

Fundamentals of Cardiovascular Molecular Imaging: a Review of Concepts and Strategies

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Published online: 23 February 2017
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Abstract

Purpose of the Review In this review, we will discuss the basic fundamentals of how to perform molecular imaging to better understand the underlying mechanisms contributing to cardiovascular disease.

Report Findings Molecular imaging combines molecular biology with in vivo imaging. Molecular probes are used to target discrete biological processes such as cell death, inflammation, and angiogenesis. These probes emit signals that are detected by traditional imaging systems. Because the same disease processes can manifest in individuals in different ways, molecular imaging may emerge as an important strategy for delivering precision medicine.

Summary Molecular imaging is a powerful tool that may help physicians provide more personalized care in the near future.

Keywords Molecular imaging · Molecular imaging probes · Cardiovascular disease

Introduction

Molecular imaging combines molecular biology with in vivo imaging, enabling clinicians and researchers to visualize

cellular processes in living subjects without disturbing them. The ability to image minute changes in molecular processes may lay the foundation for the development of novel strategies to diagnose and treat patients with cardiovascular disease earlier and more effectively. Molecular imaging may address the gaps in patient care left by our reliance on traditional imaging and allow for the development of a more personalized approach to disease management. In this review, we will discuss the basic fundamentals and current strategies for molecular imaging of cardiovascular pathology.

Basic Premise

Molecular imaging system requires the following three basic components, as discussed in detail below: (1) a biological target, (2) an imaging probe, and (3) an imaging system. As shown in Fig. 1, a probe designed to target the biological process of interest is administered to the subject. The probe emits a signal, which is detectable by the imaging modality (i.e., single photon emission tomography, positron emission tomography, ultrasound and/or magnetic resonance imaging). The key factors for successful imaging include the probe specificity to discriminate the targeted biological pathway, the probe's distribution within the tissue, its duration of retention, as well as the sensitivity of the imaging system to detect the signal generated by the probe.

Molecular Imaging Probes

A molecular imaging probe is an imaging tool that is used to qualitatively evaluate and quantitatively measure biological processes. It is comprised of a signal agent, a targeting moiety, and a linker that couples the target moiety with the signal

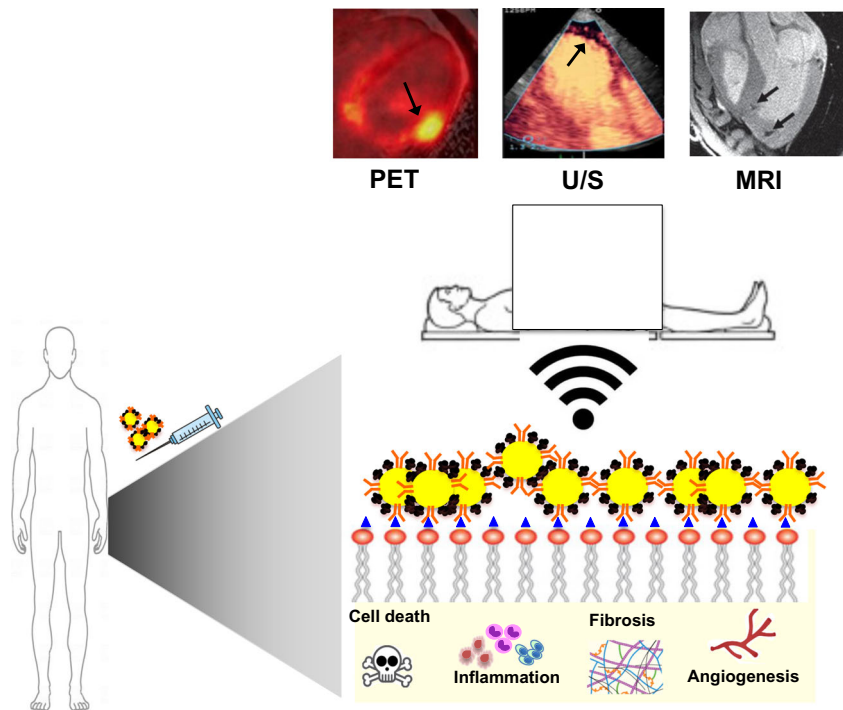
This article is part of the Topical Collection on *Molecular Imaging*

