Atherosclerosis and its sequelae have a major impact on morbidity and mortality. Not only in the Western world, but also in economically developing countries (1). The rupture of an inflamed atherosclerotic plaque is a crucial event, since it can result in acute thrombosis of an arterial vessel, resulting e.g. in myocardial infarction or stroke. Not only detection of early plaque rupture with imminent closure is therefore of clinical interest, but also timely detection of vascular inflammation and atherosclerotic plaque progression. However, plaque inflammation or even plaque rupture without vessel occlusion is not reliably detectable by current imaging techniques. Coronary angiography is the gold standard for evaluation of the coronary vessels, but only allows visualization of the vessel lumen without characterizing the important pathophysiology of the vessel wall. Therefore, highly inflamed and rupture prone plaques can be missed, or appear as a minor vessel narrowing. Although currently available techniques such as intravascular ultrasound or optical coherence tomography allow a further characterization of atherosclerotic plaques, it would be desirable to detect plaque inflammation, early plaque rupture or vascular thrombosis by non-invasive techniques such as magnetic resonance imaging (MRI), since they could allow early identification of patients at risk or triage of symptomatic patients. In this manuscript, different strategies for detection of vascular inflammation, plaque rupture and thrombosis by MRI will be discussed, with a special focus on molecular imaging contrast agents.

Keywords
Atherosclerosis, inflammation, molecular imaging

Summary
Atherosclerosis and its sequelae have a major impact on morbidity and mortality. The rupture of an inflamed atherosclerotic plaque is a crucial event, since it can result in acute thrombosis of an arterial vessel, resulting e.g. in myocardial infarction or stroke. Not only detection of early plaque rupture with imminent closure is therefore of clinical interest, but also timely detection of vascular inflammation and atherosclerotic plaque progression. However, plaque inflammation or even plaque rupture without vessel occlusion is not reliably detectable by current imaging techniques. Coronary angiography is the gold standard for evaluation of the coronary vessels, but only allows visualization of the vessel lumen without characterizing the important pathophysiology of the vessel wall. Therefore, highly inflamed and rupture prone plaques can be missed, or appear as a minor vessel narrowing. Although currently available techniques such as intravascular ultrasound or optical coherence tomography allow a further characterization of atherosclerotic plaques, it would be desirable to detect plaque inflammation, early plaque rupture or vascular thrombosis by non-invasive techniques such as magnetic resonance imaging (MRI), since they could allow early identification of patients at risk or triage of symptomatic patients. In this manuscript, different strategies for detection of vascular inflammation, plaque rupture and thrombosis by MRI will be discussed, with a special focus on molecular imaging contrast agents.

Schlüsselwörter
Magnetresonanztomographie, Atherosklerose, Inflammation, Molekulare Bildgebung

Zusammenfassung

In dieser Übersicht werden unterschiedliche Strategien zur Darstellung der vaskulären Inflammation, Plaqueruptur und Thrombose mittels MRT diskutiert, mit einem besonderen Fokus auf die molekulare Bildgebung mit entsprechenden Kontrastmitteln.

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study has demonstrated that in patients presenting with acute coronary syndrome, major adverse cardiovascular events occurring during the follow-up period were equally attributable to recurrence at the site of the initially treated culprit lesion, and to non-culprit lesions (3). These non-culprit lesions responsible for the adverse events were frequently angiographically mild in the initial coronary angiogram. Although currently available techniques such as intravascular ultrasound or optical coherence tomography allow a further characterization of atherosclerotic plaques, it would be desirable to detect plaque inflammation, early plaque rupture, or intravascular thrombosis by non-invasive techniques, since they could allow early identification of patients at risk or triage of symptomatic patients. Recent studies in humans have suggested imaging of inflamed plaques by PET-CT (positron emission computed tomography), since metabolism of radioactively marked glucose is increased in areas of inflammation (4). However, this approach is not very specific, and does not allow further functional evaluation or early detection of plaques. Dedicated molecular imaging techniques could help to overcome this limitation, since specific contrast agents could directly attach to the areas of interest in different stages of vascular inflammation, plaque rupture, or thrombosis. Figure 1 demonstrates the principles of targeted imaging contrast agents, not only for MRI but also for ultrasound or nuclear imaging (Fig. 1). Molecular imaging contrast agents consist of two components: one is a ligand, usually an antibody or a peptidomimetic addressing certain targets, for example cellular receptors. Onto this ligand, a contrast- giving moiety is conjugated. Depending upon the imaging technique, these could be paramagnetic chelates for MRI, gas-filled microbubbles for ultrasound or radionuclides for nuclear techniques.

