Faculté de Médecine et Pharmacie
Faculté des Sciences

# Development of a robust protocol for vulnerable plaque characterization by using two peptide-functionalized USPIO derivatives


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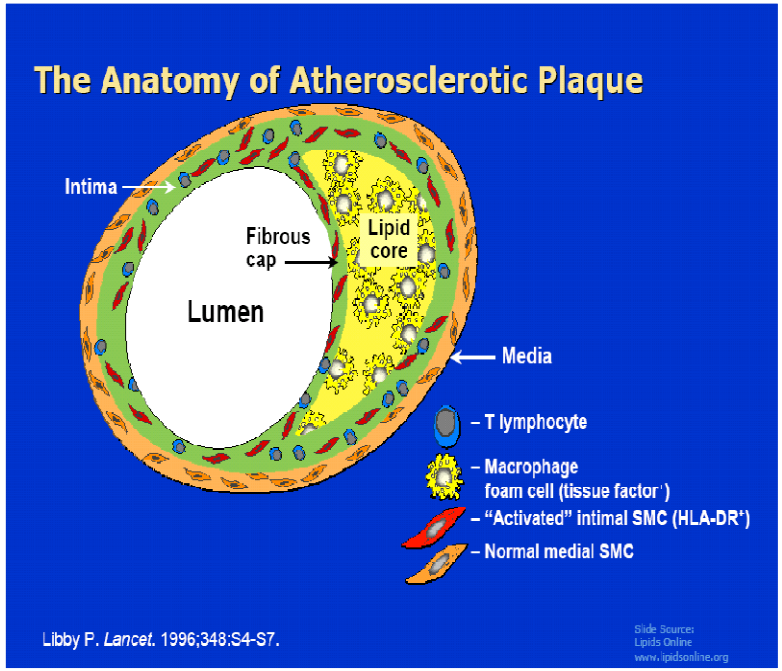
# AIM of the work

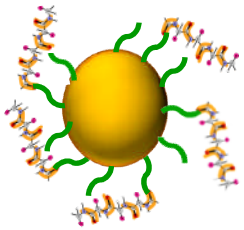
## Imaging of vulnerable atherosclerotic plaque

**Heart attack and brain stroke**




## AIM

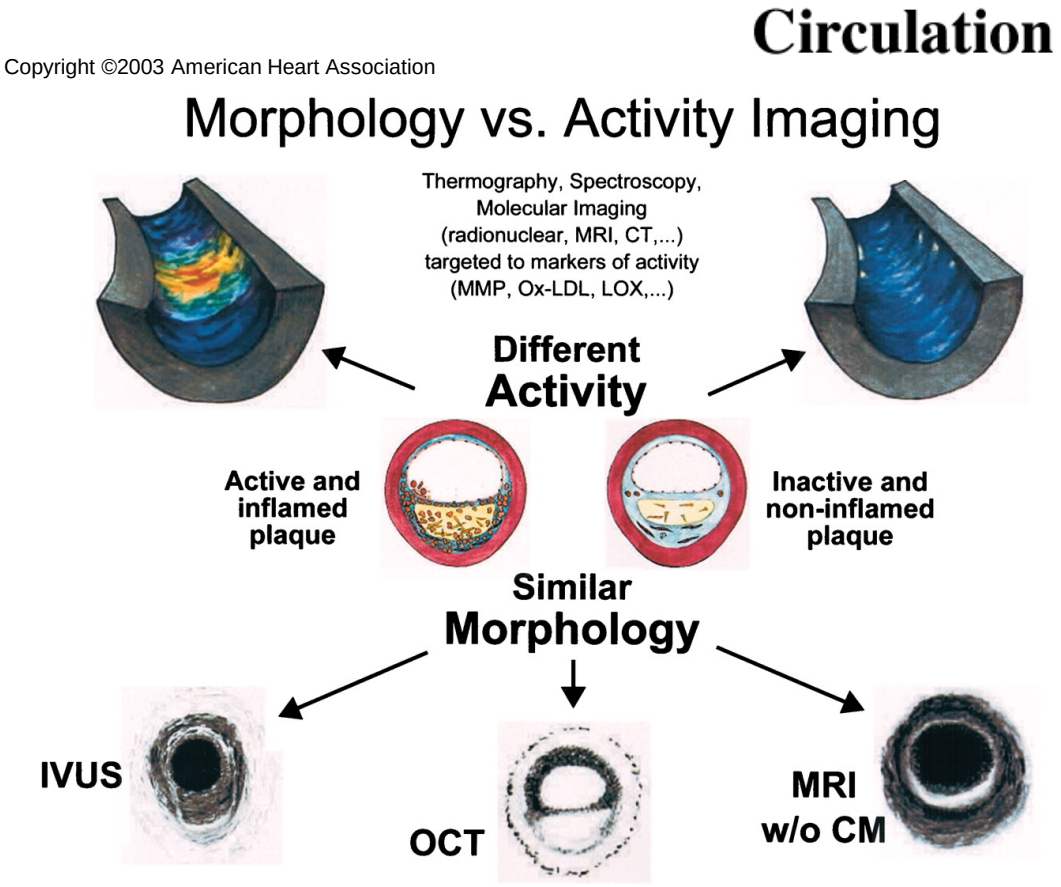
- **VCAM-1 and apoptotic cell-targeted peptides (C-NNSKSHT-C and LIKKPF)** identified by phage display and validated during our previous work (*Burtea C et al, J Med Chem, 2009, 52, 4725*; *Burtea C et al, Mol Pharm, 2009, 6, 1903*):
  - covalently conjugated to USPIO:
    - **USPIO-R832** → VCAM-1 targeting
    - **USPIO-R826** → apoptosis targeting
  - Assessed by MRI on ApoE-KO mice
  - The results were correlated with several biomarkers of plaque vulnerability which were evaluated by immunohistochemistry.



