Imaging of coronary atherosclerosis and vulnerable plaque

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CHAPTER 1

Imaging of Atherosclerosis; Invasive and Non-invasive Techniques

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INTRODUCTION

Coronary artery disease (CAD), or narrowing of the coronary arteries due to atherosclerosis, remains one of the leading causes of morbidity and mortality worldwide. However, a substantial number of patients that present with an acute coronary event due to rupture or erosion of an atherosclerotic plaque do not experience any prior symptoms. This observation emphasizes the need to improve early detection of atherosclerosis. Traditionally, imaging of the coronary arteries has focused on the assessment of luminal dimensions and the presence of severe stenosis by means of invasive coronary angiography. However, invasive coronary angiography can only assess the degree of stenosis and is less suited to evaluate the presence of atherosclerosis, including the presence of (potentially high-risk) plaques. As a result, there is an emerging need for imaging modalities that can identify atherosclerotic plaques with high-risk features indicating increased vulnerability. In this regard, particularly non-invasive techniques may be valuable as they may identify high-risk patients at a relatively early stage and may provide the opportunity for novel treatment strategies. Additionally, non-invasive imaging techniques may be used to monitor progression and/or regression of coronary atherosclerosis and thus possibly to evaluate the effectiveness of anti-atherosclerotic therapies at a larger scale. Accordingly, the present chapter will focus on invasive and non-invasive imaging modalities for the evaluation of atherosclerosis and detection of vulnerable lesions in the coronary arteries.

CHARACTERISTICS OF THE POTENTIALLY "VULNERABLE PLAQUE"

Due to the lack of prospective data and natural history studies, most details concerning the potentially vulnerable plaque have been derived from retrospective post-mortem studies.[1-3] It has been established that the majority of acute coronary events (>70%) are caused by plaque rupture followed by thrombus formation.[3] The most common substrate for superimposed thrombus formation is presumed to be the thin capped fibroatheroma; a plaque with a large necrotic core and thin fibrous cap (<65 mm thick) infiltrated by macrophages and lymphocytes (Figure 1).[4] The thin fibrous cap contains a decreased smooth muscle content which in certain circumstances can rupture and cause the thrombogenic parts of the plaque to be exposed into the lumen. This subsequently leads to the activation of the clotting cascade and the formation of a thrombus which can compromise the lumen resulting in an acute coronary syndrome (ACS). In the remaining ~30% of acute coronary events, thrombosis may be due to other causes than plaque rupture, including plaque erosion, intraplaque hemorrhage and calcified nodules.[3] The various atherosclerotic lesions and their association with thrombus are described in Table 1.

Additional characteristics of plaques prone to rupture include large plaque volume, positive remodeling, presence of microcalcifications and proximal location of the lesion. It has still not been fully elucidated which trigger actually causes the plaque to rupture, although it has been postulated that inflammation plays a critical role. Indeed, as shown

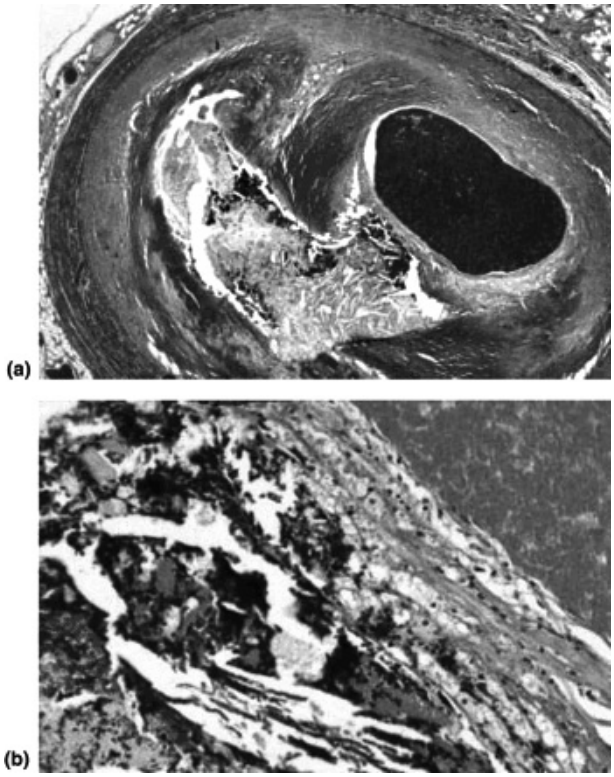

Figure 1. Histological specimen of inflamed thin capped fibroatheroma with trichrome stain, rendering lipid colorless, collagen blue and erythrocytes red. (A) Atherosclerotic coronary artery containing a large lipid core and thin fibrous cap, with post-mortem injected contrast in lumen. (B) Detail of the fibrous cap demonstrating that the cap is heavily inflamed. The fibrous cap consists of many macrophages and within the necrotic core extra-vasated erythrocytes can be seen indicating a possible plaque rupture. Reprinted with permission from Schaar et al.[4]

by studies assessing macrophage infiltration, in particular the fibrous cap is locally heavily inflamed (Figure 1).[5] Inflammation is often a result from endothelial dysfunction. Initially, endothelial dysfunction results from a disturbance in blood flow (flow reversal or oscillating shear stress) at bifurcations or tortuousness of vessels.[6] However, not only blood flow disturbances but also cardiovascular risk factors such as hypercholesterolemia, smoking and diabetes have been suggested to induce endothelial dysfunction.[7 8] Due to endothelial cell activation, increased expression of adhesion molecules (e.g. selectins, VCAMs (vascular cell adhesion molecules) and ICAMs (intercellular adhesion molecules) promote the infiltration and homing of monocytes. Consequently, the monocytes migrate into the plaque and convert into macrophages, contributing to the process of atherogenesis.[7]

At present, there is no widely accepted diagnostic technique for the identification of vulnerable plaques. However, several invasive and non-invasive imaging modalities are currently under development that may allow to some extent detection of plaques prone to rupture.

Table 1. Morphological description of atherosclerotic lesions. Table modified from Virmani et al.[3] SMC, smooth muscle cell; TCFA, thin capped fibroatheroma.

Lesion name	Lesion description	Thrombus
Non-atherosclerotic intimal lesions		
Intimal thickening	Normal accumulation of SMCs in the intima without lipid or macrophage foam cells.	Absent
Intimal xanthoma	Subendothelial accumulation of foam cells in intima without necrotic core or fibrous cap.	Absent
Progressive atherosclerotic lesions		
Pathologic intimal thickening	SMCs in proteoglycan-rich matrix with areas of extracellular lipid accumulation without necrosis	Absent
With erosion	Luminal thrombosis, plaque same as above	Thrombus most often mural and infrequently occlusive
Fibrous cap atheroma	Well-formed necrotic core with overlying fibrous cap	Absent
With erosion	Luminal thrombosis; plaque same as above, no communication of thrombus with necrotic core	Thrombus most often mural and infrequently occlusive
TCFA	Thin fibrous cap infiltrated with macrophages and lymphocytes, rare SMCs, and an underlying necrotic core	Absent, with intraplaque hemorrhage/fibrin
With rupture	Fibroatheroma with cap disruption; luminal thrombus communicates with underlying necrotic core	Thrombus usually occlusive
Calcified nodule	Eruptive nodular calcification with underlying fibrocalcific plaque	Thrombus usually non-occlusive
Fibrocalcific plaque	Collagen-rich usually with significant stenosis; large areas of calcification with few inflammatory cells, necrotic core	Absent

INVASIVE IMAGING OF ATHEROSCLEROTIC PLAQUES

Invasive coronary angiography

Invasive coronary angiography is currently the gold standard for the diagnosis of CAD and provides an accurate and detailed overview of the anatomy of the coronary artery tree, including precise quantification of the degree of stenosis. Accordingly, the technique is extensively used to guide further treatment strategies, such as coronary angioplasty or bypass surgery.

