-rich matrix tend to confer greater structural stability than those with a lack of collagen support. This suggests that plaque vulnerability in calcified lesions depends more on the type and origin of calcium rather than on the total calcium burden.

Histologically, precursor lesions are typically plaques with calcified core adjacent to collagen-rich area. Moreover, these lesions are commonly located in the proximal to mid segments of the right coronary artery or at coronary bifurcations, where hinge motion and haemodynamic mechanical stress are more pronounced. A proposed mechanism of calcified eruption is the coronary torsion of vessels during the cardiac cycle.

Eruptive calcified nodules are typically associated with luminal fibrin-rich thrombus with calcified materials and platelets protruding through the fibrous cap as also confirmed by OCT analysis (8,25).

1.3 PLAQUE HEALING

Plaque healing is a complex process that occurs after acute plaque disruption, leading to repairing of plaque morphology through histological changes. It is important to distinguish between plaque healing and plaque stabilisation. Plaque healing occurs after plaque disruption, whereas plaque stabilization refers to the transformation of plaque from lipid to a more fibrotic and necrotic phenotype. The process of plaque healing consists of three consecutive phases.

Initially, thrombus formation and platelet accumulation is stimulated by the exposure of thrombogenic plaque factors like necrotic core, collagen, and tissue factors. At the same time, endogenous fibrinolysis system, activated by endothelial cells, is activated. Molecular studies show that endogenous fibrinolytic system composed of tissue plasminogen activator and urokinase plasminogen activator from endothelial cells, elastase and cathepsin G from neutrophils and monocytes are the main mediators responsible for thrombus lysis.

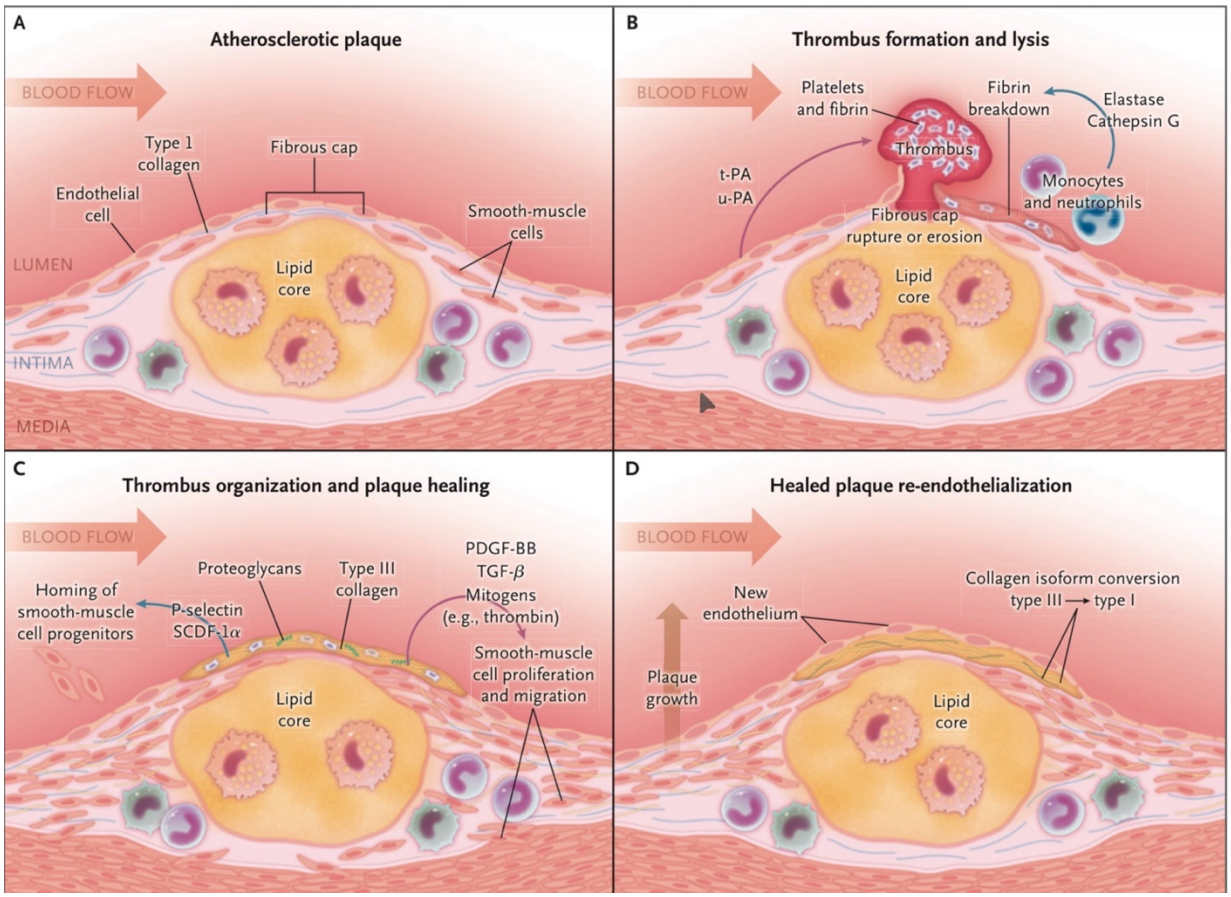


Figure 7 Vergallo R, Crea F. Atherosclerotic plaque healing. N Engl J Med. 2020

After thrombus formation and lysis, the granulation phase begins. Here smooth muscle cells play a key role. Platelet-derived growth factors (like TGF-beta) stimulate smooth-muscle cells migrate from media to the intima. The migration of these cells also recruits circulation

progenitor cells from bone marrow by the expression of arterial wall's mediator. In this setting these cells contribute to the formation of a tissue full of proteoglycans and type III collagen, replaced when plaque healing is completed by type I collagen and endothelium.

Plaque healing does not occur in all patients with ACS. Percentage of plaque healing are highly variable, ranging from 5 to 80%, depending on factors such as clinical manifestations and vessel's characteristics. Studies suggest that plaque healing is more frequently observed in patients with stable coronary syndromes than in those presenting with acute manifestation. OCT studies have shown that plaque healing, including different histological manifestation of ACS, is characterized by the presence of multi-layer structures (''onion-like'' appearance) with a core rich of lipid and progressive deposition of two production collagen type I and III. This pattern reflects repeated cycles of thrombus formation, organization and progress collagen deposition.

From a clinical and imaging perspective, identifying these features is crucial for understanding the mechanisms of plaque healing and for improving risk stratification in patients with coronary artery disease (26).

1.3.1 INFLAMMATION: A KEY DRIVER OF PLAQUE HEALING

Inflammation plays an important role in atherosclerosis both promoting plaque destabilization and modulating healing processes. Studies show that the presence of T helper 1 (Th1) lymphocytes, particularly through the secretion of interferon-γ, promote damaging of collagen created by vascular smooth muscle. This process is enhanced by cross-talking between CD40 ligand expressed by M1 macrophages and Th1 lymphocytes. This interaction enhances production of collagenases (like metalloproteinases-1, -8, -13) promoting collagen breakdown (26).

Conversely, plaque stabilisation is promoted by anti-inflammatory pathways involving M2 macrophages. In this context, regulatory T-reg cells also contribute in modulating the inflammatory response and promoting tissue repair, and their expansion has been proposed as a potential therapeutic strategy in ischemic heart disease (27).

This anti-inflammatory environment promotes tissue repair and extracellular matrix deposition due to the expression of receptors such as scavenger, mannose, galactose receptors and the release of pro-fibrotic factors (such as fibronectin, IGF-1 and TGF-beta).

In conclusion, the main factor that determinates plaque healing is macrophage phenotypic plasticity, regulating the balance between pro-inflammatory and pro-resolving pathways. This balance plays a crucial role determining whether plaque healing is effective or leads to persistent instability.

These inflammatory pathways may influence the imaging characteristics of plaque healing and may represent potential clinical predictors of plaque evolution (26).

1.3.2 FIRST AND DOUBLE HIT THEORY: A NEW LANDSCAPE FOR PLAQUE STABILITY

In vivo and OCT studies have shown that plaque disruption does not invariably result in an acute coronary syndrome. According to the single-hit model, plaque disruption is followed by an effective healing response, resulting in plaque stabilization and clinical silence. In contrast, the double-hit hypothesis proposes that plaque disruption alone is not sufficient to trigger an acute coronary syndrome; rather, an additional failure of thrombus resolution and plaque healing is required. Following plaque rupture or erosion, the clinical outcome depends on the effectiveness of the healing response. Effective healing may contain the thrombotic process and promote plaque stabilization. However, repeated cycles of subclinical plaque disruption and healing may contribute to plaque progression, increasing plaque burden and luminal narrowing, eventually leading to chronic coronary syndrome (CCS). Conversely, ineffective healing associated with persistent thrombosis may result in plaque instability and recurrent acute coronary syndromes (ACS) (26).
Studies have shown that prevalence of healed plaque is higher in segments with higher rate of stenosis, meaning the initiation of healing process after acute events occur. Moreover, OCT studies comparing patients with recurrent ACS with groups of patients with stable angina presentation and stable clinical history after AMI linked the prevalence of plaque healing in chronic coronary syndrome showing that was lower in ACS patients (16). This demonstrate that healing process is more common in patients with stable angina than unstable phenotype. Although healed plaques are often associated with a more stable clinical presentation, their presence reflects repeated subclinical plaque disruption and repair and has been linked to accelerated plaque progression and increasing plaque burden over time (28).

Furthermore, recent studies have demonstrated that healed plaque could have a higher chance to have NSTEMI presentation than STEMI. This dual behaviour suggests that plaque healing should not be interpreted solely as a stabilizing mechanism, but also as a marker of an active and progressive atherosclerotic disease process (28,29).

The localisation of healing process is more common on the proximal area of left anterior descending artery (LAD) and left circumflex artery (LCx) for the left coronary artery while diffuse localization of this process occurs in right coronary artery (16,26,29).

