Molecular Imaging of Cardiovascular Disease

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ABSTRACT The ability to visualize morphology and function of the heart plays a central role in cardiology practice today. However, clinical tools to diagnose processes in an early phase on a molecular level in order to prevent dysmorphy or dysfunction are still lacking. There is a need for early diagnosis of molecular processes on a pre-disease level and the ability to monitor existing and novel treatments enabling personalized medicine. Molecular imaging is emerging in research and early clinical trials as a very promising approach in diagnosis and monitoring of heart disease. In this review we aim to discuss the potential of molecular imaging in cardiovascular disease.
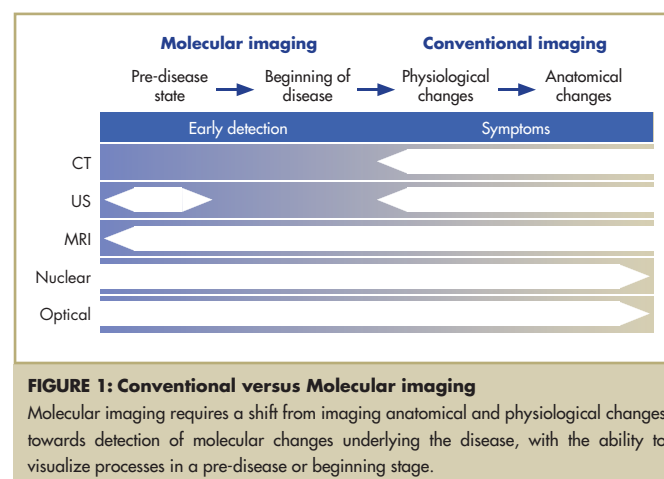
INTRODUCTION

Ischemic heart disease and cerebrovascular disease are the leading causes of death and account for about 43% and 33%, of all 16,7 million cardiovascular deaths worldwide, respectively. Of the 20 million survivors of heart attacks and strokes, a significant proportion requires costly care[1]. In anticipation of this increase in costs, there is an ongoing shift from treatment towards early detection and even prevention of cardiovascular pathology. Individual variability in response to disease or treatment stresses the need for imaging tools which are capable of early detection and therapy monitoring. Molecular imaging is emerging as one of the technologies that will be able to fulfil these needs[2, 3].

Here we review the concept and potential of molecular imaging in the cardiology clinic. This will be illustrated by examples of promising technologies in imaging of cell death in the heart and atherosclerosis that have entered the clinical trial stage.

THE CONCEPT OF MOLECULAR IMAGING

Molecular imaging can be defined as the in vivo characterization and measurement of biological processes at the cellular and molecular level within living organisms[4]. It has recently been observed with increased interest in cardiovascular disease, as conventional imaging is effective, but lacks the capability of diagnosis of the early stage of pathologic processes. Molecular imaging technology has shown to have potential to visualize processes in a pre-disease stage and monitor early therapeutic effects (Figure 1). Finally, if imaging technology could be capable of visualizing the efficacy of existing therapeutics and novel ones such as gene delivery, this could represent a great advance in personalized treatment of patients.

FIGURE 1: Conventional versus Molecular imaging
Molecular imaging requires a shift from imaging anatomical and physiological changes towards detection of molecular changes underlying the disease, with the ability to visualize processes in a pre-disease or beginning stage.

The development of molecular imaging tools is a challenging process. First, clinically relevant targets need to be found and analyzed to what extent they represent an ongoing disease-related cellular or molecular process of interest. Subsequently, a suitable probe needs to be developed, along with an appropriate tracer for in vivo application, using efficient organ and intracellular targeting and amplification strategies. Finally, evaluation of the probe in vitro and in vivo is necessary before it can be advanced into clinical trials. In Figure 2 the development of molecular imaging tools is explained in more detail.

Some of these targets have been identified for imaging of molecular and cellular processes that reflect important pathophysiological processes in cardiovascular disease. Several interesting targets have been clinically tested in atherosclerosis, in particular detection of the plaque that is vulnerable to rupture[5], and imaging of thrombi[6]. Furthermore, cell death has been visualized in patients with myocardial infarction,[7, 8] heart failure[9] and also in oncology,[10, 11] another important field for molecular imaging.

