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Detection of Atherosclerotic Plaque With Gadofluorine-Enhanced Magnetic Resonance Imaging

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Background—The purpose of this study was to visualize atherosclerotic plaques independently of luminal narrowing using T₁-weighted contrast-enhanced MRI.

Methods and Results—Eight Watanabe heritable hyperlipidemic (WHHL) rabbits, aged 9 to 18 months, and 8 age-matched controls (New Zealand White rabbits) underwent MRI of the aortic arch before and up to 48 hours after injection of 100 μmol/kg Gadofluorine (Schering AG). Additionally, 8 WHHL rabbits were examined with Magnevist (Schering AG). A half-Fourier acquisition single-shot turbo-spin-echo (HASTE) sequence and a T₁-weighted inversion-recovery turbo fast, low-angle shot sequence were used for data acquisition. Immediately after the MR examination, the animals were killed, the aorta was stained with Sudan red, and ex vivo imaging of the stained aortic specimens was performed. Additionally, gadolinium concentrations in plaque (Sudan-positive) and normal (Sudan-negative) aortic wall segments were measured. Plain MR imaging revealed no plaques in the aortic arch in either animal group. Enhancement occurred in the aortic wall of all WHHL rabbits examined with Gadofluorine but not in the vessel wall of animals examined with Magnevist and the control group. Sudan red staining demonstrated multiple plaques in the aortic arch of all WHHL rabbits. Ex vivo imaging demonstrated that the area of hyperenhancement matched the area of plaques stained with Sudan red. The gadolinium concentration was 7±5 nmol/g for normal aortic wall of the control group and 368±30 nmol/g for aortic wall with plaque in WHHL.

Conclusions—Gadofluorine enhances the imaging of atherosclerotic plaques and enables improved plaque detection of even nonstenotic lesions that are not visible on unenhanced MRI. (*Circulation*. 2003;108:605-609.)

Key Words: magnetic resonance imaging ■ contrast media ■ atherosclerosis ■ plaque

Despite advances in our understanding of the pathogenesis of atherosclerosis, cardiovascular diseases are still the leading cause of death in Western societies.¹ Early-stage atherosclerotic plaques cannot be detected with luminographic techniques because these plaques have not yet compromised the arterial lumen. This process of plaque development combined with increasing total vessel area is known as positive or outward arterial remodeling.² All luminographic techniques, both conventional angiography and magnetic resonance (MR) or computed tomography (CT) angiography, frequently underestimate the true burden of atherosclerosis. The inability of angiography to detect early plaque, which can lead to clinical events such as myocardial infarction or stroke, and to predict plaque rupture has cast a shadow of doubt over the value of this “gold standard” technique.

It is well established that the risk of an acute event mediated by plaque rupture is predicated on the composition

of the plaque rather than the degree of luminal narrowing.³ Plaques with a large necrotic lipid core and a thin fibrous cap are associated with a high risk of rupture. Several groups report that high-resolution MR is capable of detecting and characterizing atherosclerotic plaques.⁴ These promising results may lead to a change of paradigm in imaging of atherosclerosis. Although luminographic techniques are still the method of first choice, there is a growing interest in MRI of the vessel wall.^{5,6}

However, high-resolution MR techniques are restricted to superficial vessels or require the use of intravascular coils⁷ because a high contrast-to-noise ratio is a prerequisite for detection and characterization of atherosclerotic plaques. In addition to dedicated surface coils, the use of contrast agents might improve the signal-to-noise and contrast-to-noise ratios. The introduction of contrast agents has dramatically improved the sensitivity and specificity of MRI for almost all

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Drs Ebert and Weinmann and Claudia Heyer are employees of the Research Laboratories of Schering, AG, Berlin, Germany. As members of Schering Aktiengesellschaft Berlin, they have a financial interest in the development of the special paramagnetic contrast agent Gadofluorine, which is not yet commercially available.