However, evaluation of percentage diameter stenosis has limited value in predicting future cardiac events. Indeed, as demonstrated during the follow-up of patients admitted for acute myocardial infarction, almost two-thirds of plaques prone to rupture were located in non-flow limiting atherosclerotic lesions and only a minority was located in severely obstructed lesions.[9 10] Although the likelihood of occlusion for an individual

lesion is directly related to the severity of stenosis, non-obstructive lesions are far more common and thus may frequently cause coronary occlusion due to their greater number (Figure 2). Accordingly, evaluation of the percentage diameter stenosis by means of invasive coronary angiography does not allow differentiation between stable and unstable plaques.

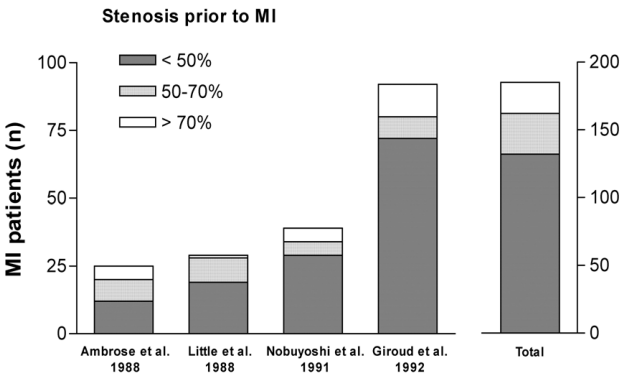

Figure 2. Bar graphs representing stenosis severity and related risk of myocardial infarction (MI) as assessed by repeated angiographic examination. As can be observed from the figure, lesions that are non-significant (< 50% stenosis) on prior angiography are frequently the underlying cause of MI. Moreover, non-significant lesions outnumber the more severely obstructive lesions and therefore account for the majority of MI. The bar graphs are constructed from data published by Ambrose et al.[9], Little et al.[96], Nobuyoshi et al.[97], and Giroud et al.[98]

Notably, novel promising angiographic acquisition approaches have been developed recently. One of these acquisition methods is rotational 3-dimensional coronary angiography, a new imaging technique in which the gantry is mechanically rotated around the patient providing a multitude of x-ray projections during a single contrast injection.[11] Using this technique, motion information of the coronary arteries can be extracted including vessel displacement and pulsation.[12] Furthermore, reconstruction of 3-dimensional images from 2-dimensional projections using specially developed dedicated software may further enhance angiographic assessment of coronary arteries (Figure 3). However, whether this novel technique will allow more accurate evaluation of atherosclerotic plaques remains to be determined more precisely.[13] Overall, it seems evident that invasive coronary angiography is an excellent modality for detecting obstructive coronary artery disease, however, detailed imaging of atherosclerosis such as determining the presence of vulnerable plaque characteristics, remodeling and inflammation, is still not feasible using this technique. Therefore other, more insightful modalities are needed for this purpose.

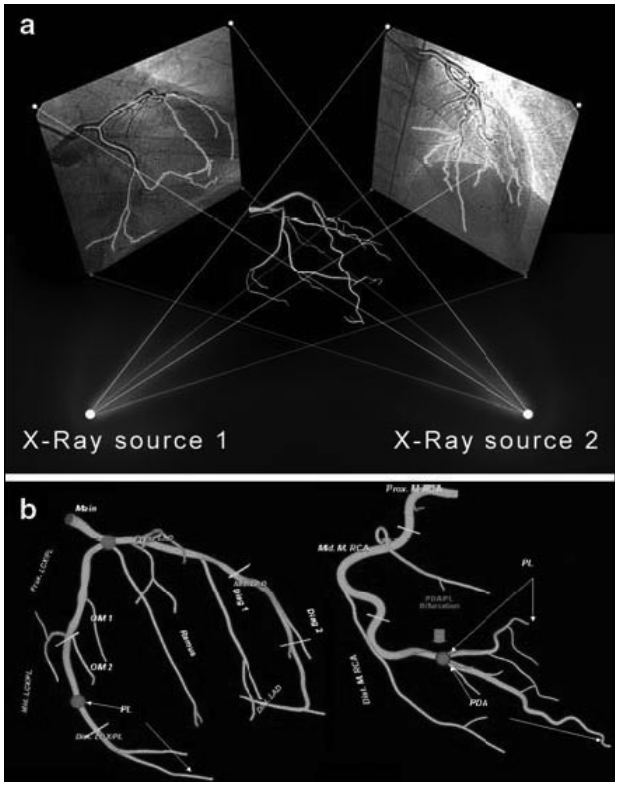

Figure 3. (A) Illustration of three-dimensional coronary modeling based on two angiograms acquired in two different projection geometries. (B) Modified American Heart Association (AHA) classification of coronary segments. Reprinted with permission from Garcia et al.[99]

Intravascular ultrasound

With respect to imaging of atherosclerosis, substantial progress has been achieved with the development of intravascular ultrasound (IVUS). IVUS is a minimally invasive imaging modality which uses miniaturized crystals incorporated at the catheter tip to provide real-time, high-resolution, cross-sectional images of the arterial wall and lumen. Axial resolution is approximately 150 μm and the lateral resolution 300 μm. As a result, the technique provides high-resolution images of the atherosclerotic process in the arterial wall.

Importantly, the technique has been extensively validated against histological autopsy specimens of human coronary arteries.[14-17] Both lumen and vessel dimensions, such as plaque and vessel area, plaque distribution, lesion length and remodeling index, can be accurately determined in vivo. In addition, semi-quantitative tissue characterization can be achieved based on plaque echogenicity. In conventional grayscale ultrasound images, calcium highly reflects ultrasound which appears as a bright and homogenous signal, resulting in acoustic shadowing.[15 18] In addition, the severity of calcifications can be quantified by measuring the angle or arc of calcium. Hypo-echoic or low reflectance in IVUS images are usually due to lipid-laden lesions (also referred to as "soft" or "sonolucent" plaques). An example is provided in Figure 4. Grayscale IVUS features of potentially vulnerable plaques have been evaluated prospectively by Yamagishi et al.[19] The investigators

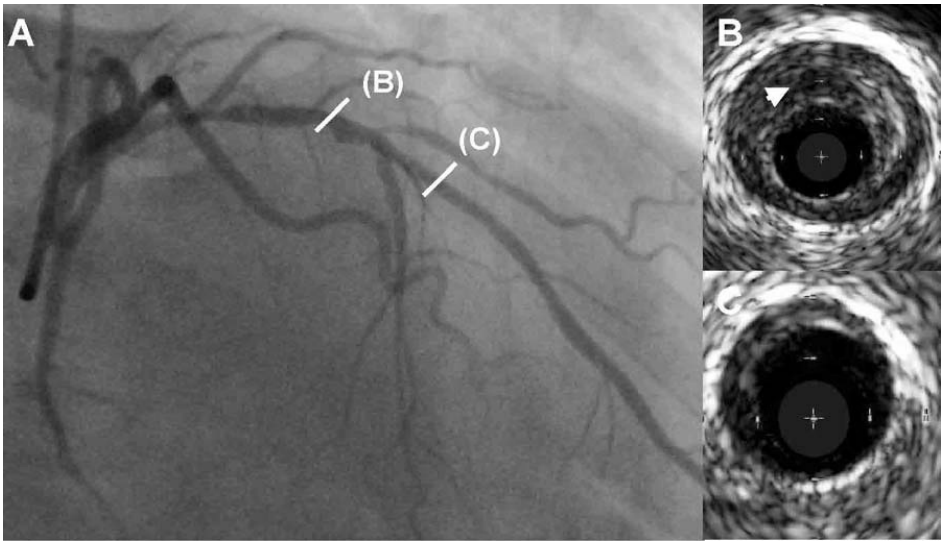

Figure 4. Coronary angiogram (A) of the left anterior descending coronary artery and corresponding intravascular ultrasound (IVUS) images (B and C) of a 55 year old patient presenting with an acute coronary syndrome. In panel B an IVUS frame is provided showing a large plaque area with an echolucent zone (arrowhead) and luminal obstruction, possibly suggesting the presence of a vulnerable plaque. Panel C shows a more distally obtained IVUS frame with minimal plaque burden.

evaluated 114 coronary plaques without luminal obstruction and assessed which plaques during a follow-up period of 21-months were related to an acute coronary event. Interestingly, it was reported that large, eccentric, positive remodeled plaques with an echolucent zone were at increased risk of instability (Figure 5). In addition, several retrospective studies confirmed that IVUS was able to identify plaques at higher risk of rupture (large echolucent area, thin fibrous cap).[20-22] Moreover, studies examining the differences between ruptured plaques and non-ruptured plaques in the same coronary artery demonstrated that the IVUS-derived lumen eccentricity index of ruptured plaques was greater.[23]

In addition, IVUS has been increasingly used as the gold standard in trials evaluating progression or regression of plaque in the coronary arteries. Indeed, unlike angiography, accurate quantification of plaque volume and area is provided by IVUS. Interestingly, Von Birgelen and co-workers performed IVUS examination of the left main coronary artery in 56 patients during initial angiography and repeated imaging after 18 months.[24] Adverse cardiovascular events occurred in 18 patients during follow-up; in patients with events, annual plaque progression was significantly greater than in the remaining asymptomatic patients. Hence, it seems feasible that IVUS-measured progression of coronary plaque may serve as a marker for future cardiovascular events.

