Imaging of myocardial infarction using ultrasmall superparamagnetic iron oxide nanoparticles: a human study using a multi-parametric cardiovascular magnetic resonance imaging approach

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Aims
The purpose of this clinical trial was to investigate whether cardiovascular magnetic resonance imaging (CMR) using ferumoxytol (Feraheme™, FH), an ultrasmall superparamagnetic iron oxide nanoparticle (USPIO), allows more detailed characterization of infarct pathology compared with conventional gadolinium-based necrosis/fibrosis imaging in patients with acute myocardial infarction.

Methods and results
Fourteen patients who had experienced an acute ST-elevation myocardial infarction were included in this study. Following coronary angiography, a first baseline study (pre-FH) was performed followed by subsequent CMR studies (post-FH) 48 h after intravenous ferumoxytol administration. The CMR studies comprised cine-CMR, T2-weighted short tau inversion recovery spin echo imaging, T2*-mapping, and T1-weighted late gadolinium enhancement (LGE) imaging. The median extent of short-axis in-plane LGE was 30% [inter-quartile range (IQR) 26–40%]. The median in-plane extent of T2-weighted ‘hypoenhancement’ in the region of myocardial infarction, which was not present prior to ferumoxytol administration in any patient, was 19% (IQR 14–22%; P < 0.001 compared with the extent of LGE). The median in-plane extent of areas showing signal void in T2*-mapping images post-FH in the region of myocardial infarction was 16% (IQR 12–18%; P < 0.001 compared with the extent of LGE; P = 0.34 compared with the extent of T2-weighted hypoenhancement). A substantial drop in absolute T2*-values was observed not only in the infarct core and peri-infarct zone, but also in the remote ‘healthy’ myocardium, although there was only a minor change in the skeletal muscle. Substantial ferumoxytol uptake was detected only in cultured macrophages, but not in peripheral blood monocytes from study patients.

Conclusion
We could demonstrate in humans that USPIO-based contrast agents enable a more detailed characterization of myocardial infarct pathology mainly by detecting infiltrating macrophages. Considering the multi-functionality of USPIO-based particles and their superior safety profile compared with gadolinium-based compounds, these observations open up new vistas for the clinical application of USPIO.

Keywords
USPIO • Ferumoxytol • Feraheme™ • CMR • Myocardial infarction • LGE
Introduction

Today, cardiovascular magnetic resonance imaging (CMR) is widely used for diagnosis and treatment stratification of cardiac diseases. Apart from functional analysis, multi-parametric CMR allows non-invasive, accurate, and repeated myocardial tissue characterization. For example, contrast-enhanced CMR (ceCMR) techniques such as late gadolinium enhancement (LGE) imaging enable accurate detection of acute and chronic myocardial infarction as well as risk stratification. More recently, T2- and T2*-weighted CMR techniques were validated as promising tools to detect myocardial oedema as well as myocardial haemorrhage (in case of acute myocardial infarction), thereby allowing a better characterization of infarct pathology and prediction of adverse outcome. However, signal loss on T2- and T2*-weighted CMR images may not only be caused by paramagnetic effects of oxidized iron (in case of myocardial haemorrhage), but also be induced by (ultra-small) superparamagnetic iron oxide nanoparticles (USPIOs) accumulating in the myocardium. Hence, USPIO-based molecular imaging agents have been developed and successfully applied among others in animal models of myocardial infarction.

Theoretically, USPIO-based targeting of macrophages infiltrating the (peri-)infarct zone and promoting infarct healing may lead to a more detailed characterization of infarct pathology in patients at risk. Recent data suggest that such infiltrating macrophages are potential diagnostic as well as therapeutic targets since both insufficient and exuberant accumulation of macrophages in the infarcted myocardium may have adverse effects regarding infarct repair.

Therefore, as part of the work programme of the German multicentre scientific network ‘NIMINI-1MMRI’ (Non-invasive myocardial inflammation based on new molecular magnetic resonance Imaging contrast agents and methods), we have conducted two prospective phase III trials (designated ‘NIMINI-1’ and ‘NIMINI-2’), performing comprehensive CMR studies (i) before and after ferucarbotran (Resovist, a SPIO; NIMINI-1 trial) administration and (ii) before and after ferumoxytol (Feraheme™, an USPIO; NIMINI-2 trial) administration in patients with acute myocardial infarction. The aim of both studies was to evaluate whether infarct imaging using USPIO/USPIO and respective T2- and T2*-weighted CMR techniques allow a more detailed characterization of infarct pathology (compared with conventional gadolinium-based necrosis/fibrosis imaging). The results of the first trial (NIMINI-1) have shown that ferucarbotran (an SPIO) does not substantially add information because it does not allow improved visualization of the (peri-)infarct zone. Here, we present the results of the second trial (NIMINI-2), using for the first time ferumoxytol (a USPIO) in patients with acute myocardial infarction.