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Fig. 1 Schematic of the fundamental components of molecular imaging systems. Molecular probes targeting specific cardiovascular disease processes (e.g., cell death, inflammation, fibrosis, and angiogenesis) are conjugated to probes that emit signals that are detected by probe-specific imaging systems (e.g., PET, U/S, and CT)



agent. Signal agents range from bioluminescent or fluorescent molecules for optical imaging, radionuclides for positron emission tomography (PET) and single photon emission computed tomography (SPECT), microbubbles for ultrasound imaging, and magnetic particles for magnetic resonance imaging (MRI). Target moieties can be small molecules, peptides, proteins, antibodies, and nanoparticles, all of which interact with a target (e.g., enzyme, receptor, DNA/RNA, physiological state) in a specific biological process. The linker agent, which joins the target moiety with the signal agent, may also modify the pharmacokinetics of the imaging agent, affecting its biodistribution and tissue retention. The ideal imaging probe has the following properties, as discussed in detail in other comprehensive reviews [1, 2]: (1) high target affinity, (2) high target specificity, (3) high sensitivity, (4) high contrast ratio, (5) high stability in vivo, (6) low immunogenicity and minimal toxicity, and (7) easy and economical production. Given these prerequisites, it is not surprising that the number of validated probes remains limited. According to the Molecular Imaging and Contrast Agents Database (MICAD), which promotes the development and application of imaging probes to advance the field of molecular imaging [3], there are approximately 1444 agents for both cardiac and noncardiac applications that are currently available (January 2013). Of these, 241 are FDA approved. Based on the modality used for imaging, the largest category of probes described in MICAD are those used for positron emission tomography (41.6%), followed by agents used for single-photon emission computed tomography (30.3%), optical imaging (12.0%), magnetic resonance imaging (9.3%), multimodality imaging

(3.4%), ultrasound (2.4%), and X-ray/computed tomography (1.0%). Details of recent advancements in probe development are highlighted below.

Radionuclide Agents

Although most radiolabeled compounds are dedicated to cancer imaging, a few radionuclide probes have been used to image cardiovascular specific markers. In general, PET tracers have advantages over SPECT tracers because some positron emission isotopes (e.g., C, N and O) are principal components of biological molecules and can be used without significantly changing the chemical properties of target moieties. Although the most commonly used radionuclide fluorine 18 (^{18}F) is not a constituent of biomolecules, it is a bioisostere of the biomolecule hydrogen. Fludeoxyglucose, the most frequently used PET probe, has a fluorine-18 substituted for the normal hydroxyl group at the C-2 position in the glucose molecule, and is taken up by metabolically active cells, enabling it to serve as a nonspecific marker for imaging inflammation in the myocardium [4] and atherosclerotic plaques [5]. More specific agents have also been investigated including ^{18}F -fluoromisonidazole (^{18}F -FMISO), a radiotracer specific for hypoxia, which was shown to correlate not only with the presence of atherosclerotic plaque, but also its progression [5]. In addition, fluorinated ^{18}F has been conjugated to annexin and integrin $\alpha_v\beta_3$ to target cell death and angiogenesis, respectively. Zhu et al. [6] recently demonstrated the feasibility of imaging apoptosis in a rat model of ischemia-reperfusion injury, showing that the area of reperfusion injury

had a 7-fold higher standard uptake value than the viable myocardium. Importantly, the imaging signal correlated with histological markers of apoptosis. Finally, Gao et al. [7] evaluated a novel one step labeling $\alpha_v\beta_3$ integrin probe targeting angiogenesis in a rat model of ischemia reperfusion injury. The tracer 18F-AIF-NOTA-PRGD2 allowed longitudinal noninvasive visualization of angiogenesis induced by ischemia-reperfusion injury, which was consistent with immunohistochemistry analysis of the distribution of CD31 and CD61 (e.g., markers of vascular density and β_3 expression, respectively).