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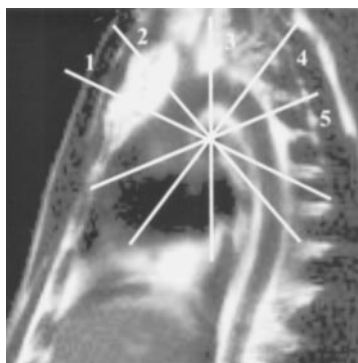


Figure 1. Planning of 5 standard views (indicated by white lines) perpendicular to aortic arch on parasagittal HASTE image.

body regions. Therefore, a contrast agent that selectively enhances atherosclerotic plaques or plaque components would be of great clinical interest. We hypothesized that contrast agents with a long plasma half-life would accumulate in atherosclerotic plaques because of enhanced endothelial permeability and increased interstitial space. The present study evaluated the potential of a new gadolinium-based contrast agent for visualization and, if possible, characterization of atherosclerotic plaques that is independent of luminal narrowing.

Methods

All experimental protocols were performed in accordance with state regulations governing animal experiments. Sixteen female Watanabe heritable hyperlipidemic (WHHL) rabbits (age 9 to 18 months, weight range 2.7 to 4.0 kg; Covance, Princeton, NJ) and 8 age-matched controls (New Zealand White rabbits) underwent MRI of the aortic arch. Animals were anesthetized with a subcutaneous injection of Rompun (xylazine 10 mg/kg; Bayer) and Ketavet (ketamine HCl 50 mg/kg; Pharmacia and Upjohn). All MRI was performed with the rabbits in the supine position inside a 1.5-T superconducting MR system (Siemens AG) equipped with a 30-mT/m gradient system. A single-loop surface coil with a diameter of 5 cm was placed on the animal's chest for data reception.

The animals were examined by a standardized protocol. After the scout scans, a parasagittal half-Fourier acquisition single-shot turbo-spin-echo (HASTE) scan covering the aortic arch was performed (Figure 1). For this sequence, a repetition time of 700 ms, echo time of 60 ms, flip angle of 150°, and slice thickness of 3 mm were used. The field of view was reduced to the minimum of 176 mm², which resulted in a spatial resolution of 0.7×0.7 mm². Based on the parasagittal scan, 5 standard views of the aortic arch were planned (Figure 1) and measured with the HASTE sequence. Thereafter, the parasagittal view and the 5 slices perpendicular to the aortic arch were obtained with a T₁-weighted inversion-recovery turbo fast, low-angle shot (IR turboFLASH) sequence with the following parameters: repetition time 300 ms, echo time 4 ms, inversion time 120 ms, flip angle 30°, field of view 176 mm², spatial resolution 0.7×0.7 mm², and slice thickness 3 mm.

In 8 WHHL rabbits and all control animals, the MR examination was performed before and 8, 24, and 48 hours after injection of 100 μmol/kg of Gadofluorine (Schering AG). Gadofluorine is a macrocyclic gadolinium-based contrast agent with a perfluorinated side chain. Such substances will create their own interface by forming very small aggregates or micelles in diluted solution. The driving force is the hydrophobic character of the fluorinated side chain. Therefore, Gadofluorine is lipophilic compared with agents such as Gd-DTPA, but it is water soluble.^{8,9} The size of the Gadofluorine micelles in water is ≈5 nm as determined by dynamic light scattering

with a photon correlation spectrometer PCS 4700c (Malvern Instruments).

The elimination of Gadofluorine from blood was investigated in WHHL rabbits up to 24 hours after intravenous injection of 100 μmol/kg. Blood samples were taken from an ear artery at 0.5, 1, 3, 5, 10, 15, 30, 60, 90, 120, 150, and 180 minutes and 24 hours after injection. The concentration of gadolinium was measured in the blood samples by means of inductively coupled plasma-atomic emission spectroscopy (ICP-AES) at a wavelength of 342.247 nm (Minitorch 3410, ARL Fisons Instruments GmbH). The plasma half-life time was calculated with a 2-compartment distribution model.

The additional 8 WHHL rabbits were examined before and after injection of 0.5 mmol/kg Magnevist (Schering AG). The high dose was applied to compensate for the lower relaxivity of Gd-DTPA compared with Gadofluorine. Because of the much shorter plasma half-life of ≈28 minutes, imaging was performed only up to 90 minutes after injection of Magnevist. The standardized imaging protocol ensured identical slice positions for the precontrast and all postcontrast scans.

After the last postcontrast MR examination, euthanasia was performed, and the thoracic aorta was excised. The perivascular fat tissue was removed, and the aorta was stained with Sudan red. Stained aortic specimens were photographed with a digital camera, followed by ex vivo MR imaging. To compare the results of the in vivo and ex vivo examinations, the IR turboFLASH sequence with identical sequence parameters was used to image the aortic specimen, although the minimum field of view of 176 mm² limited the spatial resolution.