The reason why some patients stabilized plaques and other no is unknown. Studies hypnotised that the reason is a lack of balance between prothrombotic and thrombosis-resisting factors. This is demonstrated by a study that analyse patients undergoing PCI; AMI patients had higher platelet fibrin clot strength, lead to a hypercoagulability stadium and lower activity of fibrinolytic factor compared to patient without AMI (30).

Literature shows that the enhancement of healing process could be helped by drug therapy. The use of statin could promote healing via pleiotropic effects reducing platelet aggregation and production of thromboxane having effects on vascular smooth cells. Moreover, PCSK9 and P2Y12 inhibitors are linked to higher percentage of healed plaque due to reduction of platelets aggregations. Recent evidence from the PACMAN-AMI trial demonstrated that early and intensive lipid-lowering therapy with PCSK9 inhibitors induces significant changes in coronary plaque characteristics, including reduction in plaque volume, decrease in lipid content, and increase in fibrous cap thickness, as assessed by multimodality intracoronary imaging (31).

Ant inflammatory drugs like TNF-beta inhibitors, genetic therapy or colchicine may be take advantage having an anti-inflammatory role but studies do not demonstrate the real effect of these drugs, considering this only a hypothesis. Moreover, recent evidence demonstrates that SGLT2 inhibitor, 17beta-estradiol and tamoxifen influence plaque healing process (26,28).

1.4 CORONARY ANGIOGRAPHY: PRINCIPLES AND CLINICAL APPLICATION

Coronary angiography is a commonly used invasive imaging technique, introduced in 1958 by Dr. Mason Sones, that remains the gold standard for the evaluation of coronary artery disease (CAD) despite the development of newer technique like IVUS, OCT, MRI. This procedure provides essential information on the location, the severity of coronary lesions and the extent of collateral circulation. However, despite these advantages, like any other procedure is not free of limits and complications.

Angiography is performed through arterial access, most commonly through the femoral or radial artery, and followed by selective catheterization of the coronary ostium using dedicated catheters.

Judkins or Amplatz catheters are commonly used to access coronary artery. Once the catheter is positioned, iodinated contrast medium is injected into the coronary arteries, allowing visualization of the vessel lumen under fluoroscopy.

Coronary angiography provides a two-dimensional representation of a three-dimensional vascular structure. For this reason, accurate assessment requires acquisition of multiple projections to properly evaluate coronary anatomy and lesion characteristics.

Standard angiographic views include right anterior oblique (RAO) and left anterior oblique (LAO) which visualize coronary artery from different angles, directed over the patient, and caudal and cranial view. In caudal view the image is capture towards the feet making this the best view to see left anterior descending artery (LAD) and its diagonal branches. The cranial view captures images from the head displaying circumflex artery and left main coronary artery (LMCA).

Angiographic interpretation requires a systematic evaluation of several lesion characteristics. These include:

- location of stenosis (proximal, mid, distal, or ostial),
- lesion length,

- the presence of diffuse or single disease.

Stenosis's length and diffusion have higher risk of ischemia than focal localised stenosis and are associated with more complex PCI procedure and use of longer stent (complicated by a higher risk of stenosis and restenosis). Moreover, it is important to look if the lesion involve ostium or not and the coronary dominance. Lesion morphology is also assessed, distinguishing between concentric, eccentric, smooth, irregular, or ulcerated plaques.

The severity of coronary stenosis is typically classified based on luminal diameter reduction, with lesions categorized as mild (<50%), moderate (50–70%), or severe (≥70%). Total occlusion corresponds to complete absence of flow and chronic total occlusion is documented as an absence of flow of at least three months. It's important to note that the reduction in cross-sectional area is not linearly related to the reduction in lumen diameter, due to underestimation of the second one (es. 50% diameter or luminal stenosis correspond to 75%).

Not always stenosis rate corresponds linearly to a haemodynamic instability, in fact the relationship is not linear. For this reason, cardiologist use physiological methods such as fractional flow reserve (FFR) and instantaneous wave-free ratio (iFR) and TIMI SCORE, often used to determine hemodynamical effects.

FFR is defined as the ratio of the distal stenosis pressure and aortic pressure during pharmacologically induced maximal hyperaemia. iFR measures the resting pressure gradient across a coronary lesion during mid-diastole without the need for pharmacological hyperaemia. PCI procedure is indicated if the iFR ≤ 0.89 or the FFR ≤ 0.80 giving better indications to revascularization procedures.

Moreover, coronary angiography allows assessment of coronary flow using the TIMI (Thrombolysis in Myocardial Infarction) grading system. This grading system stratify from TIMI 0 (no flow) to TIMI 3 (normal flow).

This scores and basic angiography interpretation give cardiologist a panoramic of stenosis rate, localization and functional information.

In this sense, angiography identifies many patterns of coronary disease. Thrombus is seen as intraluminal filling defect separated from adjacent wall. Calcification vessels could be seen also without injection of contrast, characteristics associated with complex PCI procedure and the use of specific adjunctive devices. Moreover, angiography could give information about coronary bridge, vessel portion that cross myocardium showing flow only in diastole, and collateral vessels that occur in CCS and vanish after PCI procedure.

Despite its central role, coronary angiography has inherent limitations. It provides only a luminal view of the coronary arteries and does not visualize the vessel wall or plaque composition. Furthermore, because it a two-dimensional representation of a three-dimensional structure, accurate interpretation depends on multiple projections and operator expertise.

The risk of angiography procedures occurs in less than 2% of cases. This includes:

- Local vascular injury like haematoma, pseudo-aneurysm, dissection and arteriovenous fistula,
- Complication due to the use of contrast like allergy, adverse reaction, contrast-induced nephropathy,
- Cholesterol emboli,
- Cerebrovascular accidents,
- Myocardial infarction,
- Cardiac tamponade,
- Mortality (32).

Beyond the classification of coronary stenosis, angiographic evaluation also requires an analysis of lesion stenosis, anatomical complexity and morphological features. For this reason, many classifications have been made to stratified lesion severity and procedural planning.

1.4.1 VISUAL ASSESTMENT OF CORONARY STENOSIS:

The first approach of an angiographic analysis is the visual appearance of coronary artery. In the visual analysis the cardiology defined the severity of a stenosis by the reduction of

lumen diameter. As the first guidelines from 1988 said, a stenosis is declared as significant if it has the potential of compromise coronary flow under physiological circumstance.

The real limit of this analysis stands with interobserver and intraobserver variability (33).

1.4.2 ACC/AHA LESION CLASSIFICATION:

The success of an angiographic procedure is determinate by operatore experience, anamnestic patient characteristics and morphological features of vessel and lesions. To stratify procedural success and complexity procedure, AHA/ACC created a classification based on morphological characteristics of vessel and lesion-specific anatomical aspects.

The classification stratify lesion into three categories to estimate rate of success or abruption:

- TYPE A LESION: this are easy to access, concentrity and discreteness lesions not located in a main branch and in a non-angulated segment. This lesion is composed of regular contour, no calcifications, absence of thrombus and total occlusion. The rate of success of procedure is > 85% and the risk of vessel closure is low.
- TYPE B LESION: this category includes lesions located in moderate tortuosity vessel with an angle > 45° but < 90°. These are eccentric lesions with a tubular shape, irregular contour, presence of thrombus and moderate calcification. Moreover, they can be in ostial area, bifurcations (requiring a double wire) or total occluded for a period inferior of 3 moths. The success of procedure in lesions with these characteristics have a range between 60 and 85% but the risk of vessel abruption remains low.
- TYPE C LESION: these types of lesions are the most complex having a diffuse length (>2 cm), excessive tortuosity and located in angulated segments (>90%). Moreover, these lesions are characterized by chronical obstructions (> 3 months), located near degenerated older graft and in areas where is not possible to protect major side branch (33).

1.4.3 AMBROSE CLASSIFICATION:

In 80s, Ambrose and his colleagues observed that only the degree of luminal narrowing was insufficient to correlate with clinical presentations of coronary artery disease. They observed

that different patients with the same rate of coronary stenosis exhibit different clinical presentations like stable angina, unstable angina and myocardial infarction.

Based on qualitative angiographic analysis, coronary lesions were classified into four morphological categories:

- Concentric lesions: symmetric and smooth narrowing of the coronary lumen.
- Eccentric type I lesions: asymmetric stenosis with smooth borders and a broad neck.
- Eccentric type II lesions: asymmetric stenosis characterized by a narrow neck resulting from one or more overhanging edges, irregular borders, or a scalloped appearance.
- Multiple irregularities: presence of three or more closely spaced severe stenoses or diffuse irregularities involving the same coronary segment.

Among these morphologies, particular attention was directed toward eccentric type II lesions. In their studies, Ambrose et al. observed that this angiographic pattern was frequently present in patients with unstable angina and acute myocardial infarction, whereas it was uncommon in patients with stable angina. Moreover, in patient with recent myocardial infarction, type II lesions were observed mainly in culprit vessels than in non-infarct coronary.

This type of lesion was related to a combination of process like disrupted plaque, occlusive thrombus and lysed thrombus. This concept was totally coherent with previous post-mortem angiographic studies linking irregular lesions and acute phenomenon like plaque rupture, plaque haemorrhage or thrombotic process.

Considering this observation, Ambrose proposed that unstable angina and acute myocardial infarction might represent different manifestations of a common pathophysiological process and a possible continuum between unstable angina and myocardial infarction linking this to imbalance of oxygen supply and demand.

Furthermore, this study highlights that plaque morphology, and angiographic aspects provide more important clinical and prognostic information beyond analysis based on coronary stenosis (34,35).

1.5 OPTICAL COHERENCE TOMOGRAPHY: PRINCIPLES, BASIC CONCEPT AND CLINICAL INDICATIONS

Optical coherence tomography (OCT) is an intravascular imaging modality based on near-infrared light and interferometry, allowing high-resolution visualization of coronary artery microstructures. Similarly to intravascular ultrasound (IVUS), OCT produces cross-sectional images of the vessel wall using backscattered light rather than acoustic waves.