The main modalities that are being used for molecular imaging in cardiovascular disease are PET (positron emission tomography) and SPECT (single photon emission computed tomography), magnetic resonance (MR), ultrasound (US) and near-infrared fluorescence (NIRF). Most of these tools are used preclinically; however, some have managed to embark in the clinical setting.
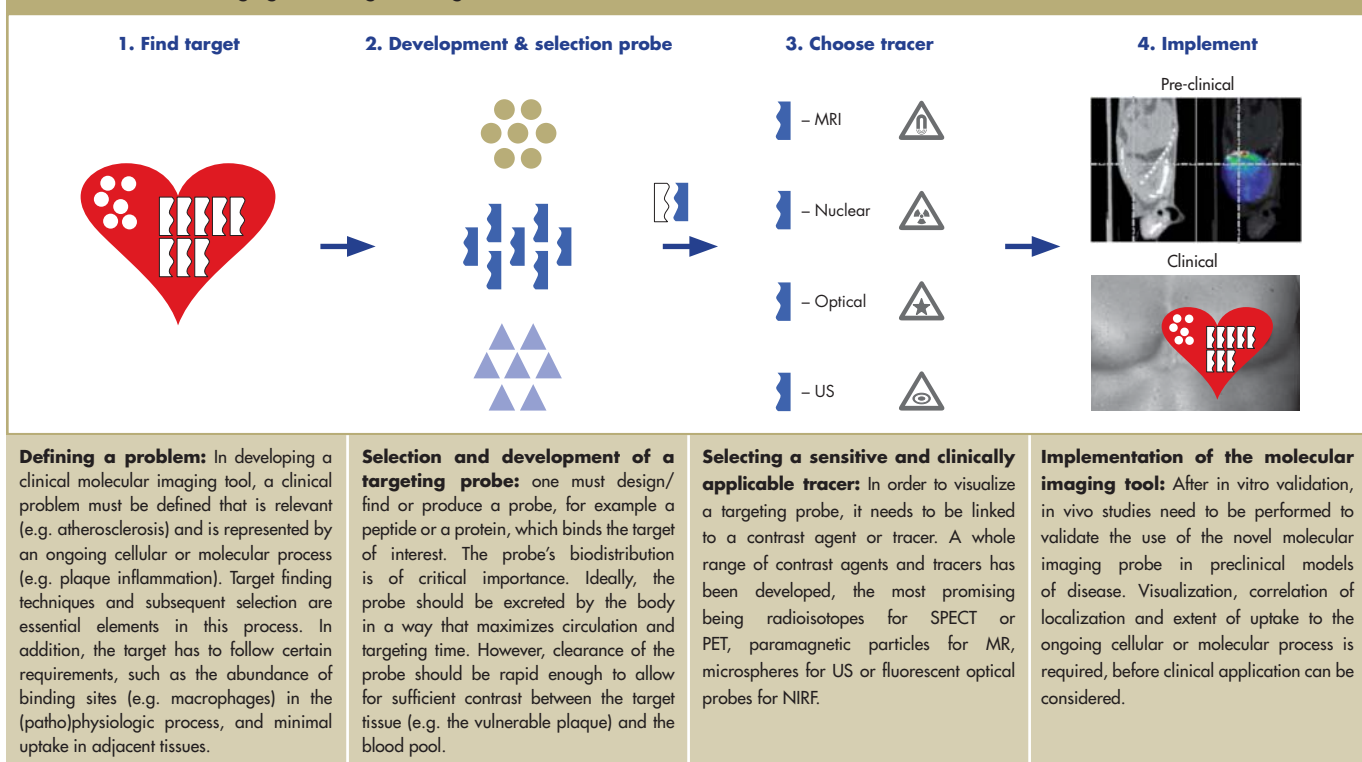
Nuclear medicine has the longest history of molecular imaging, with first specific targets being imaged over 30 years ago. It consists of molecular probes, labelled with a short-lived positron- or gamma (photon) emitting radionuclide, which are injected into the human body. Subsequently, its distribution is analyzed with PET or SPECT to understand the interaction between the radiolabeled molecular probe and its target molecules in vivo[12]. Currently, the most widely used PET radiopharmaceutical is the glucose analog ^{18}F-FDG (fluoro-2-deoxy-D-glucose). ^{18}F-FDG has been used extensively to estimate glucose utilization in heart and brain. ^{18}F-FDG stays inside the cells, since ^{18}F-FDG lacks the 2-oxygen in glucose, disabling glycolysis before radioactive decay. Therefore ^{18}F-FDG gives a good reflection of the distribution of glucose uptake in the body, and allows for imaging cells with high metabolic rate. In general, PET facilities must have cyclotrons to make ultra-short half-lived radionuclides (F-18: 108 min, C-11: 20 min, N-13: 10 min, O-15: 2 min). Like PET, SPECT is a quantitative, radionuclide-based imaging method, although it is up to two orders of magnitude less sensitive than PET. It employs longer-lived radionuclides, often requiring chelation chemistry for appropriate linking to the targeting agent. The most common gamma emitters used are technetium-99m, iodine-123, indium-111, and iodine-131 with physical half-lives of 6 hrs, 13 hrs, 3 days and 8 days, respectively.