Nevertheless, the main limitation of grayscale IVUS remains its inability to accurately differentiate plaque composition. In particular, areas with low echo reflectance such as fibrous tissue, fibro-fatty tissue and thrombus remain hard to distinguish.[14 18] More

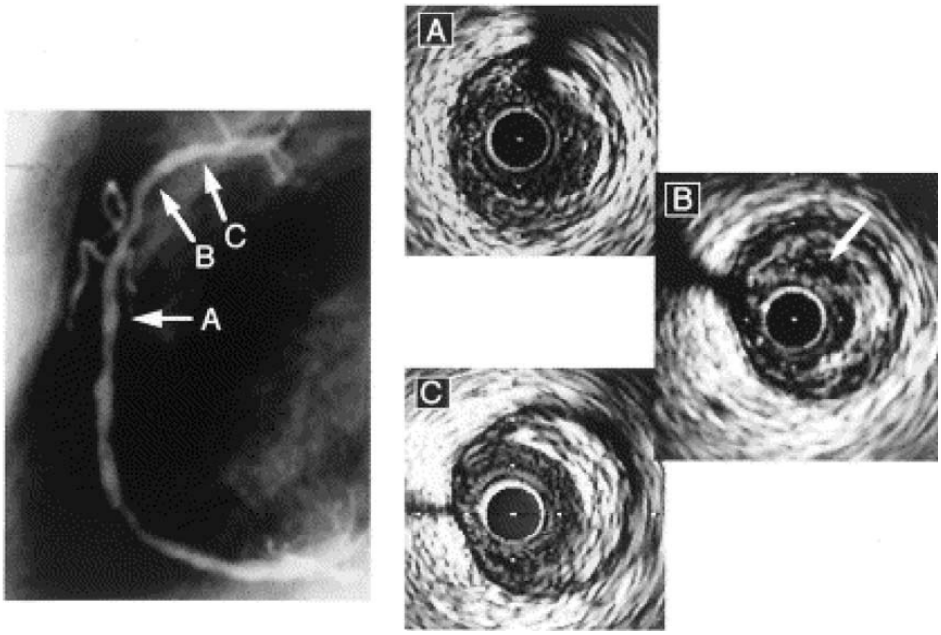

Figure 5. Coronary plaque in the right coronary artery (RCA) of a patient presenting with an acute coronary syndrome as evaluated by coronary angiography (left) and intravascular ultrasound (right). (A) A mild concentric lesion at the distal part of the RCA. (B) In the proximal portion a significant eccentric lesion with an echolucent area (arrow) and high plaque burden of 67%. (C) More proximally an eccentric lesion with high echo density. Reprinted with permission from Yamagishi et al.[19]

recently, integrated backscatter IVUS (IB-IVUS) systems have been developed to overcome this problem. Using this technique, a 2-dimensional color-coded map is constructed which reflects the tissue characteristics of the coronary arterial wall. In a prospective study by Sano et al., tissue characteristics of vulnerable plaques in patients prior to presentation with ACS were evaluated using IB-IVUS.[25] The authors demonstrated that tissue characteristics of vulnerable plaques before causing an ACS were different from those of plaques related to stable angina. However, a low positive predictive value of only 42% was reported for the identification of lipid area, indicating that further improvement is needed before application of this technique is feasible.

Virtual histology intravascular ultrasound

Virtual histology intravascular ultrasound (VH IVUS) can potentially differentiate plaque composition more accurately than conventional grayscale IVUS. The technique is based on radiofrequency analysis of intravascular ultrasound backscatter signals. A combination of spectral parameters were used to develop statistical classification schemes for analysis of in vivo IVUS data in real-time. Using these parameters, color-coded maps of plaque composition for each cross-sectional image are provided which are superimposed on the grayscale IVUS images. As illustrated in Figure 6, these tissue maps can differentiate

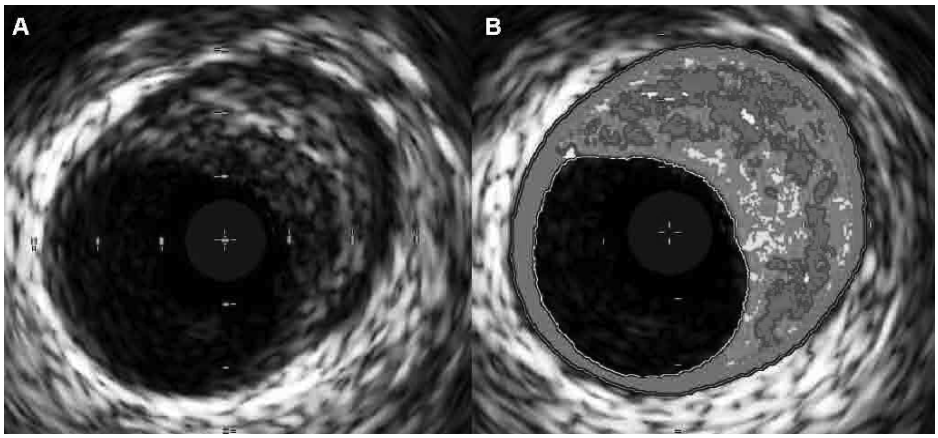

Figure 6. Plaque characterization by virtual histology intravascular ultrasound (VH IVUS). (A) Traditional grayscale intravascular ultrasound (IVUS) frame showing coronary plaque. (B) Example of VH IVUS color-coded map superimposed on grayscale IVUS frame. The colors correspond to different tissue types such as fibrous (dark green), fibro-fatty (light green), dense calcium (white) and necrotic core (red). Panel B shows a plaque with predominantly necrotic core, small dense calcium deposits and a thick fibrous cap, corresponding to a fibroatheroma.

fibrous (dark green), fibro-fatty (light green), dense calcium (white) and necrotic core (red) areas. Since its introduction, the technique has been validated with histology in several studies.[26 27] Nair and colleagues have shown accuracies of 90.4% for fibrous, 92.8% for fibro-fatty, 90.9% for calcified and 89.5% for necrotic core regions demonstrating the potential of this imaging tool for analyzing plaque composition.[26]

The ability of VH IVUS to evaluate the presence of vulnerable plaques was first demonstrated by Rodriguez-Granillo et al.[28] The investigators observed that vulnerable plaques as determined on VH IVUS were more prevalent in patients presenting with ACS than stable angina pectoris. Similar results were recently reported by Pundziute and co-workers who demonstrated that in culprit lesions of patients with ACS, the thin capped fibroatheroma was more prevalent than in plaques of patients presenting with stable symptoms (Figure 7).[29] Interestingly, the presence of positive remodeling identified by VH IVUS was found to be similarly linked to the presence of vulnerable plaques. A retrospective study using VH IVUS demonstrated that positive remodeled plaque contained significantly more necrotic core and features of high-risk plaque, whereas negative remodeled plaques showed a more stable phenotype.[30]

