

# Studying the interplay between atherosclerosis and deep vein thrombosis in a murine model using an elastin-binding contrast agent

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## Introduction:

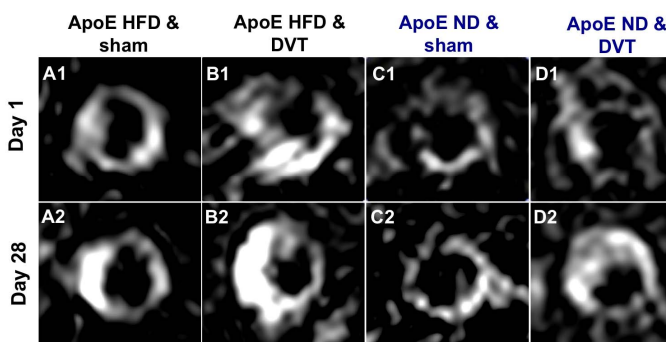
Venous thrombosis and atherosclerosis are common conditions that together cause significant morbidity and mortality worldwide. Recent evidence suggests that venous thrombosis increases the risk of myocardial infarction and stroke, which persists for many years following the thrombotic event (1). In the current work, we sought to use an experimental model of atherosclerosis and deep vein thrombosis to investigate the link between plaque growth and venous thrombosis using an elastin-specific MR contrast agent (ESMA) (2).

**Materials and Methods: Animal model:** Male apoE<sup>-/-</sup> mice were divided in four groups and were fed either a high-fat diet or normal chow diet for 8 weeks. At 8wks they underwent surgical induction of venous thrombosis in the inferior vena cava (3) or a sham operation and were returned to their respective diets for another 4wks. The plaque burden of the brachiocephalic artery was assessed at day 1 (8wks of feeding protocol) and day 28 (12wks after feeding protocol) after the thrombosis induction using MRI. **In vivo MRI:** Brachiocephalic plaque was visualized using a 3T Philips Achieva scanner. Images were acquired 2h after intravenous administration of an elastin-specific MR contrast agent (ESMA) (0.2 mmol/kg; Lantheus Medical Imaging, USA). Mice were placed prone on a single loop microscopy surface coil (diameter=23mm). Following a 3D GRE scout scan, time-of-flight (TOF) images were acquired for visualization of the vasculature. The maximum intensity projection images were used to plan the subsequent delayed enhancement (DE) and T1 mapping scans. The inversion-recovery-3D-fast-gradient echo sequence was used for DE-MRI and visualization of contrast uptake. Imaging parameters included: FOV=30x8x30mm, matrix=300, in-plane resolution=0.1x0.1, measured slice thickness=0.25mm, slices=32, TR/TE=27/8ms, TR between subsequent IR pulses=1000ms, and flip angle=30°. T1 mapping was performed using a sequence that employs two non-selective inversion pulses with inversion times ranging from 20ms to 2000ms, followed by eight segmented readouts for eight individual images. The two imaging trains result in a set of 16 images per slice with increasing inversion times. For T1 mapping the acquisition parameters were: FOV = 22x8x36, matrix = 180x171, in plane resolution= 0.2x0.2, measured slice thickness=0.5mm, slices=16, TR/TE = 9.2/4.7ms, flip angle = 10°. **Image analysis:** DE-MRI images were used to calculate the plaque burden by manually segmenting the inner and outer vessel wall contours (Osirix, Switzerland) on sequential axial slices spanning through the brachiocephalic artery. T1 values were computed on a pixel-by-pixel basis using an in-house Matlab software.

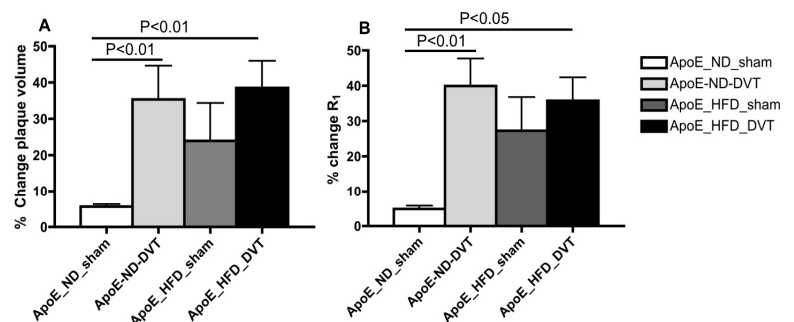
**Results and Discussion:** DE-MRI of plaque burden using an elastin-binding contrast agent acquired at day 1 and 28 after induction of deep vein thrombosis (DVT) are illustrated in Figure 1. Baseline plaque burden for each group is shown in panels A1-D1. The ApoE<sup>-/-</sup> mice fed a normal diet undergoing sham surgery did not show significant plaque progression as expected (Fig. 1C2). The ApoE<sup>-/-</sup> mice fed a high fat diet undergoing sham surgery showed significant plaque progression (Fig. 1A2) as previously demonstrated (2). Interestingly, there was an effect of venous thrombosis on atherosclerosis that was more prominent in animals fed a normal diet (Fig. 1AD2). The ApoE<sup>-/-</sup> mice fed a high-fat diet undergoing the DVT surgery (Fig. 1B2) showed similar plaque progression compared to the ApoE<sup>-/-</sup> mice fed a normal diet undergoing the DVT surgery (Fig. 1D2). Quantification of the changes in plaque volume and vessel wall relaxation rate between the 2 time points are illustrated in Figure 2.

**Conclusions:** We demonstrate an interplay between deep vein thrombosis and atherosclerosis which can be detected by MRI using an elastin-binding contrast agent. This result would explain the increased risk of atherosclerosis-related myocardial infarction and stroke in patients who also have deep venous thrombosis.

**References:** (1) Pandori P et al. NEJM 2003. (2) Makowski M et al. Nat Med 2010. (3) Saha P et al. Circulation 2013.



**Figure 1:** DE-MRI of plaque burden using an elastin-binding contrast agent at day 1 and 28 after induction of deep vein thrombosis (DVT). HFD: high fat diet, ND: normal diet.



**Figure 2:** MRI quantification of changes in plaque burden and relaxation rate.