Emergence of molecular imaging of aortic aneurysm: Implications for risk stratification and management

Reza Golestani, MD, PhD,^{a,b} and Mehran M. Sadeghi, MD^{a,b}

^a Cardiovascular Molecular Imaging Laboratory, Section of Cardiovascular Medicine and Yale Cardiovascular Research Center, Yale University School of Medicine, New Haven, CT

^b VA Connecticut Healthcare System, West Haven, CT

Received Nov 5, 2013; accepted Nov 19, 2013

doi:10.1007/s12350-013-9845-5

Imaging cellular and molecular processes associated with aneurysm expansion, dissection, and rupture can potentially transform the management of patients with thoracic and abdominal aortic aneurysm. Here, we review recent advances in molecular imaging of aortic aneurysm, focusing on imaging modalities with the greatest potential for clinical translation and application, PET, SPECT, and MRI. Inflammation (e.g., with ¹⁸F-FDG, nanoparticles) and matrix remodeling (e.g., with matrix metalloproteinase-targeted tracers) are highlighted as promising targets for molecular imaging of aneurysm. Potential alternative or complementary approaches to molecular imaging for aneurysm risk stratification are briefly discussed.

Key Words: Aneurysm • aorta • molecular imaging • inflammation • remodeling

INTRODUCTION

Over the past two decades, cardiovascular molecular imaging has moved from a concept to a field on the verge of clinical translation. Potential applications of molecular imaging in cardiovascular medicine continue to expand with some approaches advancing to the clinic, while others are just emerging. The role of molecular imaging as a research tool and its potential contribution to understanding cardiovascular pathophysiology have received considerable attention. Perhaps less emphasis has been placed on the potential of molecular imaging in transforming clinical practice by establishing novel diagnostic and management paradigms, and specific

requirements for its clinical application. Seeking to fill this gap, this is the first of a series of reviews on cardiovascular molecular imaging focused on clinical translation and application. To this end, each review starts with a clinical scenario, followed by a discussion of the scope of the problem and diagnostic gaps; pathophysiology; state of the art in molecular imaging; alternative or complementary diagnostics; pitfalls, barriers, and future directions; and potential contribution of molecular imaging to the highlighted case. We hope the format will be of interest to clinicians and scientists and serve as a trigger for dialog to bring the field closer to clinical practice.

CLINICAL SCENARIO

A 65-year-old male with history of dyslipidemia and smoking is referred for routine screening ultrasound for abdominal aortic aneurysm (AAA). Evaluation confirms that the patient is asymptomatic and the physical examination is unremarkable. Screening identifies an infrarenal fusiform AAA with a diameter of 48 mm.

Funding This work was supported by the National Institutes of Health R01HL112992 and R01HL114703, and the Department of Veterans Affairs Merit Award IOBX001750.

Reprint requests: Mehran M. Sadeghi MD, VA Connecticut Healthcare System, 950 Campbell Avenue, 111B, West Haven, CT 06516; mehran.sadeghi@yale.edu.

J Nucl Cardiol 2014;21:251–67.

1071-3581/\$34.00

Copyright © 2013 American Society of Nuclear Cardiology.

SCOPE OF THE PROBLEM AND DIAGNOSTIC GAPS

The World Health Organization estimates that twelve million deaths were caused worldwide by cardiovascular diseases in 2002. While myocardial infarction and stroke remain the leading causes of cardiovascular mortality, aneurysm rupture and dissection are responsible for a considerable number of cardiovascular deaths. As such, every year, ~10,000 deaths are caused by AAA in the US.¹

Arterial aneurysm is defined as a focal dilation of the artery to 1.5 times its normal diameter.² In the case of abdominal aorta, by convention, it is considered aneurysmal if its diameter is over 30 mm.³ The prevalence of AAA is reported to be 4.2% in US veterans aged 50-79 years⁴ and 7.2% (of which one-fifth are larger than 45 mm) in Australian men over 65 years of age.⁵ Age, gender (male), race (non-black), family history, smoking, and the presence of atherosclerotic disease are risk factors for AAA.⁴ Less information is available on the incidence of thoracic aortic aneurysm (TAA).⁶ A study in Sweden found the incidence of TAA to be increasing over time, reaching 16.3 and 9.1 per 100,000 per year, respectively, for men and women, in 2002.⁷

Most aneurysms are asymptomatic. Rupture and dissection are the main, highly lethal complications of aortic aneurysm. Aneurysm size is the best predictor of its propensity to rupture. As such, rupture occurs in 25-41% of AAA with a diameter >50 mm over a 5-year period.⁸ Rapid expansion of an aneurysm is believed to increase its propensity to rupture. Surgical repair is recommended for AAA with a diameter >55 mm, those that increase in size by >10 mm per year, or those that are symptomatic.⁹ While open surgery is the mainstay of repair interventions in aortic aneurysm, endovascular aneurysm repair (EVAR) was recently introduced as an alternative to open surgery for aortic aneurysm in a select group of patients. In the US, funding is provided through Medicare for a one-time ultrasound screening for AAA in men >65 years of age who have ever smoked or those of both genders with a family history of AAA.¹⁰ The recommended surveillance interval for AAA depends on its size and expansion rate. For AAA with a diameter of 40 to 54 mm, the surveillance interval is 6 to 12 months. The interval can be longer for smaller aneurysms.^{11,12}

While larger aneurysms are more prone to rupture, screening trials have demonstrated that a notable number of ruptures occur in smaller aneurysms which do not meet the criteria for surgical repair.^{13,14} For example, in the Multi-center Aneurysm Screening Study, 23% of ruptures in patients with known aneurysm occurred in

those who did not meet the size criteria for repair. Similarly, in the Aneurysm Detection and Management Veterans Affairs Study, there was a rupture rate of 0.6% per year in aneurysms of 40 to 54 mm diameter undergoing biannual surveillance by ultrasonography. A study of small aneurysms in the UK found a rupture rate of 1% per year for aneurysms of 40 to 55 mm in diameter.¹⁵ The risk of rupture is even higher in women, smokers, and those with a history of AAA. Mainly because of the morbidity and mortality associated with surgical repair of aneurysm, none of the randomized studies performed to date support routine surgical repair of smaller aneurysms.¹⁵ Identification of smaller aneurysms that are prone to rupture can tilt the risk-to-benefit balance toward early treatment of the high-risk small aneurysms. At the other end of the spectrum, many patients with aneurysm diameter >55 mm have comorbidities that adversely affect their outcome from invasive interventions. The ability to further risk stratify these patients based on factors other than size would help reassess their risk-to-benefit ratio of therapeutic interventions.

Existing imaging modalities for aortic aneurysm (ultrasonography, CT, and MRI) provide little information beyond anatomy, primarily the dimension of the aneurysm. The challenge is to develop novel approaches to define the risk of expansion, rupture, or dissection more accurately. This would have a major impact on clinical practice (and public health) and can possibly help track the effect of novel therapeutic interventions.

PATHOPHYSIOLOGY

The aorta is a heterogeneous organ, with regional disparities in its cellular origin and structure which are reflected in differences between TAA and AAA pathophysiology. These differences are discussed in depth in several excellent recent reviews.¹⁶⁻¹⁸ As such, while genetics-based abnormalities of matrix proteins and transforming growth factor (TGF)- β signaling appear to play a predominant role in the development of TAA, AAA is more often associated with atherosclerosis. Nevertheless, both diseases share common features (e.g., inflammation and matrix remodeling) that may be targeted for imaging. Endothelial activation, upregulation of adhesion molecules, and recruitment of leukocytes are early events in vascular pathology and probably play a similar role in AAA development. Pro-inflammatory chemokines and cytokines are locally produced and accelerate the inflammatory process. This in turn activates proteases, e.g., matrix metalloproteinases (MMPs), which through digestion of matrix proteins facilitate and promote further recruitment of

Table 1. Potential targets and representative tracers for molecular imaging of aneurysm