One of the main characteristics of OCT that has led to his widespread is superior spatial resolution, approximately 10–15 μm, which is nearly ten times higher than that of IVUS. This allows detailed assessment of superficial plaque components such as fibrous cap thickness, lipid pools, and intracoronary thrombus. However, OCT has a limited tissue penetration (1–2 mm) which is lower than other imaging tools like IVUS that can go deeper through blood and tissue (2mm vs 1 cm). The main advantage of OCT versus other technique is the high resolution leading to furthered details of stent and plaque characteristics (36).

Optical coherence tomography (OCT) allows detailed characterization of plaque morphology according to established imaging criteria. For example, plaque erosion may be categorized as definite or probable. Definite OCT-defined erosion is identified by the presence of luminal thrombus overlying an intact and visualized plaque surface. Probable erosion is defined by luminal surface irregularities in the absence of thrombus, or by attenuation of the underlying plaque caused by thrombus without evidence of superficial lipid or calcification adjacent to the lesion. These imaging criteria differ from the pathological definition of erosion, which requires attached thrombus, as thrombus dissolution may occur prior to imaging in patients treated with antithrombotic therapy (37).

OCT image acquisition is based on sequential axial scans (A-scans), which are combined to produce a two-dimensional (B-scan) image. OCT can produce hundreds of B-scan per second allowing a three-dimensional reconstruction.

OCT works by rotating the catheter with an automated pullback system that allows the capture of images along the length of the vessel. The catheter traces a helical pattern collecting data from rotation transforming the data to polar coordinates.

Unlike ultrasound, which directly measures the transit time of acoustic waves, OCT determines the depth of tissue reflectors by analysing the interference pattern of light. This

is achieved by an interferometric setup, in which a light beam is split into a reference arm and a sample arm. Light from the sample and reflected light from the reference arm mirror are recombined, generating interference that results in fringes across the detected spectrum. The frequency of these fringes is proportional to the optical path length difference which encodes the depth of the reflecting structures, with higher fringe frequencies corresponding to deeper reflectors. A catheter for OCT is composed of a single optical fibre followed by a lens and a micro prism to direct light, used with a rotation movement.

A key technical limitation of OCT is inability of infrared light to penetrate blood. Unlike IVUS, OCT require clearing of blood from lumen. For this reason, OCT can be done with two techniques. The first one is the occlusive technique that need a proximal occlusion done by a balloon and the flush of Ringer's lactate through the end-hole of the balloon catheter. The non-occlusive technique has been developed later and does not require balloon occlusion, using a standard intra coronary guide performing pull-back at the highest available speed during simultaneous infusion through the guide of viscous iso-osmolar solutions (36).

Moreover, OCT, due to low crossing profile, can cross tight lesion that IVUS may be unable to cross (38).

1.5.1 INTRAVASCULAR ULTRASOUND: PRINCIPLES AND LIMITS

IVUS is an imaging modality developed in the early 1970s, with its first use in vivo use described in 1988. IVUS is a sound-base imaging technique that provides real-time 360° vessel visualisation. Ultrasounds waves are generated by a piezoelectric crystalline mounted on a catheter which reflect differently depending on vessel characteristics. Fibrous tissue and calcification produce hyper echogenic signals; whereas lipid-rich areas produce hypoechogenic signals, resultin in grayscale imaging. The spatial resolution of IVUS depends on two factors: wavelength and beam-width and is approximately 70 μm. Penetration depth is determinate by frequency, higher frequency result in reduce penetration. IVUS can analyse plaque characteristics but its sensitivity for TCFA is lower than of OCT (37).

1.5.2 OCT VERSUS IVUS IN CORONARY IMAGING: STRENGTHS, LIMITATIONS AND CLINICAL IMPLICATIONS

The main advantages of IVUS lie in several aspects. First, IVUS does not require contrast agents, which may be contraindicated in some patients, like whom with chronic kidney disease (CKD). Moreover, IVUS is advantageous for imaging in ostial lesion (for admixture of blood with contrasts), small or big vessels and in severe stenoses or chronic total occlusion (CTO) revasculazation for risk of lumen pressurisation or suboptimal contrast opacification. Moreover, IVUS could bring advantages allowing deeper visualization of the vessel wall, specifically in the left coronary artery or for studying plaque burden at stent edge.

On the other hand, OCT offers some advantages in areas that IVUS could not detect. First of all, OCT provides superior resolution for the assessment of fibrous cap, intima and media better than IVUS due to its higher spatial resolution. Moreover, OCT is used in detection of precise location of side branch, evaluation of vulnerable plaque, TCFA, guidewire position, stent visualisation (including detection on neoatherosclerotic and restenotic in-stent lesions). In add, OCT could be used also to detect erupted calcified nodule due to attenuation of IVUS.

Intracoronary imaging has demonstrated benefits over PCI in many studies although evidence and strength are different. After the introduction of DES this has been harder to demonstrate but strong evidence come from recent studies. Trials like ULTIMATE, IVUS-XPL andCTO-IVUS have demonstrate reduction in MACE and TLR with the use of IVUS compared with angiography alone.

OCT procedure was mainly use for procedure studies. DOCTORS, ILUMIEN III and IV demonstrate that guide-OCT-PCI has lower rates of MACE and TLR. Other recent studies detect IVUS-guide-PCI and OCT-guide-PCI demonstrate the same outcomes (37,39).

1.5.3 OCT VERSUS IVUS IN PERCUTANEOUS CORONARY INTERVENTION: PRE, INTRA AND POST-PROCEDURAL APPLICATIONS

These techniques have the potential to improve procedural efficiency, outcome and cost-effectiveness. Nowadays this strategy has indications for preintervention lesion, periprocedural guidance, stent deployment strategies and post-PCI for excluding complication of stent failure and stenosis.

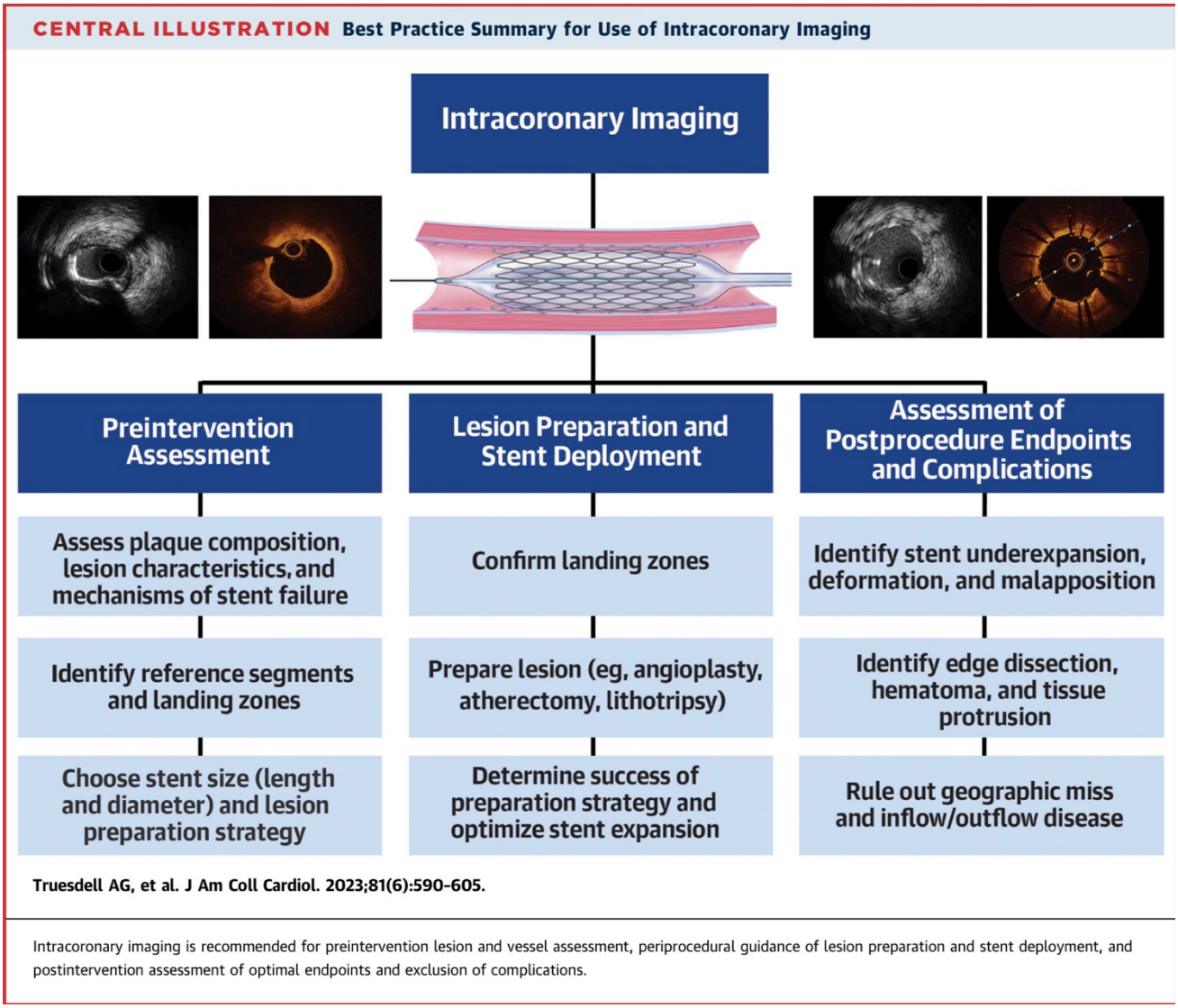


Figure 8 Best practice summary for use of intracoronary imaging. Adapted from Truesdell et al., J Am Coll Cardiol 2023;81:590–605

These instruments may be used to analyse plaque characteristics, study vessels dimensions and decide stent length and width to avoid undersizing. Moreover, IVUS and OCT could be used in coronary disease like plaque haemorrhage, aneurysm, aorto-ostial lesions, intramural hematoma and dissection and in clinical disease like Takotsubo or Myocarditis to orient clinical choice before stenting. During procedures, imaging may be used to decide length and width of stent detecting malapposition and under covered areas.

