In Vivo Characterization of a New Abdominal Aortic Aneurysm Mouse Model with Conventional and Molecular MRI

A. Klink[1], J. Heynens[2], B. Herranz[3], H. M. Sanders[2], G. J. Strijkers[2], K. Nicolay[2], M. Merkx[4], Z. Mallat[5], W. J. Mulder[1], and Z. A. Fayad[1]

[1]Translational and Molecular Imaging Institute, Mount Sinai School of Medicine, New York, NY, United States, [2]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [3]Vascular Imaging and Atherothrombosis, CNIC, Madrid, Spain, [4]Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [5]Paris Cardiovascular Research Center, INSERM, U689, Paris, France

Introduction: Abdominal aortic aneurysm (AAA) represents a life threatening condition that is responsible for 1-2% of the mortality among men over of the age of 65 in the USA [Blanchard JF, Epidemiol. Rev.1999]. Recently, an improved model of AAA has been introduced where a combination of angiotensin-II infusion (1 µg/kg/min) and TGF-β neutralization results in a high incidence of fatal AAA rupture in C57BL/6 mice [Wang Y, J Clin Invest. 2009, *in press*]. The degradation of the extracellular matrix (ECM) has been demonstrated to play a central role in AAA progression. Collagen is an essential component of the ECM in the arterial wall and its degradation is believed to be responsible for AAA rupture. Therefore imaging of collagen may represent a unique opportunity to identify vulnerable AAA prone to rupture. In the current study, we evaluated AAA temporal progression in the aforementioned model using conventional high resolution MRI, while the turnover of collagen was evaluated with nanoparticle-enhanced MR molecular imaging.

Methods: First, AAA progression was monitored in 3 mice using Time of flight (TOF), PD-, T1-, and T2-weighted MRI using a Bruker 9.4 T system over a time course of 15 days.

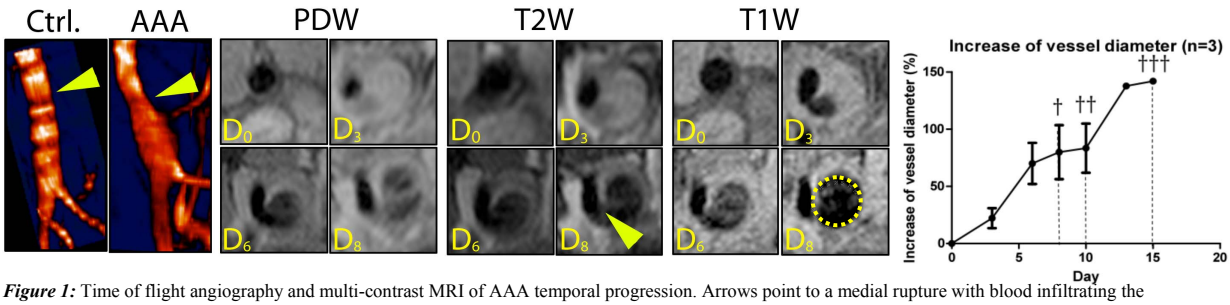

Figure 1: Time of flight angiography and multi-contrast MRI of AAA temporal progression. Arrows point to a medial rupture with blood infiltrating the adventitial tissue. The increase in diameter of the aorta over a 15 days period is reported on the graph.

Paramagnetic micelles (Figure 2A) were prepared as described previously [Mulder WJ, Magn Reson Med. 2007] and CNA35 or the non-binding mutant-CNA35 were conjugated via a sulfhydryl-maleimide coupling method. The specificity of the CNA35 micelles for collagen was first evaluated *in vitro*. CNA35 micelles (50 µmol Gd/Kg) were then injected in the tail vein of wild type mice (n=6) that had developed AAA and controls with no AAA (n=3). These animals were MR imaged pre and 32 hours post injection. We applied a spin echo T1-weighted sequence with a spatial resolution of 117 µm (1 mm slice thickness, matrix size of 256 x 256), a TR of 800 ms and a TE of 8.6 ms with 6 averages. The CNA35 mutant micelles as well as untargeted micelles were applied to AAA mice (n=6) with the same imaging protocol. After imaging, all mice were sacrificed and their aortas were excised for histology (Masson's trichrome), immunohistochemistry, as well as immunofluorescence with confocal microscopy.