Process	Target	Representative tracers	Modality	Comments
Inflammation	Cell metabolism	¹⁸ F-FDG ^{25,26,28,30,34}	PET	Pre-clinical and clinical studies Highly available, FDA-approved tracer Determinants of cellular uptake remain disputed Image acquisition protocols and image analysis methods need standardization Inconsistent data regarding predictive value for aneurysm expansion and rupture
Matrix remodeling	Phagocytosis	(U)SPIO ^{41,43,45}	MRI	Pre-clinical and clinical studies Blood clearance of SPIO may be too fast for vascular imaging Cellular determinants of uptake need further investigation Image acquisition and analysis methods need standardization Hemorrhage can complicate interpretation of images USPIO has shown promise for predicting aneurysm expansion
		¹⁸ F-nanoparticles ⁵¹	PET	Pre-clinical studies Potential alternative to (U)SPIO
	MMP activation	MMPsense 680 ^{64,65}	FMT	Slow blood clearance can result in high radiation exposure Pre-clinical studies Not applicable to non-invasive imaging due to limited depth of penetration
		p947 ⁵⁷	MRI	Pre-clinical studies Moderate binding affinity to activated MMPs
		RP782 ⁶⁹	SPECT	In vivo determinants of the tracer uptake need further validation Pre-clinical studies Good binding affinity for activated MMPs Predictive value for aneurysm expansion and rupture needs further evaluation
	Matrix proteins	CAN-35 ⁷¹	MRI	Pre-clinical studies Binds to less-organized collagen Low enhancement patterns were observed in both normal and high-risk aortae
		Elastin-specific magnetic resonance contrast agent ⁷²	MRI	Pre-clinical studies Good agreement between tracer uptake and AAA size The role of imaging in predicting expansion and rupture remains to be determined

Table 1. Continued

Process	Target	Representative tracers	Modality	Comments
Angiogenesis (and inflammation)	VEGF receptors	scVEGF-Cy5.5 ⁸⁸	NIRF	Pre-clinical studies Signal intensity correlated with aortic diameter Uptake does not co-localize with neovessels Not applicable to non-invasive imaging
	$\alpha\text{v}\beta 3$ integrin	NC100692 ⁹⁷ ^{18}F -FPPRGD ₂ ⁹⁸	SPECT PET	Pre-clinical studies Uptake correlated with neovessels and macrophage content of arterial wall Cellular determinants of tracer uptake need further evaluation

FDG, Fluorodeoxy glucose; MMP, matrix metalloproteinase; (U)SPIO, (ultrasmall) superparamagnetic iron oxide; VEGF, vascular endothelial growth factor.

inflammatory cells. AAA expansion is associated with a high density of inflammatory cells in the vessel wall. Cytokines and other mediators released by monocyte-macrophages, e.g., reactive oxygen species and prostaglandin derivatives, may induce vascular smooth muscle cell (VSMC) death and media atrophy. VSMC paucity and dysfunction adversely affect vascular repair mechanisms and contribute to AAA expansion. Inflammation, matrix degradation and disorganization, and media atrophy weaken aortic tensile strength and alter its geometry. This in conjunction with changes in wall stress can lead to aneurysm rupture.

MOLECULAR IMAGING

Molecular imaging may target key molecular and cellular players in the pathogenesis of a disease, or biomarkers that, while not direct players in the pathogenesis, potentially provide clinically (or biologically) relevant information on its course. In the case of aortic aneurysm, molecular imaging combined with anatomical imaging and assessment of vessel wall mechanics may offer a comprehensive picture of the disease, including information on its propensity for progression and morbid complications (Table 1).

Inflammation

An extensive inflammatory infiltrate is present in the media and adventitia of the aneurysm in patients undergoing surgery for AAA symptoms, rapid expansion, or rupture. This infiltrate contains components of both adaptive and innate immunity, including T and B lymphocytes, natural killer cells, and macrophages. The products of these cells, e.g., interferon- γ , tumor necrosis factor- α , and interleukin-4, can contribute to aneurysm progression and rupture by promoting inflammation, VSMC death, protease production, and extracellular matrix degradation.^{19,20} As such, inflammatory cell activity is a rational target for imaging aneurysm biology.

Metabolism. ^{18}F -fluorodeoxy glucose (FDG), a glucose analog which accumulates in cells with high metabolic activity, is often used for PET imaging of inflammation in various clinical settings.²¹ The high metabolic activity of activated leukocytes has raised the possibility of using ^{18}F -FDG PET for detection of vessel wall inflammation in vasculitis, atherosclerosis, and aneurysm. The first reports of ^{18}F -FDG PET imaging of aortic aneurysm and the possibility of an association between ^{18}F -FDG uptake and aneurysm expansion, dissection or rupture, date back to more than a decade.²² Since then, partly as a result of widespread availability and routine use of ^{18}F -FDG PET in imaging centers, a

Figure 1. ^{18}F -FDG PET and aneurysm expansion in AAA. Examples of transaxial ^{18}F -FDG PET (right) and fused PET/CT (left) images in patients with high (A) or low (B) ^{18}F -FDG uptake in AAA. (C) Correlation between average SUV_{max} on transaxial images along the aneurysmal segment of aorta and subsequent annual expansion of aneurysm. Reproduced with kind permission from Springer Science + Business Media, Kotze et al²⁸ Figures 1, 2, and 4, © Springer-Verlag.

Figure 2. ^{18}F -FDG PET and outcome in thoracic aortic dissection. (A) Transaxial PET/CT images in a patient with favorable outcome, showing low ^{18}F -FDG uptake in intramural hematoma on PET/CT obtained at 50 minutes (middle) and 100 minutes (right). (B) Transaxial PET/CT images in a patient with unfavorable outcome showing high ^{18}F -FDG uptake in the dissected aortic wall on PET/CT obtained at 50 minutes (middle) and 100 minutes (right). Aortic dissection can be seen on contrast-enhanced CT (left) on both sets of images. Arrows point to area of dissection. This research was originally published in *JNM*. (Kato et al.²³, © by the Society of Nuclear Medicine and Molecular Imaging, Inc.).

number of studies have been performed to investigate the potential role of ^{18}F -FDG PET in evaluation of human aortic diseases.²³⁻³³ As an example, the association between aortic aneurysm and ^{18}F -FDG uptake in the vessel wall was investigated in a study in 17 patients with AAA (maximal abdominal aortic diameter >3.0 cm) and 17 age- and sex-matched controls selected from a database of patients undergoing ^{18}F -FDG PET for primary lung cancer. In this study, the maximal standardized uptake value (SUV_{max}) was significantly higher in the AAA group, but ^{18}F -FDG uptake in AAA did not correlate with maximal aortic diameter.²⁶

Several small studies have linked ^{18}F -FDG uptake in AAA to histologically validated vessel wall inflammation.^{30,34} In a recent study of 18 patients who underwent AAA reparative surgery, ^{18}F -FDG PET prior to surgery showed no aneurysmal ^{18}F -FDG uptake in 10 patients and aneurysmal uptake of the tracer in 8 patients.³⁴ Aortic SUV_{max} to liver SUV_{max} ratio had a modest yet significant correlation with medial and adventitial T and B lymphocyte and macrophage content. While this association between ^{18}F -FDG uptake and vessel wall inflammation is promising, concluding that ^{18}F -FDG uptake is a result of vessel wall inflammation would probably require interventional studies aimed at modulating inflammation and demonstrating a corresponding reduction in tracer uptake in the vessel wall. One such intervention could be treatment by

statins, which are believed to act also as anti-inflammatory agents. Notably, one small study that looked into this question did not find a difference in AAA ^{18}F -FDG uptake between patients taking statins and those not taking these drugs.²⁶

A potential contribution of ^{18}F -FDG PET imaging to clinical management of aneurysm is to provide reliable information on its propensity for expansion, rupture, or dissection. Demonstration of such a capability is complicated by the average annual change in aneurysm diameter (~ 2 mm in the case of AAA),^{13,14} which is close to the accuracy of commonly used imaging techniques (ultrasound, CT). Also, the reproducibility of ^{18}F -FDG uptake in aneurysm and fluctuation of the signal over long periods of follow-up remain to be determined. The predictive value of ^{18}F -FDG PET imaging was investigated in 34 patients under routine surveillance for non-surgical AAA who were followed for 12 months after an index ^{18}F -FDG PET study. Surprisingly, in these patients, there was an inverse correlation between average SUV_{max} of transaxial images of aneurysm and future expansion determined by ultrasound (Figure 1).²⁸ Similarly, high ^{18}F -FDG uptake was reported in the so-called inflammatory AAA, which is considered a distinct entity less prone to rupture.^{25,35} These findings contrast with the results of a study of ^{18}F -FDG PET imaging performed in the acute phase of thoracic aortic dissection, in which

Figure 3. USPIO-enhanced MRI and risk of rupture in AAA. (A–F) Examples of T_2^* color maps of USPIO-enhanced (A–C) and T2W MR images (D–F) showing three different patterns of enhancement in AAA: group 1, a large change only in periluminal area (A); group 2, diffuse patchy change (B); and group 3, discrete focal area of USPIO accumulation distant from periluminal area (C). The magnitude of the change in T_2^* is shown on the color scale, with blue indicating the change below a threshold determined from non-enhanced images. (G) Percent USPIO-positive voxels in the aortic wall (solid bars) and focal areas of increased USPIO uptake (open bars) in each group of patients. USPIO-positive voxels are significantly higher in group 3 compared with the other 2 groups. (H) AAA diameter and growth rate in each group of patients. Initial aneurysm diameters (open bars) are similar for the 3 groups, but the aneurysm growth rates (solid bars) are significantly higher for patients in group 3 compared with those in groups 1 and 2. (Adapted with permission from Lippincott Williams and Wilkins/Wolters Kluwer Health, Circulation: Cardiovascular Imaging,⁴⁵ © 2011).