In this article, we will review current molecular imaging concepts in atherosclerosis, focussing on vascular inflammation and thrombosis. We will define and review targets of interest, and focus on magnetic resonance (MRI) imaging as one of the most emerging techniques in the field of cardiovascular imaging.

From inflammation to thrombosis – potential imaging targets in atherosclerosis

A detailed scheme on plaque development and potential targets is depicted (Fig. 2). One of the first events in atherosclerosis is the endothelial cell dysfunction, caused by multiple factors such as response to injury or retention of low-density lipoproteins (LDL) with its oxidative modification (5, 6). The resulting expression of cell adhesion molecules, i.e. vascular cellular adhesion molecule-1 (VCAM1) or intercellular adhesion molecule (ICAM), results in attraction of monocytes, transmigrating across the endothelium (7, 8). Subendothelial transformation of monocytes into macrophages enhances the inflammatory process. This pathology is further enhanced by oxidative modifications of subendothelial LDL, caused by smooth muscle cells and the transformed macrophages (9, 10). Furthermore, macrophages accumulate cholesteryl fatty acyl esters, forming them to foam cells, which manifest as “fatty streaks” in histology (8, 11). Another important player in further plaque progression are smooth muscle cells originating from the medial layer, migrating into the intima and producing a matrix for the fibrous cap, which covers the inflammatory process of the developing atherosclerotic plaque. During inflammation progression, matrix metalloproteinases (MMPs) secreted by macrophages digest the matrix within the fibrous cap, causing a thinning of this stabilizing entity (12, 13). Once the plaque ruptures, for example caused by mechanical stress on the shoulder of a weakened cap, fibrin and platelets accumulate at the site of injury. In minor plaque rupture, vascular wound healing can prevent further excessive thrombus formation. However, when a larger rupture of the fibrous cap occurs, a rapid superimposed thrombosis is induced which can finally result in complete occlusion of the vessel and therefore lead to acute myocardial infarction or stroke. Platelets play a pivotal role in this process, both via the formation of platelet aggregates and via activation of the coagulation cascade (14). However, platelets are not only involved in the final events leading to thrombotic vessel occlusion, but they are also involved in the earlier phases of plaque development. In particular, micro-fissures and inflamed en-
dothelium, which are typical features of vulner- able plaques, are thought to be associated with platelet adhesion (14, 15). Thus, imaging of activated adhering platelets may offer a unique opportunity to identify rupture-prone, vulnerable atherosclerotic plaques. This would allow identification of patients at risk, could guide preventive therapy, and may thus prevent myocardial infarction and/or stroke.

In the context of this review, we would like to focus on three stages of atherogen- esis and atherothrombosis: adhesion molecules, monocytes/macrophages, and fi- brin/platelets.

**Advantages of MRI for molecular imaging**

As already discussed, non-invasive detection of early thrombosis/vulnerable plaques would be of enormous value for guiding early preventive medical and interventional therapy. MRI has demonstrated substantial utility in phenotyping vascular disease, and in parallel it is possible to obtain important anatomical information of the surrounding tissue. Furthermore, MRI involves no radiation, which is of importance not only for potential patients, but also for the medical staff. Using inherent physico-chemical properties that confer particular tissue relaxivities, it is now possible to characterize the vessel wall in atherosclerosis at a sub-millimeter level (16). However, to fully capitalize on the diagnostic potential of MRI, imaging at molecular and cellular level is required (8). To achieve this, the above described molecular imaging contrast agents are needed that can identify cellular targets of interest with high specificity, while conveying sufficient signal intensity to be easily distinguished from non-enhanced tissue.

