In Vivo Magnetization Transfer and Diffusion-Weighted Magnetic Resonance Imaging Detects Thrombus Composition in a Mouse Model of Deep Vein Thrombosis

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Background—Deep vein thrombosis remains a major health problem necessitating accurate diagnosis. Thrombolysis is associated with significant morbidity and is effective only for the treatment of unorganized thrombus. We tested the feasibility of in vivo magnetization transfer (MT) and diffusion-weighted magnetic resonance imaging to detect thrombus organization in a murine model of deep vein thrombosis.

Methods and Results—Deep vein thrombosis was induced in the inferior vena cava of male BALB/C mice. Magnetic resonance imaging was performed at days 1, 7, 14, 21, and 28 after thrombus induction using MT, diffusion-weighted, inversion-recovery, and T1-mapping protocols. Delayed enhancement and T1 mapping were repeated 2 hours after injection of a fibrin contrast agent. Finally, excised thrombi were used for histology. We found that MT and diffusion-weighted imaging can detect histological changes associated with thrombus aging. MT rate (MTR) maps and percentage of MT rate (%MTR) allowed visualization and quantification of the thrombus protein content, respectively. The %MTR increased with thrombus organization and was significantly higher at days 14, 21, and 28 after thrombus induction (days 1, 7, 14, 21, 28: %MTR=2483±451, 2079±1210, 7029±2490, 10 295±4356, 32 994±25 449; P<0.05). There was a significant positive correlation between the %MTR and the histological protein content of the thrombus (r=0.70; P<0.05). The apparent diffusion coefficient was lower in erythrocyte-rich and collagen-rich thrombus (0.72±0.10 and 0.69±0.05 ×10−3 mm²/s). Thrombus at days 7 and 14 had the highest apparent diffusion coefficient values (0.95±0.09 and 1.10±0.18 ×10−3 mm²/s).

Conclusions—MT and diffusion-weighted magnetic resonance imaging sequences are promising for the staging of thrombus composition and could be useful in guiding medical intervention. (Circ Cardiovasc Imaging. 2013;6:433-440)

Key Words: diffusion weighting ■ magnetization transfer ■ MRI ■ thrombus ■ vein

Deep vein thrombosis (DVT) remains a significant cause of morbidity and mortality despite the improvements in diagnosis and treatment. Approximately 30% of patients with DVT develop pulmonary embolism, of whom 10% die, and 50% of patients with DVT develop long-term sequelae like the postthrombotic syndrome.1,2

Clinical Perspective on p 440

Treatment of DVT is usually achieved with anticoagulation (heparin, warfarin) or thrombolysis (plasminogen activators). Anticoagulants prevent thrombus expansion but have little effect on thrombus resolution, which occurs slowly through a natural process of organization that eventually leads to recanalization of the vein.1,3,4 Thrombolytic agents rapidly remove the thrombus, aiding faster vein recanalization, with less thrombus recurrence and fewer postthrombotic complications.5 Systemic thrombolysis is, however, associated with serious complications, including bleeding and pulmonary emboli. Selection criteria for identifying patients who might benefit from thrombolytic treatment are based on a subjective assessment of patient history.5-7 Catheter-directed thrombolysis has reduced the rate of complications, but the time interval between the onset of symptoms and effective thrombolysis is still unclear. Several studies have shown that thrombolysis might be ineffective if administered 10 to 21 days after the onset of symptoms.5-10 Fibrin-rich thrombus seems to be more amenable to thrombolysis, and incorporation of collagen into a fibrin clot dramatically decreases the effectiveness of fibrinolysis in experimental models.11,12 Identification of the extracellular protein milieu and structural microenvironment of the thrombus might therefore provide important prognostic information on its lysability.