SUV_{mean} on images obtained at 50 minutes (but not those obtained at 100 minutes) predicted an unfavorable outcome (Figure 2).²³ There are considerable differences in image acquisition, processing, and analysis protocols between various vascular ^{18}F -FDG PET studies, making it difficult to compare the results. Examples of these variables include the timing of imaging (1 vs 3 hours after tracer administration), fasting status, and quantification methodology (SUV_{mean} vs SUV_{max} vs

relative SUV vs target-to-background ratio), highlighting the importance of efforts aimed at standardizing the methodology for vascular ^{18}F -FDG PET imaging.^{36,37} There is also considerable debate on determinants of ^{18}F -FDG uptake by inflammatory cells, which appears to be more complicated than initially envisaged.^{38–40} As ^{18}F -FDG can accumulate in any highly metabolic cell, there is a clinical need for more specific tools to visualize and quantify vessel wall inflammation.

Phagocytosis. A potential alternative for imaging vessel wall inflammation is to target the phagocytic activity of macrophages. Superparamagnetic iron oxide (SPIO) and ultrasmall SPIO (USPIO) nanoparticles have shown promise as MR contrast agents for visualizing macrophage phagocytic activity in atherosclerosis and AAA, in small animals and humans.⁴¹⁻⁴⁵ In a recent pilot study, a change in decay of transverse magnetization (appearing as “signal void” on T_2 - and T_2^* -weighted MR images) was noted on images acquired before and 36 hours following USPIO administration in patients with aortic aneurysm, raising the possibility that this approach may be used to detect vessel wall inflammation in AAA.⁴³ The potential of this technique for predicting aneurysm expansion was evaluated in another pilot study in asymptomatic patients with AAA (with a diameter >4.0 cm on ultrasound).⁴⁵ The percent change in T_2^* values after administration of USPIO was calculated and a threshold of significance for change (determined based on the variability in T_2^* change in subjects not receiving USPIO) was applied to the dataset. In all patients, there was a non-specific periluminal USPIO signal (possibly due to uptake in thrombus). In a subset of patients, distinct areas of focal USPIO uptake could be seen beyond the periluminal area. These patients had a threefold higher aneurysm growth rate based on two ultrasounds performed 6 months apart, suggesting that the technique may be used to identify patients at higher risk for expansion (Figure 3).⁴⁵ Importantly, in patients who underwent surgical repair following imaging, iron particles co-localized with CD68+ macrophages.⁴⁵ Co-localization of USPIO particles with adventitial macrophages has also been noted in a murine model of angiotensin II-induced AAA.⁴¹

Although less sensitive than nuclear imaging for detection of vessel wall biology, MRI has the advantage of providing simultaneous information about aneurysm size, composition, and mechanics. However, iron oxide nanoparticle-based MRI faces a number of pitfalls and major challenges that need to be addressed before its routine clinical application.⁴⁶ The basis for signal change by iron oxide particles is complex and depends on their compartment (intracellular/extracellular), among other factors.⁴⁷ SPIOs (but not USPIO) clear rapidly from circulation and are not optimal for vascular imaging.⁴⁸ Coil position can affect the signal.⁴⁸ The methodology for image analysis and signal quantification is based on a number of calculations and quite variable between different studies.⁴⁸ The presence of endogenous iron (e.g., in focal loci of hemorrhage or thrombus) can complicate the interpretation of MR images.⁴⁹ Another potential limitation is USPIO uptake in not only macrophages but also endothelial cells and VSMCs.⁵⁰

Figure 4. MMP microSPECT imaging and aneurysm expansion. (A) Examples of in vivo microCT (A_1), microSPECT (A_2), and fused microSPECT/CT (A_3) images of a mouse 4 weeks after left carotid aneurysm induction showing ^{111}In -RP782 (MMP tracer) uptake in aneurysmal left carotid artery (L, left; R, right; C, coronal plane; S, sagittal plane; T, transversal plane). (B) Example of ex vivo RP782 autoradiography (right panel) and corresponding photography (left panel) of carotid arteries and aorta at 4 weeks after aneurysm induction, confirming tracer uptake in aneurysm. (C) MicroSPECT-derived quantitative analysis of background-corrected RP782 carotid uptake at 2, 4, and 8 weeks after surgery to induce left carotid aneurysm. $*P = .01$, $**P < .001$. (D) Correlation between RP782 uptake in aneurysmal carotid artery at 2 weeks and total vessel area measured at 4 weeks in the same animal. This research was originally published in *JNM*. (Razavian et al⁶⁹, © by the Society of Nuclear Medicine and Molecular Imaging, Inc.).

Radiolabeling of nanoparticles is an alternative approach for imaging macrophages based on their phagocytic activity. The feasibility of this approach was investigated in angiotensin II-induced murine aortic aneurysm using ^{18}F -labeled carbohydrate-coated nanoparticles in conjunction with microPET/CT imaging.⁵¹ On in vivo images obtained 10-12 hours after administration of ^{18}F -labeled nanoparticles, SUV_{mean} was significantly higher in aneurysmal thoracic aorta compared to the aortae of mice without aneurysm. Splenectomy to deplete macrophages reduced the incidence of aneurysm and led to a significant reduction in aortic tracer uptake. Moreover, in animals imaged at an early time point (1 week), microPET signal (expressed as target-to-background ratio) was significantly higher in mice whose aneurysm went on to expand or rupture. While promising as an alternative to (U)SPIO-based MRI discussed above, the long clearance time of radiolabeled nanoparticles could expose the subjects to high levels of ionizing radiation.

Matrix remodeling

Extracellular matrix degradation and disordered matrix synthesis are key contributors to aortic aneurysm development, expansion, and rupture. Several families of proteases, including cysteine proteases (cathepsins), serine proteases (e.g., neutrophil elastase), and MMPs are expressed at higher than normal levels in aortic aneurysm.¹⁹ In each family, there are several members that exhibit elastase activity and may directly contribute to degradation of elastic laminae, a pathological hallmark of aortic aneurysm.

MMP activation. MMPs and cathepsins have been targeted for molecular imaging of vascular remodeling.⁵²⁻⁵⁹ However, beyond a single report on fluorescence signal observed ex vivo following

administration of a cathepsin B activatable probe in spontaneous murine aortic aneurysm,⁵⁸ there is no report of cathepsin imaging in aortic aneurysm. MMPs are a family of zinc-dependent endopeptidases that can degrade extracellular matrix and other proteins.⁶⁰ In general, MMPs are produced as inactive zymogens

which undergo proteolytic or allosteric activation to expose a catalytic domain.⁶⁰ This active site may be targeted for molecular imaging of MMP activation. Inflammatory cells are a major source of MMP production and activation. Several members of MMP family, including MMP-1, -2, -3, -9, -12, and -13, are

Figure 4. continued.

upregulated in aneurysm and their activation contributes to aneurysm progression and rupture.¹⁹ As such, MMP-targeted imaging can potentially provide information on aortic aneurysm propensity for progression and risk of complications. Broad-spectrum MMP inhibitors labeled with gamma-emitters, positron emitters, fluorescent dyes, and MRI contrast agents have been produced and tested for molecular imaging of matrix remodeling (and inflammation) in various settings.⁶¹ Alternatively, it may be possible to develop tracers that detect activation of one or more specific members of MMP family. Given the common structure of MMPs, the development of selective inhibitors for this purpose is a rather challenging task. Nevertheless, as each member of MMP family is a unique protein with distinct biological functions, this approach may be of high value for tracking vascular biology in vivo. Whether imaging a specific member or, alternatively, global activation of MMPs provides the most useful information for risk stratification in aortic aneurysm remains to be empirically determined.