Specificity can be achieved through conjugation of molecular imaging contrast agent with monoclonal antibodies or their immunospecific fragments F(ab), peptides or peptide-mimetics. Previous approaches have included integrin-conjugated gadolinium-rich perfluorocarbon nanoparticles, peptide-conjugated nanoparticles of iron oxide, and fibrin-specific cyclic peptide labelled with gadolinium (19). New approaches include application of microparticles of iron oxides (MPIO) for cellular imaging and tracking. These MPIOs with a size of 1 mm convey a payload of iron that is many orders of magnitude greater than iron nanoparticles (USPIO) and cause local magnetic field inhomogeneity extending for a distance ~50 times the physical diameter of the microparticle (20).

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**Fig. 2** Simplified progression model of atherosclerosis (ICAM: intercellular adhesion molecule; LDL: low-density lipoprotein; MMP: matrix metalloprotei- nase; VCAM: vascular cell adhesion molecule); modified from (8): Development starts from a normally functioning vessel (left), ending in atherosclerotic plaque rupture with superimposed thrombosis (right). Potential molecular imaging targets for each disease stage are listed.
Imaging of inflammation and early stages of atherosclerosis

Adhesion molecules

After initiation of plaque development, endothelial cell activation occurs, expressing adhesion molecules such as VCAM-1 or ICAM; VCAM-1 is widely recognized to be an early indicator of plaque development. Multiple studies have already been published targeting VCAM-1 in atherosclerosis by molecular imaging, either using ultrasound (21), PET-CT (22), or MRI (23). Most studies have been performed using ApoE-/- mice. In a study by Nahrendorf et al, a peptide with high affinity towards VCAM-1 has been developed into a multivalent agent detectable by MRI and optical imaging (24), revealing signal enhancement in the aortic root of mice colocalizing with endothelial cells, while plaques of atorvastatin-treated mice showed reduced contrast agent deposition and VCAM-1 expression. In another approach, a molecular imaging contrast agent consisting of MPIOs and VCAM/p-selectin-antibodies was used to image atherosclerotic plaques by mimicking leukocyte binding. MPIOs (4.5 mm in diameter) were conjugated to monoclonal antibodies against vascular cell adhesion molecule-1 (VCAM-MPIO) or P-selectin (P-selectin-MPIO), and were administered in apolipoprotein E-/- mice. By light microscopy, dual-targeted MPIO binding to endothelium overlying aortic root atherosclerosis was 5- to 7-fold more than P-selectin-MPIO or VCAM-MPIO alone. Dual-targeted MPIO, injected intravenously in vivo, bound aortic root endothelium and were quantifiable by MRI ex vivo (3.5-fold increase) (Fig. 3) (23, 25). This approach represents a new strategy for imaging vascular inflammation mediating leukocyte adhesion, which is the initial step in atherosclerosis and vascular inflammation in general. Using this contrast agent, also VCAM-mediated cerebrovascular vascular inflammation has been imaged successfully in vivo in mouse cerebrovascular inflammation mimicking multiple sclerosis, confirming this concept of in vivo molecular imaging (26). In another approach for noninvasive in vivo characterization of P-selectin on active plaques, a contrast agent based on 68Ga-Fucoidan, which is a polysaccharidic ligand of P-selectin with a nanomolar affinity, was used. The construct was tested for its potential to discriminate vulnerable plaques on apolipoprotein E-deficient mice by using positron emission tomography in correlation with 17.6 T MRI. The data suggested that 68Ga-Fucoidan might serve as a versatile imaging biomarker for P-selectin with the potential to specifically detect P-selectin expression by positron emission tomography (27).