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Ultrasound examination, used in the diagnosis of DVT, provides only information about vein morphology and blood flow, but no information on the composition of the thrombus. Magnetic resonance imaging (MRI) has the potential to noninvasively image DVT, providing information on blood flow defects (time-of-flight or phase-contrast venography) and thrombus stage (T1- and T2-weighted images). MR direct thrombus imaging is a non–contrast agent–based method that has been extensively used for the detection of hematoma,13 venous,14–16 intracoronal,17 and arterial thrombus.18,19 based on the changes of T1 and T2 relaxation times of different oxygenation states of hemoglobin in erythrocytes. Proton binding to Fe3+ within accumulated methemoglobin in the thrombus is thought to result in shortening of the T1 and T2 relaxation times.20,21 The protein content of thrombus cannot, however, be directly detected using conventional MRI owing to the very short T2 relaxation times (<0.1 millisecond) of restricted protons bound to proteins. The development of fibrin-targeted MR contrast agents has allowed in vivo imaging of the fibrin content in arterial and venous thrombus in animal models and humans.22–25 The feasibility of magnetization transfer (MT) and diffusion-weighted (DW) MRI for thrombus imaging and staging has been less explored. In vivo MT has been used to detect intracranial hemorrhage,26,27 and ex vivo magnetization transfer contrast (MTC) showed the feasibility of visualizing regions of organized proteins in atherosclerosis28,29 and thrombus associated with plaque rupture.30 DW imaging (DWI) detected thrombus,31–34 and calculation of the apparent diffusion coefficient (ADC) in vitro reflected the stages of thrombus aging and organization.35 The protein content of thrombus and the diffusivity of water molecules depend on the local tissue microenvironment. We therefore hypothesized that MT and DWI could be used to generate endogenous contrast for thrombus imaging without the need of targeted agents. In this work, we tested the feasibility of in vivo MT and DWI MRI to visualize and assess thrombus composition and to stage the age of venous thrombus in a murine model of DVT.

Methods

Mouse Model of DVT

DVT was induced in the inferior vena cava (IVC) of 8- to 10-week-old male BALB/C mice (n=25) using a surgical procedure that involved a combination of reduced blood flow and endothelial damage.36 Briefly, surgical anesthesia was induced with 5% and maintained with 1% to 2% isoflurane. A midline laparotomy was performed to expose the infrarenal portion of the IVC. A 5-mm segment of the IVC just below the left renal vein was dissected. A neurosurgical vascular clip (Braun Lexington, MA) and was used for delayed-enhancement MRI imaging was used for the in vivo study. The acquisition parameters were as follows: TR=115 milliseconds, TE=16 milliseconds, flip angle=18°, FOV=30×18×14 mm, acquired matrix=148×89, slice thickness=0.4 mm, acquired resolution=0.2×0.2 mm, reconstructed resolution=0.1×0.1 mm, slices=35, averages=1, and duration=6 minutes. The MT prepulse was a binomial block (1:2:1, 90°×90°×90°) pulse with duration of 9 minutes with all other parameters maintained. The in vivo experiment was performed in accordance with the Animal (Scientific Procedures) Act 1986. Anesthesia was induced with 5% and maintained with 1% to 2% isoflurane during the MRI experiments. Mice were imaged in the prone position at days 1, 7, 14, 21, and 28 after thrombus induction. Five mice were scanned at each time point before and 2 hours after injection of a fibrin contrast agent (EP-2104R, 8 μmol/kg). The preinjection scan included time-of-flight (TOF) angiography, MT, DWI, inversion recovery (IR), and T1-mapping protocols, whereas the postinjection scan only included delayed-enhancement IR and T1-mapping scans. After a 3-dimensional (3D) gradient echo scout scan, arterial and venous TOF angiography was performed as follows: arterial TOF with repetition time (TR)=40 milliseconds, echo time (TE)=6.2 milliseconds, flip angle=60°, field of view (FOV)=20×33×17 mm, acquired matrix=68×110, slice thickness=0.3 mm, resolution=0.3×0.3 mm, reconstructed resolution=0.1×0.1 mm, slices=50, averages=2, duration=7.5 minutes, and a venous TOF with TR=50 seconds, resulting in a duration of 9 minutes with all other parameters maintained. The maximum-intensity projection images were used to visualize the abdominal aorta, the renal and iliac bifurcations, the vena cava, and the region of flow obstruction corresponding to the thrombus on the arterial and venous angiograms. These images were used for planning of the subsequent MT, DW, and delayed-enhancement scans.