Activatable near infrared fluorescent (NIRF) probes generate a signal upon cleavage of a specific substrate by an enzyme.⁶² This leads to signal amplification

in vivo. However, the fluorescent nature of the signal limits their use in vascular pathology to imaging small animals or invasive approaches based on intravascular detectors.⁶³ Also, this technique often requires a long delay between tracer administration and imaging. The potential of this technique was shown in a murine model of TAA using a protease activatable fluorescent imaging agent, MMPsense 680.⁶⁴ In vivo fluorescence molecular tomography and ex vivo fluorescence reflectance imaging 20 hours after probe administration showed higher fluorescent signal in animals with thoracic aortic aneurysm.⁶⁴ MMPsense 680 contains a peptide substrate, Pro-Leu-Gly-Val-Arg, which can be cleaved with some degree of selectivity by several MMPs, including MMP-1, -2, -7, -8, and -9.⁶² A pilot study in rat AAA used the same probe for real-time catheter-based imaging of aortic protease activity.⁶⁵

Targeted MRI could be an alternative approach to imaging protease activation in aortic aneurysm. A gadolinium-based MR contrast agent with μ M affinities to MMPs (P947⁵⁷) was evaluated for this purpose in a rat model of elastase-induced AAA.⁶⁶ The authors noted a modest mean signal enhancement on T1 spin echo MR images with this agent compared to a non-targeted control agent in aortic segments perfused with elastase. The exact target of P947 remains unclear as there was no ex vivo validation of tracer localization, and this agent has shown similar affinities to other zinc-dependent metalloproteinases including angiotensin-converting enzyme, endothelin-converting enzyme, and aminopeptidases A and N.⁶⁷ It remains to be determined whether these levels of signal enhancement change are applicable to imaging in humans.

In contrast with other targeted imaging modalities, nuclear imaging is readily applicable to imaging of human aortic aneurysm. The feasibility of MMP-targeted imaging of aneurysm was shown in a surgical mouse model of carotid aneurysm using RP782,^{56,68} an ¹¹¹In-labeled macrocyclic tracer with nM affinities for several activated MMPs.⁶⁹ In this study, specific uptake of the tracer in aneurysm could be seen on microSPECT/CT images obtained 2 hours after RP782 administration. RP782 uptake in aneurysm paralleled CD68 (macrophage marker) expression and was highest at 4 weeks after aneurysm induction, while the aneurysm diameter continued to increase over an 8-week period. Importantly, MMP tracer uptake on images obtained at an early time point after aneurysm induction correlated with aneurysm size measured at a later time point, indicating that MMP-targeted imaging can predict future aneurysm expansion in this model (Figure 4).⁶⁹ While promising, these observations, including the ability of MMP-targeted imaging to predict aneurysm expansion and rupture, will need to be replicated in additional

models of aneurysm before they are ready for clinical translation.

Matrix proteins. Collagen and elastin turnover have been targeted for imaging matrix remodeling in aortic aneurysm. CAN-35 is a collagen-binding bacterial protein that preferentially binds to less well-ordered forms of collagen with moderate affinity (500 nM).⁷⁰ In a recent study, paramagnetic (gadolinium-based)/fluorescent micellar nanoparticles functionalized with CAN-35 were used to detect and quantify collagen in a murine model of AAA (induced by angiotensin II infusion and TGF- β neutralization).⁷¹ MRI pre- and 32 hours post-injection of the tracer showed higher vessel wall enhancement in AAA compared to normal aorta, and tissue analysis showed co-localization of collagen and fluorescent micelles in AAA. On early imaging, animals with future aneurysm rupture had a lower signal enhancement (suggestive of less fibrotic response), in comparison with animals with stable aneurysm, suggesting that this technique may be used to identify high-risk AAA. The low enhancement pattern on both normal and high-risk arteries can be a limiting factor for clinical translation of this approach.

Another study used a small-molecular-weight gadolinium-based (pro)elastin-specific MRI contrast agent for MR imaging of arterial wall in a mouse model of angiotensin II-induced AAA.⁷² While this approach provided useful information on vessel wall morphometry, its usefulness with regards to risk of expansion and rupture remains to be determined. Conceptually, tracers with specificity for mature, organized elastin may be more effective for this purpose. The feasibility of molecular imaging of aorta in large animals using the same MR contrast agent was reported by the same group.^{73,74}

Angiogenesis

Extensive neovascularization (mostly medial and adventitial) is present in aortic aneurysm and is believed to contribute to aneurysm progression.^{75–78} In general, neovascularization is an integral part of inflammation and in aortic aneurysm the extent of neovascularization correlates with vessel wall inflammation.⁷⁷ Furthermore, higher levels of neovascularization are detected at the rupture edge in aortic aneurysm,⁷⁹ suggesting that imaging of molecules and processes involved in angiogenesis can potentially depict high-risk aneurysms.

Vascular endothelial growth factor (VEGF) receptors. VEGF and VEGF receptors are key mediators of angiogenesis, and molecular imaging of VEGF receptors using radio- or fluorescent dye-labeled VEGF has shown promising results for imaging angiogenesis in oncology and cardiovascular diseases.^{80–83} Relevant to imaging aneurysm, VEGF receptors are not specific for

endothelial cells and can be expressed on inflammatory cells and VSMCs.^{84–87} The feasibility of molecular imaging of VEGF receptors using a Cy 5.5-labeled single chain VEGF (scVEGF) was tested in a mouse model of angiotensin II-induced AAA. In situ and ex vivo fluorescence imaging (only two hours after tracer administration) showed a significantly higher signal in aneurysm compared with remote non-aneurysmal aorta.⁸⁸ scVEGF signal intensity correlated with aortic diameter, and while there was evidence of considerable adventitial angiogenesis on tissue analysis, scVEGF fluorescence localized along elastic fibers in the media,⁸⁸ questioning whether angiogenesis is indeed the target of scVEGF imaging in this model. Interestingly, in a study on imaging immune-mediated vascular remodeling using the same tracer, scVEGF co-localized with lymphocyte-rich areas of neointima,⁸⁹ highlighting the broad expression pattern of VEGF receptors.

$\alpha v\beta 3$ integrin. Integrins are a large family of cell membrane glycoproteins that, among many other functions, mediate cell-cell and cell-matrix interactions. Depending on environmental cues, integrins alternate between low-affinity and high-affinity states.^{90,91} The switch from the low-affinity to high-affinity state, a process called integrin activation, is associated with conformational changes that may be exploited, in conjunction with changes in integrin expression level, for imaging. $\alpha v\beta 3$ integrin is highly expressed (and activated) in proliferating endothelial cells and has been used as a target for imaging angiogenesis.⁸³ The tripeptide sequence, Arg-Gly-Asp (RGD) which binds to integrins, is the backbone of several tracers used for $\alpha v\beta 3$ -targeted imaging.^{92,93} Besides endothelial cells, VSMCs⁹⁴ and inflammatory cells express $\alpha v\beta 3$ integrin⁹⁵ and thus RGD-based imaging can also be used to detect neointimal hyperplasia⁹⁶ and inflammation in the artery.⁹⁷ The latter was demonstrated in the aforementioned murine model of carotid aneurysm, in which NC100692, a high-affinity ^{99m}Tc-labeled cyclic RGD peptide was used for in vivo microSPECT/CT imaging of vessel wall inflammation in aneurysm.⁹⁷ RGD tracer uptake in aneurysm was attributed, at least in part, to changes in integrin activation state. In another study, an ¹⁸F-labeled RGD-based compound (¹⁸F-FPPRGD₂) was used for $\alpha v\beta 3$ -targeted imaging in murine angiotensin II-induced AAA.⁹⁸ MicroPET/CT imaging performed at 21–28 days after angiotensin II infusion showed higher focal tracer uptake in aneurysmal aortae relative to aortae of saline-infused control mice. Immunostaining and autoradiography demonstrated ¹⁸F-FPPRGD₂ localization in areas of macrophage and neovessel abundance within the aneurysm. Furthermore, the number of neovessels and macrophage density, but not vessel diameter, strongly correlated with target-to-background

ratio of ^{18}F -FPPRGD₂ PET activity in AAA. Consistent with other data reviewed here, these data again indicate that size is not a good metric of underlying pathology within the arterial wall.

ALTERNATIVE OR COMPLEMENTARY DIAGNOSTICS

Genetics

Genetic factors play a role in the pathogenesis of TAA that is increasingly reflected in therapeutic decisions.^{99,100} Marfan syndrome and Loeys-Dietz syndrome are two examples of syndromic disorders with aortic involvement, including TAA and dissection, in which earlier surgical treatment is recommended. In addition, the role of TGF- β pathway dysregulation in TAA has raised the possibility of effective medical therapy for these patients.⁹⁹ In the case of AAA, the role of genetics is less well established. Fifteen percent of patients with AAA have a positive family history without known connective tissue disorder. A positive family history of AAA doubles the risk for developing AAA.^{101,102} Several genetic loci (e.g., 9p21 region and LRP1) have been linked to both familial and sporadic forms of AAA.¹⁰³ However, their contribution to aneurysm expansion or rupture and their impact on management of AAA are not clear.