Monocytes / macrophages

Compared to the above described strategies of imaging epitopes on the surface of the vessel, it is more challenging to image cells entering the plaque and therefore not being accessible to contrast agents circulating in the bloodstream. For such a "cellular imaging", multiple promising platforms have been described. Nanoparticles with different coatings have the potential to enter macrophages involved into plaque inflammation, even without specific target-oriented functionalization of their cell surface (28). Such nanoparticles include iron oxide nanoparticles (28, 29) as well as micelles with incorporated gadolinium molecules (30). For targeted imaging using peptides, peptidomimetics or antibodies, chelates of gadolinium have been incorporated into microemulsions, micelles or liposomes; these strategies usually resulted in particle sizes of 20–300 nm. Approaches...
using smaller particle sizes involve iron oxide nanoparticles 10–50 nm hydrodynamic diameter.

Imaging of macrophages using SPIOs or USPIOs is often referred to as “passive” targeting, since no antibodies are conjugated to the particle surface. For example, studies with hyperlipidemic rabbits have demonstrated that ultrasmall superparamagnetic particles of iron oxide (USPIOs) accumulate in plaques with a high macrophage content and induce susceptibility effects visible as a negative contrast on magnetic resonance (31). Important determinants of imaging efficiency are 1. the particle coating and 2. its size.

Carbohydrate coatings such as dextran have been shown to have a high affinity for macrophage scavenger receptors that are expressed on activated monocytes such as found in atherosclerotic plaques (32). Concerning particle size, in comparative in vivo-studies involving dextran-coated particles with either sizes of 180–200 nm (ferumoxides) or 15–30 nm (ferumoxtran, e.g. Sinerem), a more efficient uptake of iron oxide particles into monocytes was observed for the smaller particles. Indeed, ferumoxides are more rapidly cleared from the bloodstream into liver Kupffer cells making them more attractive e.g. for metastatic liver lesion imaging, in contrast to ferumoxtran-10 which has a long blood residence time and has therefore sufficient time to be endocytosed by macrophages thereafter entering atherosclerotic plaques (28, 31).

Another particle often used is the above described ferumoxtran-10 (Sinerem, Guerbet), providing long intravascular half-lives. In one study published by Kooi et al. (28), MRI was performed on 11 symptomatic patients scheduled for carotid endarterectomy before and 24 h as well as 72 h after administration of such USPIOs. Histological and electron microscopic analyses of the plaques showed USPIOs to be primarily located in macrophages within the plaques, and histological analysis showed USPIOs in 75% of the ruptured and “rupture-prone” lesions, but interestingly in only 7% of the stable lesions.

One step further has been published in the ATEROMA-study. Aim of this study was to evaluate the effects of low-dose and high-dose atorvastatin on carotid plaque inflammation as determined by ultrasmall superparamagnetic iron oxide (USPIO)-enhanced carotid MRI, hypothesizing that treatment with 80 mg atorvastatin would show quantifiable changes in USPIO-enhanced MRI-defined inflammation within the first 3 months of therapy. Twenty patients completed 12 weeks of treatment in each group, and a significant reduction from baseline in USPIO-defined inflammation was observed in the 80-mg group at both 6 weeks and at 12 weeks without any difference observed in the low-dose regimen (Fig. 4). Therefore, this study demonstrated that aggressive lipid-lowering therapy over a 3-month period was associated with significant reduction in inflammation as defined by USPIO infiltration of plaques, and might represent a useful imaging biomarker for the screening and assessment of therapeutic response to “anti-inflammatory” interventions in patients with atherosclerosis (33). Unfortunately, there is no further ongoing research in this field, although this approach has not been continuing such a program have not been communicated so far.