Another good candidate for molecular imaging is magnetic resonance imaging (MRI)[13]. Although the sensitivity is relatively low when compared with PET or SPECT, MRI allows high-resolution anatomical and functional images of the cardiovascular system. The detection of events at the molecular level, however, usually requires high sensitivity. In response to the inadequate sensitivity of gadolinium chelates, novel MR contrast agents have been developed with significantly higher relaxivities[14]. These include paramagnetic gadolinium-containing liposomes and superparamagnetic iron oxide nanoparticles[15].

Also in the field of ultrasound, targeted microbubbles or other acoustically active nanoparticles are being used for molecular imaging[16, 17]. Microbubbles are injected and targeted to the diseased tissue where they produce a detectable acoustic signal. Targeting of the bubbles is achieved by conjugating ligands to the microbubble surface. As the size of microbubbles is relatively large (1-2 μm), these contrast agents cannot leave the intravascular space,[17] unless the vasculature in the target organ becomes leaky[18]. Consequently, molecular imaging of contrast ultrasound has involved mainly molecular changes within the vascular compartment. Targeted contrast ultrasound has therefore been applied in angiogenesis,[19, 20] vascular inflammation[21, 22] and thrombus formation[23].

FIGURE 2: Molecular Imaging: from target finding to visualization

1. Find target
2. Development & selection probe
3. Choose tracer
   – MRI
   – Nuclear
   – Optical
   – US
4. Implement
   Pre-clinical
   Clinical

Defining a problem: In developing a clinical molecular imaging tool, a clinical problem must be defined that is relevant (e.g. atherosclerosis) and is represented by an ongoing cellular or molecular process (e.g. plaque inflammation). Target finding techniques and subsequent selection are essential elements in this process. In addition, the target has to follow certain requirements, such as the abundance of binding sites (e.g. macrophages) in the (patho)physiologic process, and minimal uptake in adjacent tissues.

Selection and development of a targeting probe: one must design/find or produce a probe, for example a peptide or a protein, which binds the target of interest. The probe's biodistribution is of critical importance. Ideally, the probe should be excreted by the body in a way that maximizes circulation and targeting time. However, clearance of the probe should be rapid enough to allow for sufficient contrast between the target tissue (e.g. the vulnerable plaque) and the blood pool.

Selecting a sensitive and clinically applicable tracer: In order to visualize a targeting probe, it needs to be linked to a contrast agent or tracer. A whole range of contrast agents and tracers has been developed, the most promising being radioisotopes for SPECT or PET, paramagnetic particles for MR, microspheres for US or fluorescent optical probes for NIRF.

Implementation of the molecular imaging tool: After in vitro validation, in vivo studies need to be performed to validate the use of the novel molecular imaging probe in preclinical models of disease. Visualization, correlation of localization and extent of uptake to the ongoing cellular or molecular process is required, before clinical application can be considered.