Circulating biomarkers

Compared with molecular imaging, blood (or urine) biomarkers provide a more global picture of the patient's status. Subtle abnormalities of the rest of the artery(ies) often accompany aortic aneurysm, supporting a potential role for circulating biomarkers in evaluation of aortic aneurysm. Despite many studies, efforts aimed at identification of sensitive and specific biomarkers for aortic aneurysm have yielded limited conclusive results.¹⁰⁴ Circulating biomarkers may be categorized into those associated with the presence of aneurysm and those used for assessment of progression and outcome.¹⁰⁴ In the case of AAA, potential biomarkers include products of extracellular matrix protein turnover, matrix proteases (MMP-9), thrombosis-related proteins, and markers of inflammation (interleukin-6, C-reactive protein).¹⁰⁴⁻¹⁰⁹ A limitation of these biomarkers is their susceptibility to interference by external factors besides aneurysm biology (e.g., systemic inflammation).

Mechanics

Aneurysm rupture occurs when mechanical stress (the main component of which is systemic blood pressure)

exceeds aortic wall tensile strength diminished as a result of matrix remodeling. Patient-specific computational modeling based on aortic geometry (derived from non-invasive imaging), wall stress computation, wall thickness, strength, and compliance, and the presence and extent of intraluminal thrombus may help identify patients who are at risk for aortic aneurysm expansion or rupture.^{110,111} Many of these variables are best measured ex vivo, but can be replaced with more or less accuracy with surrogate in vivo measurements. While a difference in this predicted wall stress is reported between ruptured and non-ruptured AAA,^{112,113} the predictive value (as well as reproducibility) of these measurements for aneurysm expansion, rupture, or dissection remains to be determined. Labor intensity and complexity of these calculations in their current form are major barriers to their routine application in clinical practice. Interestingly, in a small study in 5 patients with thoracic or AAA, regions of high predicted wall stress co-localized with region of high ^{18}F -FDG uptake on PET images obtained 60 minutes after tracer administration,³³ raising the specter of redundancy (rather than complementarity) of biomechanics assessment and molecular imaging for risk stratification in aortic aneurysm.

PITFALLS, BARRIERS, AND FUTURE DIRECTIONS

Advances in molecular imaging can potentially have a major impact on management of patients with aortic aneurysm. Nuclear imaging techniques, with their high sensitivity and quantitative nature (in conjunction with high resolution anatomic imaging by CT or MRI), can be quite useful for molecular imaging of aneurysm. Like any new field, enthusiasm for novelty is tempered with the necessity of sound methodology and a stepwise approach toward clinical translation. Lack of optimal pre-clinical models of aneurysm, especially in large animals, is a hurdle to be addressed.¹¹⁴ The nature of the target (size, proximity to blood, etc.) complicates imaging protocol, its reproducibility, and data analysis and may lead to misleading conclusions. As an example, non-specific uptake of tracers is very common in inflammatory diseases such as aortic aneurysm; abdominal aorta in the mouse can be easily mistaken with adjacent structures; and the size of the aorta in small animals and vessel wall thickness in large animals and humans (which are close to spatial resolution of corresponding imaging systems) complicate signal quantification in aneurysm. The variability in image acquisition and analysis methodology, especially for clinical studies, is quite challenging and needs standardization. For any new imaging technique, reproducibility and fluctuation of the signal over time will need to be established.

The long-term outcomes of EVAR are not yet well-defined and traditional imaging modalities do not provide sufficient information to predict the need for re-intervention, a gap that may be addressed by molecular imaging. While new techniques for risk stratification of small aneurysms are under development, it is possible that a reduction in procedural complications with EVAR compared to traditional open surgical repair will shift the threshold for intervention toward smaller aneurysms.¹¹⁵

As we discussed, a number of tracers are under development and evaluation for imaging aortic aneurysm, and conceptually others (e.g., targeted at TGF- β pathway in the case of TAA) may be suitable for this purpose. The economics of novel tracer development entail a large market for a new tracer to be economically viable. One way to address this is to focus on tracers with broad application (e.g., targeting inflammation or remodeling) which can be used in different settings. Existing approved tracers, e.g., ¹⁸F-FDG, need further evaluation for this purpose. In the modern era, introduction of new imaging techniques may require demonstration of not only diagnostic accuracy but also an effect on outcome.

The path forward should include additional basic and pre-clinical studies to better address the biological basis of ¹⁸F-FDG uptake in the vessel wall, as well as adequately powered clinical studies to evaluate ¹⁸F-FDG PET imaging for risk stratification in aneurysm. Imaging and image analysis methodology will need to be standardized. In parallel, the effectiveness and safety profile of existing investigational tracers will need further evaluation in pre-clinical and ultimately clinical studies. Potential pitfalls of existing tracers and progress in our understanding of pathophysiology warrant the quest for new tracers aimed at the same and potentially novel pathways for imaging. Finally, comparative effectiveness and cost analysis research should ascertain the potential contribution of emerging targeted and non-targeted imaging techniques to management of aortic aneurysm.

CASE DISCUSSION

Routine screening is recommended by the ACC/AHA and the U.S. Preventive Services Task Force in males between 65–75 years of age who have ever smoked.^{3,12} In addition, aortic aneurysms are often discovered incidentally on diagnostic tests performed for other indications. In the case of this patient, current US guidelines recommend follow-up with serial imaging every 6 months,¹¹ and a referral for open surgery or EVAR for symptoms, rapid growth, or a diameter more than 55 mm.⁹ The risk of rupture of $\sim 1\%$ per year for

aneurysms of 40 to 55 mm in diameter¹⁴ (or possibly higher with a history of smoking¹⁵) is less than the risks of surgery ($\sim 5\%$ thirty-day mortality for elective and $\sim 30\%$ for emergency open surgery¹¹⁶), but not negligible. Molecular imaging (or other emerging approaches discussed above) may reclassify this patient in a higher-risk group, warranting referral for invasive therapies. Molecular imaging may also help assess the effectiveness of emerging medical therapies, when they become available for clinical use.

Acknowledgments

We thank Drs George Tellides and Alan Dardik for their critical review and thoughtful suggestions.

Disclosures

Mehran M. Sadeghi received experimental tracers from Lantheus Medical Imaging.

References

1. Minino AM, Murphy SL, Xu J, Kochanek KD. Deaths: Final data for 2008. Natl Vital Stat Rep 2011;59:1-126.
2. Sterpetti AV, Schultz RD, Feldhaus RJ, Cheng SE, Peetz DJ Jr. Factors influencing enlargement rate of small abdominal aortic aneurysms. J Surg Res 1987;43:211-9.
3. U.S. Preventive Services Task Force. Screening for abdominal aortic aneurysm: Recommendation statement. Ann Intern Med 2005;142:198-202.
4. Lederle FA, Johnson GR, Wilson SE, Chute EP, Hye RJ, Makaroun MS, et al. The aneurysm detection and management study screening program: Validation cohort and final results. Aneurysm Detection and Management Veterans Affairs Cooperative Study Investigators. Arch Intern Med 2000;160:1425-30.
5. Norman PE, Jamrozik K, Lawrence-Brown MM, Le MT, Spencer CA, Tuohy RJ, et al. Population based randomised controlled trial on impact of screening on mortality from abdominal aortic aneurysm. BMJ 2004;329:1259.
6. Kuzmik GA, Sang AX, Elefteriades JA. Natural history of thoracic aortic aneurysms. J Vasc Surg 2012;56:565-71.
7. Olsson C, Thelin S, Stahle E, Ekblom A, Granath F. Thoracic aortic aneurysm and dissection: Increasing prevalence and improved outcomes reported in a nationwide population-based study of more than 14,000 cases from 1987 to 2002. Circulation 2006;114:2611-8.
8. Ernst CB. Abdominal aortic aneurysm. N Engl J Med 1993;328:1167-72.
9. Cosford PA, Leng GC. Screening for abdominal aortic aneurysm. Cochrane Database Syst Rev 2007;2:CD002945.
10. Lederle FA. Screening for AAA in the USA. Scand J Surg 2008;97:139-41.
11. Chaikof EL, Brewster DC, Dalman RL, Makaroun MS, Illig KA, Sicard GA, et al. SVS practice guidelines for the care of patients with an abdominal aortic aneurysm: Executive summary. J Vasc Surg 2009;50:880-96.
12. Hirsch AT, Haskal ZJ, Hertzner NR, Bakal CW, Creager MA, Halperin JL, et al. ACC/AHA 2005 Practice Guidelines for the

