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# Magnetic resonance imaging (MRI) of inflamed myocardium using iron oxide nanoparticles in patients with acute myocardial infarction — Preliminary results

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ABSTRACT

*Objectives:* Superparamagnetic iron oxide nanoparticle (SPIO)-based molecular imaging agents targeting macrophages have been developed and successfully applied in animal models of myocardial infarction. The purpose of this clinical trial was to investigate whether magnetic resonance imaging (MRI) of macrophages using ferucarbotran (Resovist®) allows improved visualisation of the myocardial (peri-)infarct zone compared to conventional gadolinium-based necrosis/fibrosis imaging in patients with acute myocardial infarction.

*Material and methods:* The clinical study NIMINI-1 was performed as a prospective, non-randomised, non-blinded, single agent phase III clinical trial (NCT0088644). Twenty patients who had experienced either an acute ST-elevation or non-ST-elevation myocardial infarction (STEMI/NSTEMI) were included to this study. Following coronary angiography, a first baseline cardiovascular magnetic resonance (CMR) study (pre-SPIO) was performed within seven days after onset of cardiac symptoms. A second CMR study (post-SPIO) was performed either 10 min, 4 h, 24 h or 48 h after ferucarbotran administration. The CMR studies comprised cine-CMR, T2-weighted "edema" imaging, T2*-weighted cardiac imaging and T1-weighted late-gadolinium-enhancement (LGE) imaging.

*Results:* The median extent of short-axis in-plane LGE was 28% (IQR 19–31%). Following Resovist® administration the median extent of short-axis in-plane T2*-weighted hypoenhancement (suggestive of intramyocardial haemorrhage and/or SPIO accumulation) was 0% (IQR 0–9%; $p = 0.68$ compared to pre-SPIO). A significant in-slice increase (>3%) in the extent of T2*-weighted "hypoenhancement" (post-SPIO compared to pre-SPIO) was seen in 6/16 patients (38%). However, no patient demonstrated "hypoenhancement" in T2*-weighted images following Resovist® administration that exceeded the area of LGE.

*Conclusions:* T2/T2*-weighted MRI aiming at non-invasive myocardial macrophage imaging using the approved dose of ferucarbotran does not allow improved visualisation of the myocardial (peri-) infarct zone compared to conventional gadolinium-based necrosis/fibrosis imaging.



## 1. Introduction

Cardiovascular magnetic resonance imaging (CMR) has gained wide acceptance as a useful tool for diagnosis and treatment-stratification of cardiac diseases [1,2]. Particularly contrast-enhanced CMR (ceCMR) is a well-established and widely accepted method in the clinical work-up of patients with acute and chronic myocardial infarction [2] while techniques without the need for contrast agent application are still under evaluation [3]. The transmural extent of myocardial injury after myocardial infarction is a strong predictor of clinical outcome [4] and can be exactly evaluated with ceCMR [5–7]. Accurate diagnosis of the degree of myocardial injury is clinically essential as therapeutic procedures reducing the degree of damage improve clinical prognosis [8]. However, although ceCMR is able to depict even small infarcts with high accuracy, it does not allow

*Abbreviations:* ceCMR, contrast-enhanced CMR; CMR, cardiovascular magnetic resonance imaging; EF, ejection fraction; FOV, field-of-view; GRE, gradient echo; LAD, left anterior descending artery; LGE, late-gadolinium-enhancement; MRI, magnetic resonance imaging; NIMINI-MMRI, **N**on-**i**nvasive **m**yocardial **in**flammation **i**maging based on new **m**olecular **m**agnetic **r**esonance **i**maging contrast agents and methods; NSTEMI, non-ST-elevation myocardial infarction; PBMC, peripheral blood mononuclear cells; PCI, percutaneous coronary intervention; RCA, right coronary artery; RCX, circumflex coronary artery; SPIO, superparamagnetic iron-oxide nanoparticle; STEMI, ST-elevation myocardial infarction; STIR, short tau inversion recovery; TE, echo time; TI, inversion time; TR, repitition time; USPIO, ultra-small SPIO.

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distinguishing an acute myocardial infarction from a chronic one and represents a rather unspecific method.