Finally, near infrared fluorescence (NIRF) has emerged as one of the most sensitive applications that is widely applied in small animal research. The main restriction of using NIRF is the limited penetration depth[24, 25]. Despite this limitation, NIRF imaging may become useful in the detection of superficial signal in tissues such as the breast and the carotid artery. In addition, a promising approach of NIRF is fiberoptical imaging to characterize vulnerable components of atheroma in the cath lab[26].

The abovementioned molecular imaging tools have been listed in an overview (Table 1) to provide information on the spatial and temporal resolution, sensitivity, depth and dosing of each modality. The combination of molecular imaging tools with existing anatomical modalities, such as computed tomography (e.g. PET/SPECT-CT) and expected in the future, magnetic resonance imaging (e.g. PET-MR), enables localization of the uptake and improved quantification, specificity and sensitivity.

TABLE 1: Characteristics of imaging modalities

Modality	Spatial resolution	Depth	Temporal resolution	Sensitivity (mol/L)	[Molecular probe]
CT	50–200 µm	No limit	min	–	N/A
Ultrasound	50–500 µm	mm-cm	sec-min	–	µg-mg
MRI	25–100 µm	No limit	min-hrs	10^{-3}-10^{-5}	µg-mg
Nuclear					
PET	1–2 mm	No limit	10 s-min	10^{-11}-10^{-12}	ng
SPECT	0.5–1.5 mm	No limit	min	10^{-10}-10^{-11}	ng
Optical					
Bioluminescence	3–5 mm	1–2 mm	sec-min	10^{-15}-10^{-17}	µg-mg
Fluorescence	2–3 mm	<1 mm	sec-min	10^{-9}-10^{-12}	µg-mg

CLINICAL MOLECULAR IMAGING IN CARDIOVASCULAR DISEASE

Imaging of cell death

Programmed cell death plays a crucial role in the pathogenesis of cardiovascular disease. A noninvasive imaging technique capable of localizing and quantifying cell death would permit assessment of disease progression or regression and similarly define the efficacy of therapy designed to inhibit or induce cell death. Necrosis and apoptosis are two distinct types of cell death which are hallmarks of injury after myocardial infarction[27, 28]. Necrosis occurs after exogenous insults such as ischemic, or inflammatory injury, and manifests itself by cellular swelling and rupture, disintegration of subcellular organelles, and their release of factors to extracellular vicinity that incites inflammatory response[27]. In contrast, apoptosis occurs by activation of a suicide program that leads to contractile protein fragmentation and condensation, along with DNA fragmentation and chromatin condensation; the cell shrinks and forms small apoptotic bodies, which are cleared without disruption of surrounding tissue architecture or induction of inflammation[27]. It has been traditionally believed that ischemia is associated with necrotic cell death. Recently it has been demonstrated that ischemic cell damage initiates the cascade of the apoptotic cell death program, which is an energy-requiring process in contrast to necrosis. As the energy production ceases in ischemia, a large proportion of the apoptotic cells die by the passive process of secondary necrosis.