Fourteen patients who had experienced an acute ST-elevation myocardial infarction (STEMI) were included in this study between September 2010 and August 2011. Following coronary angiography with percutaneous coronary intervention (PCI), a first baseline CMR study (pre-FH) was performed within 7 days—but at least 48 h—after the onset of cardiac symptoms. Patients were eligible for this study if (i) coronary angiography confirmed coronary artery occlusion or plaque rupture as an underlying cause for acute STEMI and if (ii) ceCMR (as part of the first CMR study) revealed transmural or subendocardial distribution of LGE suggestive of an ischaemic cause of myocardial damage. Then, 24 h after the first CMR study, ferumoxytol (FH; USPIO) was intravenously administered as recommended by the manufacturer (bolus injection of 17 mL FH containing 510 mg Fe). In the first three patients, serial CMR studies were performed 6, 24, 48, and 96 h after ferumoxytol administration (post-FH), considering the blood half-life of ferumoxytol and previous animal experiences. After the analysis of CMR images in these first three patients, subsequent patients underwent post-FH studies 48 h after ferumoxytol administration only. Major patient exclusion criteria were described elsewhere previously. In addition, patients with severe cardiovascular compromise and those with signs of extensive microvascular obstruction (MVO) were also excluded.

Cardiovascular magnetic resonance imaging: protocol

ECG-gated CMR imaging was performed in breath-hold with the use of a 1.5 T Aera (Siemens Medical Solutions, Erlangen, Germany) as described previously. The first CMR study (pre-FH) comprised cine-CMR, T2*-weighted ‘oedema’ imaging, T2*-mapping, and T2*-weighted LGE imaging after intravenous contrast administration (0.15 mmol/kg Magnetvist™). The second (and subsequent) CMR study following ferumoxytol administration (post-FH) comprised the same protocol without repeated LGE imaging (in order to avoid repeated exposure to a gadolinium-based contrast agent). Both cine and LGE short-axis CMR images were prescribed every 10 mm (slice thickness 6 mm) from base to apex. In addition, two-, three-, and four-chamber long-axis views were acquired.

Cine-CMR was performed with the use of a steady-state free precession sequence [repetition time (TR) 2.3 ms; echo time (TE) 1.15 ms; flip angle 74°; matrix 256 × 192; field-of-view (FOV) ranging from 320–400 mm]. T2*-weighted oedema imaging was performed with the use of a T2*-weighted short-tau inversion recovery (STIR) black-blood segmented turbo spin echo sequence (T2*-weighted STIR-SE) which is sensitive to increased myocardial free water content and also to paramagnetic T2-effects of iron ions (TR 2 R-to-R intervals; TE 52 ms; flip angle 180°; TI 170 ms; matrix 248 × 256; FOV 320–400). At least three contiguous short-axis slices were obtained covering the area of myocardial infarction (identified by the presence of wall motion abnormalities during cine-CMR) in addition to at least one long-axis view. T2*-mapping was performed with the use of a T2*-weighted multi-echo/gradient echo sequence (T2*-MAP) in order to depict myocardial areas with potential USPIO accumulation (TR 200 ms; TE 2.2/4.4/6.6/8.8/11 ms; flip angle 20°; matrix 320 × 240; FOV 320–400). T2*-mapping images were acquired in the same views as were chosen for previous T2*-weighted STIR-SE imaging. For LGE imaging, inversion recovery segmented turbo FLASH gradient echo (T1-weighted IR-FLASH) images were acquired, on average, 5–10 min after contrast administration with the adjustment of inversion time to null normal myocardium (TR 2 R-to-R; TE 4 ms; flip angle 25°; TI 240–320 ms; matrix 256 × 192; FOV 320–400 mm). During the second (and subsequent) CMR study
Cardiovascular magnetic resonance imaging: data analysis

All CMR image analyses were performed off-line by two experienced investigators. Endocardial and epicardial borders were outlined on the short-axis cine-CMR images. Volumes and ejection fraction (EF) were derived by summation of epicardial and endocardial contours. The diagnosis of ‘ischaemic’ LGE (in the first pre-FH study) required visual identification of elevated subendocardial or transmural signal within the myocardium in two orthogonal views. On the short-axis LGE images, the number of left ventricular segments with positive LGE was first quantified using a standard left ventricular 17-segment model. Classification of myocardial segments with respect to the presence of myocardial damage was made dichotomously based on visual identification of LGE. In addition, the extent of LGE was planimetered on the short-axis contrast images with the use of the ImageJ software (National Institutes of Health, Bethesda, MD, USA), and an image intensity level ≥3 SD above the mean of the remote myocardium was chosen to define LGE indicative of the infarcted myocardium as described previously.15 Microvascular obstruction was defined as a dark zone within the infarcted segments, typically located in the subendocardium.

Short-axis T2*-weighted STIR-SE images at the level of maximal oedema as well as corresponding short-axis cine-CMR and T2*-mapping images (pre-FH as well as corresponding post-FH) were chosen for subsequent analysis. The extent of ‘hyperenhancement’ observed in cine-CMR images particularly after ferumoxytol administration (post-FH) was calculated by the use of a threshold-based approach (signal intensity ≥2 SD above the mean of the remote non-infarcted myocardium (see Supplementary material online, Figure S1A)).