Optical Imaging Agents

Promising optical imaging probes include organic fluorophores, nanoparticle-based contrast agents, and semiconductor crystals. Because of their low molecular weight, high quantum yield, low cost, and well-validated conjugation strategies, organic fluorophores are an ideal optical imaging label. Dyes in the near fluorescent range are of greatest interest owing to their depth of penetration. Using a NIR dye conjugated to an antibody targeting oxidized LDL, for instance, Khamis et al. [8] successfully visualized the atherosclerotic plaques of mice lacking the LDL receptor. In vivo application of these fluorophores, however, is limited by their shallow depth of penetration and the difficulty achieving high target to background ratios [8]. One approach to reduce background noise is to use a smart fluorescent probe, which incorporates a protease that is subsequently cleaved. In the absence of the protease, these probes do not fluoresce due to self-quenching. Cleavage of the peptide linker, in contrast, results in fluorescence. Using this approach, Abd-Ehrahman et al. [9] demonstrated that macrophages in murine atherosclerotic plaques had elevated levels of cathepsin protease activity. The administration of a cathepsin inhibitor induced macrophage apoptosis, suggesting that cathepsin targeting is a promising approach for the treatment of plaque inflammation. An alternative strategy is to use metallic nanoparticles composed of gold or silver that efficiently scatter light. Gold nanoparticles, for example, have been used to label macrophages injected into an apolipoprotein E-deficient mouse to track their recruitment using computed tomography (CT) [10]. Gold nanoparticles have also been conjugated to annexin and Technetium 99M to target apoptotic macrophages in the vulnerable plaque [11]. A final strategy is to use quantum dots, which are semiconductor nanocrystals with diameters smaller than the Bohr excitation diameter (2–10 nm). Advantages over organic fluorophores include improved brightness due to a high extinction coefficient, enhanced photostability, and increased flexibility to label multiple biomarkers. Recently, Chen et al. [12] developed functionalized quantum dots with VCAM-1 binding peptides to selectively target VCAM-1 expressing endothelial cells to image inflammation as a marker of early atherosclerosis. Although

results are preliminary, they demonstrate the feasibility of quantum dots to detect early signs of atherosclerosis.

Ultrasound Imaging Agents

Ultrasound molecular imaging is achieved by using microbubbles containing targeting ligands on the bubble shell. These microbubbles remain intravascular, aggregate in regions expressing the disease biomarker, and appear as bright areas of signal that localize the target. One of the primary advantages of microbubble imaging is that it allows real-time in vivo imaging of evolving disease processes. By conjugating specific anti-GP IIb/IIIa single-chain antibodies to microbubbles, for example, Wang et al. [13] visualized thrombi and its subsequent resolution after the administration of thrombolytics in the diseased carotid arteries of mice. Importantly, microbubbles can also be conjugated to multiple ligands to evaluate different components of the same disease process. To image both platelet adhesion and vascular inflammation in a mouse model of atherosclerosis, for example, Liu et al. [14] used microbubbles that targeted platelet glycoprotein Ib alpha (GPIb α), P selectin, and VCAM-1, respectively. More recently, studies have investigated the feasibility of using ultrasound-targeted microbubble destruction (UTMD) to enhance the transfection efficiency of naked plasmid DNA for delivering cardiovascular gene or drug therapy [15]. Chen et al. [16] used UTMD to deliver the thymosin beta 4 (TB4) gene into the normal rat heart to enhance the differentiation of resident adult cardioprogenitor cells. After the microbubbles reached the target organ, the payload attached to the microbubbles was destroyed by ultrasound. Although results are promising, the duration of exposure, frequency, mode, mechanical index, and payload amounts need further optimization.

Magnetic Resonance Imaging (MRI) Contrast Agents

MRI contrast agents modify the magnetic properties of T1, T2, and susceptibility. The positive contrast agents are typically gadolinium- or magnesium-containing paramagnetic chelates, which predominantly shorten the longitudinal relaxation time (T1) and increase the relaxation rate of water (e.g., relaxivity). Tissues that take up this contrast agent become bright or appear hyperintense on T1-weighted MRI. Negative contrast agents, otherwise known as superparamagnetic or susceptibility contrast agents, are small iron oxide particles that generate local magnetic field gradients that disrupt the primary magnetic field over the tissue, causing a T2* shortening or susceptibility effect and resulting in hypointense signals on T2-, T2*-, and T1-weighted MR images.