Results: The *in vitro* binding experiment revealed a strong affinity of the CNA35-micelles for collagen, while minimal binding occurred in case of mutant-CNA35 and unconjugated micelles. Conventional MRI allowed us to monitor AAA progression at the anatomical level (Figure 1). Multi-contrast sequences revealed a rupture of the medial layer of the aorta with blood consequently infiltrating the adventitial tissue (arrows), which resulted in the formation of an intramural thrombus (indicated by dotted circle) As depicted by the graph in Figure 1, further analyses showed a dramatic increase in the diameter of the aorta reaching a 50% growth – which is the minimal increase necessary to define AAA - at day 5. However, conventional multi-contrast MRI did not allow us to visualize molecular biological processes involved in AAA progression and eventual rupture. To this end, paramagnetic and fluorescent micelles targeted towards collagen (Figure 2A) were applied. T1-weighted images measured before (Figure 2B) and 32 hours after (Figure 2C) the injection of CNA35 micelles images revealed a bright region appearing at the periphery of the aneurysm. Further histological examinations of the corresponding slices (Figure 2D) confirmed the presence of AAA and stained positively for a thin layer of collagen in regions of MR signal enhancement. Fluorescent confocal microscopy depicted in Figure 2E permitted the precise colocalization of the CNA35 micelles (red) with collagen-I marked by immunofluorescence (green). Importantly, despite showing a larger collagen content in the vessel wall, animals with early stages of AAA did not display any significant augmentation in MR signal after injection of CNA35 micelles, suggesting an increased affinity of the nanoparticles for cleaved collagen. Finally, CNA35 mutant and untargeted micelles did not cause a significant signal enhancement in the MR images post injection.

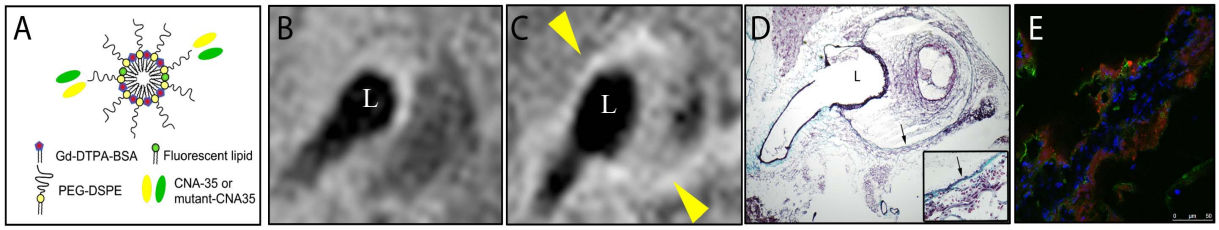

Figure 2: A- Fluorescent and paramagnetic micelles; B- Pre scan of an AAA."L" indicates the lumen; C- Corresponding slice 32hours after injection of CNA35 micelles. Arrows indicates areas of signal enhancement; D- Trichrome Masson's staining displaying presence of collagen in blue (arrow); E- Confocal microscopy representing an overlay of collagen-I marked by immunofluorescence (green), CNA35 micelles (red) and DAPI (blue).

Conclusion: This study describes the *in vivo* characterization of a novel mouse model of AAA with both conventional and molecular MR imaging. While multi-contrast MRI enabled the clear identification of the anatomical progression of AAA, MR molecular imaging of collagen offered an opportunity to image the turnover of collagen, which is believed to be key in AAA progression and rupture.