T1-weighted spoiled 3D gradient-echo images were acquired without and with an on-resonance MT prepulse. Phantom work using samples with different protein composition in our laboratory showed that saturation of the bound proton pool by exploiting the differences in the T2 relaxation properties between the pools using a binomial on-resonance MT prepulse was more effective in detecting the protein composition of agar samples and in vitro thrombus compared with selectively saturating the bound pool using off-resonance MT prepulse (online-only Data Supplement). Therefore, the on-resonance implementation was used for the in vivo study. The acquisition parameters were as follows: TR=115 milliseconds, TE=16 milliseconds, flip angle=18°, diffusion echo time=333 milliseconds, FOV=18×30×12 mm, acquired matrix=88×150, slice thickness=0.5 mm, acquired resolution=0.2×0.2 mm, reconstructed resolution=0.1×0.1 mm, slices=24, averages=2, and duration=36 minutes. The ADC was calculated for 4 b values of 0, 333, 667, and 1000 mm²/s. Diffusion gradients were applied parallel and perpendicular to the external magnetic field. 2. A 2-dimensional DW spin-echo images were acquired with TR=2 seconds, TE=105 milliseconds, flip angle=90°, diffusion echo time=333 milliseconds, FOV=18×30×12 mm, acquired matrix=88×150, slice thickness=0.5 mm, acquired resolution=0.2×0.2 mm, reconstructed resolution=0.1×0.1 mm, slices=24, averages=2, and duration=36 minutes. The ADC was calculated from 4 b values of 0, 333, 667, and 1000 mm²/s. Diffusion gradients were applied parallel and perpendicular to the external magnetic field.

In Vivo MRI Protocol at 3 T

In vivo MRI of thrombus was performed using a 3-T Achieva Gyroscan MR scanner (Philips Healthcare, Best, the Netherlands) equipped with a clinical gradient system (30 mT/m, 200 mT/m per millisecond) and a single-loop surface coil (diameter=47 mm). Anesthesia was induced with 5% and maintained with 1% to 2% isoflurane during the MRI experiments. Mice were imaged in the prone position at days 1, 7, 14, 21, and 28 after thrombus induction. Five mice were scanned at each time point before and 2 hours after injection of a fibrin contrast agent (EP-2104R, 8 μmol/kg). The preinjection scan included time-of-flight (TOF) angiography, MT, DWI, inversion recovery (IR), and T1-mapping protocols, whereas the postinjection scan only included delayed-enhancement IR and T1-mapping scans. After a 3-dimensional (3D) gradient echo scout scan, arterial and venous TOF angiography was performed as follows: arterial TOF with repetition time (TR)=40 milliseconds, echo time (TE)=6.2 milliseconds, flip angle=60°, field of view (FOV)=20×33×17 mm, acquired matrix=68×110, slice thickness=0.3 mm, resolution=0.3×.3 mm, reconstructed resolution=0.1×0.1 mm, slices=50, averages=2, duration=7.5 minutes, and a venous TOF with TR=50 seconds, resulting in a duration of 9 minutes with all other parameters maintained. The maximum-intensity projection images were used to visualize the abdominal aorta, the renal and iliac bifurcations, the vena cava, and the region of flow obstruction corresponding to the thrombus on the arterial and venous angiograms. These images were used for planning of the subsequent MT, DW, and delayed-enhancement scans.

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Histology

After completion of the MRI experiments, the IVC containing thrombus (from immediately above the suture to the bifurcation of the iliac veins) was harvested, fixed in formalin, and embedded in paraffin. Serial 5-μm-thick cross sections were stained with hematoxylin and eosin to detect cellular components and Martius scarlet blue to detect fibrin and collagen.