The extent of T2*-weighted ‘hyperenhancement’ (suggestive of intramyocardial USPIO accumulation and/or haemorrhage) was calculated from T2*-weighted STIR-SE images by the use of a threshold-based approach (signal intensity <2 SD below the mean of the remote non-infarcted myocardium (see Supplementary material online, Figure S1B)) as described previously.15

To get T2*-mapping images, a pixel-by-pixel analysis was performed of T2*-weighted multi-echo/gradient echo sequence images, using MRmap v1.2 (http://sourceforge.net/projects/mrmap/) with fitting to a simple monoexponential decay. In order to allow one-to-one comparison of T2*-mapping results to cine-CMR as well as T2*-weighted STIR-SE images, the extent of areas showing signal void in T2*-mapping images (suggestive of intramyocardial USPIO accumulation and/or haemorrhage) was first calculated by the use of a threshold-based approach (signal intensity >1.5 SD below the mean of the remote non-infarcted myocardium) (see Supplementary material online, Figure S1C). In addition, regions of interest (ROIs) were manually drawn in short-axis T2*-mapping images in order to acquire absolute T2*-values (Figure 2A–D). These ROIs corresponded to the infarct core, the peri-infarct zone (derived by subtraction of the hyperenhanced area on T2*-weighted STIR-SE images from the hyperenhanced area on LGE images), the remote myocardium, the blood pool, the skeletal muscle, the liver, and the spleen.

Identification of the optimal time point for performing cardiovascular magnetic resonance imaging after ferumoxytol administration

As illustrated in Figure 1A and B, the first three patients underwent serial CMR studies prior to (pre-FH) and 6, 24, 48, and 96 h after ferumoxytol administration (post-FH). Considering cine-CMR images (see Supplementary material online, Figure S1A), the maximal extent of hyperenhancement observed in the region of myocardial infarction after ferumoxytol administration (post-FH) was identified 24 h after ferumoxytol administration, with a slight decrease of hyperenhancement 48 h after ferumoxytol administration. Considering both T2*-weighted STIR-SE (see Supplementary material online, Figure S1B) and T2*-mapping images (see Supplementary material online, Figure S1C), the maximal extent of T2*-weighted hyperenhancement (suggestive of intramyocardial USPIO accumulation and/or haemorrhage) as well as the maximal extent of areas showing signal void in T2*-mapping images (again suggestive of intramyocardial USPIO accumulation and/or haemorrhage) was measured 48 h after ferumoxytol administration, respectively. Therefore, subsequent patients included in this study underwent their second CMR study 48 h after labelling, (iv) histochemical detection of (U)SPIO, and (v) cell viability assays can be found in Supplementary material online.
Figure 1 Exemplary cardiovascular magnetic resonance (CMR) images [(A) in the short-axis view; (B) in the long-axis view] of a study patient with a septal myocardial infarction who underwent pre-Feraheme™ (FH) (baseline) and post-Feraheme™ (after 6, 24, 48, 96, and 3 months) cardiovascular magnetic resonance imaging studies, respectively. The first line shows cine-cardiovascular magnetic resonance images at different time points with proof of hyperenhancement in the septal wall at 6–48 h post-Feraheme™. The second line shows $T_2$-weighted short-tau inversion recovery-spin echo images at different time points with proof of hypoenhancement in the septal wall at 6–48 h post-Feraheme™. The third line shows $T_2^*$-mapping images at different time points with proof of "signal void" in the septal wall at 6–48 h post-Feraheme™. The fourth line shows LGE images at baseline and 3 months later.
ferumoxytol administration in order to simplify the study protocol and complexity in favour of patient comfort.

**Comparison of pre-Feraheme™ and post-Feraheme™ cardiovascular magnetic resonance images**

As illustrated in Figure 3A, the extent of short-axis in-plane LGE (prior to ferumoxytol administration, pre-FH) was compared (i) with the extent of hyperenhancement observed in the same short-axis plane in cine-CMR images, (ii) with the extent of $T_2$-weighted hypoenhancement observed in the same short-axis plane, and (iii) with the extent of areas showing signal void in $T_2^*$-mapping images again in the same short-axis plane each 48 h after ferumoxytol administration (post-FH). The median extent of short-axis in-plane LGE was 30% (IQR 26–40%).

As shown in Figure 3B, hyperenhancement in cine-CMR images in the region of myocardial infarction, which was not present prior to ferumoxytol administration in any patient, was observed in all 14 patients 48 h after ferumoxytol infusion with a median in-plane extent of 37% (IQR 28–41%; $P < 0.01$ compared with the extent of LGE).
The median in-plane extent of $T_2^*$-weighted hypoenhancement in the region of myocardial infarction, which was not present prior to ferumoxytol administration in any patient, was 19% (IQR 14–22%; $P < 0.001$ compared with the extent of LGE). There was only one patient in whom no area of hypoenhancement was observed after ferumoxytol administration.

The median in-plane extent of areas showing signal void in $T_2^*$-mapping images in the region of myocardial infarction—by the use of a threshold-based approach—was 16% (IQR 12–18%; $P < 0.001$ compared with the extent of LGE; $P = 0.34$ compared with the extent of $T_2^*$-weighted hypoenhancement). There were two patients demonstrating small subendocardial areas of signal void (<5% in-plane extent) in $T_2^*$-mapping images in the region of myocardial infarction already prior to ferumoxytol administration. These two patients also showed a dark zone within the infarcted LGE-positive segments indicative of MVO. The other two patients with signs of MVO in LGE-CMR images did not demonstrate areas of signal void in $T_2^*$-mapping images prior to ferumoxytol administration.