The principal challenge of molecular MRI is its relatively low sensitivity (micromolar range) compared to radiotracer

and fluorescence techniques (picomolar range). To compensate for this, most approaches using MRI have either selected to image highly expressed molecular targets that are very abundant (e.g., fibrin or thrombi) or delivered contrast agents packaged in liposomes or micelles (e.g., gadolinium chelate) or aggregated as nanoparticles (e.g., iron oxide). Dellinger et al. [17], for example, used liposomes containing gadolinium encased in a carbon shells to increase longitudinal relaxivity 25-fold. Liposomes were coupled with a ligand that binds the surface of macrophage foam cells on atherosclerotic plaques to improve target specificity. Time-dependent accumulation of gadolinium was found in aortas of ApoE^{-/-} mice, which was confirmed by histology and not observed in controls. No toxicity was reported. Similarly, Chen et al. [18] labeled high-density lipoprotein nanoparticles bound to a collagen-specific peptide with gadolinium chelates to target intraplaque macrophages during plaque regression in a mouse model of atherosclerosis. While MRI probes continue to improve, they still lack the sensitivity required to image discrete molecular processes and remain inferior to optical and radionuclide probes.

Multimodal Imaging Agents

First introduced a decade ago, multimodality imaging agents consist of a target moiety that is conjugated to two signal agents, enabling simultaneous or sequential imaging of biological processes using two complementary imaging systems. Radionuclide and MRI probes joined to optical imaging agents are most frequently used for multimodality imaging. Withana et al. [19], for example, used a dual modality optical and PET/CT probe to noninvasively image activated macrophages in a murine stroke model as well as ex vivo samples of diseased human carotid arteries (Fig. 2). Multimodality imaging has also been used to monitor stem cell fate after transplantation. Transplanted cells transfected with triple fusion reporter genes that incorporate optical (e.g., firefly luciferase for bioluminescence imaging) and radionuclide probes (herpes simplex virus type 1 thymidine kinase, HSV1-tk, for PET imaging) in addition to green fluorescent protein for histological confirmation have been used to evaluate cellular proliferation, migration, and death after injection into small and large animals [20]. By incorporating multiple modality imaging probes, studies can harness the strengths and address the weaknesses of each molecular imaging approach.

Imaging Modalities

Like traditional imaging, molecular imaging uses standard equipment to visualize the interaction of the probe and target including nuclear, optical, ultrasound, and magnetic resonance imaging systems. Recent advances in technology have improved the application of these imaging systems for molecular

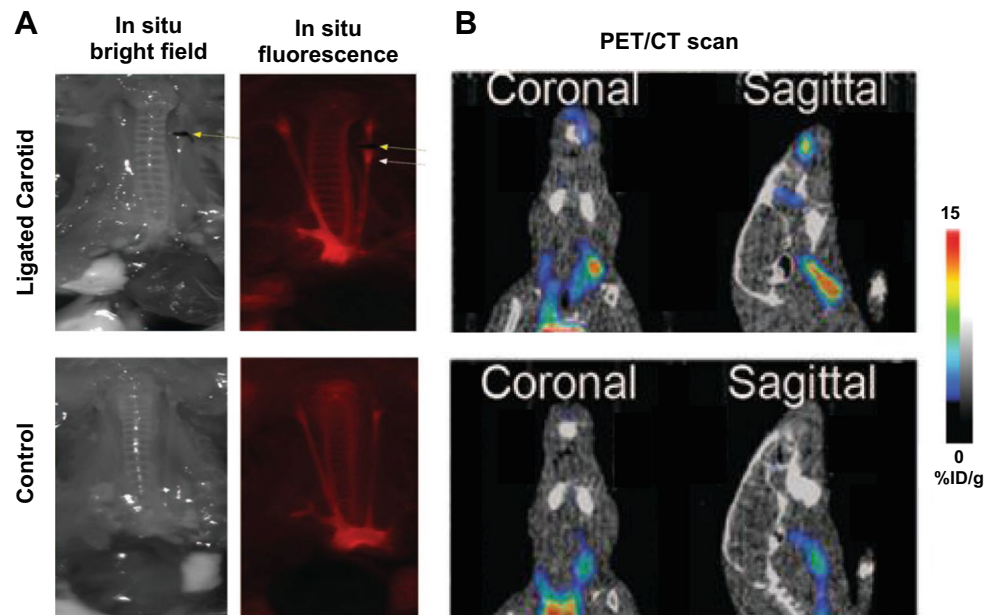
imaging. For additional information on instrumentation, we refer the reader to other comprehensive reviews [21].