Of note, in addition to remodeling, Valgimigli et al. demonstrated that plaque composition on VH IVUS was influenced by the location of the plaque in the coronary artery tree.[31] As shown by VH IVUS, proximal segments of coronary arteries had a larger necrotic core area when compared to distal coronary segments whereas the other plaque components (fibrous, fibro-fatty and dense calcium) were distributed evenly along the coronary artery tree. Accordingly, distance from the ostium was demonstrated to be inversely associ-

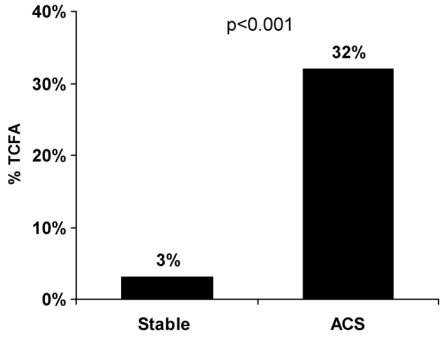

Figure 7. The prevalence of thin capped fibroatheroma (TCFA) in patients presenting with stable symptoms versus patients presenting with an acute coronary syndrome (ACS) evaluated by virtual histology intravascular ultrasound. TCFA were more frequently observed in plaques of patients with ACS (32%) as compared to patients with stable symptoms (3%). The bar graph is constructed with data from Pundziute et al.[29]

ated to plaque vulnerability, possibly explaining the higher incidence of culprit lesions in proximal parts of the coronary artery tree.

Interestingly, in addition to evaluating progression or regression in plaque burden, VH IVUS may also have the ability to monitor changes in plaque composition (and possibly even plaque vulnerability) after treatment with anti-atherosclerotic therapy. Serruys et al. assessed the effect of the direct lipoprotein-associated phospholipase A2 (Lp-PLA2) inhibitor darapladib on plaque composition by VH IVUS.[32] The investigators showed that necrotic core size increased in patients receiving placebo. In contrast, Lp-PLA2 inhibition prevented further progression of necrotic core, suggesting stabilization of atherosclerosis.

Although VH IVUS is a promising imaging modality for plaque characterization, some limitations remain. Importantly, detection of the thin fibrous cap (<65 µm) is not yet feasible as VH IVUS has limited radial resolution of only 100 µm. However, with the introduction of 40 MHz catheters imaging of the thin fibrous cap may eventually become possible.

Optical coherence tomography

Optical coherence tomography (OCT) is an unique high-resolution imaging technique which uses low coherence, near infrared light for intravascular imaging of the coronary artery wall. It has excellent spatial resolution of 10-20 µm which is ten times higher than the resolution of IVUS. Furthermore, using histological controls, it has been demonstrated that OCT is superior than IVUS in detecting important features of vulnerable plaque components including thickness of fibrous cap, thrombus and density of macrophages.[33-35]

One of the first investigations to demonstrate the feasibility of plaque characterization with OCT in vivo was performed by Jang et al.[36] Furthermore, using this technique the authors reported a higher frequency of thin capped fibroatheroma in patients with ACS as compared to patients with stable angina pectoris. In addition, Kubo et al. compared assessment of culprit plaque morphology on OCT to grayscale IVUS and coronary angioscopy.[37] The authors concluded that OCT was superior in identifying the thin capped fibroatheroma and thrombus, and that OCT was the only modality that could distinguish the thickness of fibrous cap (Figure 8).

Another interesting feature of OCT is that it enables quantification of macrophages within fibrous caps. Tearney and colleagues showed in vitro, by comparing OCT images

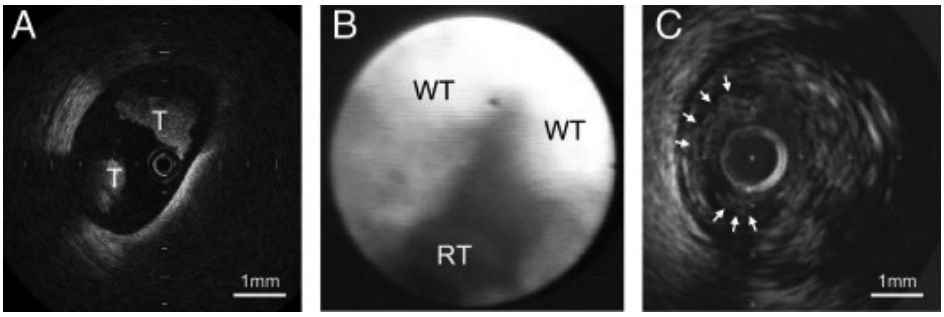

Figure 8. Intraluminal thrombi in corresponding images of optical coherence tomography (A), coronary angioscopy (B), and intravascular ultrasound (C). (A) Thrombus with optical coherence tomography signal attenuation (T). (B) Large white thrombus (WT) and small red thrombus (RT) adhering to a rough surface of yellow plaque. (C) Thrombus (arrows) identified as a mass protruding into the vessel lumen from the surface of the vessel wall. Reprinted with permission from Kubo et al.[37]

to histological specimens, that a high positive correlation exists between OCT measurements and fibrous cap macrophage density (r=0.84).[38] In vivo, Raffel and colleagues demonstrated a significant relationship between systemic inflammation (white cell blood count) and macrophage density in fibrous caps identified by OCT.[39]

At present, it is important to realize that there are some important limitations in the use of OCT. Blood leads to significant attenuation of the emitted infrared light, therefore regular saline flushes or balloon occlusion of the artery is necessary for adequate imaging. Consequently, data acquisition is time-consuming and is therefore limited to focal lesion exploration. Furthermore, the penetration depth of near infrared light is only 1-2 mm. As a result, OCT is not able to visualize the complete plaque and vessel wall and quantitative measurements of plaque and/or lipid volume are currently not possible. However, a second-generation OCT technology, namely optical frequency domain imaging (OFDI), has recently been developed, which enables imaging of the coronary arteries with a short, non-occlusive saline flush and rapid spiral pullback.[40]

OTHER INTRA-CORONARY TECHNIQUES

Intravascular Ultrasound Palpography

Intravascular palpography is a technique based on intravascular ultrasound. This imaging modality allows assessment of local mechanical tissue properties by assessing tissue deformation or strain. At a given pressure limit, fatty tissue components will show more deformation than fibrous components. Accordingly, palpography uses these differences in tissue deformation to differentiate between various plaque components. Indeed, differences of strain between fibrous, fibro-fatty and fatty components of the plaque of coronary and femoral arteries have been reported in vitro.[41] In addition, a distinctive

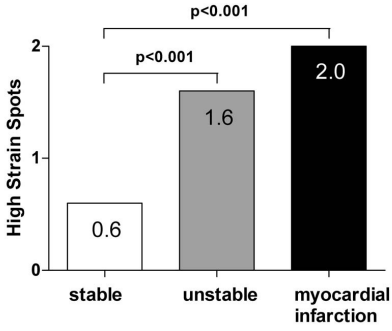

Figure 9. Bar graph representing the relation between the number of high strain spots as assessed by palpography and clinical presentation in 55 patients. High strain spots correspond to the more vulnerable plaques. More high strain spots were demonstrated by palpography in patients presenting with unstable angina pectoris and acute myocardial infarction as compared to patients presenting with stable angina pectoris. Bar graph constructed with data from Schaar et al.[42]

strain pattern was found with a high sensitivity and specificity (89%) for the detection of thin capped fibroatheroma in postmortem coronary arteries. Schaar et al. performed the first clinical study using palpography in patients to assess the incidence of vulnerable plaque.[42] In 55 patients presenting with stable symptoms, unstable symptoms and acute myocardial infarction, palpography was performed and the number of deformable plaques was assessed. The investigators reported that patients with stable angina pectoris had significantly fewer deformable plaques (high strain spots) per vessel as compared to patients presenting with unstable angina pectoris or acute myocardial infarction (Figure 9). Thus, although additional validation is required, intravascular ultrasound palpography appears to have potential for the identification of vulnerable plaque characteristics.