- management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): A collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): Endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. *Circulation* 2006;113:e463-654.
13. Thompson S, Brown L, Sweeting M, Bown M, Kim L, Glover M, et al. Systematic review and meta-analysis of the growth and rupture rates of small abdominal aortic aneurysms: Implications for surveillance intervals and their cost-effectiveness. *Health Technol Assess* 2013;17:1-118.
 14. RESCAN Collaborators, Bown MJ, Sweeting MJ, Brown LC, Powell JT, Thompson SG. Surveillance intervals for small abdominal aortic aneurysms: A meta-analysis. *JAMA* 2013;309:806-13.
 15. Powell JT, Brady AR, Brown LC, Forbes JF, Fowkes FGR, Greenhalgh RM, et al. Mortality results for randomised controlled trial of early elective surgery or ultrasonographic surveillance for small abdominal aortic aneurysms. *Lancet* 1998;352:1649-55.
 16. El-Hamamsy I, Yacoub MH. Cellular and molecular mechanisms of thoracic aortic aneurysms. *Nat Rev Cardiol* 2009;6:771-86.
 17. Nordon IM, Hinchliffe RJ, Loftus IM, Thompson MM. Pathophysiology and epidemiology of abdominal aortic aneurysms. *Nat Rev Cardiol* 2011;8:92-102.
 18. Ruddy JM, Jones JA, Ikonomidis JS. Pathophysiology of thoracic aortic aneurysm (TAA): Is it not one uniform aorta? Role of embryologic origin. *Prog Cardiovasc Dis* 2013;56:68-73.
 19. Shimizu K, Mitchell RN, Libby P. Inflammation and cellular immune responses in abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol* 2006;26:987-94.
 20. HELLenthal FA, Buurman WA, Wodzig WK, Schurink GW. Biomarkers of abdominal aortic aneurysm progression. Part 2: Inflammation. *Nat Rev Cardiol* 2009;6:543-52.
 21. Zhuang H, Alavi A. 18-Fluorodeoxyglucose positron emission tomographic imaging in the detection and monitoring of infection and inflammation. *Semin Nucl Med* 2002;32:47-59.
 22. Sakalihasan N, Van Damme H, Gomez P, Rigo P, Lapiere CM, Nussgens B, et al. Positron emission tomography (PET) evaluation of abdominal aortic aneurysm (AAA). *Eur J Vasc Endovasc Surg* 2002;23:431-6.
 23. Kato K, Nishio A, Kato N, Usami H, Fujimaki T, Murohara T. Uptake of 18F-FDG in acute aortic dissection: A determinant of unfavorable outcome. *J Nucl Med* 2010;51:674-81.
 24. Menezes LJ, Kotze CW, Hutton BF, Endozo R, Dickson JC, Cullum I, et al. Vascular inflammation imaging with 18F-FDG PET/CT: When to image? *J Nucl Med* 2009;50:854-7.
 25. Kotze CW, Menezes LJ, Endozo R, Groves AM, Ell PJ, Yusuf SW. Increased metabolic activity in abdominal aortic aneurysm detected by 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography/computed tomography (PET/CT). *Eur J Vasc Endovasc Surg* 2009;38:93-9.
 26. Truijers M, Kurvers HA, Bredie SJ, Oyen WJ, Blankensteijn JD. In vivo imaging of abdominal aortic aneurysms: Increased FDG uptake suggests inflammation in the aneurysm wall. *J Endovasc Ther* 2008;15:462-7.
 27. Kuehl H, Eggebrecht H, Boes T, Antoch G, Rosenbaum S, Ladd S, et al. Detection of inflammation in patients with acute aortic syndrome: Comparison of FDG-PET/CT imaging and serological markers of inflammation. *Heart* 2008;94:1472-7.
 28. Kotze CW, Groves AM, Menezes LJ, Harvey R, Endozo R, Kayani IA, et al. What is the relationship between (1)F-FDG aortic aneurysm uptake on PET/CT and future growth rate? *Eur J Nucl Med Mol Imaging* 2011;38:1493-9.
 29. Sakalihasan N, Hustinx R, Limet R. Contribution of PET scanning to the evaluation of abdominal aortic aneurysm. *Semin Vasc Surg* 2004;17:144-53.
 30. Reeps C, Essler M, Pelisek J, Seidl S, Eckstein HH, Krause BJ. Increased 18F-fluorodeoxyglucose uptake in abdominal aortic aneurysms in positron emission/computed tomography is associated with inflammation, aortic wall instability, and acute symptoms. *J Vasc Surg* 2008;48:417-23 discussion 24.
 31. Blockmans D, Coudyzer W, Vanderschueren S, Stroobants S, Loeckx D, Heye S, et al. Relationship between fluorodeoxyglucose uptake in the large vessels and late aortic diameter in giant cell arteritis. *Rheumatology* 2008;47:1179-84.
 32. Reeps C, Pelisek J, Bundschuh RA, Gurdan M, Zimmermann A, Ockert S, et al. Imaging of acute and chronic aortic dissection by 18F-FDG PET/CT. *J Nucl Med* 2010;51:686-91.
 33. Xu XY, Borghi A, Nchimi A, Leung J, Gomez P, Cheng Z, et al. High levels of 18F-FDG uptake in aortic aneurysm wall are associated with high wall stress. *Eur J Vasc Endovasc Surg* 2010;39:295-301.
 34. Courtois A, Nussgens BV, Hustinx R, Namur G, Gomez P, Somja J, et al. 18F-FDG uptake assessed by PET/CT in abdominal aortic aneurysms is associated with cellular and molecular alterations prefacing wall deterioration and rupture. *J Nucl Med* 2013;54:1740-7.
 35. Hellmann DB, Grand DJ, Freischlag JA. Inflammatory abdominal aortic aneurysm. *JAMA* 2007;297:395-400.
 36. Rudd JH, Fayad ZA, Machac J, Weissberg PL, Davies JR, Warburton EA, et al. Response to 'Laurberg JM, Olsen AK, Hansen SB, et al. Imaging of vulnerable atherosclerotic plaques with FDG-microPET: No FDG accumulation' [Atherosclerosis 2006]. *Atherosclerosis* 2007;192:453-4; author reply 1-2.
 37. Rudd JH, Narula J, Strauss HW, Virmani R, Machac J, Klimas M, et al. Imaging atherosclerotic plaque inflammation by fluorodeoxyglucose with positron emission tomography: Ready for prime time? *J Am Coll Cardiol* 2010;55:2527-35.
 38. Tavakoli S, Zamora D, Ullevig S, Asmis R. Bioenergetic profiles diverge during macrophage polarization: Implications for the interpretation of 18F-FDG PET imaging of atherosclerosis. *J Nucl Med* 2013;54:1661-7.
 39. Christen T, Sheikine Y, Rocha VZ, Hurwitz S, Goldfine AB, Di Carli M, et al. Increased glucose uptake in visceral versus subcutaneous adipose tissue revealed by PET imaging. *JACC Cardiovasc Imaging* 2010;3:843-51.
 40. Folco EJ, Sheikine Y, Rocha VZ, Christen T, Shvartz E, Sukhova GK, et al. Hypoxia but not inflammation augments glucose uptake in human macrophages implications for imaging atherosclerosis with (18)fluorine-labeled 2-deoxy-D-glucose positron emission tomography. *J Am Coll Cardiol* 2011;58:603-14.
 41. Turner GH, Olzinski AR, Bernard RE, Aravindhan K, Boyle RJ, Newman MJ, et al. Assessment of macrophage infiltration in a murine model of abdominal aortic aneurysm. *J Magn Reson Imaging* 2009;30:455-60.
 42. Morris JB, Olzinski AR, Bernard RE, Aravindhan K, Mirabile RC, Boyce R, et al. p38 MAPK inhibition reduces aortic ultra-small superparamagnetic iron oxide uptake in a mouse model of atherosclerosis: MRI assessment. *Arterioscler Thromb Vasc Biol* 2008;28:265-71.
 43. Sadat U, Taviani V, Patterson AJ, Young VE, Graves MJ, Teng Z, et al. Ultras-small superparamagnetic iron oxide-enhanced