Finally, the entire aorta of 5 WHHL and 5 control animals examined with Gadofluorine and 5 WHHL rabbits examined with Magnevist was divided into normal segments and Sudan red-stained segments that indicated atherosclerotic plaques. For each of these animals, the samples were weighted, and the gadolinium concentrations were measured in nonstained (Sudan-negative) aortic wall segments and in Sudan-positive segments by means of ICP-AES.

Results

With T₂-weighted HASTE and T₁-weighted IR turboFLASH sequences, plain MR imaging detected no plaques in the aortic arch of either group of animals. At 8 and 24 hours after injection of Gadofluorine, the vessel lumen appeared bright on T₁-weighted images because of a still-pronounced T₁ shortening of blood, which indicates a long blood half-life of the contrast agent. Delineation of the vessel wall and detection of mural contrast enhancement was impossible within the first 24 hours after contrast injection.

The calculated average elimination plasma half-life time of Gadofluorine in the WHHL rabbit was 10.0 hours. Therefore, 48 hours after injection of Gadofluorine, the majority of the contrast agent had cleared from the blood, and the vessel lumen appeared dark on T₁-weighted IR turboFLASH images. Pronounced enhancement occurred in the aortic wall of all WHHL rabbits but not in the vessel wall of the control animals (Figures 2 through 4). In WHHL rabbits, 37 of 40 slices perpendicular to the aortic arch showed areas with increased signal intensity of the vessel wall compared with plain MRI. The pattern of enhancement was circular in 13 slices; incomplete enhancement including >50% of the circumference was observed in 15 slices, whereas 9 slices included <50% of the circumference. In WHHL rabbits, only 3 slices through the ascending aorta showed no enhancement. In the control animals, none of the 40 slices showed any enhancement of the aortic wall.

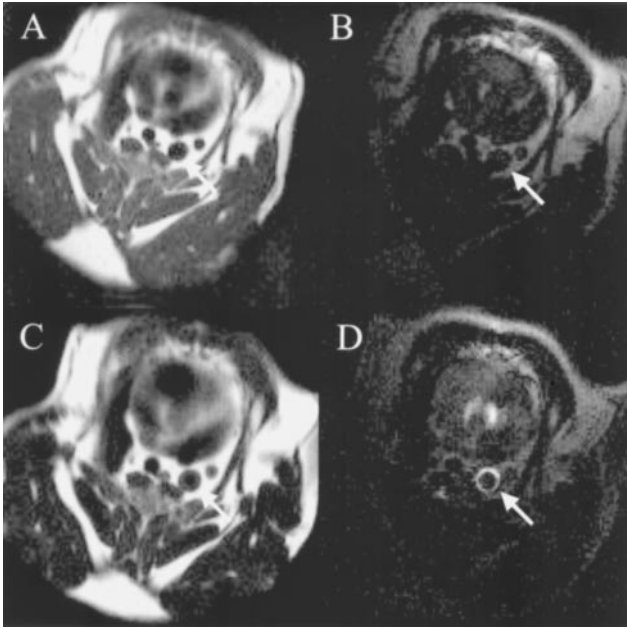


Figure 2. HASTE (A, C) and IR turboFLASH (B, D) images before (A, B) and 48 hours after (C, D) application of Gadofluorine in 18-month-old WHHL rabbit at identical slice positions. Wall of descending aorta (arrows) shows marked enhancement after contrast injection (D). Sudan red-stained aorta of this animal is shown in Figure 6A.

After injection of Magnevist, the vessel lumen appeared bright on T₁-weighted IR turboFLASH images for ≈60 minutes. The vessel wall and the surrounding tissues showed a diffuse contrast enhancement, but wall segments with and without plaque could not be differentiated. After the contrast had cleared from the blood, no aortic wall segments with increased enhancement could be detected (Figure 5).