Intracoronary angioscopy

Intracoronary angioscopy is an imaging technique which uses optic fibers to allow direct visualization of the plaque surface, presence of thrombus and color of the luminal surface. A normal artery appears as glistening white, whereas a plaque can be categorized based on its angioscopic color such as yellow or white. Additionally, thrombus can be identified as white (platelet rich) or red (platelets and erythrocytes) (Figure 8B). Uchida and co-workers performed intracoronary angioscopy in 157 patients presenting with stable angina.[43] In a 12-month follow-up period, ACS occurred more frequently in patients with glistening yellow plaques (69%) than in those with white plaques (3%).

Of interest, intracoronary angioscopy can also be applied as a tool for monitoring changes in plaque morphology following pharmaceutical therapy. Using this technique, Takano and colleagues were able to demonstrate an effect of preventive treatment with atorvastatin.[44] Interestingly, lipid-lowering therapy with atorvastatin changed plaque color and morphology as determined by angioscopy, thereby suggesting plaque stabilization.

A major limitation of angioscopy remains that, similar to OCT, the technique requires a blood-free field while investigation is restricted to a limited part of the vessel.

NON-INVASIVE IMAGING OF ATHEROSCLEROTIC PLAQUES

Calcium score

It has been well established that the presence of coronary artery calcifications (CAC) confirms the presence of atherosclerosis. In fact, an association between visible CAC on invasive coronary angiography and the risk of cardiovascular events has been demonstrated in the early 1980s.[45] The introduction of electron beam computed tomography (EBCT) allowed non-invasive evaluation of CAC and resulted in the development of the widely established quantification method by Agatston.[46] More recently, assessment of CAC is performed by means of multislice computed tomography (MSCT) (Figure 10).

The relation between the presence and extent of CAC and presence of coronary artery stenosis has been assessed in several studies.[47-49] As expected, a high sensitivity of CAC for the presence of obstructive CAD has been reported. However, extensive calcifications can be present in the absence of luminal narrowing. As a result, specificity for obstructive CAD is low. Accordingly, the technique may be more suited to provide an estimate of total plaque burden rather than stenosis severity.

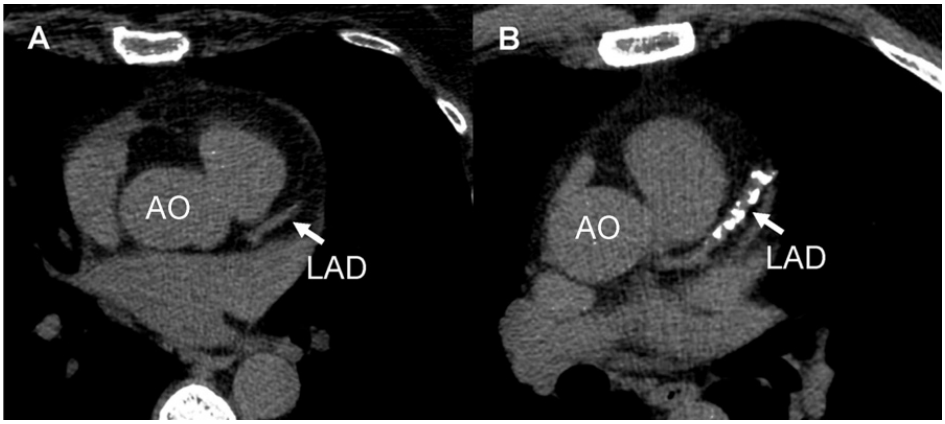

Figure 10. Example of coronary calcium on non-contrast enhanced multislice computed tomography (MSCT) axial images. Calcifications appear as bright white dense structures on MSCT. Panel A shows a 57 year old patient without evidence of coronary calcifications in the left anterior descending coronary artery (LAD). Panel B shows a 53 year old patient with calcifications in the LAD. AO - Aorta.

Importantly, the information on calcified plaque burden has been shown to translate in prognostic information. Indeed, the value of CAC scoring for risk stratification has been extensively studied. A large clinical trial by Greenland and colleagues showed the distinct incremental value of CAC scoring over the Framingham risk score in asymptomatic patients.[50] In addition, Detrano et al. demonstrated that CAC performed equally well among the four major racial and ethnical groups.[51] In a even larger cohort of 25,253 asymptomatic individuals, Budoff and colleagues confirmed that CAC was an independent

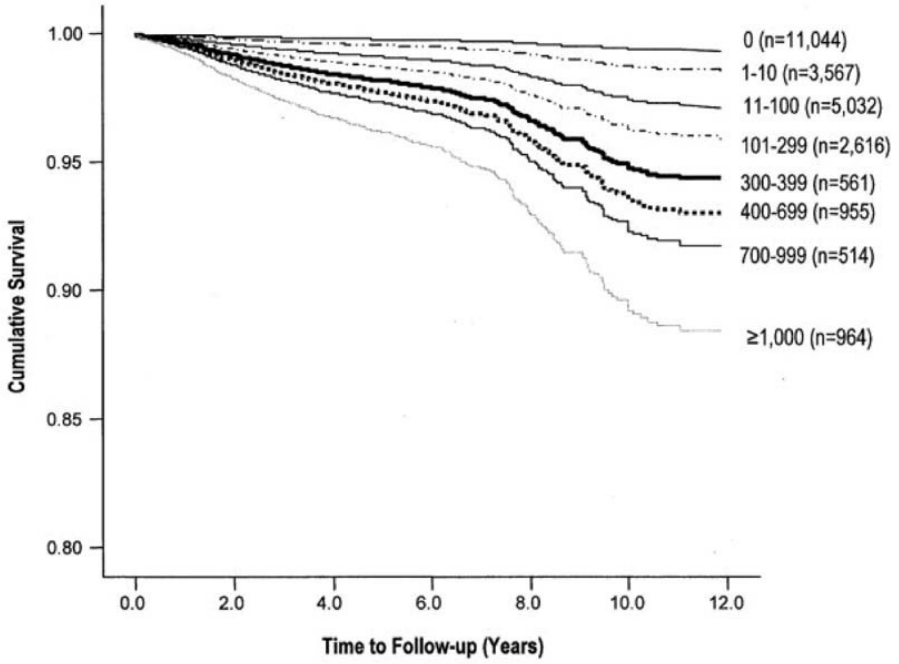

Figure 11. Cumulative survival by coronary artery calcification score adjusted for risk factors such as age, hypercholesterolemia, diabetes, smoking, hypertension, and a family history of premature coronary artery disease. Increasing calcium scores were associated with worse survival and each increment of calcium score was associated with significant increased risk of all-cause mortality. Reprinted with permission from Budoff et al.[52]

predictor of mortality and that risk scores increased proportionally with higher CAC scores (Figure 11).[52] Particularly in patients initially classified as being at intermediate risk, knowledge of the extent of CAC may be valuable to refine risk stratification and determine further management.

In addition to risk stratification, it has been suggested that CAC scoring may allow non-invasive monitoring of changes in atherosclerotic plaque burden. Several investigations have demonstrated a halt in progression or even regression of coronary calcifications as a result of reductions in serum low-density lipoprotein (LDL) cholesterol concentrations.[53] However, other investigations failed to show such effect despite effective reductions in systemic inflammation or LDL cholesterol concentrations. Possibly, changes in calcified plaque burden may not adequately reflect changes in total atherosclerotic plaque burden. Moreover, it has been suggested that plaque stabilization may even be associated with a relative increase of coronary calcifications rather than decrease. Indeed, it remains important to realize that the presence or absence of calcium itself is not a direct marker for vulnerability. Since no information is obtained on the presence of non-calcified plaques, CAC scoring does not allow for reliable distinction between potentially unstable versus stable plaques.[54]

Multislice computed tomography angiography

MSCT is a rapidly evolving imaging tool that allows non-invasive visualization of coronary atherosclerosis. Since the introduction of 4-slice scanners, the technique has developed rapidly and 64-slice and even 320-slice systems are currently available. Accordingly, the temporal and spatial resolution have improved resulting in superior image quality and diagnostic accuracy for the detection of CAD. Although the resolution of MSCT remains inferior as compared to invasive coronary angiography, high diagnostic accuracies have been demonstrated for the detection of significant CAD.[55] Additionally, the technique may be of use in the work-up of patients presenting to the emergency department with suspected ACS. Promising results were reported by Hoffmann and co-workers who demonstrated that the absence of significant coronary artery stenosis (73 of 103 patients) and non-significant coronary atherosclerotic plaque (41 of 103 patients) on MSCT accurately ruled out ACS.[56] Accordingly, a high negative predictive value was observed indicating that MSCT angiography may be a valuable gatekeeper for invasive coronary angiography.