Apoptosis leads to even redistribution of phospholipids across the lipid bilayer, with abundant expression of phosphatidylserine (PS) on the outer surface of the cell membrane[29, 30]. Annexin A5 (Anx A5), an endogenous human protein, has high affinity for exteriorized PS,[31, 32] and has been employed for SPECT imaging following intravenous injection after labelling with technetium-99m. The first clinical use of technetium-99m labelled Annexin A5 has been to visualize cell death in patients with acute myocardial infarction[7]. In this study, the Anx A5 SPECT scan showed uptake that was clearly confined to the region of perfusion loss as demonstrated by ^{201}thallium perfusion imaging (see Figure 3A). Based on earlier studies in mice, the explanation of Anx A5 positivity in the myocardial region is likely to reflect the aggregate of cell death, both necrosis as well as apoptosis[7].
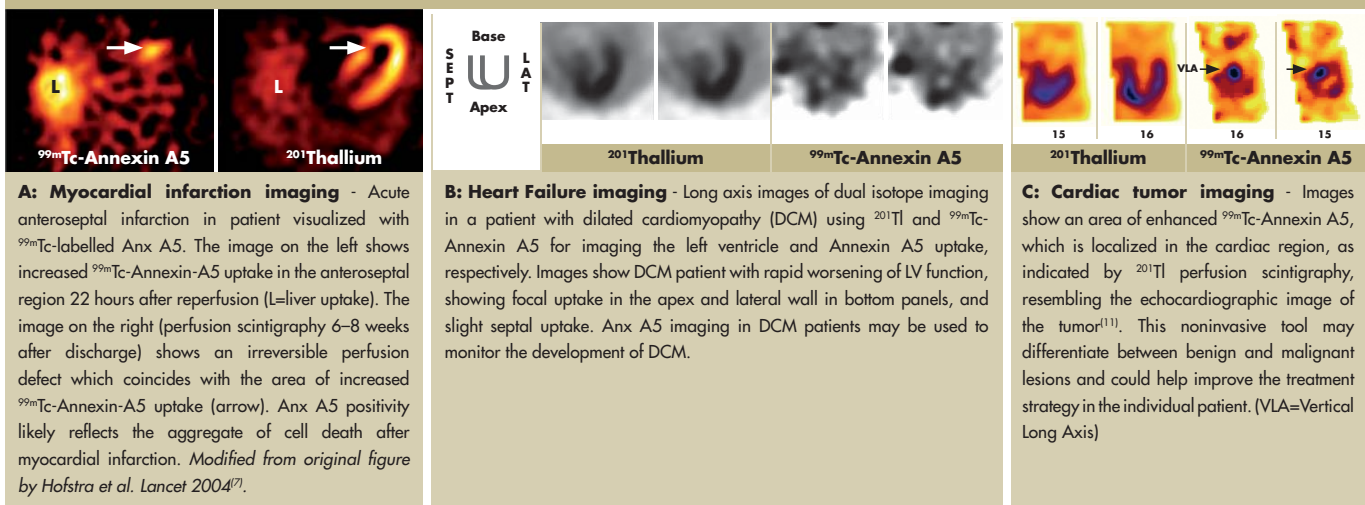
In a recent study, Annexin A5 imaging has also been performed in 9 patients with non-ischemic cardiomyopathy and advanced heart failure[9]. Of the nine patients, five showed Annexin A5 uptake in the left ventricular myocardium, without uptake in the right ventricle (Figure 3B). Uptake was found in focal regions in the myocardium in three patients, whereas one patient showed diffuse cardiac uptake of the technetium-99m labeled Annexin. Myocardial perfusion was essentially normal in the patients and did not match the areas of Annexin A5 uptake, contrasting with the infarction perfusion defect and uptake. These four patients had dilated cardiomyopathy (DCM) and had recently experienced worsening of heart failure in the last 3 months. The fifth patient had a myosin gene mutation and demonstrated a positive, diffuse uptake explained by a substantial decrease in LVEF in the past 6 months. Imaging cell death in heart failure may be used to monitor and compare therapeutic interventions aimed at reducing the degree or rate of development of DCM.

The presence of cardiac masses poses a diagnostic problem, since biopsies are associated with a high complication rate. Malignant tumors are characterized by an increased mitotic cell count as well as the rate of apoptosis, as compared to benign tumors[33]. In an early clinical report, SPECT imaging of an intracardiac sarcoma was performed in a patient using ^{99m}Tc-Anx A5 along with a thallium-201 perfusion scintigram[11]. Molecular imaging using Anx A5 might therefore distinguish between benign and malignant lesions and could provide prognostic information noninvasively and determine the strategy in the individual patient.

Imaging the unstable atherosclerotic plaque

Most of the acute coronary syndromes are thought to be the result of thrombosis of the coronary artery[34]. The etiology of thrombosis is three-fold: plaque rupture (55-60% of cases), erosion (30-35%) and

FIGURE 3: Molecular imaging of cell death

A: Myocardial infarction imaging - Acute anteroseptal infarction in patient visualized with 99mTc-labelled Anx A5. The image on the left shows increased 99mTc-Annexin-A5 uptake in the anteroseptal region 22 hours after reperfusion (L=liver uptake). The image on the right (perfusion scintigraphy 6–8 weeks after discharge) shows an irreversible perfusion defect which coincides with the area of increased 99mTc-Annexin-A5 uptake (arrow). Anx A5 positivity likely reflects the aggregate of cell death after myocardial infarction. *Modified from original figure by Hofstra et al. Lancet 2004*[7].