Port M, Corot C, Raynal I, Rousseaux O. US Patent 2004/0253181 A1.

- **Vulnerable plaques** = rupture-prone plaques characterized by a **large lipid core** (cholesterol and lipids remaining from dead foam cells) and **a thin fibrous cap**, being highly thrombogenic (Hellings WE et al, Trends Cardiovasc Med 2007;17:162–171).
- "**Plaque rupture**" = a structural defect in the fibrous cap that separates a necrotic core from the lumen resulting in its exposure to the blood via a gap in the cap (Schwartz SM et al, Arterioscler Thromb Vasc Biol, 2007, 27, 705).
- Rupture of atherosclerotic plaque is **the primary cause of sudden cardiac death** mainly in the industrialized countries.
- Acute ischemic events are caused by the **disruption of type IV and Va lipid-rich lesions**, which are often not angiographically visible (Frank H, Am Heart J 001;141:S45-8).

## Plaques with nearly similar morphology in terms of lipid core and fibrous cap (middle panel) may look similar with diagnostic imaging aimed at morphology only (bottom panel)




**Naghavi, M. et al. Circulation 2003;108:1664-1672**

American Heart Association
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**IVUS** = intravascular ultrasonography
**OCT** = optical coherence tomography

# Features of Rupture-Prone Plaques

**Kullo I J et al. Ann Intern Med 1998;129:1050-1060**



Structural
- Large lipid-rich core
- Thin fibrous cap
- Reduced collagen content

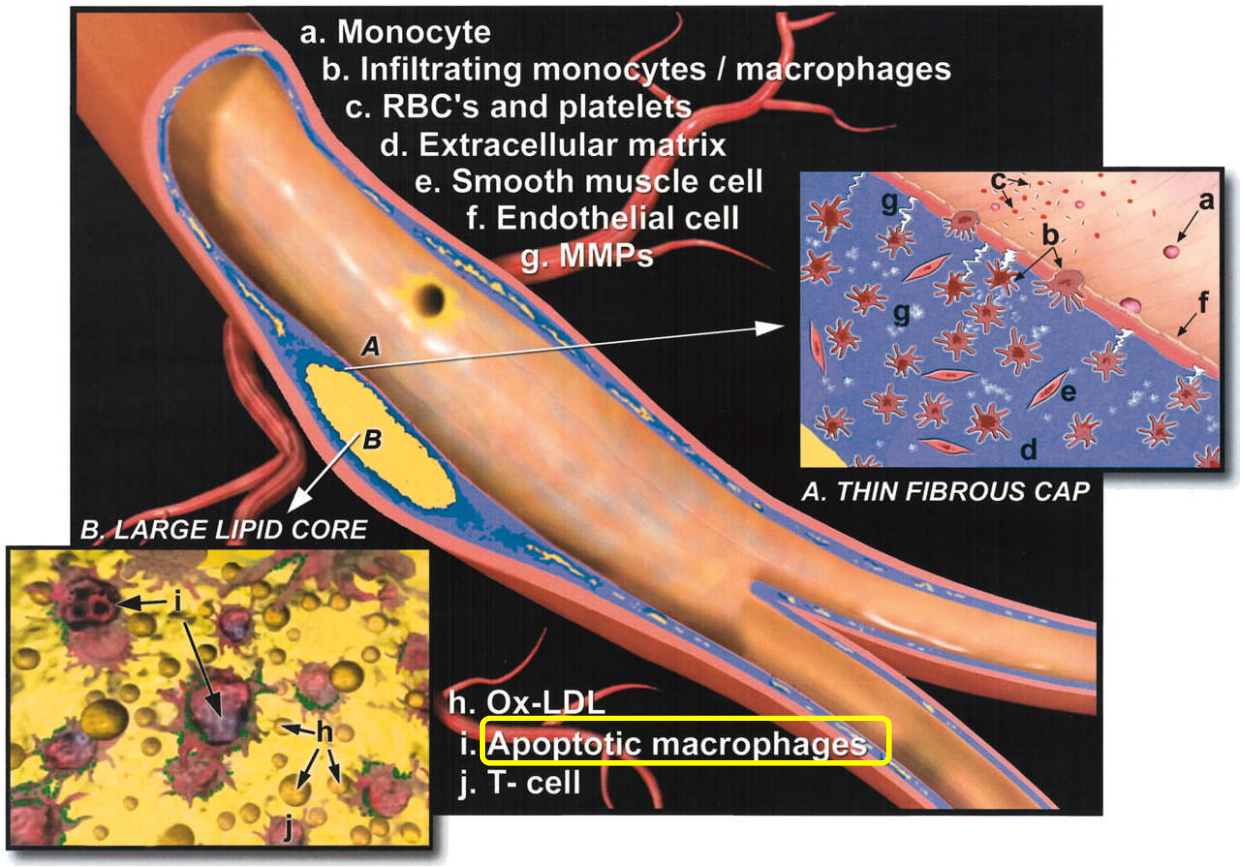
Cellular
- Local chronic inflammation
- Increased macrophage density and activity
- T-lymphocyte accumulation near sites of rupture
- Increased neovascularization
- Reduced density of smooth-muscle cells
- Increased number and activity of mast cells
- Expression of markers of inflammatory activation

Molecular
- Matrix metalloproteinase secretion
- Increased tissue-factor expression

Annals of Internal Medicine

**Schematic figure illustrating the most common type of vulnerable plaque characterized by thin fibrous cap, extensive macrophage infiltration, paucity of smooth muscle cells, and large lipid core, without significant luminal narrowing**