Sudan red staining (Figure 6) demonstrated plaques in the ascending aorta (n=5), aortic arch (n=8), and descending aorta (n=8) of the WHHL rabbits examined with Gadofluorine. Only the 3 animals without enhancement of the ascending aorta showed no plaques in this vessel segment after Sudan red staining. In New Zealand White rabbits, no plaques could be detected after Sudan red staining (Figure 6). Ex vivo imaging showed that the area of hyperenhancement matched the area of plaques stained with Sudan red in all examined

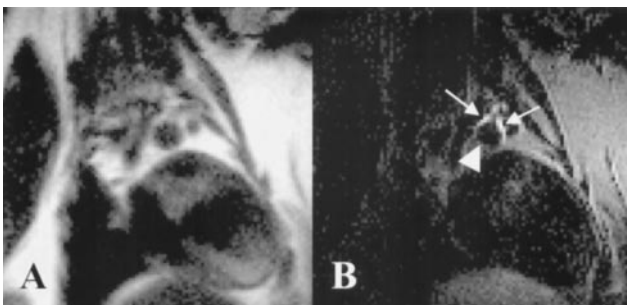


Figure 3. HASTE (A) and IR turboFLASH (B) images 48 hours after application of Gadofluorine in 9-month-old WHHL rabbit. After contrast injection, wall segments with enhancement (arrows) and wall segments without enhancement (arrowhead) could be distinguished.

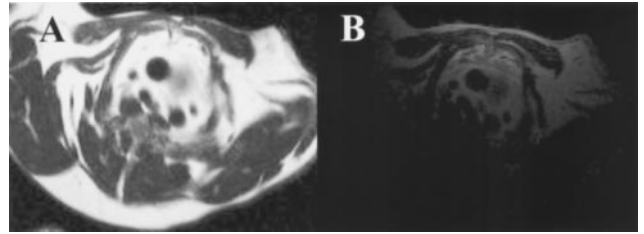


Figure 4. HASTE (A) and IR turboFLASH (B) images in 18-month-old control animal 48 hours after application of Gadofluorine. Aortic wall of control animal does not show any enhancement.

specimens (Figure 7). Although no enhancement could be depicted in vivo, all WHHL rabbits examined with Magnevist showed focal plaques in the thoracic aorta after Sudan red staining.

The gadolinium concentration at 48 hours after injection was 7 ± 5 nmol/g for normal aortic wall in the New Zealand White rabbits. In Watanabe rabbits examined with Gadofluorine 48 hours after injection, a gadolinium concentration of 95 ± 41 nmol/g was measured for wall segments not stained with Sudan red, whereas the gadolinium concentration in plaques stained with Sudan red was 368 ± 30 nmol/g tissue. Ninety minutes after injection of Magnevist, the gadolinium concentration was 427 ± 132 nmol/g for aortic wall segments with plaque and 401 ± 200 nmol/g for normal aortic wall segments in WHHL rabbits.

Discussion

This study has yielded 3 important findings: (1) Gadofluorine enhances aortic wall segments with plaque burden on

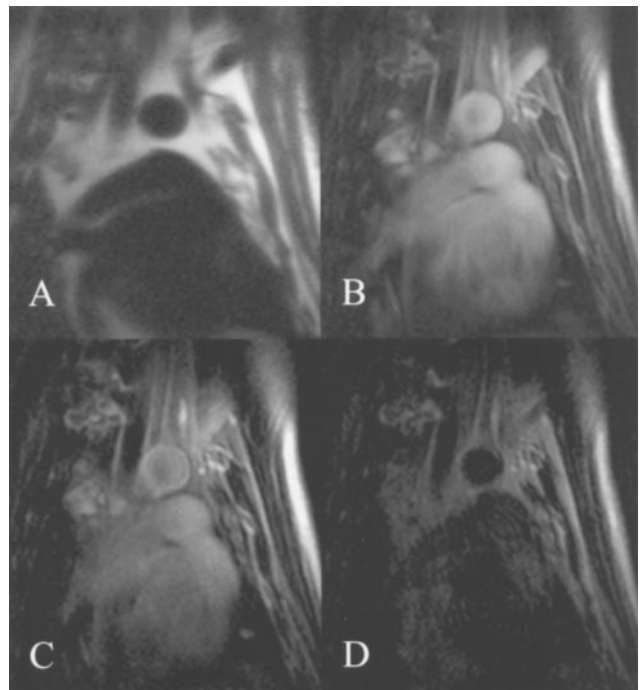


Figure 5. HASTE (A) and IR turboFLASH (B through D) images 1 (B), 30 (C), and 65 (D) minutes after application of Magnevist in 18-month-old WHHL rabbit.