The two most common imaging systems to detect nuclear-based probes are SPECT and PET. These imaging systems have superb sensitivity (nanomolar to picomolar range), excellent depth of penetration (unrestricted) and field of view (centimeters to submeter scales), good temporal resolution (seconds to minutes), short examination times (seconds to minute), and a wide selection of probes for cardiovascular disease processes. Importantly, the same probes can be used in humans as well as small animals although modified scanners are required for small animal imaging. The main disadvantage of nuclear imaging, however, is its low spatial resolution, resulting in difficulty identifying the anatomical source of emission. SPECT cameras actually offer the poorest spatial resolution of current imaging techniques. In recent years, the addition of an increasing number of detectors and modified collimator designs have improved sensitivity 4–8-fold, paving the way for clinical use in cardiovascular molecular imaging.

In comparison to nuclear imaging, optical imaging offers similar sensitivity with greater spatial resolution. Despite lower costs and strides in the past 20 years, techniques such as bioluminescence and fluorescence continue to be limited by poor depth of signal penetration (few millimeters to centimeters) due to scatter and attenuation. These factors limit optical imaging to the whole body and intravascular imaging of small animals and humans, respectively. Although the development of 3D optical imaging with diffuse optical tomography and fluorescence molecular tomography offers improved depth sensitivity than planar imaging, imaging depth continues to be limited by photon scatter. Optoacoustic imaging ameliorates this situation by utilizing ultrasound transducers to detect the signal released by the thermal expansion of fluorophores [22]. Because biological tissues are more transparent to sound than light, the depth of imaging is, thus, expanded.

The advantages of ultrasound imaging systems over other imaging modalities include 1) its low cost, portability, and real-time capabilities, 2) improved imaging depth relative to optical imaging, and 3) high temporal resolution. It also possesses the unique ability to remove contrast agents, manipulate contrast distribution with radial force, and combine therapy with imaging. Challenges, however, remain including its limited sensitivity (due to poor contrast retention at the target site and high background signal), relatively small field of view, and inability to accurately quantify signal. One approach to address these challenges is to minimize microbubble destruction by using low amplitudes and higher frequencies. To detect these microbubble echoes that have a low mechanical index, pulse inversion imaging and contrast pulse sequencing can be used. These strategies, however, may add to scan time and reduces frame rates, limiting the clinical translation of ultrasound for molecular imaging.

Fig. 2 Dual modality optical and PET/CT probe, ^{64}Cu -BMV101, for imaging activated macrophages in a murine stroke model. **a** In situ fluorescence imaging ^{64}Cu -BMV101 in mice with and without ligated carotid arteries. **b** Coronal and sagittal PET/CT images of the ^{64}Cu -BMV101 in mice with and without ligated carotid arteries. This figure was originally published in *JNM*. Withana et al. Dual-activity vascular inflammation imaging. *J Nucl Med*. 2016;57:1583–1590. © by the Society of Nuclear Medicine and Molecular Imaging, Inc.



Unlike ultrasound, MRI provides exquisite high-resolution anatomical information with excellent soft tissue contrast; however, it also suffers from low detection sensitivity, requiring 10 μM to 10 mM of contrast agent for visualization. To compensate for its low sensitivity, MRI can be combined with nuclear and optical imaging to improve target localization and detection of less ubiquitous targets. Recent development of superconducting magnet technology that does not require cryogenic fluid for cooling has also enabled less costly installation and improved accessibility to molecular magnetic resonance molecular imaging.

Biological Processes

Cell Death

The major modes of cell death in the heart include oncosis (ischemic cell death), apoptosis (programmed cell death 1), and autophagic cell death (programmed cell death 2), which have all been detected using electron microscopy, molecular techniques, and immunohistochemical approaches. While readers may be more familiar with the first two processes, autophagy has emerged as an important contributor of myocyte death. Autophagic death is mediated by excessive intracellular lysosome catabolic metabolism that is normally responsible for the degradation and recycling of damaged or dysfunctional cytoplasmic components and intracellular organelles [23, 24]. In essence, autophagy results from the cell “canabilizing” itself from the inside.