### Quantification of absolute $T_2^*$-values in different cardiac and non-cardiac tissues

Absolute $T_2^*$-values were calculated in $T_2^*$-mapping images acquired pre-FH as well as 6, 24, 48, and 96 h after ferumoxytol administration in the first three patients (Figure 2E) and also pre-FH as well as 48 h post-FH in each of the 14 study patients (Figure 2F and Table 2). The $T_2^*$-value time curve obtained in the first three patients revealed a marked drop of $>40\%$ in absolute $T_2^*$-values in all tissues/organs—apart from the skeletal muscle. Moreover, the change in absolute $T_2^*$-values, which was observed already 6 h after ferumoxytol administration, remained rather constant for the first 48 h after ferumoxytol administration in all tissues/organs, and a substantial recovery in $T_2^*$-values was only observed 96 h after ferumoxytol administration (Figure 2E).

The detailed $T_2^*$-values and their changes after ferumoxytol administration are given in Table 2. A substantial drop in the $T_2^*$-value was observed not only in the infarct core and peri-infarct zone, but also in the remote ‘healthy’ myocardium, although there was only a minor change (of 11%) in the $T_2^*$-value of the skeletal...
Figure 3 (A) Comparative illustration of the extent of short-axis in-plane late gadolinium enhancement (LGE) [pre-Feraheme\textsuperscript{TM} (FH)] compared with (i) to the extent of hyperenhancement observed in the same short-axis plane in cine-cardiovascular magnetic resonance (CMR) images, (ii) to the extent of \( T_2 \)-weighted hypoenhancement observed in the same short-axis plane, and (iii) to the extent of areas showing signal void in \( T_2^* \)-mapping images again in the same short-axis plane each 48 h after ferumoxytol administration (post-Feraheme\textsuperscript{TM}). (B) The diagram (according to Figure 4A) showing the values for the extent of late gadolinium enhancement in \( T_1 \)-weighted late gadolinium enhancement-cardiovascular magnetic resonance images, the extent of hyperenhancement in SSFP-cine-cardiovascular magnetic resonance images, the extent of \( T_2 \)-weighted hypoenhancement, and for the extent of areas showing signal void in \( T_2^* \)-mapping images for each study patient. The red line represents the median values.
Table 2 Changes in absolute $T_2^*$-values in different tissues/organs

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pre-FH ($T_2^*$ in ms)</th>
<th>48 h post-FH ($T_2^*$ in ms)</th>
<th>Change in $T_2^*$</th>
<th>P-value</th>
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<tr>
<td>Infarct core (2)</td>
<td>25 (16–31)</td>
<td>9 (8–12)</td>
<td>−64%</td>
<td>&lt;0.001</td>
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<tr>
<td>Peri-infarct zone (3)</td>
<td>34 (30–43)</td>
<td>17 (15–19)</td>
<td>−50%</td>
<td>&lt;0.001</td>
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<td>Remote myocardium (4)</td>
<td>32 (29–35)</td>
<td>18 (16–23)</td>
<td>−44%</td>
<td>&lt;0.001</td>
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<td>Blood pool (5)</td>
<td>54 (46–60)</td>
<td>11 (10–12)</td>
<td>−80%</td>
<td>&lt;0.001</td>
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<tr>
<td>Skeletal muscle (6)</td>
<td>27 (26–28)</td>
<td>24 (23–26)</td>
<td>−81%</td>
<td>&lt;0.001</td>
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<tr>
<td>Liver (7)</td>
<td>26 (21–30)</td>
<td>5 (5–8)</td>
<td>−83%</td>
<td>&lt;0.001</td>
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<tr>
<td>Spleen (8)</td>
<td>30 (23–25)</td>
<td>5 (3–6)</td>
<td>−83%</td>
<td>&lt;0.001</td>
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Pre-FH, prior to Feraheme™ administration; post-FH, after Feraheme™ administration; IQR, inter-quartile range. Analysis was performed using the Wilcoxon signed-rank test.

Ex vivo ultrasmall superparamagnetic iron oxide nanoparticle vs. superparamagnetic iron oxide uptake by blood monocytes

Monocytes from healthy donors that did not undergo incubation with SPIO or USPIO did not show any positive staining for iron, as expected (Figure 4A, first line). Less than 1% of the monocytes incubated in the presence of ferumoxytol 0.5 mg Fe/mL for up to 24 h contained intracellular iron (Figure 4A, second line). In contrast, monocytes incubated with (a lower concentration of) ferucarbotran 0.25 mg Fe/mL showed intracellular iron deposition already after 1 h incubation and a positive iron staining in ~80% of monocytes after 24 h incubation (Figure 4A, third line). When ferucarbotran was used in the same concentration as ferumoxytol (0.5 mg Fe/mL), a positive iron staining in almost 100% of monocytes was observed already after 1 h incubation and an extracellular clustering of SPIO particles was clearly documented after 4 h incubation with a lethal effect on monocytes that was most impressive after 24 h of incubation (Figure 4A, fourth line).

The effect of USPIO vs. SPIO uptake on monocyte viability was further analysed by measuring the percentage of apoptotic monocytes by the number of annexin V-positive cells using a FACScan flow cytometer. Although there was no relevant difference in the number of annexin V-positive monocytes between the control (neither ferumoxytol nor ferucarbotran incubation), the ferumoxytol (0.5 mg Fe/mL), and the lower concentration ferucarbotran (0.25 mg Fe/mL) treated monocytes after 24 h of incubation, there was an apparent increase in apoptotic monocytes when ferucarbotran was given in the concentration of 0.5 mg Fe/mL (Figure 4B).