Data Analysis

MRIs were analyzed using the software Osirix (OsiriX Foundation, Geneva, Switzerland). MRI slices spanning the region of luminal obstruction, as seen on TOF venous angiography, were used for analysis. Regions of interest encompassing the thrombus were manually segmented on images acquired without the MT prepulse and then copied to images acquired with the MT prepulse and to DW images. The mean signal intensity and area (mm²) of each region of interest were recorded. Images acquired with and without MT were used to calculate the percentage of MT rate (MTR) based on the following formula: %MTR=(M₀−Mₚ)/M₀×100, where M₀ is the signal intensity without the MT prepulse, and Mₚ is the signal intensity with the MT prepulse. The average %MTR of thrombus was then normalized to the volume of the thrombus for each animal at each time point to acquire the protein content or density of the thrombus expressed as %MTR/cm³. MTR maps were generated on the basis of the same formula using an Osirix plug-in for visualization of protein-rich areas within the thrombus. The same regions of interests were used to calculate the ADC from the following equation: ADC=ln(S₀−Sᵢ)/(b⋅b₀) (mm²/s), where Sᵢ is the signal intensity of the area of interest obtained with a b value of b, To have an internal control, we also estimated the ADC values of muscle and free water (represented by the urine from the bladder) in each mouse. T1 values were computed on a pixel-by-pixel basis using in-house Matlab (Mathworks, Natick, MA) software.

Thrombus cross-sectional area (mm²) was measured on Martius scarlet blue–stained sections by computerized planimetry (ImageJ, NIH, Bethesda, MD). Computer-assisted color image analysis (Color Threshold plug-in, ImageJ) was used to quantify the collagen and fibrin area on Martius scarlet blue–stained sections. These measurements were used to calculate the total histological protein content of the thrombus as follows: percent thrombus protein content on histology = (fibrin+collagen area)/thrombus area×100. For registration of the in vivo MRIs and histological sections, the distances from the renal and iliac bifurcations were used as internal landmarks.

Statistical Analysis

The statistical package SPSS 19.0 (IBM Corp, Somers, NY) was used for all analyses. Multiple-group comparisons were performed by 1-way ANOVA followed by a Tukey highest-significant-difference post hoc test. The correlation between %MTR and the histological protein composition of the thrombus was assessed using the Pearson correlation. Receiver-operating curve analysis was used to evaluate the predictive value of %MTC and ADC to estimate the thrombus age. Data are presented as means±SD. Values of P<0.05 were considered significant.

Results

Thrombus Evolution and Organization Detected by MT and a Fibrin-Binding MR Contrast Agent

Thrombi were successfully induced in the IVC of all mice as seen by MRI. Examples of venograms and images acquired without MR (no MT) and with MR (with MT), corresponding MT maps (MTR), and pre- and post–contrast-enhanced IR images of the thrombus at 1, 7, and 28 days after surgery are illustrated in Figure 1. The area of the thrombus was identified as the filling defect (absence of flow) in the venogram and was used to plan the subsequent scans. The images acquired with MT prepulses usually contained areas of lower signal intensity within the thrombus compared with the images acquired without MT prepulses. This effect was better visualized in the MTR maps. At day 1 (d1; Figure 1A) after ligation, central and peripheral areas of increased MTC were observed in the MTR map. There was a lack of signal in the precontrast IR image and the presence of high signal intensity in the periphery of the thrombus in the postcontrast image. The corresponding Martius scarlet blue histology showed an erythrocyte-dense area in the center and deposition of fibrin in the periphery of the thrombus. These data suggest that the central region of the MTR map showing high %MTR corresponds to the erythrocyte-rich area, whereas the periphery corresponds to fibrin. There was good agreement in the spatial distribution of the region identified as fibrin on the MTR map and the area of signal enhancement on the post-IR image. At day 7 after thrombus induction (d7; Figure 1B), high %MTR was also observed in the center and periphery of the thrombus. The region of high signal intensity in the precontrast IR image suggested the formation of methemoglobin in the core but not in the periphery the thrombus. The post–contrast-enhanced IR image showed signal enhancement in the center and the periphery of the thrombus, indicating the deposition of fibrin in these 2 regions. The corresponding histology verified the presence of a fibrin network intermixed with erythrocytes in the central part of the thrombus and the deposition of fibrin in the periphery. At days 14, 21 (data not shown), and 28 after ligation (d28; Figure 1C), regions of higher %MTR (red pixels) are visualized in both the periphery and the center of the thrombus compared with days 1 and 7. The lack of signal on both the pre– and post–contrast-enhanced IR images suggests the absence of both methemoglobin and fibrin. The corresponding histology showed an occlusive collagen-rich thrombus at day 28. There was a good correlation between the spatial distribution of collagen as visualized by the MTR map and histology.