In complex procedures, this tools are used to analyse stent deformations, bifurcation of left coronary and complicated procedure such as kissing balloon, side branch coverage and bifurcation interventions (37).

Overall, the integration of intracoronary imaging into PCI has significantly improved procedural precision and clinical outcomes, supporting a more tailored and mechanism-based approach to coronary artery disease

1.6 OCT ANALISIS OF PLAQUE CHARACTERISTICS AND PREVALENCE LINKED TO PATIENT CLINICAL PRESENTATIONS AND OUTCOMES

OCT imaging can identify plaque characteristics and provide information about what plaques are composed (18).

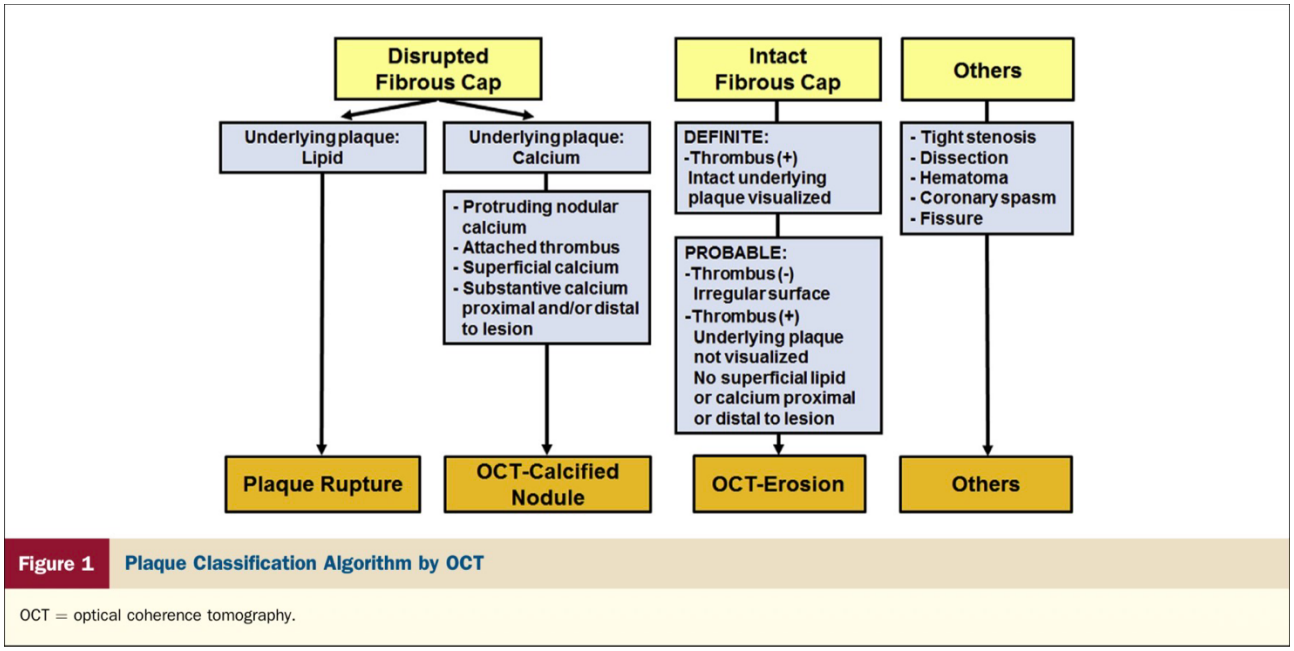


Figure 9 Plaque classification algorithm based on optical coherence tomography (OCT). Culprit lesions were classified as plaque rupture, OCT-defined erosion, OCT-defined calcified nodule, or other causes according to fibrous cap integrity, thrombus presence, and

Normal vessels appear in OCT as a three-layer structure, composed of a dark band (the medium layer) between internal elastic lamina (IEL) and external elastic lamina (EEL) that generate a signal-rich band of 20 μm (38).

In OCT a plaque is defined as a region with loss of the normal three-layer structure (intima, media and adventitia) of the vessel wall.

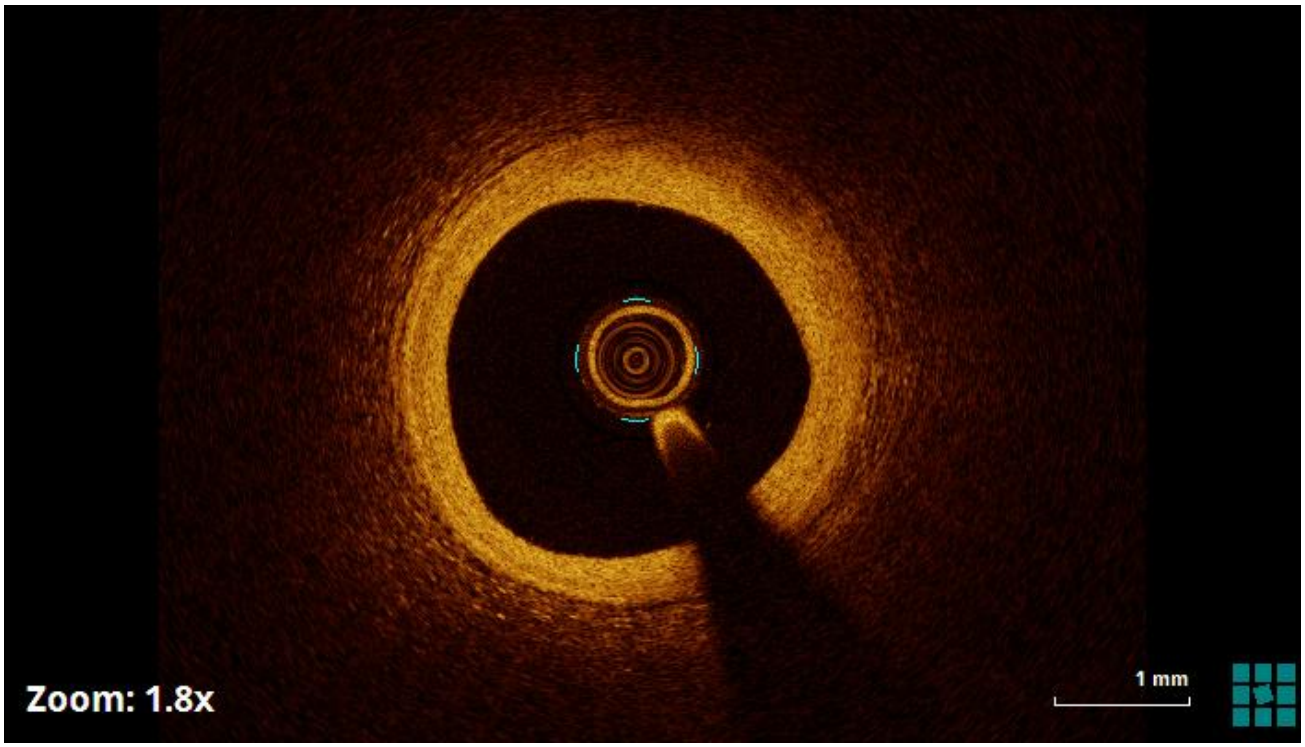


Figure 10 This photo represents a normal vessel with a fibrous layer

Them earliest detectable change that OCT could visualize is diffuse thickening of intima, a common process of adult different from atherosclerosis that appear as bright, homogeneous, with a texture like fibrous plaque (38).

Based on OCT findings, plaques can be classified into different pathological phenotypes. Plaque rupture is defined by fibrous cap discontinuity associated with the presence of an intraplaque cavity communicating with the lumen occurring with or without the presence of a thrombus. When rupture or ulceration appear without sign of thrombosis, the lesion cannot

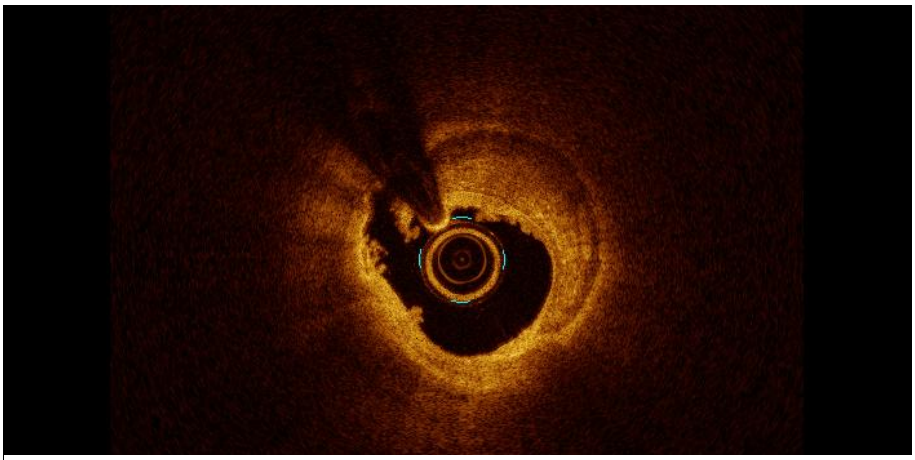


Figure 11 This OCT photos captured a plaque rupture and the presence of mixed thrombus. In the same image it is possible to see a plaque haemorrhage.

be identified as a culprit lesion unless clinical criteria that provide is responsible for acute events(38).

Plaque erosion on OCT is defined by the absence of fibrous cap disruption and the presence of a thrombus.