Furthermore, MSCT is not only able to identify coronary artery stenosis but also has the potential to provide information on lesion morphology and plaque composition. As illustrated in Figure 12, the technique can distinguish non-calcified, mixed and calcified plaques. Due to the substantially higher density values, identification of calcified plaque is relatively simple on MSCT. However, identification of non-calcified plaque is more

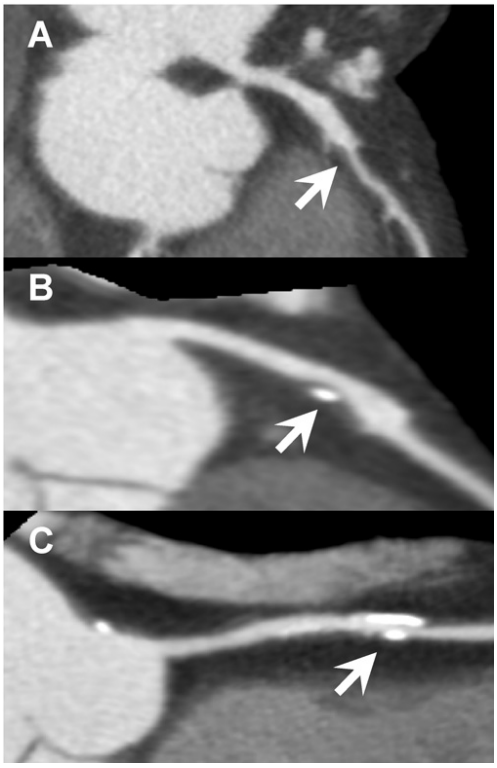

Figure 12. Example of plaque imaging performed on 320-slice multislice computed tomography coronary angiography. (A) Curved multiplanar reconstruction of the left anterior descending artery (LAD) with non-calcified plaque (arrow). (A) Curved multiplanar reconstruction of the LAD demonstrating mixed plaque (arrow). (C) Curved multiplanar reconstruction of the right coronary artery demonstrating calcified plaque (arrow).

demanding because of the more subtle difference in attenuation and relatively larger influence of body-mass index, cardiac output and amount of contrast injected. Interestingly, comparison of density measurements of non-calcified plaques on MSCT to invasive IVUS showed that the attenuation within hyper-echoic (fibrous) plaques was higher than within hypo-echoic (lipid-rich) plaques (mean attenuation values of 121 ± 34 HU versus 58 ± 43 HU).[57] However, for individual lesions a substantial overlap between hyper-echoic and hypo-echoic attenuation values was observed, indicating that, at this stage, further characterization of non-calcified plaque is not yet feasible.

Plaque composition as evaluated by MSCT has been linked to clinical presentation. Motoyama and colleagues compared plaque morphology on MSCT in 38 patients with ACS versus 33 patients with stable angina pectoris and demonstrated that plaques associated with ACS showed lower density values, positive remodeling and spotty calcification.[58] Moreover, Pundziute and colleagues compared plaque characteristics on 64-slice MSCT and VH IVUS in patients with ACS and stable angina pectoris and demonstrated that non-calcified (32%) and mixed plaques (59%) were more frequently present in ACS.[29] In line with these findings, using 64-slice MSCT, Henneman et al. demonstrated in 40 patients suspected of ACS that CAC was absent in a large proportion of patients (33%). However, as illustrated in Figure 13, in these patients non-calcified plaques were highly prevalent (39%).[59] As a result, atherosclerosis and even obstructive CAD were frequently observed, even in the absence of detectable calcium. Thus, the investigators suggested that in patients presenting with ACS, absence of CAC does not reliably exclude CAD.

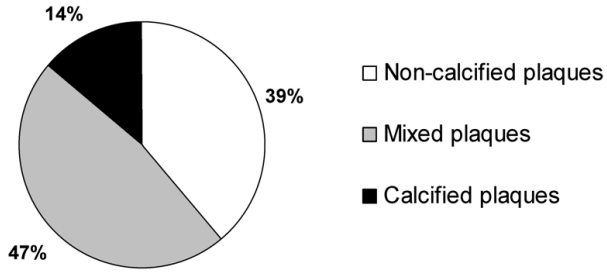

Figure 13. Prevalence of different plaque types in patients presenting with suspected acute coronary syndrome (ACS). A high prevalence of non-calcified and mixed plaques was observed in patients presenting with suspected ACS. Pie graph constructed with data from Henneman et al.[59]

Preliminary studies have suggested that information on atherosclerosis derived from MSCT angiography may also provide prognostic information.[60 61] Interestingly, van Werkhoven et al. demonstrated that the presence of substantial non-calcified plaque burden was an independent predictor of events (all-cause mortality, non-fatal myocardial infarction, and unstable angina requiring revascularization).[62] However, further investigations are required in larger patient populations to confirm these observations.

In addition, MSCT may potentially be applied to monitor progression and/or regression of coronary plaque burden. Preliminary results from an experimental animal model reported that MSCT could accurately document serial changes in aortic plaque burden which correlated well with measurements derived from magnetic resonance imaging (MRI).[63] In humans, Burgstahler and co-workers studied the effect of lipid-lowering therapy on coronary plaque burden with MSCT after one year.[64] Although no differences were found in total plaque burden and CAC, significantly lower non-calcified plaque burden was demonstrated after lipid-lowering therapy.

While MSCT angiography may have potential for non-invasive evaluation of plaque composition and subsequent identification of patients at higher risk of events, several important limitations remain. Firstly, the technique is associated with radiation exposure, although significant dose reductions have been achieved with recent advances in scanner hardware and acquisition protocols.[65-67] In addition, the resolution remains inferior as compared to invasive atherosclerosis imaging techniques and no validated algorithms are currently available for quantification of observations. Further improvement in plaque characterization however, is expected by the development of dual-energy MSCT or dedicated contrast agents.

Magnetic resonance imaging

MRI is a versatile imaging technique with a high potential to visualize vessel anatomy. The technique is able to differentiate atherosclerotic tissue without exposure to radiation using features such as chemical composition, water content, molecular motion-, or diffusion. Due to recent improvements in MR techniques such as high-resolution and multi-contrast MR (time-of-flight (TOF) imaging T1- and T2- weighted and proton density (PD) weighted imaging), plaque characterization has become possible as demonstrated in experimental models, histological specimens, human carotid arteries and the aortic wall in vivo (Table 2).[68-71] Fayad and colleagues assessed aortic wall plaque composition with MR images matched to transesophageal echocardiograms, demonstrating a strong correlation for plaque composition, thickness and extent.[68] In several studies the potential of MRI to characterize different plaque characteristics, including the fibrous cap, lipid core and even the presence of hemorrhage in human carotid atherosclerotic plaques (Figure

Table 2. Multicontrast weightings and corresponding plaque characterization on magnetic resonance imaging (MRI). Intensities are relative to that of the sternomastoid muscle. Table modified from Yuan et al.[94] TOF, time of flight; PD, proton density.