MTR Detects the Protein Content of Thrombus

Volumetric analysis of the %MTR (%/cm³) of the thrombus showed an increase at different time points after ligation (Figure 2A). The %MTR increased with thrombus organization and was significantly higher at days 14, 21, and 28 after thrombus induction compared with day 1 (days 1, 7, 14, 21, 28: %MTR [%/cm³]=2483±451, 2079±1210, 7029±2490, 10 295±4356, 32 994±25 449; PANOVA<0.05). In addition, the %MTR measured at days 21 and 28 was significantly higher compared with that measured at day 7. There was a significant linear correlation between the %MTR and the protein content of the thrombus (r=0.70; P<0.05; Figure 2B). These data suggest that the %MTR can be used to identify organized thrombus rich in collagen and with just small fibrin content, from thrombus rich in erythrocytes and intermixed with small amounts of fibrin.

Thrombus Evolution and Organization Detected by DW MRI and Measurement of the ADC

The same thrombus volume segmented on the MTC images and used for the calculation of the MTR was also used to estimate the ADC values. Figure 3A shows the changes in the signal intensity (S/S₀) for different b factors in the DW images. Free water, characterized by nonrestricted water diffusion, showed the fastest signal decay, whereas muscle, characterized by more restricted water diffusion, showed slower signal decay.
The signal decay curve for thrombus was between that of muscle and free water and varied depending on thrombus stage of organization. Figure 3B shows representative thrombus ADC maps at different time points of thrombus organization. The average ADC values at different time points of thrombus organization are illustrated in Figure 3C. In the early stages (day 1), the high erythrocyte content of the thrombus may hinder water diffusion, resulting in low ADC values. As soon as the thrombus is cleared of dead erythrocytes and the fibrin matrix becomes its main component, there is a slight increase of water diffusion that reaches a maximum ADC value between days 7 and 14. In the latest stages (days 21 and 28), when...
a collagen-rich scar characterizes thrombus composition, the diffusivity of water within thrombus follows a restricted pattern with low ADC values. These data suggest that low ADC values could represent either acute (high erythrocyte content) or chronic (high collagen content) thrombus and could be used to differentiate them from thrombus with intermediate organization, characterized by higher fibrin content.

### Sensitivity and Specificity of %MTR and ADC in Measuring Thrombus Age

MTR quantification can accurately differentiate between young (days 1 and 7) and old (>day 14) thrombus. A cutoff value of 5150%/cm³ for the %MTR can differentiate between these subgroups with a sensitivity of 92% and specificity of 100%. The ADC did not accurately differentiate between these 2 groups (young versus old thrombi). A cutoff value of 0.87×10⁻³ mm²/s for the ADC has an area under the curve of 0.67 (95% confidence interval, 0.42–0.92) to differentiate between the 2 groups. Both the %MTR and ADC alone are poor indicators for the identification of thrombus at intermediate organization time (between days 7 and 14), when the fibrin content is higher. However, the combinations of both measurements can successfully identity thrombus between days 7 and 14 (Figure 4). ADC values >0.81×10⁻³ mm²/s and %MTR values <7620%/cm³ had a sensitivity of 87.5% (95% confidence interval, 47–99) and specificity of 83.3% (95% confidence interval, 51–97) to identify thrombus between 7 and 14 days old, with a positive likelihood ratio of 5.3 and a negative likelihood ratio of 0.15.