- magnetic resonance imaging of abdominal aortic aneurysms: A feasibility study. *Eur J Vasc Endovasc Surg* 2011;41:167-74.
44. Nchimi A, Defawe O, Brisbois D, Broussaud TK, Defraigne JO, Magotteaux P, et al. MR imaging of iron phagocytosis in intraluminal thrombi of abdominal aortic aneurysms in humans. *Radiology* 2010;254:973-81.
45. Richards JM, Semple SI, MacGillivray TJ, Gray C, Langrish JP, Williams M, et al. Abdominal aortic aneurysm growth predicted by uptake of ultrasmall superparamagnetic particles of iron oxide: A pilot study. *Circ Cardiovasc Imaging* 2011;4:274-81.
46. Fayad ZA, Razzouk L, Briley-Saebo KC, Mani V. Iron oxide magnetic resonance imaging for atherosclerosis therapeutic evaluation: Still "rusty"? *J Am Coll Cardiol* 2009;53:2051-2.
47. Stoll G, Bendszus M. Imaging of inflammation in the peripheral and central nervous system by magnetic resonance imaging. *Neuroscience* 2009;158:1151-60.
48. Tang TY, Muller KH, Graves MJ, Li ZY, Walsh SR, Young V, et al. Iron oxide particles for atheroma imaging. *Arterioscler Thromb Vasc Biol* 2009;29:1001-8.
49. Yao Y, Wang Y, Zhang Y, Li Y, Sheng Z, Wen S, et al. In vivo imaging of macrophages during the early-stages of abdominal aortic aneurysm using high resolution MRI in ApoE mice. *PloS One* 2012;7:e33523.
50. Kooi ME, Cappendijk VC, Cleutjens KB, Kessels AG, Kitslaar PJ, Borgers M, et al. Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by in vivo magnetic resonance imaging. *Circulation* 2003;107:2453-8.
51. Nahrendorf M, Keliher E, Marinelli B, Leuschner F, Robbins CS, Gerszten RE, et al. Detection of macrophages in aortic aneurysms by nanoparticle positron emission tomography-computed tomography. *Arterioscler Thromb Vasc Biol* 2011;31:750-7.
52. Schafers M, Riemann B, Kopka K, Breyholz HJ, Wagner S, Schafers KP, et al. Scintigraphic imaging of matrix metalloproteinase activity in the arterial wall in vivo. *Circulation* 2004;109:2554-9.
53. Deguchi JO, Aikawa M, Tung CH, Aikawa E, Kim DE, Ntziachristos V, et al. Inflammation in atherosclerosis: Visualizing matrix metalloproteinase action in macrophages in vivo. *Circulation* 2006;114:55-62.
54. Fujimoto S, Hartung D, Ohshima S, Edwards DS, Zhou J, Yalamanchili P, et al. Molecular imaging of matrix metalloproteinase in atherosclerotic lesions: Resolution with dietary modification and statin therapy. *J Am Coll Cardiol* 2008;52:1847-57.
55. Lancelot E, Amirbekian V, Brigger I, Raynaud JS, Ballet S, David C, et al. Evaluation of matrix metalloproteinases in atherosclerosis using a novel noninvasive imaging approach. *Arterioscler Thromb Vasc Biol* 2008;28:425-32.
56. Zhang J, Nie L, Razavian M, Ahmed M, Dobrucki LW, Asadi A, et al. Molecular imaging of activated matrix metalloproteinases in vascular remodeling. *Circulation* 2008;118:1953-60.
57. Amirbekian V, Aguinaldo JG, Amirbekian S, Hyafil F, Vucic E, Sirol M, et al. Atherosclerosis and matrix metalloproteinases: Experimental molecular MR imaging in vivo. *Radiology* 2009;251:429-38.
58. Chen J, Tung CH, Mahmood U, Ntziachristos V, Gyurko R, Fishman MC, et al. In vivo imaging of proteolytic activity in atherosclerosis. *Circulation* 2002;105:2766-71.
59. Nahrendorf M, Waterman P, Thurber G, Groves K, Rajopadhye M, Panizzi P, et al. Hybrid in vivo FMT-CT imaging of protease activity in atherosclerosis with customized nanosensors. *Arterioscler Thromb Vasc Biol* 2009;29:1444-51.
60. Hadler-Olsen E, Fadnes B, Sylte I, Uhlin-Hansen L, Winberg JO. Regulation of matrix metalloproteinase activity in health and disease. *FEBS J* 2011;278:28-45.
61. Schafers M, Schober O, Hermann S. Matrix-metalloproteinases as imaging targets for inflammatory activity in atherosclerotic plaques. *J Nucl Med* 2010;51:663-6.
62. Bremer C, Tung CH, Weissleder R. In vivo molecular target assessment of matrix metalloproteinase inhibition. *Nat Med* 2001;7:743-8.
63. Suter MJ, Nadkarni SK, Weisz G, Tanaka A, Jaffer FA, Bouma BE, et al. Intravascular optical imaging technology for investigating the coronary artery. *JACC Cardiovasc Imaging* 2011;4:1022-39.
64. Kaijzel EL, van Heijningen PM, Wielopolski PA, Vermeij M, Koning GA, van Cappellen WA, et al. Multimodality imaging reveals a gradual increase in matrix metalloproteinase activity at aneurysmal lesions in live fibulin-4 mice. *Circ Cardiovasc Imaging* 2010;3:567-77.
65. Sheth RA, Maricevich M, Mahmood U. In vivo optical molecular imaging of matrix metalloproteinase activity in abdominal aortic aneurysms correlates with treatment effects on growth rate. *Atherosclerosis* 2010;212:181-7.
66. Bazeli R, Coutard M, Duport BD, Lancelot E, Corot C, Laissy JP, et al. In vivo evaluation of a new magnetic resonance imaging contrast agent (P947) to target matrix metalloproteinases in expanding experimental abdominal aortic aneurysms. *Invest Radiol* 2010;45:662-8.
67. Ouimet T, Lancelot E, Hyafil F, Rienzo M, Deux F, Lemaitre M, et al. Molecular and cellular targets of the MRI contrast agent P947 for atherosclerosis imaging. *Mol Pharm* 2012;9:850-61.
68. Su H, Spinale FG, Dobrucki LW, Song J, Hua J, Sweterlitsch S, et al. Noninvasive targeted imaging of matrix metalloproteinase activation in a murine model of postinfarction remodeling. *Circulation* 2005;112:3157-67.
69. Razavian M, Zhang J, Nie L, Tavakoli S, Razavian N, Dobrucki LW, et al. Molecular imaging of matrix metalloproteinase activation to predict murine aneurysm expansion in vivo. *J Nucl Med* 2010;51:1107-15.
70. Sanders HM, Iafisco M, Pouget EM, Bomans PH, Nudelman F, Falini G, et al. The binding of CNA35 contrast agents to collagen fibrils. *Chem Commun* 2011;47:1503-5.
71. Klink A, Heynens J, Herranz B, Lobatto ME, Arias T, Sanders HM, et al. In vivo characterization of a new abdominal aortic aneurysm mouse model with conventional and molecular magnetic resonance imaging. *J Am Coll Cardiol* 2011;58:2522-30.
72. Makowski M, Wiethoff A, Ebersberger U, Blume U, Warley A, Jansen C, et al. Molecular assessment of aortic aneurysm wall integrity using an elastin-specific MR imaging probe. *J Cardiovasc Magn Reson* 2013;15:1-3.
73. von Bary C, Makowski M, Preissel A, Keithahn A, Warley A, Spuentrup E, et al. MRI of coronary wall remodeling in a swine model of coronary injury using an elastin-binding contrast agent. *Circ Cardiovasc Imaging* 2011;4:147-55.
74. Makowski MR, Wiethoff AJ, Blume U, Cuello F, Warley A, Jansen CH, et al. Assessment of atherosclerotic plaque burden with an elastin-specific magnetic resonance contrast agent. *Nat Med* 2011;17:383-8.
75. Holmes DR, Liao S, Parks WC, Thompson RW. Medial neovascularization in abdominal aortic aneurysms: A histopathologic marker of aneurysmal degeneration with pathophysiologic implications. *J Vasc Surg* 1995;21:761-71 discussion 71-2.
76. Paik DC, Fu C, Bhattacharya J, Tilson MD. Ongoing angiogenesis in blood vessels of the abdominal aortic aneurysm. *Exp Mol Med* 2004;36:524-33.
77. Thompson MM, Jones L, Nasim A, Sayers RD, Bell PR. Angiogenesis in abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 1996;11:464-9.