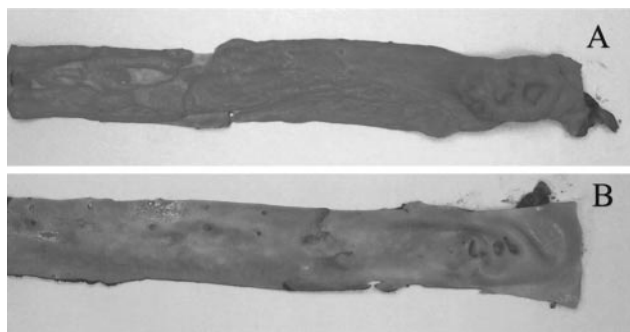


Figure 6. Aorta of 18-month-old WHHL rabbit (A) and age-matched control animal (B) stained with Sudan red. Aorta of WHHL is stained bright red, indicating atherosclerotic plaques, whereas aorta of control animal does not show any plaques.

strongly T_1 -weighted images in WHHL rabbits and distinguishes these segments from the normal vessel wall; (2) Gadofluorine does not enhance the aortic wall of healthy control animals; and (3) Magnevist leads to a diffuse enhancement of the vessel wall, but segments with and without plaque burden cannot be distinguished in WHHL rabbits.

Atherosclerosis is a chronic inflammatory disease of the vessel wall associated with thickening of the intima, structural disorganization, and accumulation of lipids, cells, and matrix components in the vessel wall.^{1,10,11} Although atherosclerosis is a systemic disease that occurs in the carotid arteries, the aorta, the coronaries, and the run-off vessels, high-risk plaques have different characteristics depending on their location. Whereas vulnerable plaques of the carotid arteries are usually severely stenotic, high-risk plaques of the coronary arteries are often nonstenotic and therefore not visible by luminographic techniques.¹² Acute coronary syndromes frequently evolve from only mild or even nonstenotic plaques, which are characterized by thin fibrous caps and a large necrotic core.³ Therefore, the noninvasive detection of these early nonstenotic plaques might be of great clinical interest.

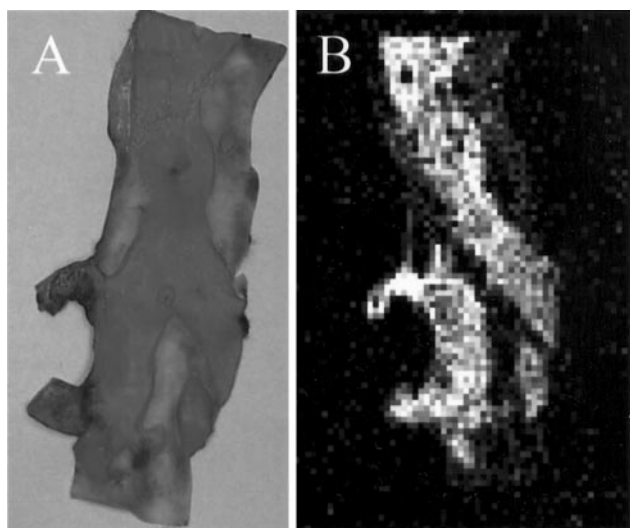


Figure 7. A, Aortic wall specimen of WHHL rabbit stained with Sudan red. B, Ex vivo MR scan of same specimen. Area of hyperenhancement matches plaques stained with Sudan red.

Vessel Wall Imaging

Different imaging modalities can be used for direct visualization of plaques and the normal vessel wall, but the search for a perfect method is not over yet. Ultrasound is limited to superficial vessels like the carotid arteries unless a catheter-based ultrasound system (intravascular ultrasound) is used, which makes the procedure invasive. Ultrafast CT can detect plaques and distinguish calcified and noncalcified lesions, but the radiation exposure is a major limitation of this technique. Additionally, both ultrasound and CT have limited value for the analysis of plaque composition.^{12,13} Because of its ability to distinguish different plaque components such as lipids, the fibrous cap, calcium, and thrombi, high-resolution multicontrast MRI is considered the most promising imaging technique.¹²

Contrast-Enhanced MRI of Plaque

Because the contrast-to-noise ratio is an important limitation of plain MRI of the vessel wall, several groups use contrast agents to improve the detectability and characterization of atherosclerotic plaques. Ultrasmall superparamagnetic iron oxide agents have been used to detect and characterize atherosclerotic plaques,¹⁴ but this technique requires very high doses of the contrast agent, and plaques are only indirectly visualized by susceptibility artifacts. Other groups have shown that paramagnetic contrast agents with high avidity for fibrin may be used to characterize plaques,¹⁵ but this technique is limited to advanced plaques with thrombi on the surface.