In vivo ferumoxytol (ultrasmall superparamagnetic iron oxide nanoparticle) uptake by blood monocytes

Peripheral blood mononuclear cells (PBMCs) from study patients were stained with Prussian blue in order to visualize potential intracellular ferumoxytol uptake following blood withdrawal 4, 24, and 48 h after ferumoxytol administration. Based on visual qualitative assessment, even 48 h after ferumoxytol infusion, no substantial ferumoxytol uptake could be detected in human PBMCs (Figure 4C). Monocyte viability was checked by mitochondrial outer membrane permeabilization (MOMP) analysis, which revealed no differences between monocytes isolated prior to and after ferumoxytol administration. In addition, there was no difference in the percentage of apoptotic monocytes (measured by the number of annexin V-positive cells) when monocytes isolated prior to ferumoxytol administration were compared with those isolated thereafter. No side effects were observed after intravenous bolus injection of ferumoxytol.

Ex vivo ultrasmall superparamagnetic iron oxide nanoparticle vs. superparamagnetic iron oxide uptake by cultured human macrophages

Since the behaviour (phagocytic activity) of tissue-resident macrophages is different from that of non-activated blood monocytes, we directly cultured freshly isolated human macrophages and induced macrophage differentiation ex vivo (Figure 5). Light microscopic images clearly revealed successful differentiation of monocytes into macrophages (Figure 5A). In addition, flow cytometry using a FITC-labelled antibody against the macrophage-specific surface protein EMR1 (human homologue of mouse F4/80) confirmed macrophage differentiation (Figure 5B). Light microscopic images revealed that the incubation of macrophages for 24 h with ferumoxytol (0.5 mg Fe/mL) and the lower concentration ferucarbotran (0.25 mg Fe/mL) did not result in significant morphological changes and was not toxic, whereas incubation with higher concentration ferucarbotran (0.5 mg Fe/mL) resulted in rapid cell death of most macrophages (Figure 5C).

Finally, USPIO and SPIO uptake of macrophages were evaluated (Figure 5D). Macrophages that did not undergo incubation with SPIO or USPIO did not show any positive staining for iron, as expected (Figure 5D, first line). In contrast to monocytes, ~20% of the macrophages incubated in the presence of ferumoxytol (0.5 mg Fe/mL) demonstrated intracellular iron, but not before 24 h of incubation (Figure 5D, second line). Macrophages incubated with (a lower concentration of) ferucarbotran (0.25 mg Fe/mL) showed intracellular iron deposition already after 4 h incubation and a positive iron staining in ~90% of macrophages after 24 h incubation (Figure 5D, third line). When ferucarbotran was used...
Figure 4

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in the same concentration as ferumoxytol (0.5 mg Fe/mL), a positive iron staining in almost 100% of macrophages was observed already after 1 h incubation with a lethal effect on macrophages starting already after 1 h incubation and being most impressive after 24 h of incubation (Figure 5D, fourth line).

Discussion

In the present clinical study, the diagnostic value of a USPIO-based agent (ferumoxytol) for in vivo CMR imaging was evaluated in patients with acute myocardial infarction. The clinical study NIMINI-2 was designed as a proof-of-principle trial and comprised 14 patients with acute myocardial infarction who underwent comprehensive multi-parametric CMR studies prior to and after intravenous ferumoxytol administration. Comparing $T_2^*$-weighted STIR-SE and $T_1^*$-mapping images that were acquired prior to and after ferumoxytol administration, we could demonstrate in humans that USPIO-based contrast agents open up new vistas in the characterization of infarct pathology by causing hypoenhancement (in $T_2^*$-weighted STIR-SE images) and signal void (in $T_2^*$-mapping images), respectively, mainly by detecting infiltrating macrophages and most likely by altered tissue partitioning of USPIOs in different layers of the infarcted myocardium. Moreover, additional information regarding the extent and composition of the peri-infarct zone could be obtained when comparing SSFP-cine-CMR images that were acquired prior to and after ferumoxytol administration.

Essential characteristics of Feraheme™

Feraheme™ consists of ferumoxytol, which is a USPIO with a diameter of $\approx 30$ nm, a neutral surface charge, a magnetic relaxivity of $r_1 = 15$mmol/L/s and $r_2 = 89$ mmol/L/s (at 1.5 T) and a blood half-life of $\sim 15$ h. It has been developed by AMAG Pharmaceuticals, Inc., in the USA and was approved for iron replacement therapy in patients with anemia due to chronic renal failure in June 2009 by the FDA. In June 2012, the company Takeda received European marketing authorization for Rienso® (i.e. 510 mg ferumoxytol) and intends to launch Rienso® across Europe in the near future. As described previously, after cellular internalization, iron oxide nanoparticles accumulate in lysosomes in which the low pH breaks the iron oxide core down into iron ions. These ions are then incorporated back into the haemoglobin pool. However, ferumoxytol is also attractive as an MRI contrast agent because of its relaxivity properties and because it can be given as a bolus (with 510 mg Fe) and is safe (and therefore approved by the FDA) even in patients with chronic kidney disease. In contrast to gadolinium-based contrast agents, there is no renal elimination of ferumoxytol, which accounts for the enhanced safety profile compared with gadolinium-based compounds in patients with renal dysfunction who appear to be at increased risk for contrast-induced nephropathy or nephrogenic systemic fibrosis.