78. Scott DJ, Allen CJ, Honstvet CA, Hanby AM, Hammond C, Johnson AB, et al. Lymphangiogenesis in abdominal aortic aneurysm. *Br J Surg* 2013;100:895-903.
79. Choke E, Thompson MM, Dawson J, Wilson WR, Sayed S, Loftus IM, et al. Abdominal aortic aneurysm rupture is associated with increased medial neovascularization and overexpression of proangiogenic cytokines. *Arterioscler Thromb Vasc Biol* 2006;26:2077-82.
80. Jaffer FA, Sosnovik DE, Nahrendorf M, Weissleder R. Molecular imaging of myocardial infarction. *J Mol Cell Cardiol* 2006;41:921-33.
81. Cai W, Chen X. Multimodality molecular imaging of tumor angiogenesis. *J Nucl Med* 2008;49(Suppl 2):113S-28S.
82. Dobrucki LW, Sinusas AJ. PET and SPECT in cardiovascular molecular imaging. *Nat Rev Cardiol* 2010;7:38-47.
83. Dobrucki LW, de Muinck ED, Lindner JR, Sinusas AJ. Approaches to multimodality imaging of angiogenesis. *J Nucl Med* 2010;51(Suppl 1):66S-79S.
84. Ishida A, Murray J, Saito Y, Kanthou C, Benzakour O, Shibuya M, et al. Expression of vascular endothelial growth factor receptors in smooth muscle cells. *J Cell Physiol* 2001;188:359-68.
85. Wang H, Keiser JA. Vascular endothelial growth factor upregulates the expression of matrix metalloproteinases in vascular smooth muscle cells: Role of flt-1. *Circ Res* 1998;83:832-40.
86. Zhang J, Silva T, Yarovsky T, Manes TD, Tavakoli S, Nie L, et al. VEGF blockade inhibits lymphocyte recruitment and ameliorates immune-mediated vascular remodeling. *Circ Res* 2010;107:408-17.
87. Sawano A, Iwai S, Sakurai Y, Ito M, Shitara K, Nakahata T, et al. Flt-1, vascular endothelial growth factor receptor 1, is a novel cell surface marker for the lineage of monocyte-macrophages in humans. *Blood* 2001;97:785-91.
88. Tedesco MM, Terashima M, Blankenberg FG, Levashova Z, Spin JM, Backer MV, et al. Analysis of in situ and ex vivo vascular endothelial growth factor receptor expression during experimental aortic aneurysm progression. *Arterioscler Thromb Vasc Biol* 2009;29:1452-7.
89. Zhang J, Razavian M, Tavakoli S, Nie L, Tellides G, Backer JM, et al. Molecular imaging of vascular endothelial growth factor receptors in graft arteriosclerosis. *Arterioscler Thromb Vasc Biol* 2012;32:1849-55.
90. Hynes RO. Integrins: Bidirectional, allosteric signaling machines. *Cell* 2002;110:673-87.
91. Shattil SJ, Kim C, Ginsberg MH. The final steps of integrin activation: The end game. *Nat Rev Mol Cell Biol* 2010;11:288-300.
92. Dijkgraaf I, Beer AJ, Wester HJ. Application of RGD-containing peptides as imaging probes for $\alpha v \beta 3$ expression. *Front Biosci* 2009;14:887-99.
93. Danhier F, Le Breton A, Preat V. RGD-based strategies to target $\alpha v \beta 3$ integrin in cancer therapy and diagnosis. *Mol Pharm* 2012;9:2961-73.
94. Hoshiga M, Alpers CE, Smith LL, Giachelli CM, Schwartz SM. $\alpha v \beta 3$ integrin expression in normal and atherosclerotic artery. *Circ Res* 1995;77:1129-35.
95. Huang S, Endo RI, Nemerow GR. Upregulation of integrins $\alpha v \beta 3$ and $\alpha v \beta 5$ on human monocytes and T lymphocytes facilitates adenovirus-mediated gene delivery. *J Virol* 1995;69:2257-63.
96. Sadeghi MM, Krassilnikova S, Zhang J, Gharaei AA, Fassaei HR, Esmailzadeh L, et al. Detection of injury-induced vascular remodeling by targeting activated $\alpha v \beta 3$ integrin in vivo. *Circulation* 2004;110:84-90.
97. Razavian M, Marfatia R, Mongue-Din H, Tavakoli S, Sinusas AJ, Zhang J, et al. Integrin-targeted imaging of inflammation in vascular remodeling. *Arterioscler Thromb Vasc Biol* 2011;31:2820-6.
98. Kitagawa T, Kosuge H, Chang E, James ML, Yamamoto T, Shen B, et al. Integrin-targeted molecular imaging of experimental abdominal aortic aneurysms by ^{18}F -FPPRGD2 positron emission tomography. *Circ Cardiovasc Imaging* 2013;6:950-6.
99. Gillis E, Van Laer L, Loeys BL. Genetics of thoracic aortic aneurysm: At the crossroad of transforming growth factor-beta signaling and vascular smooth muscle cell contractility. *Circ Res* 2013;113:327-40.
100. Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE Jr, et al. ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease: A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. *Circulation* 2010;121:e266-369.
101. Kuivaniemi H, Shibamura H, Arthur C, Berguer R, Cole CW, Juvonen T, et al. Familial abdominal aortic aneurysms: Collection of 233 multiplex families. *J Vasc Surg* 2003;37:340-5.
102. Larsson E, Granath F, Swedenborg J, Hultgren R. A population-based case-control study of the familial risk of abdominal aortic aneurysm. *J Vasc Surg* 2009;49:47-50 discussion 1.
103. Golledge J, Kuivaniemi H. Genetics of abdominal aortic aneurysm. *Curr Opin Cardiol* 2013;28:290-6.
104. Golledge J, Tsao PS, Dalman RL, Norman PE. Circulating markers of abdominal aortic aneurysm presence and progression. *Circulation* 2008;118:2382-92.
105. Dawson J, Cockerill GW, Choke E, Belli AM, Loftus I, Thompson MM. Aortic aneurysms secrete interleukin-6 into the circulation. *J Vasc Surg* 2007;45:350-6.
106. Jones KG, Brull DJ, Brown LC, Sian M, Greenhalgh RM, Humphries SE, et al. Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. *Circulation* 2001;103:2260-5.
107. Norman P, Spencer CA, Lawrence-Brown MM, Jamrozik K. C-reactive protein levels and the expansion of screen-detected abdominal aortic aneurysms in men. *Circulation* 2004;110:862-6.
108. Takagi H, Manabe H, Kawai N, Goto SN, Umamoto T. Circulating matrix metalloproteinase-9 concentrations and abdominal aortic aneurysm presence: A meta-analysis. *Interact Cardiovasc Thorac Surg* 2009;9:437-40.
109. Wilson WR, Anderton M, Choke EC, Dawson J, Loftus IM, Thompson MM. Elevated plasma MMP1 and MMP9 are associated with abdominal aortic aneurysm rupture. *Eur J Vasc Endovasc Surg* 2008;35:580-4.
110. Malkawi AH, Hinchliffe RJ, Xu Y, Holt PJ, Loftus IM, Thompson MM. Patient-specific biomechanical profiling in abdominal aortic aneurysm development and rupture. *J Vasc Surg* 2010;52:480-8.
111. Pappu S, Dardik A, Tagare H, Gusberg RJ. Beyond fusiform and saccular: A novel quantitative tortuosity index may help classify aneurysm shape and predict aneurysm rupture potential. *Ann Vasc Surg* 2008;22:88-97.
112. Fillinger MF, Raghavan ML, Marra SP, Cronenwett JL, Kennedy FE. In vivo analysis of mechanical wall stress and abdominal aortic aneurysm rupture risk. *J Vasc Surg* 2002;36:589-97.

113. Vande Geest JP, Di Martino ES, Bohra A, Makaroun MS, Vorp DA. A biomechanics-based rupture potential index for abdominal aortic aneurysm risk assessment: Demonstrative application. *Ann N Y Acad Sci* 2006;1085:11-21.
114. Trollope A, Moxon JV, Moran CS, Golledge J. Animal models of abdominal aortic aneurysm and their role in furthering management of human disease. *Cardiovasc Pathol* 2011;20: 114-23.
115. Ouriel K. The PIVOTAL study: A randomized comparison of endovascular repair versus surveillance in patients with smaller abdominal aortic aneurysms. *J Vasc Surg* 2009;49:266-9.
116. Thompson SG, Ashton HA, Gao L, Scott RA, Multicentre Aneurysm Screening Study G. Screening men for abdominal aortic aneurysm: 10 year mortality and cost effectiveness results from the randomised Multicentre Aneurysm Screening Study. *BMJ* 2009;338:2307.