### Discussion

In this study, we demonstrate the use of in vivo MT and DW MRI for the visualization and detection of the thrombus protein composition, thereby allowing staging of the age of venous thrombus in a murine model of DVT. We found that MT maps allow visualization of thrombus. The %MTR varied with thrombus stage and reflected the protein (fibrin and collagen) content of thrombus. The %MTR increased with thrombus organization and was significantly higher at days 14, 21, and 28 after thrombus induction compared with days 1 and 7. There was a significant positive linear correlation between the %MTR and the protein content of thrombus (r=0.70; P<0.05).

Venous thrombus undergoes a process of organization and recanalization that is similar to the formation of granulation tissue in healing wounds.38,39 The formation, organization, and degradation of the fibrin matrix followed by the deposition of collagen are key events in the natural history of thrombus organization. These proteins therefore represent informative imaging targets. The changes in the levels of these proteins during thrombus resolution could be informative of the susceptibility to lysis, and noninvasive detection of these changes by MT, together with the assessment of the thrombus microenvironment by DWI, may improve thrombus staging and patient selection for thrombolytic therapy.

Although direct thrombus MRI without the use of a contrast agent allows thrombus detection through visualization of methemoglobin, it may lead to underestimation of thrombus size and extent and thus provides incomplete information about the stage of thrombus organization. Methemoglobin is usually formed in the acute stages of thrombosis because of the local hypoxic environment of occlusive thrombus but is not present during the chronic stage. Conversely, use of a fibrin-binding contrast agent allows visualization of all the stages of thrombus evolution, with quantification of fibrin contrast uptake using T1 mapping or the estimation of the enhanced...
thrombus volume after administration, allowing identification of thrombus that may be more amenable to thrombolysis.41

The feasibility of using MT as an endogenous contrast mechanism to image the protein content of thrombus was demonstrated ex vivo using a catheter-based imaging MRI coil28 and at high field.30 Ex vivo measurement of the %MTR in human endarterectomy specimens showed that old (rich in protein debris) and recent (fibrin-rich) intraplaque hemorrhage had higher MTR compared with fresh intraplaque hemorrhage (rich in intact erythrocytes).29 Similarly, ex vivo application of MT permitted the distinction between platelet-rich and protein-rich thrombus in thrombosed rabbit plaque.30

Here, we demonstrated the in vivo feasibility of MT for monitoring the evolution of venous thrombus at a clinically relevant field strength. MT uses saturation prepulses to destroy the magnetization of the bound-proton pool, resulting in images with reduced signal intensity that is proportional to the number of macromolecules.42–44 In our study, binomial radiofrequency pulses transmitted on-resonance with a resultant 0° pulse angle (jump and return) were used. These binomial pulses can be considered transparent for the free pool. During such a pulse train, the magnitude of the free proton magnetization is unaltered, but its direction is changed according to the flip angle of each radiofrequency element. We found that the quantitative measure of %MTR correlated with the protein content of the thrombus, allowing the distinction between erythrocyte-rich and protein-rich thrombus. Interestingly, we found a strong linear correlation between the %MTR and the protein content of the thrombus as measured by histology. In vivo applications of MT in human diseases45,46 have also reported a linear correlation between %MTR and tissue collagen concentration. Although collagen comprises only ≈40% of the macromolecules in cartilage, it is responsible for most of the MT effect observed.42,44 Moreover, several other mechanisms contribute to the degree of MT, including the mobility, the hydration state, and the number of macromolecules.
hydroxyl and amide groups, as well as the concentration of the macromolecules or water.