Component	TOF	T1-weighted	T2-weighted	PD-weighted
Hemorrhage	High	High - moderate	Variable	Variable
Lipid-rich necrotic core	Moderate	High	Variable	High
Calcification	Low	Low	Low	Low
Fibrous tissue	Moderate - low	Moderate	Variable	High

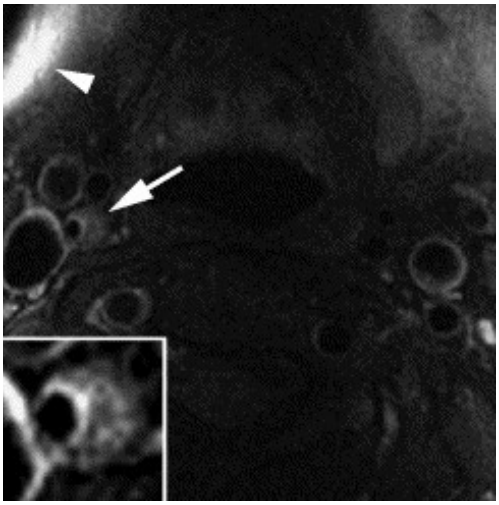

Figure 14. Example of MR image (T2-weighted) of the carotid arteries. A stenotic lesion of the right internal carotid artery can be observed just distal of the bifurcation (arrow). The arrowhead indicates a high signal artifact of the close-placed superficial phase-array coil. Finally, in the enlargement, a hypo-intense signal within the plaque corresponding to lipid accumulation can be observed. Reprinted with permission from Corti et al.[100]

14), has been demonstrated.[72 73] In addition, a good correlation has been identified between fibrous cap integrity on MRI and histopathological specimens.[69]

Plaque imaging with MRI of the coronary arteries remains challenging as deep location, motion and respiratory artifacts and small caliber vessels remain obstacles for accurate coronary visualization and plaque differentiation. Nevertheless, several novel approaches for coronary plaque imaging are currently under development and may potentially allow accurate evaluation of atherosclerotic plaque in the coronary arteries.[74] In particular 'black-blood' techniques (an imaging approach in which the blood appears black and the arterial wall can be seen) are promising for accurately portraying plaque presence, size and morphology with sub-millimeter resolution and high reproducibility.[75] Kim et al. recently applied a novel 3D free breathing black-blood fast gradient technique with real-time motion correction developed by Botnar et al. to evaluate patients with non-significant CAD and compared these patients to a control group without CAD.[76 77] The investigators demonstrated that MRI could identify significantly increased vessel wall thickness with preserved lumen size in patients with non-significant CAD.

High-resolution MRI in combination with molecular contrast agents targeted to specific cells or molecules offers an interesting alternative approach for more detailed plaque characterization.[78-80] In particular contrast agents dedicated to the identification of vulnerable plaque components are of considerable interest. Paramagnetic contrast agents such as gadolium (T1 shortening contrast with a high affinity for lipid-rich lesions) are able to assess the more subtle differences in plaque composition.[79] Furthermore, T2 shortening contrast agents such as ultra small superparamagnetic particles of iron oxide (USPIOs) have been studied both in vitro and in vivo. Interestingly, these particles were found to accumulate in plaques with high macrophage content and cause signal decrease in MR images.[81] Additionally, promising results have been achieved with fibrin targeted contrast agents, which have the potential to allow non-invasive molecular imaging of thrombus. Spuentrup and colleagues demonstrated in an experimental animal model that

using these agents, acute pulmonary, cardiac and coronary thrombosis could be accurately visualized by MR imaging.[82] Furthermore, continued advances in radiofrequency hardware have resulted in an increase in the operating field strength from 1.5T (tesla) to 3T and even 7T. At 3T an approximately two-fold increase in signal-to-noise-ratio can be obtained, resulting in a four-fold reduction in scanning time and significant increase in temporal resolution.

Moreover, recent studies support MRI as an effective tool to evaluate plaque regression following lipid-lowering therapy. Corti et al. demonstrated in 18 hypercholesterolemic patients that MRI could document a marked reduction in atherosclerotic lesion size induced by statin therapy in humans.[83] Accordingly MRI may become a particular attractive modality to non-invasively monitor the effect of anti-atherosclerotic interventions in vivo.

Nevertheless, detailed characterization of plaque including the identification of high-risk features remains difficult at present. Although much is expected from current developments, evidently more data are needed before plaque characterization with MRI may be clinically used for identification and management of patients at risk.

Molecular imaging with nuclear techniques

Using dedicated tracers, nuclear imaging techniques such as single photon emission tomography (SPECT) and positron emission tomography (PET) can target distinct mediators and regulators involved in the cascade of atherosclerosis. As a result of increasing knowledge regarding the pathophysiology of atherosclerosis, several radionuclide-labeled tracers that serve as markers of inflammation, angiogenesis, apoptosis and lipid metabolism have been developed for plaque imaging (Table 3).

Matrix metalloproteinases (MMP) are released by activated macrophages and are therefore used to identify proteolytic activity in atherosclerotic lesions. MMPs modulate the degrading of the extracellular matrix and the thin fibrous cap of an atherosclerotic lesion, contributing to the vulnerability of the plaque. In several animal models the feasibility of in vivo imaging of MMP activity using radionuclide-labeled MMP inhibitors has been shown.[84-86]

Additionally, it has been proposed that apoptosis is one of the features of an atherosclerotic unstable lesion and that apoptosis consequently leads to growth of the necrotic core and influences plaque stability. Annexin A5 has a high affinity for phospatidylserine (exposed on the plasma membrane of apoptotic cells) and therefore radionuclide-labeled Annexin A5 can be used as a marker of apoptotic cells in atherosclerotic lesions. In experimental models, a direct correlation was demonstrated between Annexin A5 uptake, macrophage burden and histologically demonstrated apoptosis.[87] In a small patient cohort with a history of transient ischemic attack, Annexin A5 imaging of carotid atherosclerosis was performed by Kietselaer et al.[88] Imaging was performed before carotid surgery and correlated to histopathology findings. The investigators reported that Annexin A5 uptake in carotid lesions correlated highly with plaque instability. However, only preliminary data are available and further research in humans is necessary.

Table 3 . Targets for molecular imaging of plaque vulnerability. Table modified from Narula et al.[95] FDG - fluorodeoxyglucose; HLA - human leukocyte antigen; ICAM - intercellular adhesion molecule 1; MCP-1 - monocyte chemotactic protein 1; MMP - matrix metalloproteinase; PS - phosphatidylserine; SRA - scavenger receptor A; VCAM - vascular cell adhesion molecule; VEGF - vascular endothelial growth factor.

Process targeted	Targets	Target agents
Monocyte migration		
Reversible prelude with intima	Selectins	Microbubbles with antibodies
Receptors for chemotactic peptides	MCP-1	Radiolabeled MCP-1
Activation-dependent receptors	ICAM or VCAM	Antibodies; radiolabeled or on microbubbles
Subintimal activation of monocytes		
Lipid scavenging receptors	SRA I,II	Oxidized LDL
	FcγRIII	Radiolabeled non-specific IgG or Fc fragments
Other phagocyte receptors	PS receptor	PS-rich microbubbles
	Others	Superparamagnetic iron (USPIOs); nanoparticulate CT contrast
Immune activation	HLA expression	Radiolabeled antibody
Heightened metabolic activity	FDG	Positron-labeled FDG
Macrophage apoptosis		
Cell membrane	PS expression	Radiolabeled Annexin A5
Cell apoptosis pathways	Caspase substrate	Radiolabeled DEVD
Collateral products from macrophages		
Cytokines	MMPs	MMP inhibitor or substrate; radiolabeled or fluorochromes
Vasa vasorum or neovascularization	Integrins VGEF	Radiolabeled RDG peptide Radiolabeled VGEF

Finally, PET imaging with F18-fluorodeoxyglucose (FDG) is currently considered to be one of the most promising imaging modalities for the identification of vulnerable lesions. FDG is a radionuclide tracer that competes with glucose for uptake into metabolically active cells, especially macrophages, and enables quantification via PET. Within carotid artery atherosclerotic plaques, Rudd et al. demonstrated with FDG PET that FDG was taken up by resident macrophages in atherosclerotic plaque but not by surrounding cellular plaque components.[89] The authors suggested that FDG may be capable of imaging and possibly even quantification of plaque inflammation. In addition, FDG PET could potentially be used to serially monitor changes in atherosclerotic plaque macrophage content. In an experimental rabbit model, Worthley and co-workers demonstrated that assessment of progression and/or regression of macrophage content in atherosclerotic plaques was feasible using this non-invasive technique.[90] In addition, Tahara et al. showed in 43 patients that FDG PET, co-registered with computed tomography data, was able to visualize significantly reduced plaque inflammation following 3 month treatment with simvastatin.[91]