A number of strategies have been proposed to image cell death. By far, the most experience over the past two decades

has been the use of annexin as the target moiety. Annexin binds to phosphatidylserine residues expressed on the outer leaflet of the plasma membranes of dying cells. Annexin V has been coupled with radionuclides for nuclear imaging, magnetic nanoparticles, and gadolinium-containing liposomes for MRI, and fluorescent markers for optical imaging [25]. A major limitation is the large size of annexin derivatives, which may impact its long-term storage, increase its immunogenicity, and limit its access to target tissue. Furthermore, annexin cannot distinguish between oncosis and apoptosis because both processes can exhibit externalization of phosphatidylserine residues while still maintaining cell membrane integrity [26].

Over the last 5 years, studies have evaluated the feasibility of using intracellular rather than extracellular apoptosis markers such as the expression of caspases, which play an important role in the initiation and execution of cell death, and the loss of membrane potential across the inner mitochondrial membrane ($\Delta\psi\text{m}$), which accompanies the induction of cell death. The most common strategy is to image caspase activation using optical imaging approaches (e.g., fluorescence or bioluminescence imaging). To detect apoptosis associated with doxorubicin toxicity, for example, Su et al. [27] synthesized an ^{18}F -labeled tetrapeptidic caspase substrate, ^{18}F -CP18, which contains the caspase 3-specific recognition sequence DEVD. DEVD is cleaved intracellularly in the presence of activated caspase 3, resulting in the accumulation of radioactive DEVD in the cytoplasm of apoptotic cells. A 2-fold increase in uptake of CP18 was observed in mice treated with doxorubicin compared to controls, which was verified by ex vivo measures of caspase 3-enzymatic activity and apoptosis. A promising alternative approach is using isatins, which are small molecules that selectively bind and covalently

inhibit caspase 3 activation. Thukkani et al. [28], for example, successfully synthesized a radiolabeled istatin 5-sulfonamide ([18F]WC-4116) and demonstrated a 2-fold higher uptake of [18F]WC-4-116 in animals after ischemia reperfusion injury compared to control animals.

Apoptosis can also be quantified by measuring changes in $\Delta\psi_m$. Changes in the $\Delta\psi_m$ can be assessed using fluorescent or radiolabeled lipophilic cations such as triphenylphosphonium (TPP), whose large surface area enables rapid, several hundred fold accumulation in cells [29]. Although TPP has been shown to accurately measure $\Delta\psi_m$ in vitro [30] and ex vivo [31], an initial study in a swine model of ischemia reperfusion injury using TPP radiolabeled with fluorine (18F-TPP) overestimated $\Delta\psi_m$, suggesting that these measures are not yet ready for clinical application [32]. Improved accuracy may be achieved with the use of two TPP compounds simultaneously, which react together to form a stable product that accumulates in the cytosol, thereby, increasing sensitivity to small changes in membrane potential [33••].

While many strategies have been explored to image oncosis and apoptosis, imaging of autophagy has been restricted to reporter imaging models using transgenic mice that express microtubule-associated protein 1A/1B-light chain 3 (e.g., LC3, a marker protein for autophagosomes) and green fluorescent protein. Using an alternative approach of imaging a cathepsin-activated fluorochrome, Chen et al. [34] demonstrated the feasibility of in vivo fluorescent tomography of the upregulation of autophagy after rapamycin therapy. Studies of autophagy in humans, however, have not yet been performed.

Inflammation

Inflammation is a major component of many types of cardiovascular disease including atherosclerosis, myocarditis, and remodeling. One of the major strategies for imaging inflammation at the molecular level is to target the migration of immune cells as they interact with noxious stimuli. A favorite target in cardiovascular disease is imaging monocyte/macrophage trafficking. Although initial approaches have been nuclear based, magnetic resonance molecular imaging alone or in combination with PET approaches has emerged as promising alternatives. Because of their phagocytic ability, monocytes and macrophages readily take up MRI contrast agents. Macrophage molecular MRI has been used to study atherosclerosis [35, 36, 37•], aortic aneurysm [38], infarction [39–41], and myocarditis [42]. In a recent study, Pedersen et al. [37•] used a novel PET ligand [(64)Cu] [1,4,7,10-tetraazacyclododecane-N,N',N',N'-tetraacetic acid]-d-Phe1,Tyr3-octreotate ((64)Cu-DOTATATE) to target macrophages, enabling PET-MRI of carotid plaques in ten patients prior to carotid endarterectomy. The study reported higher uptake of the tracer in symptomatic plaques compared to

contralateral controls in the same patient, suggesting that the tracer can be used to identify vulnerable plaques.