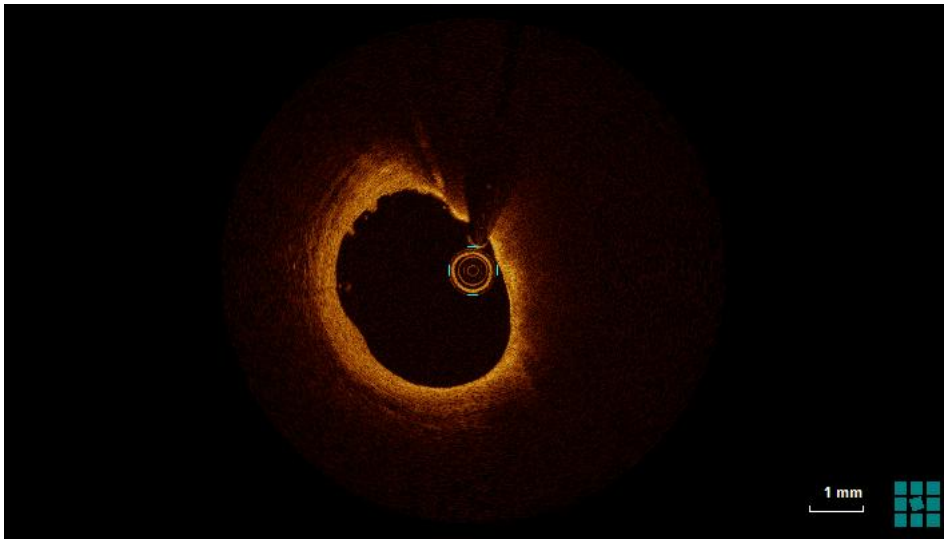


Figure 12 In this it is possible to see plaque erosion with thrombus on the endothelium layer

Calcified nodules are identified when cap disruption occurs over a calcified plaque with protruding or superficial calcium and substantial calcification in the surrounding segments.

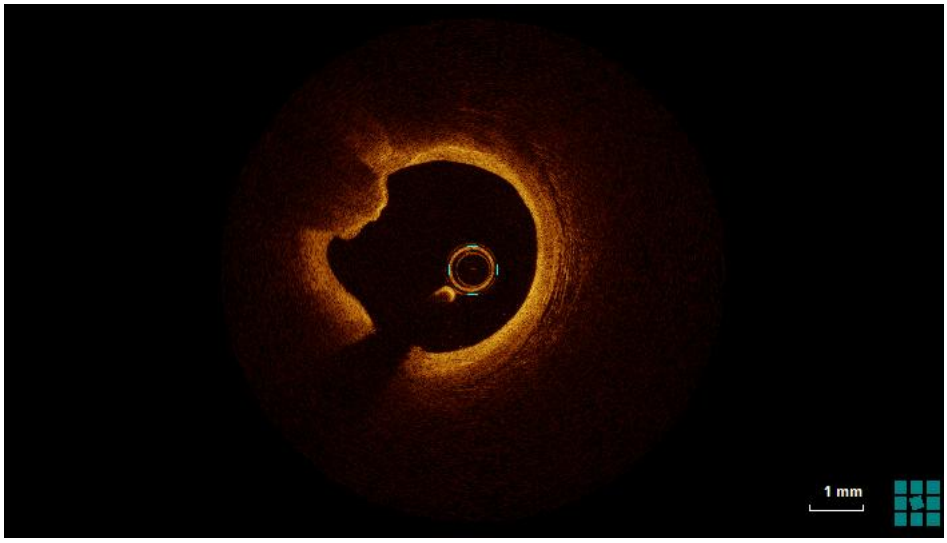


Figure 13 This photo represent a calcified nodule protruding into the lumen

In addition to plaque phenotype, OCT allows characterization of plaque composition.

With this technique plaques could be classified as fibrous, appearing as homogeneous regions with high backscattering, or lipid-rich, defined as diffuse signal-poor regions with diffuse borders and an overlying signal-rich band. In lipid plaques, the maximal lipid arc and longitudinal lipid length can be measured. A lipid rich plaque is defined when lipid arc is >90° (8,16,18).

Among these features, thin cap fibroatheroma (TCFA) represents the most clinically relevant high-risk phenotype. A thin-cap fibroatheroma (TCFA) is traditionally defined as a lipid-rich plaque, involving at least two quadrants, with a fibrous cap thickness <65 μm. Community do not consider the extension of plaque but only the histology-derived threshold of 65 μm (moreover scientists include 70,75 and 80 μm). Fibrous plaque describes as homogeneous high-back scattering signal. OCT represent the main technique of imaging which could be used to investigate TCFA plaque (8,16,18).

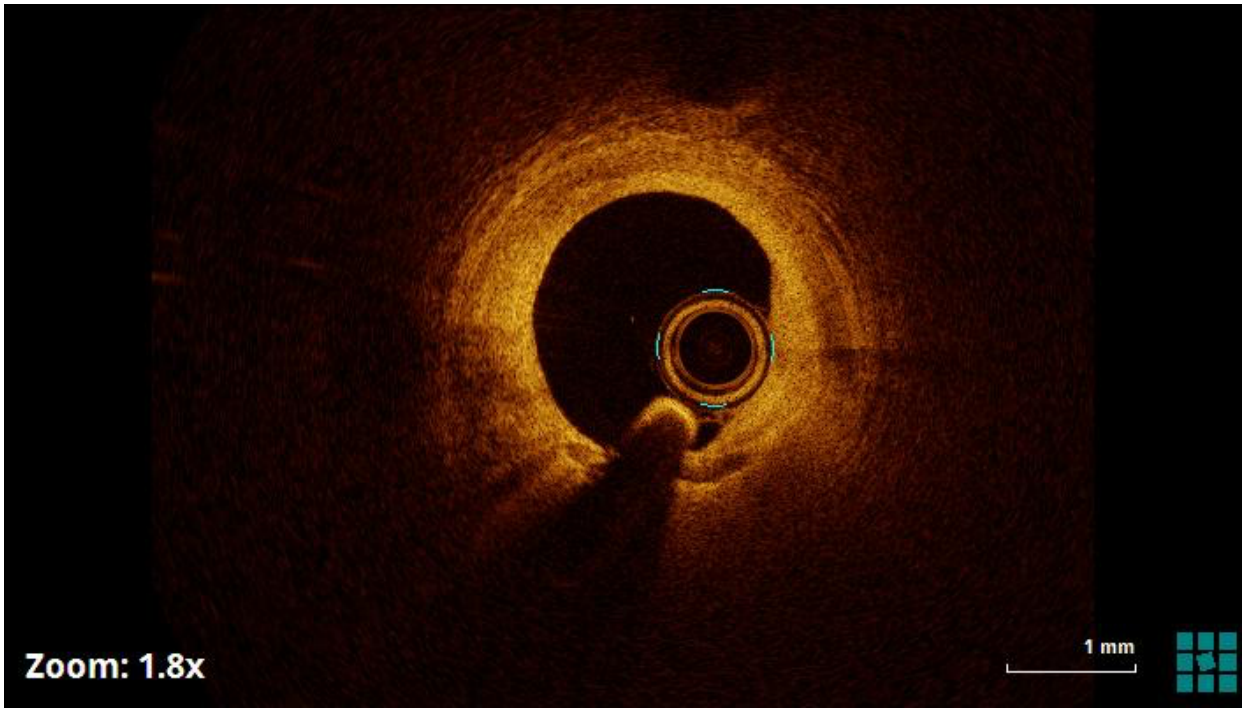


Figure 14 This OCT photo represent a healed plaque. It is possible to understand this phenotype because this plaque protrudes into the lumen and it has an onion-layer phenotype

Healed plaques are identified as one or more heterogenous signal layer located to luminal surface and distinct from underlying tissue.

OCT include additional features. Calcifications areas are defined as sharply delineated regions with heterogeneous or signal-poor area with low-back-scattering. If areas are less

than 4 mm are considered spotty calcifications. By a semi-quantitative grading, calcium can be classified according to the number of quadrants it is visualized (38).

Moreover, macrophage areas are defined as spotty-bright areas creating a backward shadowing (16).

Intracoronary thrombus is identified as a mass (>250 μm) attached to the luminal surface or floating within the lumen and may be categorized as red thrombus, characterized by high backscattering and strong signal attenuation due to erythrocyte content, or white thrombus, which appears as a homogeneous structure with low attenuation and is predominantly platelet rich. Additional OCT features include calcification, appearing as signal-poor regions with sharply delineated borders, and microchannels, defined as signal-poor voids visible across multiple contiguous frames (18).

Furthermore, plaque dissection is a common finding specifically with plaque rupture, appearing as a rim of tissue protruding into the lumen (38).

Beyond morphological assessment, OCT findings can be correlated with clinical presentation and outcomes.

Recent studies using OCT technique in vivo demonstrated the linkage between low endothelial shear stress areas and plaque characteristics. This analysis proves that in areas with low endothelial shear stress there is an higher prevalence of TCFA, lipid-rich plaque and higher macrophage density (40).

Contemporary studies linked OCT characteristics with clinical feature explaining clinical and prognosis differences. A comparison between three groups patients composed of ACS recurrent patients, single IMA and patients with stable presentations shows that the prevalence of TCFA plaque, spotty calcifications and macrophage accumulation is higher in whom with recurrent ACS than the other two groups. Moreover, phenomenon of plaque healing and fibrous plaque are higher in patients with stable presentation and after a single IMA (16). Moreover, OCT studies analysing non culprit lesion between patients with plaque rupture and plaque erosion shows that in the first group of patients the prevalence of TCFA, lipid-rich plaque and plaque extension is higher. In add this study shows that pancoronary destabilisation of no-culprit plaque was higher in patient undergoing to plaque rupture presentations, preferentially located in infarct-related artery (41). This information add to information taken from PROSPECT study showing that TCFA plaque are the higher risk

phenotype associated with major cardiovascular events demonstrating that drug therapy and marker to identification markers are necessary to change clinical prognosis(12,41).