In the time-course of myocardial infarction, abrupt termination of blood supply leads to swelling of cardiomyocytes causing texture changes described as myocytic weaving [9]. Such changes are followed by release of intracellular enzymes, break-down of myocyte homeostasis and development of coagulation necrosis in the core of the infarcted region. Within 24 h after onset of ischemia, proliferation of lymphocytes and macrophages with a significant inflammatory reaction in the peri-infarct zone is taking place. Then, progressive granulation tissue formation starts (around day four) from the peri-infarct zone and leads to fibrotic remodelling of the infarcted core region. Thus, infiltration with and accumulation of macrophages is a main characteristic of the (peri-)infarct zone starting as early as within 24 h after onset of ischemia.

Molecular imaging agents targeting macrophages have been developed [10] and successfully applied amongst others in animal models of myocardial infarction [11,12]. For example, Chapon et al. performed high-field magnetic resonance imaging (MRI) in a rat model of myocardial infarction evaluating the potency of superparamagnetic iron oxide nanoparticles (SPIO) to discriminate infarcted from non-infarcted tissue. A significant contrast increase between normal and infarcted myocardium could be demonstated on T2-weighted images [11]. Recently, Sosnovik et al. demonstrated successful non-invasive imaging of macrophages infiltrating the infarcted myocardium by both fluorescence tomography and MRI using magnetofluorescent nanoparticles [12]. However, no clinical studies targeting macrophages in the human myocardium have been conducted so far and none of the contrast agents used in animal models of myocardial infarction is approved for human use.

One of the first SPIOs which has already been approved for human use is ferucarbotran (Resovist®) [13,14]. Ferucarbotran has been developed for T2-weighted imaging of liver diseases. Labelling of monocytes by phagocytosis of SPIO-based contrast agents has been analysed extensively in vitro, and ferucarbotran has turned out to be superior to other SPIO including ultrasmall SPIO (USPIO) with regard to the degree of monocyte uptake and impairment of cell viability [10]. Therefore, ferucarbotran is expected to be a promising contrast agent for targeting of inflammatory processes not only in the liver, but also in the myocardium, e.g. following acute myocardial infarction. Since the extent and texture of the peri-infarct zone in patients with myocardial infarction has been shown to be an important determinant of the risk for sudden cardiac death caused by malignant arrhythmias originating from this peri-infarct zone [15,16], SPIO-based targeting of macrophages infiltrating the peri-infarct zone and promoting infarct healing may theoretically lead to additional insights in and better characterisation of infarcted myocardium in patients at risk. Therefore, as part of the German multi-centre scientific network "NIMINI-MMRI" (**N**on-**i**nvasive **m**yocardial **in**flammation **i**maging based on new **m**olecular **m**agnetic **r**esonance **i**maging contrast agents and methods), we have conducted a prospective phase III trial (designated "NIMINI-1") performing comprehensive CMR studies pre- and post ferucarbotran administration in patients with acute myocardial infarction. The aim of this study was to evaluate whether macrophage imaging using ferucarbotran allows delineating the region of myocardial infarction (compared to conventional gadolinium-based necrosis/fibrosis imaging).

## 2. Methods

### 2.1. Study design

NIMINI-1 was performed as a prospective, non-randomised, non-blinded, single agent phase III clinical trial (NCT0088644). The German Federal Institute for Drugs and Medical Devices (BfArM) and the ethics committee of the University of Tübingen approved the study protocol, and all participating patients provided written informed consent.

Twenty patients who had experienced either an acute ST-elevation or non-ST-elevation myocardial infarction (STEMI/NSTEMI) were included to this study between April 2009 and March 2010. Following coronary angiography with percutaneous coronary intervention (PCI) a first baseline CMR study (pre-SPIO) was performed within seven days – but at least 48 h – after onset of cardiac symptoms. Patients were eligible for this study, if 1) coronary angiography confirmed coronary artery occlusion or plaque rupture as underlying cause for acute STEMI/NSTEMI and if 2) ceCMR (as part of the first CMR study) revealed transmural or subendocardial distribution of late-gadolinium-enhancement (LGE) suggestive of an ischemic cause of myocardial damage. Then, four to 12 h after the first CMR study ferucarbotran (Resovist®) was intravenously administered as recommended by the manufacturer (bolus injection of 0,9 ml Resovist® in patients with body weight <60 kg and of 1,4 ml Resovist® in those with body weight >60 kg). A second CMR study (post-SPIO) was performed either 10 min, 4 h, 24 h or 48 h after ferucarbotran administration considering previous animal experience [11,12].