In line with these studies, it seems intuitive that uptake efficiency of SPIOs/USPIOs into plaque macrophages may depend upon cellular activity. Litovski et al. describe a fourfold increased of SPION uptake into atherosclerotic plaques of apoE (-/-) mice after acute administration of tumour necrosis factor (TNF)-α, interleukin (IL)-1β and interferon-γ confirming the importance of inflammation and macrophage activation in plaques for SPIO uptake. Recent in vitro-studies have described the scavenger receptor (SRA-1) and the macrophage integrin MAC-1 (CD11b/CD18) as important mediators in iron oxide uptake (35, 36).

Concerning molecular imaging of macrophages using the properties of the MAC1-receptor (CD11b/CD18), we have previously generated a contrast agent tar-
targeting CD11b (CD11b-SPIOs) for improved macrophage detection in plaques. Aortic arches and vessel branches of ApoE(-/-)-knockout mice on a Western-type diet were imaged before and 48 h after contrast agent injection of either CD11b-SPIOs or a control construct, using a 9.4 T animal MRI system. The SPIO-induced change in the MRI signal was quantified, showing a non-significant trend towards an improved uptake of CD11b-SPIOs in the subclavian artery and subsections of the aortic arch. However, results were not sufficient to allow for a definite identification of inflamed plaques, potentially due to the challenging experimental setup and the difficulty to obtain sufficient spatial in vivo resolution of atherosclerotic plaques in murine disease for the detection of nano-sized particles (37). Research on the use of larger particles is currently ongoing.

When looking at applications involving gadolinium, another study with gadolinium-immunomicelles targeted against the macrophage scavenger receptor was able to image atherosclerotic lesions in ApoE-/- and WT mice by using in vivo MRI, revealing that at 24 hours after injection, immunomicelles provided a superior in vivo enhancement of atherosclerotic plaques (30).

Altogether, as macrophage activity results in variable contrast agent uptake/binding in these different studies described in this paragraph, they provide a promising tool for the detection and monitoring of the inflammatory activity of human and animal atherosclerotic plaques. However, factors such as size, contrast-agent payload, biocompatibility on the contrast giving particles, and economical feature are important prerequisites for a further transfer into human approaches.

**Imaging of vascular thrombosis**

Concerning the non-invasive imaging of atherothrombosis with platelet and fibrin accumulation, promising pilot studies have used functional imaging with gadolinium contrast agents targeting fibrin as the end product of the activated coagulation system (38, 39). However, these studies mostly use large occlusive thrombi, and thus fibrin-targeted magnetic resonance imaging (MRI) seems to be highly suitable for the detection of strong fibrin accumulation. Imaging of platelets promises to be a suitable approach for sensitive detection of small thrombi and vulnerable plaques in the arterial system.

**Fibrin**

Fibrin is primarily found in thrombi and, at very low concentrations, also in the blood. In contrast to other targets of the clotting cascade, fibrin can be found in thrombi of all ages and any location, making it an ideal target for imaging of vascular thrombosis. However, this also limits the ability to use fibrin as a specific marker of acute thrombosis, such as found in acute plaque rupture and subsequent acute coronary syndromes.

Several studies have focussed on imaging of fibrin in vascular thrombosis. A construct called EP-2104R is a fibrin specific contrast agent, composed of a small peptide with 4 Gd-chelate moieties that selectively and reversibly binds fibrin (40). In one of his first studies with EP-2104, fresh thrombi were engineered ex vivo from human blood and delivered in the lungs and coronary arteries of swine. Subsequent molecular MR imaging was performed before and after systemic administration of the contrast agent. After contrast administration, pulmonary emboli, emboli in the right heart, and coronary thrombi were selectively visualized as white spots (41). So far, EP-2104R is also the most investigated MRI contrast agent for imaging intravascular thrombosis in humans, and has already advanced to clinical translation after extensive pre-clinical evaluation (42). Phase II trials have demonstrated its safety and specificity for the target of interest (42, 43): in cases of vascular thrombosis, thrombi were detectable before and 36 hours after contrast agent administration.