However, thus far FDG imaging of the coronary arteries has been challenging because of cardiac motion, FDG uptake in the myocardium and limited resolution of PET. Possibly, co-registration of the functional images with high-resolution anatomical data obtained with MSCT in combination with dedicated protocols to suppress myocardial uptake could possibly overcome this limitation (Figure 15).[92 93]

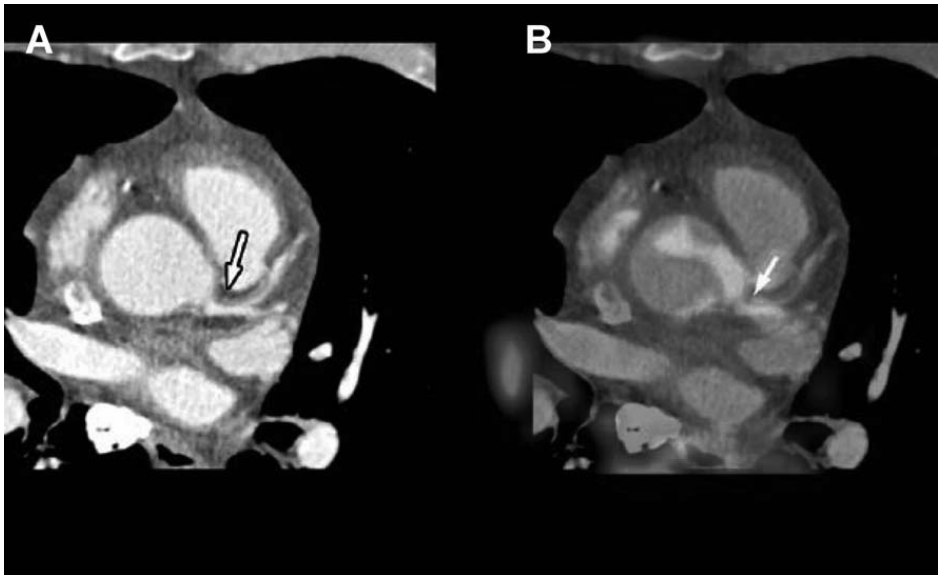

Figure 15. Example of the co-registration of functional imaging with F-18 fluorodeoxyglucose (FDG) positron emission tomography (PET) and anatomical imaging with multislice computed tomography (MSCT). (A) On MSCT axial images a non-calcified plaque in left main coronary artery (arrow) was identified. (B) Corresponding image after fusion with F-18 FDG PET, localizing the inflammatory PET signal with a maximal standard uptake value of 2.1 to the non-calcified plaque seen in the left coronary artery (arrow). Reprinted with permission from Alexanderson et al.[92]

SUMMARY AND CONCLUSION

Plaque rupture followed by coronary occlusion due to thrombosis is responsible for a large number of acute coronary events. Identification of lesions before they rupture would allow initiation of aggressive systemic or even local therapy and could potentially improve outcome. Due to absence of natural history data, the precursor of vulnerable lesions remains largely unknown and most details have been derived from retrospective post-mortem studies. On the basis of these investigations, it has been suggested that the most common substrate for superimposed thrombus formation is the thin capped fibroatheroma; a plaque with a large necrotic core and an inflamed thin fibrous cap (<65 mm thick) infiltrated by macrophages and lymphocytes.

As discussed in the current chapter, extensive effort is invested in the development of imaging tools to characterize coronary atherosclerosis with the ultimate goal of detecting vulnerable lesions. To this end, several techniques are currently under investigation, with each technique having specific advantages as well as limitations. Importantly, the clinical relevance in terms of predicting outcome and changing management remains to be established for all currently available techniques.

At present, invasive techniques, such as OCT and VH IVUS, provide the most detailed information and are currently employed in prospective natural history studies. Application of these techniques will remain largely restricted to symptomatic high-risk patients due to their invasive nature and a non-invasive technique would allow application on a wider scale. At present, non-invasive approaches cannot provide detailed characterization of the individual vulnerable coronary plaque. However, direct in vivo comparisons with invasive modalities may substantially improve our understanding and interpretation of non-invasive observations. Consequently, this information may be translated into enhanced strategies for risk stratification. In addition, the measurements of plaque vulnerability obtained with either invasive or non-invasive imaging techniques may be used as surrogate endpoint for prospective anti-atherosclerotic therapy trials.[32] Possibly, the combination of imaging techniques targeting both morphological and functional characteristics may be of particular value.

Evidently, large prospective studies are needed to further define the potential role of each imaging technique in the identification of vulnerable plaques. Moreover, much uncertainty remains on how these vulnerable lesions should be treated. In addition to increased intensity of systemic therapy, such as aspirin and statin therapy, also local or regional therapeutic approaches (such as plaque sealing) have been suggested. However, no robust data are currently available to support their effectiveness. Potentially, imaging techniques may be proven of great value in the development of such individually targeted treatment strategies.

OUTLINE OF THE THESIS

The aim of this thesis was to evaluate the role of imaging in the assessment and characterization of atherosclerosis and vulnerable plaque in the coronary arteries. Non-invasive computed tomography coronary angiography (CTA) is a relatively new technique for the evaluation of coronary atherosclerosis. Therefore, the performance of CTA in characterizing coronary atherosclerosis was assessed and was compared to invasive imaging techniques, in addition to determining the impact on clinical management.

In **Part 1**, the current advances of coronary CTA in characterizing atherosclerosis and vulnerable plaque were explored. **Chapter 2** explores the ability of the novel 320-row CTA to characterize different plaque components as compared to plaque imaging with invasive virtual histology intravascular ultrasound (VH IVUS). In **Chapter 3**, differences in

plaque composition were evaluated in relation to the degree of stenosis as assessed both non-invasively by CTA and invasively by VH IVUS. In **Chapter 4**, the spatial relationship between the site of greatest stenosis and site of greatest vulnerability was evaluated with VH IVUS on a per-vessel basis. The aim of **Chapter 5** was to systematically investigate the diagnostic performance of CTA for two endpoints, namely detecting significant stenosis (using invasive coronary angiography as the reference standard) versus detecting the presence of atherosclerosis (using IVUS as the reference of standard). Assessment of the length of coronary lesions was compared between CTA and quantitative coronary angiography in patients who underwent subsequent percutaneous coronary intervention (PCI) in **Chapter 6**. The purpose of the next chapters was to systematically compare high-risk plaque features on CTA, such as the pattern of calcifications (**Chapter 7**) and presence of positive remodeling (**Chapter 8**) and relate these characteristics on CTA to vulnerable plaque characteristics on VH IVUS.

In **Part 2**, the relation between characterization of atherosclerosis on CTA and the effect on clinical management was evaluated. **Chapter 9** focuses on the evolving role of coronary CTA (including coronary calcium scoring) on the diagnosis of patients with acute chest pain. In addition, an overview of a wide range of other CT applications is provided, including triple rule-out, evaluation of plaque composition, myocardial function, and perfusion. As CTA is inherently associated with patient radiation exposure, **Chapter 10** addresses effective strategies for radiation dose reduction. The diagnostic accuracy of 320-row CTA in the non-invasive evaluation of significant stenosis and atherosclerosis in patients referred for CTA as well as in patients presenting with acute chest pain is evaluated in **Chapter 11** and **Chapter 12**, respectively. **Chapter 13** evaluates the relation between the value of the calcium score and plaque characteristics (on CTA and VH IVUS) in patients with suspected acute coronary syndrome. The aim of **Chapter 14** was to evaluate the role of non-invasive CTA as a gatekeeper before invasive coronary angiography. Lastly, **Chapter 15** evaluates the value of CTA variables of atherosclerosis to predict the presence of ischemia on myocardial perfusion imaging.

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