**Naghavi, M. et al. Circulation 2003;108:1664-1672**

Circulation

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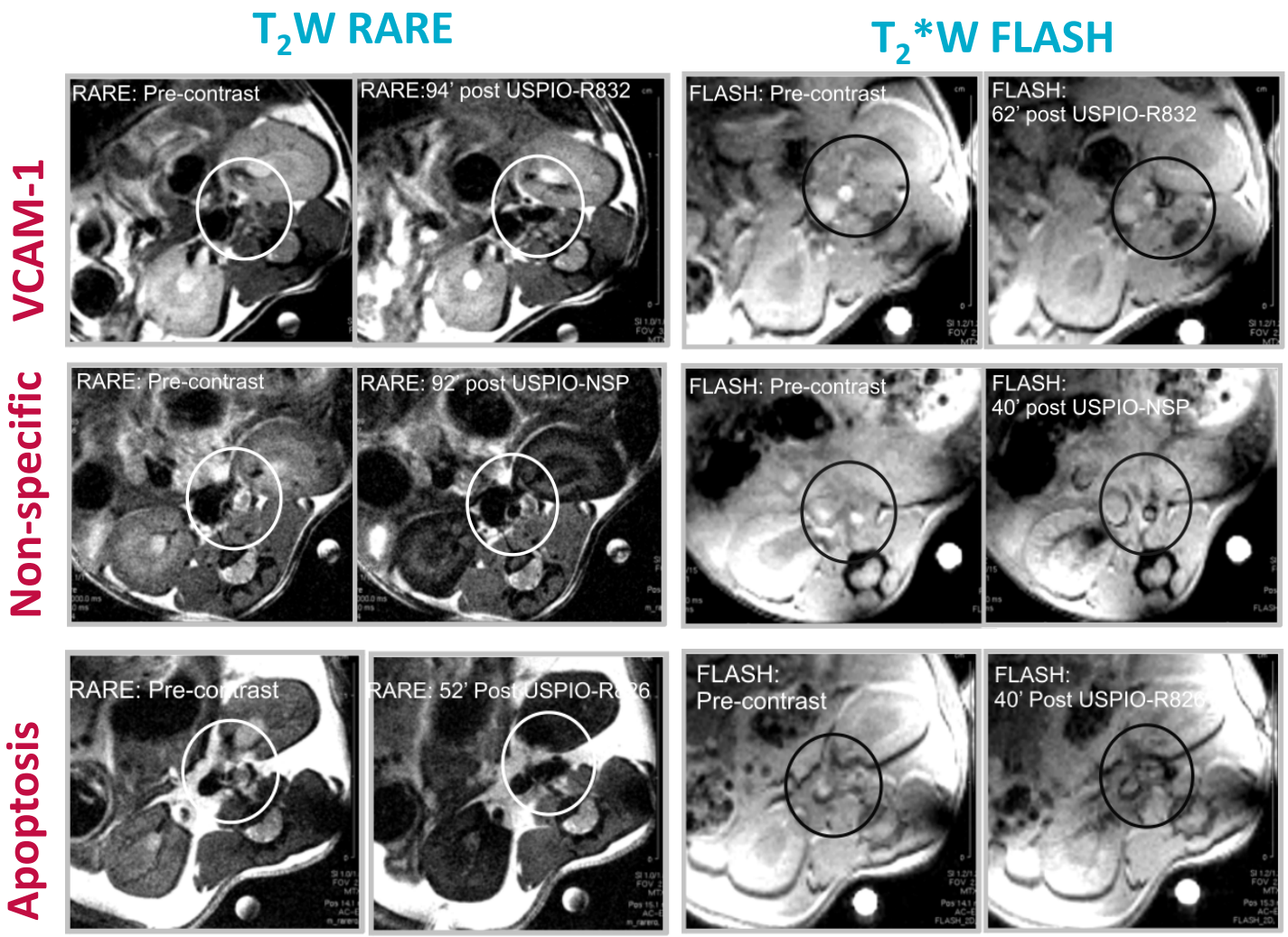
# Materials and Methods

- Female ApoE-KO mice (aged 11 – 12 months, 2 – 3 months cholesterol before imaging), n = 6/group
- Dose: 100 µmol Fe/kg
- 4.7 T Bruker MRI, $T_2$W RARE (TR/TE = 3000/20 ms, spatial resolution = 90 µm) and $T_2$*W FLASH (TR/TE = 175/1.88 ms, flip angle = 90°, spatial resolution = 172 µm) imaging protocols
- Aortic samples examined by histochemistry for the binding of contrast agent (Perl's-DAB staining protocol), the presence of collagen and thrombus (Masson's trichrome staining), of angiogenic blood vessels (VCAM-1), apoptotic cells (caspase-3), macrophages (Mac 1), neutral lipids (Nile blue), and smooth muscle cells ($\alpha$-actin staining)
- The MR images and histological pictures analyzed with ImageJ software