Alternative approaches of an anti-fibrin contrast agents involved antibodies conjugated to nanoparticles of Gd-DTPA-bis-oleate (BOA) (43), Gd-DTPA-phosphatidylethanolamine (PE) (44), and manganese(III)-labeled nanobialys (45), which were all investigated in-vitro. Nevertheless, there are still difficulties in transferring this technique into the clinical setting due to the toxic potential of manganese in humans.

**Platelets**

Platelets are much more challenging for molecular imaging, since they are very small cells and therefore allow no passive targeting such as described above for monocytes/macrophages. In addition, platelets and especially their glycoprotein surface receptors are the most abundant receptor-types in the body, and therefore potentially non-specific. However, as already mentioned, platelets can be found in different stages of vascular inflammation, plate rupture and atherothrombosis, which makes them an interesting target for molecular imaging.

Already in 1996 Yamada and Kidéra (46) mutated echistatin, a disintegrin with a high affinity for integrins, to increase its binding to the platelet glycoprotein gpIIb/IIIa receptor (αⅡbβ3, CD61/CD41), which resulted in the agent P977. Later, Klink et al. (47) explored the ability of P975, a conjugate of P977 and gadolinium-DOTA, to detect intravascular thrombi by T1-weighted MRI in vivo using a murine model of carotid artery thrombosis. Two hours after injection of P975 or Gd-DOTA, an increase in the contrast-to-noise ratio (CNR) for P975 was detected. Furthermore, competitive inhibition with a glycoprotein IIb/IIIa (GPIIb/IIIa) receptor antagonist suppressed the MRI signal enhancement, demonstrating specific binding of P975 to activated platelets in vivo (47). Future studies need to evaluate its clinical translatability.

Schwarz et al. (46) described the development of a monoclonal single-chain antibody against the activated form of GPIIb/IIIa receptor, which is of interest due to the above described fact that the overall GPIIb/IIIa-receptor is the most abundant one in the human body. This single-chain antibody binds to ligand-induced binding sites (LIBS) of platelets, which only become exposed upon activation e.g. by fibrin. In multiple studies conducted in our working group, we conjugated the LIBS-antibody to...
the already described MPIOs, which resulted in the LIBS-MPIO contrast agent. In initial studies, we proved the concept of binding of the contrast agent construct to human platelets in vivo, and could also show its binding properties under flow conditions (48). We further transferred the LIBS-MPIO contrast agent into animal models of atherothrombotic disease and vascular inflammation. A wire injury model of the femoral arteries was performed in mice, and LIBS-MPIO injected afterwards. After sacrificing the animals, ex vivo-MRI demonstrated the typical MPIO-induced signal voids at the vascular wall, which corresponded well with findings in histology (49). Another interesting model constituted the ferric-chloride induced thrombosis of the carotid artery. In order to simulate the clinical situation of a ruptured plaque, we modified the model and the dose of the endothelial-toxic ferric chloride in a way that we reliably obtained non-obstructive and wall-adherent thrombi. After performing this type of surgery in mice, LIBS-MIO was injected and typical signal voids detected in MRI. After performing thrombolysis, these signal voids disappeared, demonstrating the specificity and diagnostic potential of this approach (Fig. 5A) (50). Since coronary vessels with wall-adherent thrombosis also constitute a potential target for imaging with LIBS-MPIO, we also applied this model of wall-adherent arterial thrombosis in animals after a corresponding injury of the left anterior descending artery, which also allowed an identification of LIBS-MPIO binding in MRI (Fig. 5b). However, due to the enormous motion artefacts with a heart rate of up to 600/min in mice, detection was only possible ex vivo (51).

In other approaches, it was also possible to detect early platelet aggregates in cerebral malaria by this technique in in vivo MRI. This is of interest since animals with vascular platelet adhesion were neurologically asymptomatic, but activated platelets already detectable (Fig. 5C) (52) – an interesting finding if we think about detection of atherosclerotic plaques with only minor rupture and minor symptoms, which might cause severe symptoms and morbidity in the long run.