The other main strategy is to imaging tissue-specific markers of inflammation such as endothelial cell adhesion molecules (e.g., selectins, ICAM-1, and VCAM-1). These proteins signal the presence of endothelial cell activation and attract inflammatory cells into vulnerable atherosclerotic plaques. One limitation of this approach is the low target to background ratios of available agents. In an effort to identify a more sensitive agent, Dimastromatteo et al. [43] tested several derivatives of VCAM-1-specific major histocompatibility complex 1-derived peptide B2702p. They found 2–3-fold increased activity in target tissues in a mouse model of stroke using (99m)Tc-B2702p1, suggesting that this may be a sensitive marker for detecting inflammation in the vulnerable plaque.

Because calcification is the final process of inflammation, calcium activity has also been imaged using SPECT or PET imaging with 99mTc-phosphonate or 18F-fluoride, respectively. In a promising prospective clinical trial, Joshi et al. [44••] demonstrated the highest uptake of 18F-NaF, which is adsorbed into calcified deposits with high affinity and selectivity [45], at the site of culprit lesion in 37/40 patients presenting with myocardial infarction and 18/40 patients with high-risk coronary arterial plaque (Fig. 3). Whether identifying inflammation within the vulnerable plaque can alter morbidity and mortality needs further exploration.

Fibrosis

Fibrosis is a pathological process characterized by an increased collagen within the extracellular matrix and is a feature of almost all cardiovascular diseases. Following myocyte loss, fibrosis replaces dying myocytes (e.g., replacement fibrosis). Alternatively, stress or inflammation can result in a reactive fibrosis that could lead to ventricular stiffening, functional compromise, and heart failure.

Although traditionally, late gadolinium contrast MRI has been used to identify fibrosis, T1 mapping has emerged as a surrogate measure for fibrosis by evaluating changes in the extracellular matrix. This approach typically uses a modified Look-Locker inversion recovery (MOLLI), which obtains diastolic single-shot images with multiple different T1 sensitivities that are incorporated into a T1 recovery function to create a map. The T1 time has been shown to inversely correlate with histological fibrosis on endomyocardial biopsy in transplant patients [40] and cardiac events in patients with heart failure [46].

For molecular imaging of fibrosis, the most common targets for imaging fibrosis include collagen, myofibroblasts, intracardiac renin-angiotensin axis, matrix metalloproteinases, and matricellular proteins [47]. Collagen can be directly targeted using collagen avid peptide such as CNA35-based probes. Although CNA35 has been successfully used to image collagen in animal models of abdominal aortic aneurysm [48] and

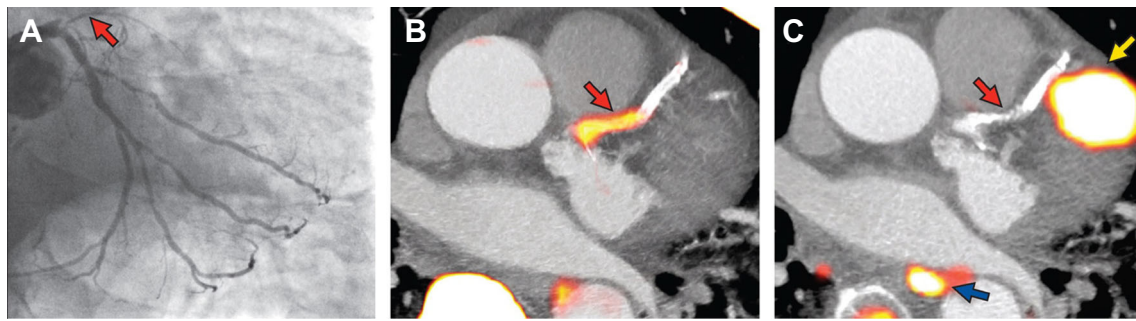


Fig. 3 PET/MRI comparing focal ^{18}F -NaF and ^{18}F -FDG in a patient with an acute ST elevation myocardial infarction. **a** Invasive angiography showing proximal occlusion of the left anterior descending artery. **b** Focal