DWI has been used ex vivo to identify thrombus in human and animal samples. In vivo application of DWI in human carotid plaque showed that changes in the ADC, which represent the magnitude of diffusion of water molecules, vary in parallel to the evolution of thrombus organization. Restricted water diffusion within the thrombus was observed in 1-week-old in vitro thrombus, reflecting the maturation of fibrin fibers, the presence of an extensive collagen network, and the entrapment of erythrocytes. Conversely, resolution of the thrombus results in increased water diffusion. The ADC values of organized hemorrhage in carotid plaque in vivo were characterized by the most restricted diffusion, whereas the lysed thrombus showed increased diffusivity. Similarly, we found that the most restricted water diffusion was observed within the erythrocyte-rich (day 1) and collagen-rich (days 21 and 28) thrombus. However, the mechanism causing restricted water diffusion in erythrocyte-rich and collagen-rich thrombus may be different. Previous studies have shown that thrombus with these histological compositions is associated with a decreased efficacy of thrombolytic therapies.

The structural characteristics of blood clots are associated with their susceptibility to thrombolysis, and the importance of calculating the thrombus ADC might be used for the assessment of the retraction of the clot and to predict the outcome of clot thrombolysis before treatment. After 2 hours of thrombolysis using a recombinant tissue-type plasminogen activator in plasma, whole-blood in vitro clot volumes were efficiently dissolved in regions with ADC $\geq 0.8 \times 10^{-3}$ mm$^2$/s, whereas dissolution was poor and prolonged in regions with ADC $<0.8 \times 10^{-3}$ mm$^2$/s.

**Limitations**

In this study, we did not test the sensitivity and specificity of MT and DW MRI to predict the effectiveness of thrombolysis in the murine DVT model. Further studies are needed to elucidate this important question. Finally, 5 animals were used at each time point, which, because of the high reproducibility of thrombus formation in the vena cava, represent a sufficient number of animals for statistical analysis.

**Conclusions**

We demonstrate that MT and DW MRI allows the identification and staging of venous thrombus in a murine model at clinical field strengths, thereby facilitating clinical translation of non-contrast-enhanced thrombus detection and characterization.

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Supplementary Methods

MRI of agar phantoms and in vitro thrombus at 3T

Prior to imaging thrombus in vivo a series of experiments were performed to identify the best acquisition parameters for the MT pre-pulse and DWI protocols using agar phantoms and in vitro thrombus. Agar phantoms with concentrations of 0, 5, 10% were prepared and used as controls. Blood was collected from volunteers by venipuncture. In vitro thrombi were washed with saline, placed in sealed eppendorf tubes and imaged at 4hr (fresh), 11 (organized) and 17 (lysed) days after preparation [1]. Between collection and imaging thrombi were stored at 4°C to prevent dehydration.

MRI at 3T was performed using a 3T Achieva Gyroscan MR scanner (Philips Healthcare, Best, The Netherlands) equipped with a clinical gradient system (30mT m⁻¹, 200mT/m/ms) and a single–loop surface coil (diameter = 47 mm). T1-weighted 3D-fast-gradient echo images were acquired (a) without an MT prep-pulse, (b) with an on-resonance binomial MT pre-pulse, (c) with an off-resonance MT pre-pulse, and (d) with multiple off-resonance MT pre-pulses. For the on resonance scan the parameters were: TR=115s, TE=6.8ms, flip angle=18°, NEX=1, slice thickness=2.0mm, matrix=100x100, FOV=30x50x50mm, (rec. resolution=0.4x0.4mm), duration=3min. The MT pre-pulse was a binomial block (1:2:1, 90°x 90°-x 90°-x90°) pulse with a duration=1.92ms, repetitions=1, and offset=0Hz. These binomial pulses can be considered as transparent for the ‘free pool’. From the Bloch equations its known that higher order binomial pulses, with a shorter duration and a larger B1, will result in a larger transparent bandwidth. This is the range of frequencies for which the ‘free spins’ are not affected after the pulse.