Major patient exclusion criteria were defined as follows: 1) clinical contraindications to CMR (e.g. cerebral clips or claustrophobia); 2) renal insufficiency with creatinine clearance <60 ml/min; 3) history of previous myocardial infarction and/or known coronary artery disease (CAD); 4) history of myocarditis and/or malaise for more than one week and/or history of respiratory and/or gastrointestinal infection, both within six months prior to admission; 5) history of severe valve disease and/or history of cardiomyopathy and/or history of infiltrative or connective tissue diseases; 6) history of hypersensitivity reactions (e.g. anaphylaxis, skin rash to medication, hypersensitivity to iodinated contrast media, allergies to dextran and iron salts); 7) history of ongoing liver disorders or chronic viral hepatitis; 8) history of malignancy; 9) age <18 yrs or >80 yrs and 10) pregnancy in female patients.

### 2.2. Cardiovascular magnetic resonance imaging — protocol

ECG-gated CMR imaging was performed in breath-hold with the use of a 1.5-T Magnetom Sonata (Siemens Medical Solutions, Erlangen, Germany) as described previously [17,18]. Time to the first CMR study (pre-SPIO) after emergent admission to our hospital was four days for the median (range 2–5 days). The first CMR study (pre-SPIO) comprised cine-CMR, T2-weighted "edema" imaging, T2*-weighted cardiac imaging and T1-weighted LGE imaging after intravenous contrast administration (0.15 mmol/kg Magnevist®). The second CMR study (post-SPIO) following ferucarbotran administration comprised the same protocol without repeated LGE imaging (Fig. 1a). Both cine and LGE short-axis CMR-images were prescribed every 10 mm (slice thickness 6 mm) from base to apex. In addition, 2-, 3- and 4-chamber long-axis views were acquired.

Cine-CMR was performed with the use of a steady-state free precession sequence (repetition time (TR) 41 msec; echo time (TE) 1.0 msec; flip angle 72°; matrix 256×192; field-of-view (FOV) ranging from 320 to 400 mm). T2-weighted "edema" imaging was performed with the use of a T2-weighted short tau inversion recovery black-blood segmented turbo spin echo sequence (T2-weighted STIR-SE) which is sensitive to increased myocardial free water content (TR 2 R-to-R intervals; TE 60 msec; flip angle 180°; TI 170 msec; matrix 248×256; FOV 340–400). At least three contiguous short-axis slices were obtained at the area of myocardial infarction (identified by the presence of wall motion abnormalities during cine-CMR) in addition to at least two different long-axis views. T2*-weighted cardiac imaging was performed with the use of a T2*-weighted single-echo/gradient echo sequence (T2*-weighted GRE) in order to depict myocardial areas with potential SPIO-accumulation (TR 217 msec; TE 19 msec; flip angle 35°; matrix 384x512; FOV 255–400). We used a single-echo T2*-weighted sequence that was optimised for visualisation of hypoenhancement caused by magnetic susceptibility-inducing structural changes (such as intramyocardial accumulation of SPIO particles). T2*-weighted GRE 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 five to ten minutes after contrast administration with adjustment of inversion time to null normal myocardium (TR 2 R-to-R intervals; TE 4 msec; flip angle 25°; TI 240–320 msec; matrix 256x192; FOV 320-400 mm). During the second CMR study (post-SPIO) anatomical characteristics were used to choose exactly the same level of short- and long-axes for T2-weighted STIR-SE and T2*-weighted GRE imaging (Fig. 1b-c).

### 2.3. Cardiovascular magnetic resonance imaging – data analysis

All CMR image analyses were performed off-line by one experienced investigator. 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 "ischemic" LGE (in the first pre-SPIO 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 [19–21]. 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 ImageJ software (National Institutes of Health, Bethesda, Md, USA) and an image intensity level ≥3 SD above the mean of remote myocardium to define LGE indicative of infarcted myocardium as described