uptake of ^{18}F -NaF in the culprit vessel. **c** No uptake of ^{18}F -FDG is observed in the culprit vessel. Adapted and reprinted with permission from Joshni et al., *Lancet*. 2014;383:705–13

atherosclerosis [49], in vivo cardiac imaging has not yet been demonstrated. Alternatively, the renin angiotensin system (RAS) and/or myofibroblasts can be targeted because cardiomyocyte stretch induces RAS, which promotes fibroblast differentiation into myofibroblasts that secrete collagen. The feasibility of imaging RAS was demonstrated in a study by Dilsizian et al. [50] who showed increased uptake of technetium-99m-labeled lisinopril (an angiotensin receptor blocker) in transgenic rats that overexpress angiotensin-converting enzyme compared to control animals. Because a discussion of all the potential targets for imaging fibrosis is beyond the scope of this review, we refer the reader to other comprehensive reviews for information on other molecular targets for imaging fibrosis [47]. Successful visualization of these targets involved in the development of myocardial fibrosis may help us better understand the mechanism regulating this pathological process.

Angiogenesis

Angiogenesis, the new growth of capillaries from preexisting vessels, is a complex process that is an integral part of atherosclerosis. Although vessels are generally post-mitotic in adults, endothelial cells have retained the ability to become mobile, invade, and sprout into tissue. Perhaps the most well-studied target for imaging angiogenesis is integrin $\alpha v \beta 3$, which is a cell adhesion molecule that is low in normal tissue but is highly expressed in activated endothelial cells. Integrin $\alpha v \beta 3$ contains a binding site for cyclic Arg-Gly-Asp (RGD) peptide, which has been radiolabeled with SPECT or PET tracers to image angiogenesis in several animal and human studies. In one recent clinical study in 21 patients 2 weeks post ST elevation myocardial infarction, Jenkins et al. [51] demonstrated increased uptake of a novel PET RGD-radiotracer, ^{18}F -fluciclatide, in areas of new myocardial infarction compared to areas of old myocardial infarction and control patients. The tracer preferentially accumulated in areas of subendocardial injury with associated regional wall hypokinesia that subsequently recovered, suggesting that it can serve as a marker of cardiac repair. Integrin $\alpha v \beta 3$ expression, however, is not

restricted to endothelial cells and is found in macrophages [52]; thus, its specificity for angiogenesis has been questioned. Moreover, these integrins are not required for angiogenesis and can be compensated by the upregulation of VEGF-R2 expression [53]. A promising alternative is to image the expression of integrin $\alpha 5 \beta 1$, which is only found on endothelial cells. Comparing the specificity of integrin $\alpha 5 \beta 1$ and $\alpha v \beta 3$ using ^{68}Ga -based tracers for imaging tumor angiogenesis, Notni et al. [54] confirmed the reliability of targeting $\alpha 5 \beta 1$ as a marker of angiogenesis. Although studies in myocardial infarction models are still forthcoming, this study suggests that targeting integrin $\alpha 5 \beta 1$ may be a viable approach to image myocardial angiogenesis.

Future Directions

Although much has been achieved over the last decade in molecular imaging, it still remains a research tool that has not yet made its debut in clinical practice. One potential reason for its lack of routine use is that large-scale studies have yet to demonstrate a clear mortality and morbidity benefit in incorporating molecular imaging into the care of patients with cardiovascular disease. The push toward personalized medicine may present an opportunity to bring these valuable tools to the bedside. With its potential to complement the information obtained from genetic data, molecular imaging may help better characterize individual disease phenotypes and determine who may or may not benefit from therapy. In the next decade, molecular imaging may be incorporated in clinical trials of novel cardiovascular therapeutics to better understand individual variation in response treatment.

Compliance with Ethical Standards

Conflict of Interest All of the authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of major importance

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