B: Heart Failure imaging - Long axis images of dual isotope imaging in a patient with dilated cardiomyopathy (DCM) using 201Tl and 99mTc-Annexin A5 for imaging the left ventricle and Annexin A5 uptake, respectively. Images show DCM patient with rapid worsening of LV function, showing focal uptake in the apex and lateral wall in bottom panels, and slight septal uptake. Anx A5 imaging in DCM patients may be used to monitor the development of DCM.

C: Cardiac tumor imaging - Images show an area of enhanced 99mTc-Annexin A5, which is localized in the cardiac region, as indicated by 201Tl perfusion scintigraphy, resembling the echocardiographic image of the tumor[11]. This noninvasive tool may differentiate between benign and malignant lesions and could help improve the treatment strategy in the individual patient. (VLA=Vertical Long Axis)

calcified nodules (3-7%)[35-38]. The ruptured plaque is characterized by a lesion with a necrotic core, with an overlying thin ruptured fibrous cap that results in obstruction of the coronary artery. The eroded plaque has a thrombus which adheres to a proteoglycan-rich layer and smooth muscle cells with minimal inflammation. Lastly, the calcified nodule is the least common of all lesions that cause thrombosis. It shows a calcified plate with superimposed bony nodules that result in discontinuity of the fibrous cap without endothelial cells with overlying luminal thrombus.

The knowledge that a large fraction of ruptured plaques have non-significant stenoses warrant the move beyond stress testing and coronary angiography in order to attempt identification of high risk patients with potentially vulnerable plaques that are not flow-limiting[39-41]. Multi-slice computed tomography (MSCT) is one of the important candidates for detection of atherosclerotic lesions in the coronary artery wall following an intravenous injection of a contrast agent[42]. MSCT technology has dramatically increased the ability to detect coronary artery plaque noninvasively in patients at risk, detecting both soft and calcified plaques for imaging. However, the ability to identify the biological characteristic of high risk plaques using MSCT remains limited. Therefore, the challenge remains to find a method that detects the potentially lethal plaque. To achieve this, quantifiable information regarding the cellular, biochemical and molecular composition of lesions needs to be obtained. Various imaging techniques, such as angioscopy[43] and intravascular ultrasonography[44, 45] or more recently with optical coherence tomography,[46, 47] thermography,[48, 49] elastography[50, 51] and magnetic resonance imaging,[52] have attempted to characterize these plaques using tissue characteristics such as fibrous cap thickness, necrotic core and the severity of the inflammatory component in the lesions. Although promising, these technologies have shown limited clinical success.

To collect information about the molecular and cellular composition of plaques, PET and SPECT nuclear imaging have emerged as potential tools to find a plaque vulnerable to rupture. Potential targets for the detection of inflammation in vulnerable plaque are LDL and macrophage content[53]. Although the application of nuclear imaging to characterize atherosclerotic lesions is still in its infancy, one of the first promising attempts was imaging the unstable plaque using 99mTc-Anx A5[54]. In a preliminary study, 4 patients were imaged with carotid vascular disease, 2 of whom had suffered a recent transient ischemic attack (TIA). Anx A5 uptake was seen in the carotid region in the two patients, with recent TIA. No uptake was discernable in the other two patients who were being treated with statins after suffering a TIA longer than six months prior to imaging (Figure 4A). One day after Anx A5 SPECT imaging, patients underwent carotid endarterectomy. Positive images correlated with plaque macrophage content and Anx A5 uptake, whereas both patients with negative annexin scans had smooth muscle cell-rich lesions. One of the two positive patients with recent TIA had a large atheroma in the contralateral carotid artery, however without Anx A5 uptake. These data indicate a promising potential for Anx A5 imaging for the detection of the vulnerable plaque.