The present study introduces a new gadolinium-based contrast agent that accumulates in atherosclerotic plaques and allows for direct visualization of plaque burden. The significant uptake of the contrast agent into atherosclerotic plaques combined with a high relaxivity leads to an increased signal intensity of plaques on strongly T_1 -weighted images. A similar finding has not been reported for other higher molecular blood pool agents. Although the exact mechanism of Gadofluorine uptake and enhancement in atherosclerotic plaques is not completely understood, we hypothesize that the aggregated contrast agent (micelles) slowly leaks off the vessel lumen into plaques because of an increased endothelial permeability in atherosclerotically damaged wall segments.¹⁶ The changes in endothelial integrity occur in a very early stage of atherosclerotic damage, which may explain the increased gadolinium concentration in wall segments not stained with Sudan red in Watanabe rabbits compared with the control animals.

However, this increased gadolinium concentration in wall segments not stained with Sudan red did not result in an increased signal intensity on IR turboFLASH images. Compared with these unstained vessel areas, the gadolinium concentration was significantly higher in macroscopically detected areas with plaque, which resulted in pronounced enhancement of these vessel segments on IR turboFLASH images.

Additional possible explanations for the increased gadolinium concentration in the Sudan-stained areas of the vessel wall of WHHL rabbits include accumulation in the enlarged interstitial space, necrotic areas, and lipid plaque components

or phagocytosis of the agent by macrophages. With fluorescence labeling techniques, it could be demonstrated that Gadofluorine accumulated in the lymph nodes in cells with macrophage activity.⁸

We assume that a similar uptake mechanism may take place in the atherosclerotic plaque. However, the rate of accumulation is remarkably different; lymph node uptake is a relatively fast process, whereas accumulation in the atherosclerotic plaques follows a much slower process of several hours. It remains to be seen whether the Gadofluorine micelles diffuse passively into the intima or accumulate via a cellular entrapment mechanism (monocytes or foam cells) similar to ultrasmall superparamagnetic iron oxide as described by Ruehm et al.¹⁴ Further work is necessary to evaluate these issues and the potential ability to characterize atherosclerotic plaques or plaque components with Gadofluorine.

MR Technique

The IR turboFLASH sequence used in the present study has been developed recently to visualize myocardial infarction. After injection of standard extracellular contrast agents, it dramatically improves the contrast between infarcted and normal myocardium compared with standard turbo spin-echo and gradient-echo techniques.¹⁷ The present study demonstrates that an optimized modification of this strongly T₁-weighted sequence is well suited for displaying contrast enhancement of atherosclerotic plaques. The excellent contrast properties of this sequence combined with a delay of 48 hours after contrast injection minimizes the signal from the vascular lumen and the perivascular fat tissue, which allows for sensitive detection of contrast enhancement within the vessel wall.

Our approach improves the contrast of plaques and surrounding tissues and therefore offers several advantages compared with other MR techniques. In contrast with MR angiography, plaques can be visualized in earlier stages because their detection does not depend on luminal narrowing. Compared with plain MR, plaque detection does not depend on high spatial resolution, and therefore a large area of interest can be covered. Because of the fast data acquisition capabilities of the IR turboFLASH sequence, ECG-triggered data sets can be collected in a single breath hold. Therefore, even detection of plaques in the coronary arteries appears possible with this approach.

Clinical Implications

This approach appears suitable for detection of atherosclerosis at a very early stage. Even in 9-month-old animals, very early plaques (Stary class I and II) were well visualized. The ability to detect atherosclerotic plaques noninvasively before

they become symptomatic may allow for the identification of high-risk patients and early treatment. This imaging technique can visualize potential targets for new interventional therapy concepts such as local gene delivery. Direct visualization of plaques may also enhance the understanding of the natural history of atherosclerosis.

Conclusions

Gadofluorine enhances atherosclerotic plaques and improves plaque detection compared with plain MRI. This approach may be suitable for detection of atherosclerosis at a very early stage and may allow identification of high-risk patients.

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