The distinctiveness of the NIMINI-2 study

After overcoming some challenging regulatory issues (FH was not approved in Europe when the study was performed), we were allowed to perform a prospective, non-randomized, non-blinded, single-agent phase III trial (NIMINI-2) applying FH intravenously as recommended by the manufacturer (bolus injection of 17 mL FH containing 510 mg Fe) in patients with acute myocardial infarction. Hence, compared with the previous NIMINI-1 trial, we were now able to administer almost the 13-fold dose of iron (510 mg Fe in FH compared with only 39 mg Fe in Resovist), resulting, for example, in a concentration of 6.4 mg Fe/kg in a 80 kg weighing patient (the median dose of iron was 5.4 mg Fe/kg in the present study). Sosnovik et al. already demonstrated in a mouse model that this concentration should be sufficient for infarct imaging. A magnetofluorescent USPIO (CLIO-Cy5.5) which has a diameter of $\approx 50$ nm, a magnetic relaxivity of $r_1 = 19$ mmol/L/s and $r_2 = 48$ mmol/L/s (at 0.47 T), and a blood half-life of $\sim 10$ h—similar to Feraheme™—was used, and high-field MRI (9.4 T) 48 h after LDL- ligation was performed in a mouse model. Accumulation of the administered USPIO in the study of Sosnovik et al. resulted in a dose-dependent hypoenhancement in the injured myocardium.

Figure 4 (A) Microscopic images (200-fold and 1000-fold magnification as indicated) of peripheral blood mononuclear cells from healthy donors: human monocytes incubated neither with Feraheme™ (FH) (ultrasmall superparamagnetic iron oxide nanoparticle) nor with Resovist (SPIO) showed no positive staining for iron (first line). Less than 1% of the monocytes incubated in the presence of ferumoxytol 0.5 mg Fe/mL for up to 24 h contained intracellular iron (second line). In contrast, monocytes incubated with (a lower concentration of) ferucarbotran 0.25 mg Fe/mL showed intracellular iron deposition already after 1 h incubation and a positive iron staining in 80% of monocytes after 24 h incubation (third line). When ferucarbotran was used in the same concentration as ferumoxytol (0.5 mg Fe/mL), a positive iron staining in almost 100% of monocytes was observed already after 1 h incubation and an extracellular clustering of SPIO particles was clearly documented after 4 h incubation with a lethal effect on monocytes that was most impressive after 24 h of incubation (fourth line). (B) FACS measurement of the percentage of apoptotic monocytes by the number of annexin V-positive and/or propidium iodide (PI) positive cells. Although there was no relevant difference in the number of annexin V-positive monocytes between the control (neither ferumoxytol nor ferucarbotran incubation), the ferumoxytol (0.5 mg Fe/mL), and the lower concentration ferucarbotran (0.25 mg Fe/mL) treated monocytes after 24 h of incubation, there was an apparent increase in apoptotic monocytes when ferucarbotran was given in the concentration of 0.5 mg Fe/mL. (C) Exemplary results of one study patient. Monocytes were stained with Prussian blue in order to visualize potential intracellular ferumoxytol uptake following blood withdrawal prior to and 48 h after ferumoxytol administration. Based on visual qualitative assessment, 48 h after ferumoxytol infusion, no substantial ferumoxytol uptake could be detected in human PBMCs. Monocyte viability was checked by MOMP analysis, which revealed no differences between monocytes isolated prior to and after ferumoxytol administration. In addition, there was no difference in the percentage of apoptotic monocytes (measured by the number of annexin V-positive cells) when monocytes isolated prior to Feraheme™ administration were compared with those isolated thereafter.
Figure 5
in $T_2^*$-weighted images, with a dose of 3 mg Fe/kg being sufficient for successful MRI. Moreover, uptake of USPIOs by infiltrating macrophages in the infarcted myocardium was proven by ex vivo immunohistochemical analyses. Hence, this pre-clinical study suggested that passive uptake of USPIOs by infiltrating macrophages leads to the accumulation of these USPIOs in the infarcted myocardium and consequently enables the non-invasive detection of the infarcted myocardium by visualization of regional hypoenhancement in $T_2^*$-weighted MRI images.

**Effect of ferumoxytol on $T_2^*/T_2^*$-weighted imaging**

In the present study, the pre-clinical results of Sosnovik et al.\(^8\) were confirmed in humans: the infarcted myocardium was visualized non-invasively based on $T_2^*$-weighted STIR-SE and $T_2^*$-mapping images that were obtained prior to and after ferumoxytol administration by the detection of hypoenhancement (in $T_2^*$-weighted STIR-SE images) and signal void (in $T_2^*$-mapping images), respectively. These changes were detectable already 6 h after ferumoxytol administration and peaked \(\approx 48\ h\) after ferumoxytol administration, in agreement with the pre-clinical results of Sosnovik et al. Ferumoxytol-induced hypoenhancement (in $T_2^*$-weighted STIR-SE images) and signal void (in $T_2^*$-mapping images) in the infarcted myocardium already disappeared 96 h after ferumoxytol administration.