Concerning the transfer into a human setting, we could also show that LIBS-MPIO binds to atherosclerotic plaques of patients with symptomatic carotid artery stenosis, which corresponded well to findings in histology (Fig. 6A) (50). This shows us that the LIBS-antibody itself binds to activated human platelets, but a definite drawback is the biocompatibility of the MPIO-particles themselves – which will be discussed in the „limitations“-section of this article.
Fig. 6 MRI and histology of symptomatic human carotid plaques; modified from (53, 57). Transversal MRI sections show carotid artery endarterectomy specimens before and after incubation with LIBS MPIOs (A). Black arrows depict areas of contrast agent binding within the vessel lumen on the surface of the plaque. No luminal binding can be observed in a plaque incubated with control-MPIOs (B) before and after contrast agent incubation. Dual imaging with LIBS-MPIO and gadolinium (Gd) (C). For animals injected with LIBS-MPIOs (top row) and control-MPIOs (bottom row), baseline scans are shown on the left. After contrast agent injection, a constant signal decrease can be observed in ischemic areas of animals with LIBS-MPIO injection as the typical susceptibility artifact induced by MPIOs (red arrows), while no signal effect is visible in animals with control-MPIO injection. After total imaging for 37 minutes, Gd was injected, and late gadolinium enhancement observed as a marker of myocardial necrosis, which was present in both treatment groups (yellow arrows).

Last but not least, we also performed a study combining the diagnostic potential of platelet imaging in inflammation and vascular obstruction observed after total occlusion of a coronary vessel. Therefore, we developed a dual, noninvasive imaging approach using molecular magnetic resonance imaging in an in vivo mouse model of myocardial ischemia and reperfusion injury, which was induced in mice by temporary ligation of the left anterior descending coronary artery. After injection and imaging of LIBS-MPIOs, late gadolinium enhancement was further used to depict myocardial necrosis. In in vivo MRI, activated platelets were detectable via a significant signal voids in the area of the left anterior descending coronary artery occlusion 2 hours after reperfusion, and in parallel late gadolinium enhancement identified the extent of myocardial necrosis (Fig. 6B). Such a non-invasive imaging strategy might be of clinical interest for both diagnostic and prognostic purposes, and further highlights the potential of molecular MRI for characterizing ischemia/reperfusion injury (53).

Limitations
Numerous approaches have described the possibility of detecting targets at the cellular level (28, 54–56). A major problem of these approaches is the translation into human models. Targeted contrast agents involving antibodies and conjugated particles can be toxic in human applications. This is either caused by the
• immunogenicity of the antibody produced in other species than human,
• method of the conjugation between the antibody and the contrast molecule (e.g. biotin-avidin bonds revealing toxicity in vivo and human approaches), or
• contrast molecule itself (coating of iron oxide particles with toxic polystyren, intrinsic toxicity of gadolinium).

However, these problems need to be addressed before any targeted contrast agent can be applied in human disease. Besides intrinsic toxicity, targeted contrast agents may remain bound to biologically vulnerable and sensitive areas for many hours, compromising an additional toxic effect on such areas of risk, potentially resulting in a detrimental outcome in such patients. However, as seen with carotid plaque imaging with SPIOs in the context of the ATEROMA-study, molecular imaging with dedicated contrast agents is possible, delivering important diagnostic and prognostic informations.

Conclusion
Molecular imaging of atherosclerosis is a challenging but fascinating technique for disease characterization. Recent progress in magnetic resonance imaging technique and contrast agent preparations allows numerous approaches for detection of different stages of plaque development, e.g. vascular inflammation, endothelial activation, or atherothrombosis. Some strategies have already been applied in humans, such as shown for monocye/macrophage or fibrin imaging. In murine disease, many more epitopes can be targeted by MRI contrast agents, but transfer into human approaches is still challenging. It will be exciting to see the progress and newly developing potentials of this technique over the next years, and how the technique can help in the clinical setting in diagnosis, therapy, and risk stratification.

Conflict of interest
The authors declare no conflict of interest.

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