2 THE PURPOSE OF THE STUDY:

Over the years, several studies have focused on coronary plaque characteristics, investigating plaque phenotype, vulnerability, and the relationship between clinical and imaging findings. More recently, increasing attention has been directed toward plaque healing, highlighting its potential role in the biological processes underlying plaque stabilization and disease progression (26).

The evolution of vulnerable plaques and the healing phenomenon are multifactorial. Previous studies have shown a higher prevalence of healed plaques in patients with long-term clinical stability compared with those experiencing recurrent acute coronary syndromes, suggesting a possible association between plaque healing and a more stable clinical course (16). Furthermore, plaque healing appears to be influenced by several factors, including inflammatory and biological mechanisms, clinical characteristics, and pharmacological therapies, and may reflect the vascular response to repeated episodes of plaque destabilization and repair (26).

Optical coherence tomography (OCT) is currently considerate as the most accurate intracoronary imaging modality for the identification and characterization of healed plaques, allowing the correlation of specific morphological features with patients' clinical profiles (16). Such detailed assessment cannot be achieved using angiography alone, which provides limited information on plaque composition and healing patterns (34,35).

Therefore, the aim of the present study is to evaluate clinical, angiographic, and OCT-derived characteristics associated with coronary plaque healing and to identify potential predictors of this phenomenon.

3 MATERIALS AND METHODS:

3.1 STUDY PARTICIPANTS:

Clinical and imaging data comes from registry of Ospedale Policlinico San Martino - Istituto di Ricovero e Cura a Carattere Scientifico (IRCSS), from this database a total of 74 patients were selected.

The patients included in this study were affected of stable presentation (including stress test, coro CT, stable angina and elective PCI) and unstable presentation (including STEMI, NSTEMI and unstable angina) analysed with angiography and OCT imaging. A total of 74 patients were divided into two groups:

Group 1 – 33 patients having evidence of at least one healed plaque

Group 2 – 41 patients showing any evidence of plaque healing.

In these patients were analysed 1272 OCT run and culprit lesion with angiography.

3.2 CLINICAL DATA:

Via Information System of Policlinico San Martino Hospital - Istituto di Ricovero e Cura a Carattere Scientifico (IRCSS), a series of bio-humoral data and drug therapy were collected for each patient.

The blood analysis include: Hemoglobin (g/dL), Platelets (n/µL), White Blood Cells (n/µL), Creatinine (mg/dL), eGFR (CG), Low-density lipoprotein (mg/dL), High density lipoprotein (mg/dL), Triglycerides (mg/dL), aPTT (sec),INR, D-dimer (µg/L), Glucose (mg/dL), HbA1c (%), TnI (ng/mL), PCR (mg/L).

This data was obtained before any angiography or OCT procedure on the patient. The study analysed drug therapy data before and after hospital access including: B-block, ACE-i, Sartans, ARNI, Statins, Ezetimibe, PCSK9-i, Inclisiran, Bempedoic Acid, ASA, Clopidogrel, Prasugrel,Ticagrelor, Heparin, Fondaparinux, NAO, VKA, Ca-channel blocke, SGLT2-i, oral hypoglycemic drugs, and Insulin).

3.3 ANGIOGRAPHY ANALISIS:

Coronary angiography was retrospectively reviewed using the angiographic images available in the institutional database. For each patient, the culprit vessel and the presence of coronary stenting were recorded. The angiographic severity of the target lesion was assessed by visual estimation and expressed as percentage diameter stenosis.

Lesion complexity was classified according to the American College of Cardiology/American Heart Association (ACC/AHA) classification into type A, B1, B2, and C lesions. In addition, lesion morphology was evaluated according to the angiographic classification proposed by Ambrose et al.,[36] categorizing lesions as concentric, eccentric type I, eccentric type II, or multiple irregularities.

All angiographic variables were collected and subsequently correlated with OCT-derived plaque characteristics to investigate the relationship between angiographic lesion morphology and plaque features identified by intracoronary imaging.

3.4 OCT ANALISIS:

OCT analysis was performed offline using registered software (LightLab Imaging) by XX independent investigators who were unaware of the clinical and laboratory data reported by the patients. In case of disagreement between observers, consensus was sought with a third investigator.

Pullbacks were obtained after intravascular administration of 200 µg nitroglycerin using an FD-OCT system (C7-XR OCT Intravascular Imaging System, St. Jude Medical, St. Paul, MN). Automatic pullbacks were acquired simultaneously with contrast injection according to blood clearance.

Analysis was performed by dividing the vessels into 3-mm segments. Only non-culprit segments were included in the study.

Atherosclerotic plaque appears on OCT as a region with loss of the typical three-layer structure (intima, media, and adventitia) that normally characterizes the vessel wall or as a mass-forming lesion.

The lipid plaque is defined as a region with blurred edges and low signal intensity superimposed on a layer of intense signal corresponding to the fibrous cap. The lipid arc was calculated for each mm, and the lipid length was calculated as the distance between the first and last section where lipids were visible in the context of the 3-mm segment. The lipid index was calculated as the product of lipid arc and lipid length. A lipid plaque with a lipid arc > 90° was considered lipid rich.

The thickness of the fibrous cap was measured three times at the thinnest point, and the average of the three measurements was calculated. If the calculated fibrous cap thickness is > 65 μm, the lipid-rich plaque is defined as TCFA (thin-cap fibroatheroma).

Fibrous plaque is defined as a region of intense and homogeneous signal at least 600 μm thick.

Pathologic intimal thickening (PIT) is defined as an intimal thickening of at least 300 μm.

Healed plaque is defined as a plaque with at least one signal-intensive heterogeneous layer with different optical properties adjacent to the luminal surface and clearly demarcated from the underlying tissue.

Calcifications are areas of hypointense or heterogeneous signal demarcated by well-defined borders. Calcifications that subtend an arc > 90° and are less than 4 mm in length are classified as spotty calcified nodules.

Macrophage accumulation is defined as the presence of bright punctate signals (bright spots), either distinct or confluent, of intense signal that exceeds background signal intensity and creates a shadow posteriorly.

Neovascularization is defined as the presence of microchannels (diameter 50-330 μm) appearing as small circular or ovoid structures (small black holes), of low signal intensity, located in the intima and without connection to the vessel lumen, present in at least 3 consecutive image segments

4 RESULTS

4.1 Baseline clinical characteristics according to plaque healing status

A total of 74 patients undergoing OCT evaluation were included in the analysis. Overall, 33 patients had evidence of at least one healed plaque, whereas 41 patients showed no evidence of plaque healing. Baseline clinical characteristics according to plaque healing status are reported in **Table 1**.

Patients with healed plaques showed similar age (66.2 ± 10.4 vs. 67.6 ± 11.0 years; p=0.582), sex distribution (male sex: 75.8% vs. 78.0%; p=0.816), and BMI (28.4 ± 5.3 vs. 26.0 ± 3.9 kg/m²; p=0.055) compared with patients without healed plaques. No significant differences were observed in traditional cardiovascular risk factors, including hypertension, current smoking, dyslipidaemia, or family history of coronary artery disease.

Conversely, diabetes mellitus was significantly less prevalent among patients with healed plaques compared with those without evidence of plaque healing (12.1% vs. 56.1%; p<0.001). Accordingly, patients with healed plaques had significantly lower glucose levels (112.0 ± 21.8 vs. 139.8 ± 53.8 mg/dL; p=0.009) and HbA1c values (5.9 ± 0.8% vs. 6.8 ± 1.6%; p=0.008).

Regarding clinical presentation, patients with healed plaques more frequently presented with chronic coronary syndrome (48.5% vs. 34.1%) and less frequently with acute coronary syndrome (51.5% vs. 65.9%), although this difference did not reach statistical significance (p=0.212). Previous cardiovascular events, including prior ACS, PCI, CABG, and stroke, were similar between groups.

Baseline cardiovascular therapies were generally balanced between groups. Patients with healed plaques had lower use of oral antidiabetic therapy (9.1% vs. 36.6%; p=0.006), while other cardiovascular medications including antiplatelet agents, statins, beta-blockers, ACE inhibitors/ARBs, insulin, and SGLT2 inhibitors did not significantly differ.

Table 1. Patient baseline characteristics

	Patients without healed plaque (n=41)	Patients with healed plaque (n=33)	P value
Age, years	67.6 ± 11.0	66.2 ± 10.4	0.582
Male sex	32 (78.0%)	25 (75.8%)	0.816
BMI, kg/m^2	26.0 ± 3.9	28.4 ± 5.3	0.055
Hypertension	30 (73.2%)	25 (75.8%)	0.800
Current smoking	13 (31.7%)	8 (24.2%)	0.479
Dyslipidemia	22 (53.7%)	16 (48.5%)	0.658
Diabetes mellitus	23 (56.1%)	4 (12.1%)	<0.001
Family history of CAD	12 (29.3%)	9 (27.3%)	0.850
Clinical presentation			
CCS	14 (34.1%)	16 (48.5%)	0.212
ACS	27 (65.9%)	17 (51.5%)	
Prior ACS	16 (39.0%)	7 (21.2%)	0.100
Prior stroke	2 (4.9%)	0 (0.0%)	0.198
Prior PCI	17 (41.5%)	9 (27.3%)	0.204
Prior CABG	2 (4.9%)	1 (3.0%)	0.268
Creatinine, mg/dl	1.37 ± 1.42	1.05 ± 0.59	0.223
Glucose, mg/dl	139.8 ± 53.8	112.0 ± 21.8	0.009
HbA1c, %	6.8 ± 1.6	5.9 ± 0.8	0.008
Medications on admission			
Aspirin	18 (43.9%)	14 (42.4%)	0.898
P2Y12i	8 (19.5%)	9 (27.3%)	0.430
Statin	21 (51.2%)	14 (42.4%)	0.451
Beta-blocker	19 (46.3%)	15 (45.5%)	0.939
ACEi/ARBs	24 (58.5%)	14 (42.4%)	0.168
Oral antidiabetic drugs	15 (36.6%)	3 (9.1%)	0.006
Insulin	9 (22.0%)	2 (6.1%)	0.056
SGLT2i	5 (12.2%)	2 (6.1%)	0.370

Data are reported as mean ± standard deviation or number (percentages).