The first studies to show that 18F-FDG might have a role in imaging atherosclerosis were performed in a rabbit model, showing uptake by macrophages in plaques in the aortic arch. 18F-FDG uptake appeared to be related to macrophage content of the plaque[55]. Consequently, a clinical study using FDG-PET imaging was performed by Rudd et al.[56], where FDG-PET scans were performed on 8 patients who had suffered a recent TIA and in whom there was evidence of internal carotid artery stenosis. Co-registration with CT angiograms confirmed significant FDG uptake coinciding with identified stenotic plaques. In the contralateral asymptomatic plaques, significantly less FDG uptake was found (p=0.005) and normal arteries did not show any uptake (Figure 4B).

FIGURE 4: Unstable Plaque imaging

A: Imaging unstable plaque lesions of the carotid artery using 99mTc-Annexin A5. Panel A (upper) shows images obtained by SPECT in patient 1, who experienced a left-sided TIA 3 days before imaging. This patient had significant stenoses of both carotid arteries. Only one side showed uptake of 99mTc-Annexin A5, which coincided with the culprit lesion (see arrows). Histopathology of the plaque performed after endarterectomy showed substantial infiltration of macrophages into the neointima, with extensive binding of annexin A5 (anti-Annexin A5 antibody, brown). Lower panel shows Anx A5 imaging in a second patient, who had experienced a right-sided TIA three months before, did not show uptake in the carotid region, neither left or right. Histologic analysis showed a lesion rich in smooth-muscle cells, with negligible binding of Annexin A5. This preliminary study suggests that Anx A5 may provide a valuable tool in assessing plaque vulnerability. *Modified from original figure by Kietselaer et al. NEJM 2004[54].*

B: FDG-PET (left), CT angiography (middle) and fused (right) images from a patient with carotid stenosis who was symptomatic (top row) and with a contralateral asymptomatic carotid stenosis (bottom row). The arrows point at FDG uptake corresponding to stenotic carotid plaque. Note that FDG uptake into symptomatic plaque was significantly higher. *Modified with permission from original figure by Rudd et al. Circulation 2002[56].*

C: PET / CT images show transaxial slices through heart. Enhanced uptake of FDG, reflecting inflammation (arrow-heads), is present proximal and distal to the calcified left anterior descending artery (arrow). A tumor is localized adjacent to esophagus. *Modified from Dunphy et al. JNM 2005, with permission[57].*

In another study by Dunphy et al. patients with suspected cancer were scanned by CT and FDG-PET (Figure 4C)[57]. Atherosclerotic coronary arteries were found in a subset of these patients. FDG uptake was found in the coronary artery with a proximal and multifocal pattern, which agreed with autopsy findings. One limitation of coronary 18F-FDG imaging was reflected by the fact that myocardial and hepatic FDG uptake prevented evaluation in coronary arteries in approximately half of the patients. The use of beta-blockers prior to imaging may be necessary to suppress this uptake in the myocardium.

The abovementioned glucose metabolism imaging studies provide proof of the principle that FDG-PET can image atherosclerotic plaque inflammation and is able to quantify plaque inflammatory cell activity. If confirmed, these observations suggest that FDG-PET could be used to identify potentially unstable plaques and to monitor effects of drug therapy on plaque inflammation.

OUTLOOK

Almost one-third of total global deaths are caused by the various forms of cardiovascular disease. Around 80% of these deaths take place in low and middle-income countries. Consequently, cardiovascular disease is estimated to be the leading cause of death in developing countries by 2010. Until recently, the diagnosis of cardiovascular disease has been principally focused on the early detection of anatomical and mechanical changes. However, molecular imaging techniques have advanced to the detection of early and pre-disease molecular and cellular changes and

may prove useful for early diagnosis in such a way that events can be reduced or disease can even be prevented.

Another opportunity for molecular imaging lies in clinical trials for the evaluation of new drugs. By using a molecular imaging approach, a reduction in the number of patients is needed for a trial, since molecular imaging could be able to identify a high-risk subgroup in which most events would be expected to occur.

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