The off-resonance MT pre-pulse was an adiabatic (sinc-Gauss) pulse with an angle= 620°, duration= 17.5ms, repetitions=1, and offset=1100Hz. For the multiple off-resonance MT pre-pulses adiabatic pulses with an angle= 700°, duration= 20ms, repetitions=4, and offsets=1, 200, 500, 800, 1000 Hz were used. Finally, off-resonant adiabatic MT pre-pulses with an angle= 700°, duration= 20ms, repetitions=4,6,8, and fixed offset frequency=500Hz were used.
2D DW spin echo images were acquired with: TR=1.6s, TE=90ms, NEX=1, slice thickness=2mm, MTX=100x100, FOV=30x50x50mm (rec. resolution=0.4x0.4mm), diffusion echo time=70.5ms. The apparent diffusion coefficient (ADC) was calculated from 4 b-values=0, 377, 639, 1000 s/mm².

Supplementary Data

To select the most appropriate MT pre-pulse scheme three parameters were tested: an on resonance MT pre-pulse, an off-resonance MT pre-pulse, and multiple off-resonant MT pre-pulses. An example of the application of an on-resonant MT pre-pulse is illustrated in Figure 1. As expected, an incremental MT effect (increased signal reduction) was observed in samples with a higher concentration of agar. Similarly, a higher MT effect was observed in the organized compared to the fresh and the lysed thrombus. The corresponding histology shows that the fresh thrombus is rich in erythrocytes, the organized thrombus contains abundant collagen fibers (blue staining), and the lysed thrombus contains degraded collagen fibers and lysed erythrocytes. Quantitation of the %MTR revealed a linear correlation between the %MTR and the agar concentration for both the on-resonant and off-resonant MT pre-pulse (Figure 2A). However, the MT effect was more prominent when the MT pre-pulse was applied on-resonance. Importantly, for pure water samples the %MTR=0.8±0.6 and the $M_{sat}/M_o=0.99±0.006$ suggesting no direct magnetization transfer when on-resonance binomial MT pre-pulse were used. Similarly, a significantly higher %MTR was observed for the organized compared to the fresh and the lysed thrombus and this effect was also more pronounced when the MT pre-pulse was applied on-resonance (Figure 2B).

The effect of the offset frequency and the number of repetitions (number of pre-pulses) was investigated when multiple MT pre-pulses were applied. Figure 3A shows that a direct MT effect exists for offset frequencies<500Hz. Therefore, if an offset MT pre-pulse scheme is chosen then it should be applied 500Hz away from the resonance frequency of the free water.
peak to avoid direct MT effect. **Figure 3B** shows that when the offset frequency is set to 500Hz the MTR increased with the number of repetitions in the agar samples and the organized thrombus.

The application of DWI in agar phantoms and in vitro thrombus is illustrated in **Figure 4**. The ADC maps and calculated ADC showed a more restricted diffusion with increasing concentration of agar. Organized thrombus showed the most restricted diffusion, followed by fresh and lysed thrombus as previously shown [1].

Supplementary Figure 2

A

\[ y = 5.7206x \]
\[ R^2 = 0.97324 \]

\[ y = 2.5943x \]
\[ R^2 = 0.91747 \]

% MTR vs. % Agar

B

% MTR vs. Condition

On resonance all \( P < 0.01 \)

\( P = 0.02 \)
Supplementary Figure 4

ADC map

Organized thrombus
Lysed thrombus

Fresh thrombus

D=2.13x10^{-3} mm^2/s
D=2.0x10^{-3} mm^2/s
D=1.22x10^{-3} mm^2/s
D=1.18x10^{-3} mm^2/s
D=0.88x10^{-3} mm^2/s
D=1.45x10^{-3} mm^2/s

0% agar
2% agar
5% agar

b-factor (s/mm^2)
S/So (au)