ACEi, angiotensin-converting enzyme inhibitor; ACS, acute coronary syndrome; ARB, angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CCS, chronic coronary syndrome; HbA1c, glycated hemoglobin; PCI, percutaneous coronary intervention; P2Y12i, P2Y12 receptor inhibitor; SGLT2i, sodium–glucose cotransporter 2 inhibitor.

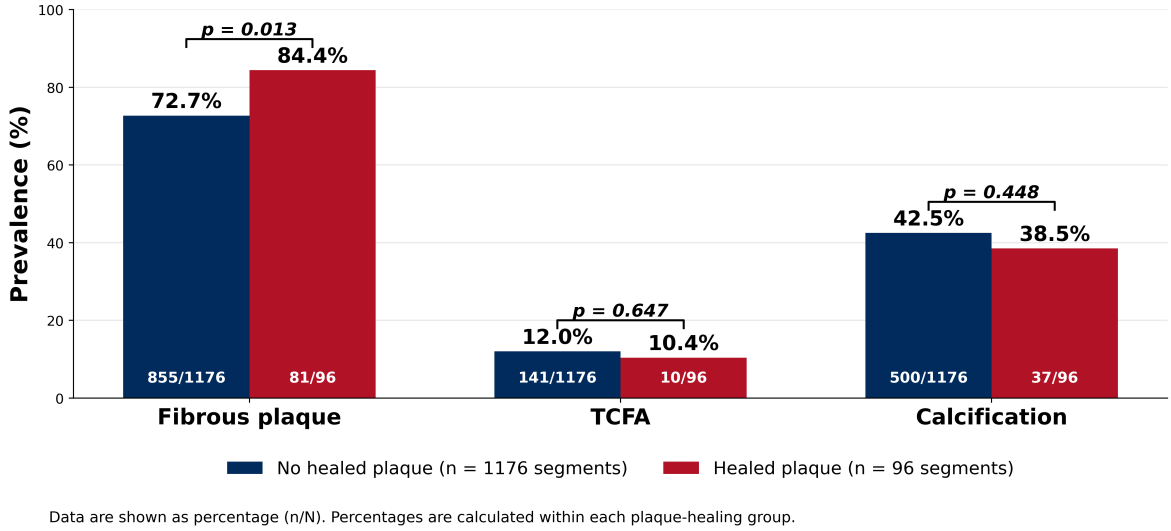
4.2 OCT plaque phenotype according to healing status

Segment-based OCT analysis included 1272 coronary segments, including 96 segments with healed layered plaques and 1176 segments without evidence of plaque healing.

Compared with non-healed segments, healed plaques were characterized by a significantly higher prevalence of fibrous plaque phenotype (84.4% [81/96] vs. 72.7% [855/1176]; p=0.013).

Conversely, no significant differences were observed in the prevalence of thin-cap fibroatheroma (10.4% [10/96] vs. 12.0% [141/1176]; p=0.647) or calcification (38.5% [37/96] vs. 42.5% [500/1176]; p=0.448) (**Figure 1**).

FIGURE 1 **OCT Plaque Phenotype According to Healing Status**
(segment-based analysis)



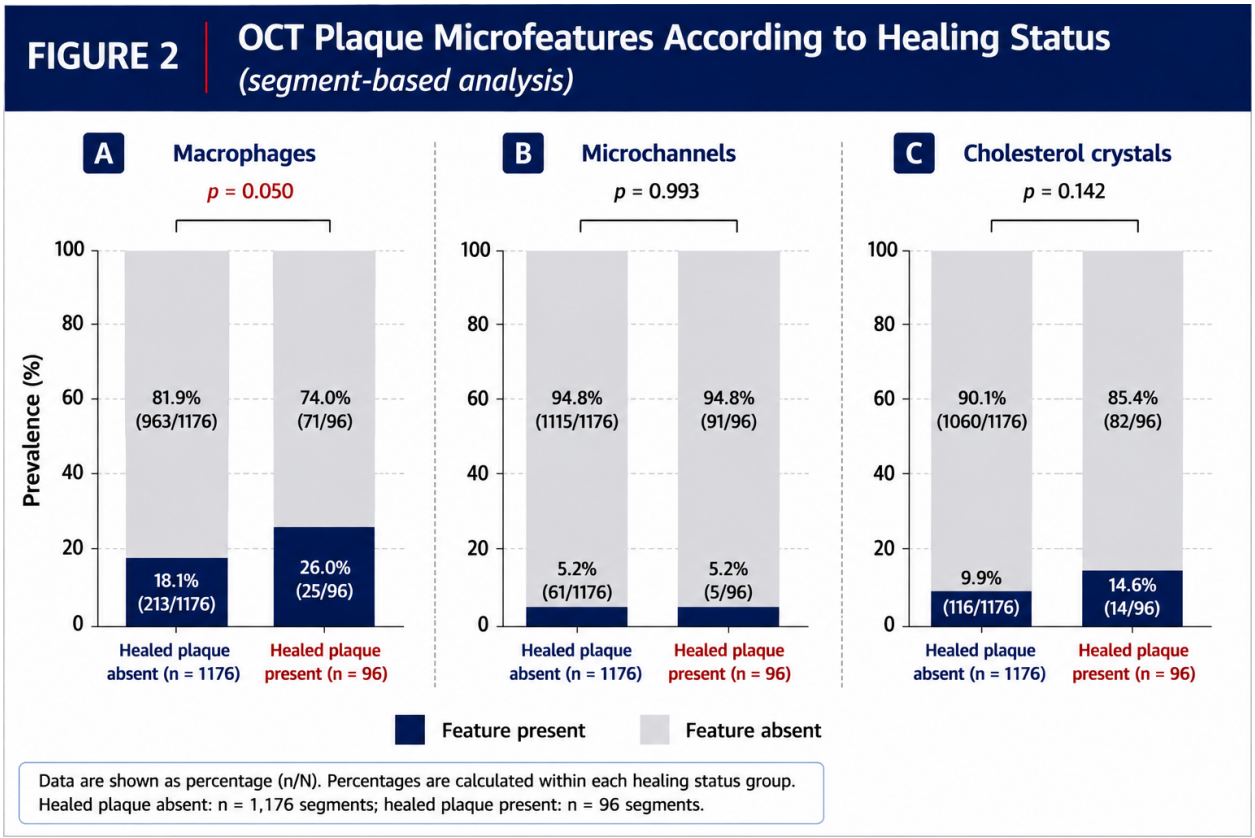
Data are shown as percentage (n/N). Percentages are calculated within each plaque-healing group.

4.3 OCT plaque microfeatures according to healing status

OCT assessment of plaque microfeatures demonstrated a higher prevalence of macrophage accumulation among healed plaques compared with non-healed plaques (26.0% [25/96] vs. 18.1% [213/1176]), reaching borderline statistical significance (p=0.050).

No differences were observed in microchannels, which were equally distributed between groups (5.2% [5/96] vs. 5.2% [61/1176]; p=0.993).

Cholesterol crystals were numerically more frequent in healed plaques compared with non-healed plaques (14.6% [14/96] vs. 9.9% [116/1176]), although this association did not reach statistical significance (p=0.142) (**Figure 2**).



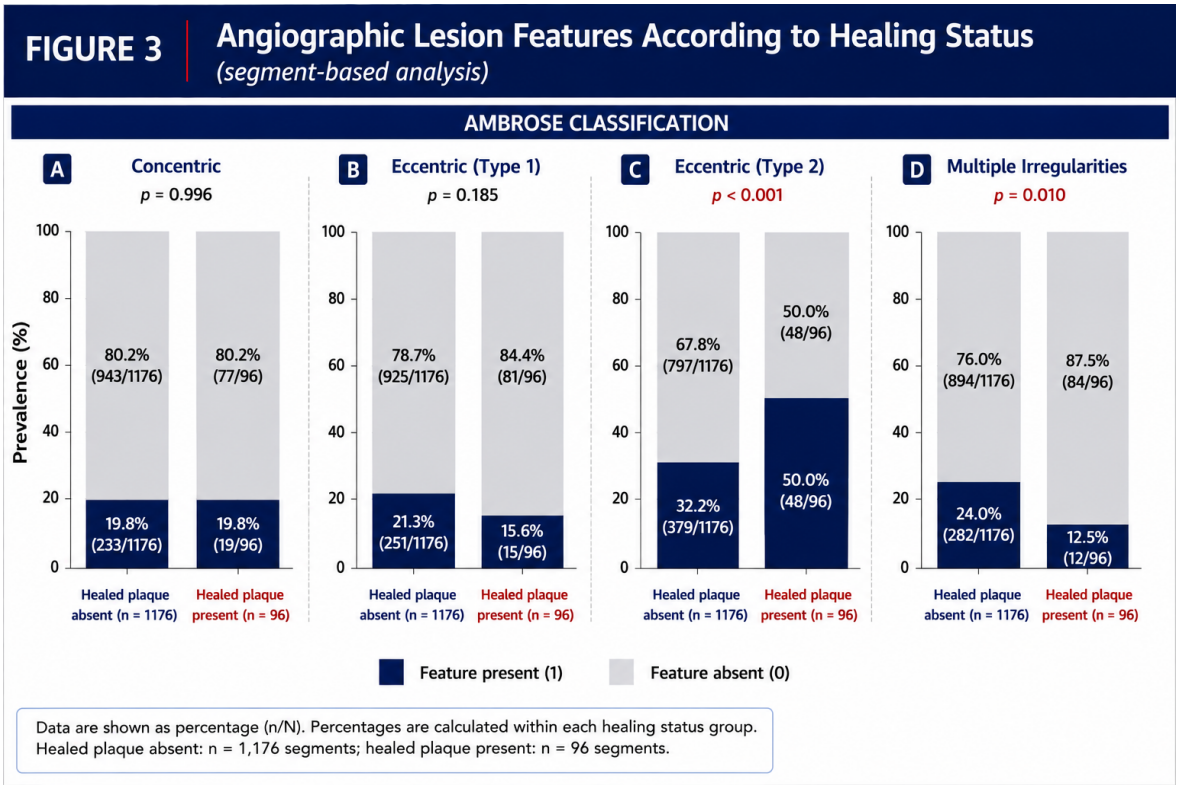
4.4 Angiographic lesion morphology according to healing status

Angiographic lesion morphology was assessed according to the Ambrose classification (**Figure 3**).