The explanation of these observations is quite challenging, since no histopathological data were available in this study. Since ex vivo analyses revealed that ferumoxytol was taken up only by activated macrophages—but not undifferentiated blood monocytes (!)—the assumption that intravenously administered ferumoxytol would be absorbed by blood monocytes and then ‘transported’ into the infarcted myocardium was rendered unlikely. Moreover, pre-clinical small-animal studies suggested that USPIOs are directly absorbed by macrophages infiltrating the infarcted myocardium during myocardial repair.\(^8,18\) These studies also showed that the accumulation of blood monocytes in the infarcted myocardium starts already in the first hours after infarction and macrophages are the most abundant and dominant cells during myocardial repair between days 1–8 after myocardial infarction (e.g. induced by coronary ligation).\(^18,19\) Our study results are in line with those pre-clinical data since we observed a substantial drop in absolute $T_2^*$-values in the (peri-)infarct zone already 6 h after ferumoxytol administration. Considering ex vivo data that demonstrated substantial uptake of ferumoxytol by activated macrophages, this drop in $T_2^*$-values is expected to be caused by macrophages which had infiltrated the (peri-)infarct zone. This drop in $T_2^*$-values remained rather constant for the first 48 h after ferumoxytol administration and disappeared only 96 h after ferumoxytol administration (corresponding in the median to day 8 (!) after myocardial infarction), which is in line with the data from a recent study by Leuschnner et al.\(^20\) in which the monocyte/macrophage resident time in the infarcted myocardium was shown to be only 20 h and the exit rate of macrophages from infarcted tissue between 5 and 13% within 24 h.

Two contradictory observations on the dynamics of iron uptake deserve commenting. Our ex vivo studies suggested that differentiated human macrophages incubated in the presence of ferumoxytol demonstrated iron uptake not before 24 h of incubation. However, the drop in absolute $T_2^*$-values as well as hypoenhancement (in $T_2^*$-weighted STIR-SE images) and signal void (in $T_2^*$-mapping images) were already observed 6 h after ferumoxytol administration. This suggests that macrophages infiltrating the infarcted myocardium in vivo could be more aggressive/active than cultured macrophages ex vivo and/or that there might be an (additional) direct ferumoxytol effect caused by altered tissue partitioning of USPIOs in different layers of the infarcted myocardium with varying severity of structural damage (already 6 h after ferumoxytol administration) as suggested by our $T_2^*$-mapping analyses in Figure 2E and F.

Intriguingly, a substantial drop in absolute $T_2^*$-values was observed not only in the area of myocardial infarction, but also (to a smaller extent) in the non-infarcted remote myocardium (Figure 2E and F), which is in line with the data from a recent clinical study using a similar approach.\(^21\) This observation suggests that the infiltration of macrophages not only takes place in the (peri-)infarct zone but also in the non-infarcted remote myocardium, which is in line with the recent data from Lee et al.\(^22\) Whether iron uptake imaging using USPIOs might enable a better differentiation of the infarct core from the peri-infarct zone (compared with established techniques) has to be evaluated in future studies with appropriately study size.

Moreover, signal loss on $T_2^*$- and $T_2^*$-weighted CMR images was shown to be caused by paramagnetic effects of oxidized iron in the area of myocardial haemorrhage in previous infarct studies.\(^7\) However, in the present study, the recovery of absolute $T_2^*$-values and the disappearance of hypoenhancement (in $T_2^*$-weighted STIR-SE images) and signal void (in $T_2^*$-mapping images) in the infarcted myocardium already 96 h after ferumoxytol administration

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**Figure 5** Light microscopic images (400-fold magnification) revealed successful differentiation of monocytes into macrophages. Flow cytometry using an FITC-labelled antibody against the macrophage-specific surface protein EMR1 (human homologue of mouse F4/80) confirmed macrophage differentiation. Cells were stained with either rat IgG2a-FITC as an isotype control (blue line) or rat anti-F4/80-FITC (red line). Light microscopic images (200-fold magnification) revealed that the incubation of macrophages for 24 h with ferumoxytol (0.5 mg Fe/mL) and the lower concentration ferucarbotran (0.25 mg Fe/mL) did not result in significant morphological changes and was not toxic, whereas incubation with higher concentration ferucarbotran (0.5 mg Fe/mL) resulted in rapid cell death of most macrophages. Ultrasound superparamagnetic iron oxide nanoparticle and superparamagnetic iron oxide uptake of macrophages were evaluated by light microscopy (200-fold magnification) and Prussian blue staining. About 20% of the macrophages incubated in the presence of ferumoxytol (0.5 mg Fe/mL) demonstrated intracellular iron, but not before 24 h of incubation (second line). Macrophages incubated with (a lower concentration of) ferucarbotran (0.25 mg Fe/mL) showed intracellular iron deposition already after 4 h incubation and a positive iron staining in \(\sim 90\%\) of macrophages after 24 h incubation (third line).
argue against such a paramagnetic effect of oxidized iron coming from erythrocytes' haemoglobin since the erythrocytes' iron should continue to stay in the myocardium and cause such signal effects even after 96 h. Our observation rather supports the conception that ferumoxytol intriguingly allows visualization of infiltrating macrophages and of altered perfusion kinetics in the infarcted myocardium, which was not possible with previous iron-based contrast agents.