The prevalence of concentric lesions was identical between healed and non-healed plaques (19.8% vs. 19.8%; p=0.996), while eccentric type I lesions were numerically less frequent among healed plaques (15.6% vs. 21.3%; p=0.185).

Conversely, healed plaques showed a significantly higher prevalence of eccentric type II morphology compared with non-healed plaques (50.0% [48/96] vs. 32.2% [379/1176]; p<0.001).

Multiple irregularities were significantly less frequent among healed plaques compared with non-healed plaques (12.5% [12/96] vs. 24.0% [282/1176]; p=0.010).



4.5 Morphological factors associated with plaque healing

Multivariable logistic regression analysis was performed to evaluate morphological features independently associated with healed plaque presence (**Table 2**).

Among OCT-derived plaque characteristics, fibrous plaque phenotype was independently associated with plaque healing (OR 2.42, 95% CI 1.35–4.32; p=0.003).

Macrophage accumulation showed a trend toward an association with healed plaque presence (OR 1.61, 95% CI 0.93–2.78; p=0.088).

Conversely, Ambrose angiographic characteristics, TCFA, calcification, microchannels, and cholesterol crystals were not independently associated with plaque healing after multivariable adjustment.

Table 2. Morphological predictors of plaque healing (multivariate logistic regression)

	Odds ratio	95% CI	P value
Concentric (Ambrose)	1.12	0.25 – 5.16	0.878
Eccentric type I (Ambrose)	0.88	0.19 – 4.09	0.869
Eccentric type II (Ambrose)	1.91	0.43 – 8.42	0.392
Multiple irregularities (Ambrose)	0.59	0.12 – 2.83	0.506
Fibrous plaque	**2.42**	**1.35 – 4.32**	**0.003**
TCFA	0.81	0.40 – 1.64	0.553
Calcification	0.82	0.52 – 1.27	0.372
Macrophages	1.61	0.93 – 2.78	0.088
Microchannel	0.90	0.34 – 2.34	0.821
Cholesterol crystals	1.26	0.65 – 2.45	0.500

CI, confidence interval; TCFA, thin-cap fibroatheroma.

4.6 Clinical factors associated with plaque healing

Clinical variables associated with plaque healing were evaluated using multivariable logistic regression analysis (**Table 3**).

Diabetes mellitus was independently associated with a lower prevalence of healed plaques (OR 0.03, 95% CI 0.01–0.52; p=0.015), suggesting that metabolic dysregulation may be linked to impaired mechanisms of plaque repair.

Male sex was independently associated with the presence of healed plaques (OR 2.24, 95% CI 1.02–4.94; p=0.046), suggesting potential sex-related differences in the mechanisms underlying plaque destabilization and subsequent repair. Differences in plaque composition, inflammatory response, and vascular healing processes may contribute to distinct patterns of plaque evolution between men and women.

Clinical presentation was also associated with plaque healing, with chronic coronary syndrome independently associated with healed plaque presence (OR 2.65, 95% CI 1.07–6.58; p=0.036). This observation supports the concept that healed plaques may represent the morphological footprint of previous episodes of plaque disruption followed by repair, contributing over time to plaque progression and a more chronic clinical phenotype.

Table 3. Clinical predictors of plaque healing (multivariate logistic regression analysis)

	Odds ratio	95% CI	P value
Age, years	1.00	0.95 – 1.06	0.849
Male sex	**2.24**	**1.02 – 4.94**	**0.046**
Hypertension	0.42	0.17 – 1.08	0.072
Dyslipidemia	1.69	0.65 – 4.43	0.285
Current smoking	2.43	0.99 – 5.96	0.053
Diabetes mellitus	**0.03**	**0.01 – 0.52**	**0.015**
Family history of CAD	**0.13**	**0.04 – 0.41**	**0.001**
Presentation with CCS	**2.65**	**1.07 – 6.58**	**0.036**
HbA1c, %	1.00	0.99 – 1.00	0.893
Aspirin	0.54	0.09 – 3.27	0.502
Statin	**6.35**	**1.17 – 34.64**	**0.033**
Beta-blocker	**2.78**	**1.16 – 6.63**	**0.022**
ACEi/ARBs	0.28	0.07 – 1.19	0.085
Oral antidiabetic drugs	5.47	0.39 – 76.33	0.206
Insulin	1.90	0.16 – 22.26	0.610
SGLT2i	0.71	0.15 – 3.44	0.671

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CAD, coronary artery disease; CCS, chronic coronary syndrome; CI, confidence interval; HbA1c, glycated hemoglobin; SGLT2i, sodium–glucose cotransporter 2 inhibitor.

Among baseline therapies, statin treatment (OR 6.35, 95% CI 1.17–34.64; p=0.033) and beta-blocker therapy (OR 2.78, 95% CI 1.16–6.63; p=0.022) were independently associated with healed plaque presence. Given the observational nature of the study, these findings should be interpreted as associations rather than evidence of a causal effect. However, they may reflect the relationship between established secondary prevention strategies, plaque stabilization, and vascular reparative processes.

Overall, these findings suggest that plaque healing represents a distinct coronary phenotype resulting from the interaction between previous plaque destabilization and subsequent repair. Healed plaques were associated with fibrotic plaque organization, chronic clinical

presentation, and exposure to cardiovascular therapies, whereas diabetes identified a phenotype characterized by reduced plaque healing, potentially reflecting impaired reparative capacity.

5 DISCUSSION AND CONCLUSION

A total of 74 patients undergoing OCT evaluation were included in the analysis. Overall, 33 patients had evidence of at least one healed plaque, whereas 41 patients showed no evidence of plaque healing. Patients with healed plaques showed similar age, sex distribution, and BMI compared with patients without healed plaques. No significant differences were observed in traditional cardiovascular risk factors, including hypertension, current smoking, dyslipidaemia, or family history of coronary artery disease.

Conversely, diabetes mellitus was significantly less prevalent among patients with healed plaques compared with those without evidence of plaque healing.

Accordingly, patients with healed plaques had significantly lower glucose levels and HbA1c values. Regarding clinical presentation, patients with healed plaques more frequently presented with chronic coronary syndrome and less frequently with acute coronary syndrome, although this difference did not reach statistical significance. Previous cardiovascular events, including prior ACS, PCI, CABG, and stroke, were similar between groups.

Baseline cardiovascular therapies were generally balanced between groups. Patients with healed plaques had lower use of oral antidiabetic therapy, while other cardiovascular medications including antiplatelet agents, statins, beta-blockers, ACE inhibitors/ARBs, insulin, and SGLT2 inhibitors did not significantly differ.

Segment-based OCT analysis included 1272 coronary segments, including 96 segments with healed layered plaques and 1176 segments without evidence of plaque healing.

Compared with non-healed segments, healed plaques were characterized by a significantly higher prevalence of fibrous plaque phenotype.

Conversely, no significant differences were observed in the prevalence of thin cap fibroatheroma.

OCT assessment of plaque microfeatures demonstrated a higher prevalence of macrophage accumulation among healed plaques compared with non-healed plaques, reaching borderline statistical significance.

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The prevalence of concentric lesions was identical between healed and non-healed plaques, while eccentric type I lesions were numerically less frequent among healed.

Conversely, healed plaques showed a significantly higher prevalence of eccentric type II morphology compared with non-healed plaques.

Multiple irregularities were significantly less frequent among healed plaques compared with non-healed plaques.

Multivariable logistic regression analysis was performed to evaluate morphological features independently associated with healed plaque presence.

Among OCT-derived plaque characteristics, fibrous plaque phenotype was independently associated with plaque healing.

Macrophage accumulation showed a trend toward an association with healed plaque presence.

Conversely, Ambrose angiographic characteristics, TCFA, calcification, microchannels, and cholesterol crystals were not independently associated with plaque healing after multivariable adjustment.

Clinical variables associated with plaque healing were evaluated using multivariable logistic regression analysis.

Diabetes mellitus was independently associated with a lower prevalence of healed plaques, suggesting that metabolic dysregulation may be linked to impaired mechanisms of plaque repair.

Male sex was independently associated with the presence of healed plaques, suggesting potential sex-related differences in the mechanisms underlying plaque destabilization and subsequent repair. Differences in plaque composition, inflammatory response, and vascular healing processes may contribute to distinct patterns of plaque evolution between men and women.

Clinical presentation was also associated with plaque healing, with chronic coronary syndrome independently associated with healed plaque presence. This observation supports

the concept that healed plaques may represent the morphological footprint of previous episodes of plaque disruption followed by repair, contributing over time to plaque progression and a more chronic clinical phenotype.

Among baseline therapies, statin treatment and beta-blocker therapy were independently associated with healed plaque presence. Given the observational nature of the study, these findings should be interpreted as associations rather than evidence of a causal effect. However, they may reflect the relationship between established secondary prevention strategies, plaque stabilization, and vascular reparative processes.

Overall, these findings suggest that plaque healing represents a distinct coronary phenotype resulting from the interaction between previous plaque destabilization and subsequent repair. Healed plaques were associated with fibrotic plaque organization, chronic clinical presentation, and exposure to cardiovascular therapies, whereas diabetes identified a phenotype characterized by reduced plaque healing, potentially reflecting impaired reparative capacity.

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