**Effect of ferumoxytol on SSFP-cine imaging?**

Surprisingly, hyperenhancement, but not hypoenhancement, was observed in (balanced) SSFP-cine-CMR images in all 14 patients 48 h after ferumoxytol administration (in the region of myocardial infarction), which was not prior to ferumoxytol administration in any patient. Moreover, the maximal extent of hyperenhancement in SSFP-cine-CMR images was measured 24 h after ferumoxytol administration. The exact reason for this observation is so far unclear. In principle, SSFP is a gradient-echo sequence in which a non-zero steady state develops for both components of magnetization (longitudinal, $T_1$; transverse, $T_2$). The SSFP signal depends on $T_2$ as well as $T_1$, unless measures are taken to destroy signal refocusing and prevent the development of steady-state free precession. In case of a predominant $T_2$-effect, one would again expect rather a hyperenhancement caused by ferumoxytol in SSFP-cine-CMR images. Consequently, a predominant $T_1$-effect has to be assumed. Alternatively, the preceding administration of a gadolinium-based contrast agent (Magnevist) during the first CMR study may have resulted in hyperenhancement in subsequent SSFP-cine-CMR studies due to the ‘delayed’ wash-in and wash-out kinetics of gadolinium in the infarcted myocardium, as suggested by Engblom et al. Moreover, previous studies suggested an overestimation of the infarct size by SSFP-cine-CMR (following gadolinium administration) early after myocardial infarction due to swelling of the ischaemic myocardium caused by acute inflammation, oedema, and haemorrhage. This would explain the observation of slightly (but significantly) increased areas of hyperenhancement following ferumoxytol administration in the region of myocardial infarction in SSFP-cine-CMR images compared with conventional gadolinium-based contrast images.

However, the gadolinium-based contrast agent is not likely to be the cause of the observed hyperenhancement on SSFP-cine images. Instead, the effect of $T_1$-relaxivity (of USPIO-based contrast agents) is still substantial at 1.5 T and will thus diminish signal attenuation caused by $T_1$/$T_2$-effects. Only when imaging at field strengths $>3$ T, additional confounding $T_1$-effects can be neglected in $T_2$- or $T_2^*$-weighted MRI. Therefore, we assume that hyperenhancement observed in SSFP-cine-CMR images in all 14 patients 48 h after ferumoxytol administration is at least partly due to the ferumoxytol infusion itself.

**Clinical implications**

As pointed out previously, successful characterization of the infarcted myocardium—in particular by the detection of infiltrating macrophages—based on USPIO imaging may be of great clinical value, since (i) infiltrating macrophages are potential diagnostic as well as therapeutic targets, (ii) USPIOs may also be used in patients with contraindications to conventional gadolinium-based contrast agents such as in those with advanced renal insufficiency. (iii) USPIOs may help to differentiate acute myocardial infarction from chronic myocardial fibrosis, and (iv) further modification of the coating properties of USPIOs (e.g. coupling with specific antibodies) may allow targeted molecular imaging. Hence, the present proof that ferumoxytol—an already clinically approved USPIO (I)—(ii) allows non-invasive detection of the infarcted myocardium based on a multi-parametric CMR approach and (ii) can be applied safely even in patients with acute myocardial infarction, could be of paramount and far-reaching consequence for future developments of USPIO-based contrast agents.

Another potentially important aspect of this study is that hyperenhancement (in $T_2$-weighted STIR-SE images) and signal void (in $T_2^*$-mapping images) can be detected in the region of myocardial infarction following ferumoxytol administration. Such a hypointense infarct core caused by intramyocardial haemorrhage was shown to be related to infarct size and impaired left ventricular function and to be a strong predictor of major adverse cardiovascular events. The detection of hyperenhancement (in $T_2$-weighted STIR-SE images) and signal void (in $T_2^*$-mapping images) in the region of myocardial infarction following ferumoxytol administration may be related to the degree of myocardial inflammation (to the degree of macrophage infiltration) and therefore be of superior clinical and prognostic value. This aspect is currently evaluated in ongoing clinical studies.

**Study limitations**

In the present study, image analyses were performed ‘automatically’ using a threshold-based planimetric approach. Although the thresholds used for analysis were chosen in agreement with previous studies (if possible), no optimal threshold has yet been defined for ferumoxytol-contrasted CMR images. In addition, there is no validated method for automatic image analysis. Alternatively, even ‘manual’ planimetric analysis is used at many centres supposing an excellent reproducibility and a low inter-observer and intra-observer variability.

Obviously, we did not perform endomyocardial biopsy following ferumoxytol administration to prove myocardial iron accumulation due to ethical reasons. Hence, the exact localization and distribution of USPIOs in the myocardium (capillary bed? interstitium? macrophages?) could not be assessed using histopathological analyses.

**Conclusions**

We could demonstrate for the first time in humans that USPIO-based contrast agents lead to signal changes in the infarct area, using different CMR pulse sequences. Drop in absolute $T_1$-values as well as hyperenhancement (in $T_2$-weighted STIR-SE images) and signal void (in $T_2^*$-mapping images) are mainly due to the accumulation of USPIOs in infiltrating macrophages. Considering the multi-functionality of USPIO-based particles and their superior safety profile compared with gadolinium-based compounds, these observations open up new vistas not only in the characterization of infarct pathology.
Supplementary material

Supplementary material is available at European Heart Journal online.

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