# Results

## VCAM-1 and apoptosis imaging by MRI

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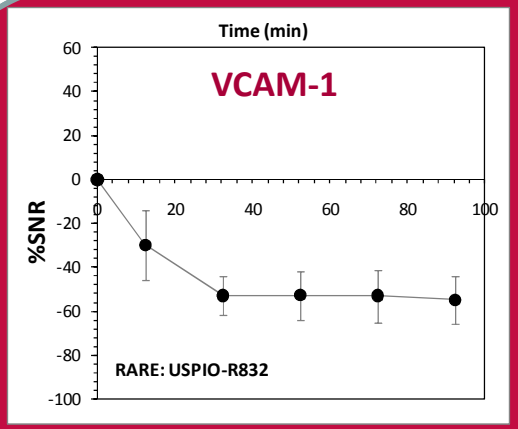
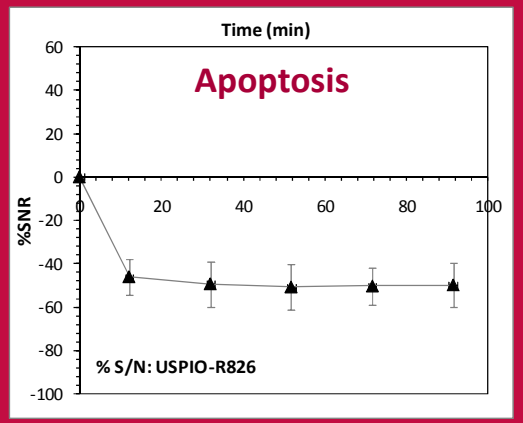
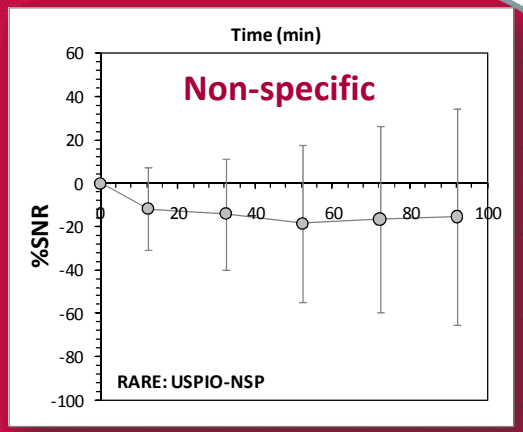
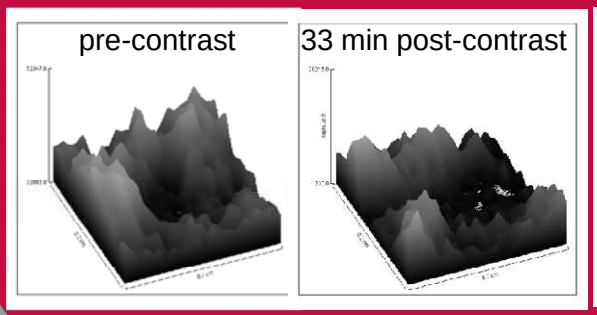
# Results

## MRI SNR evolution and black pixel distribution

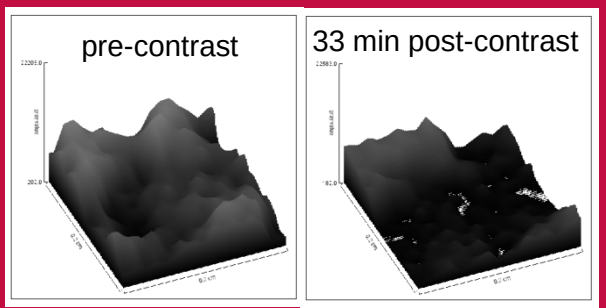


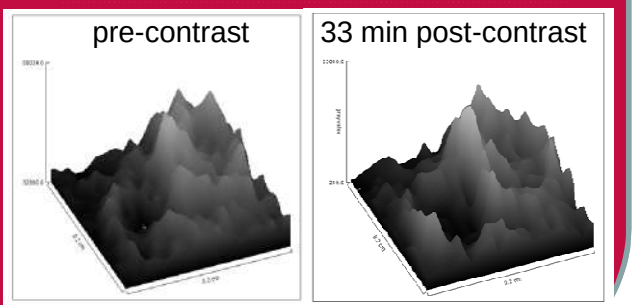




USPIO-R832




USPIO-R826



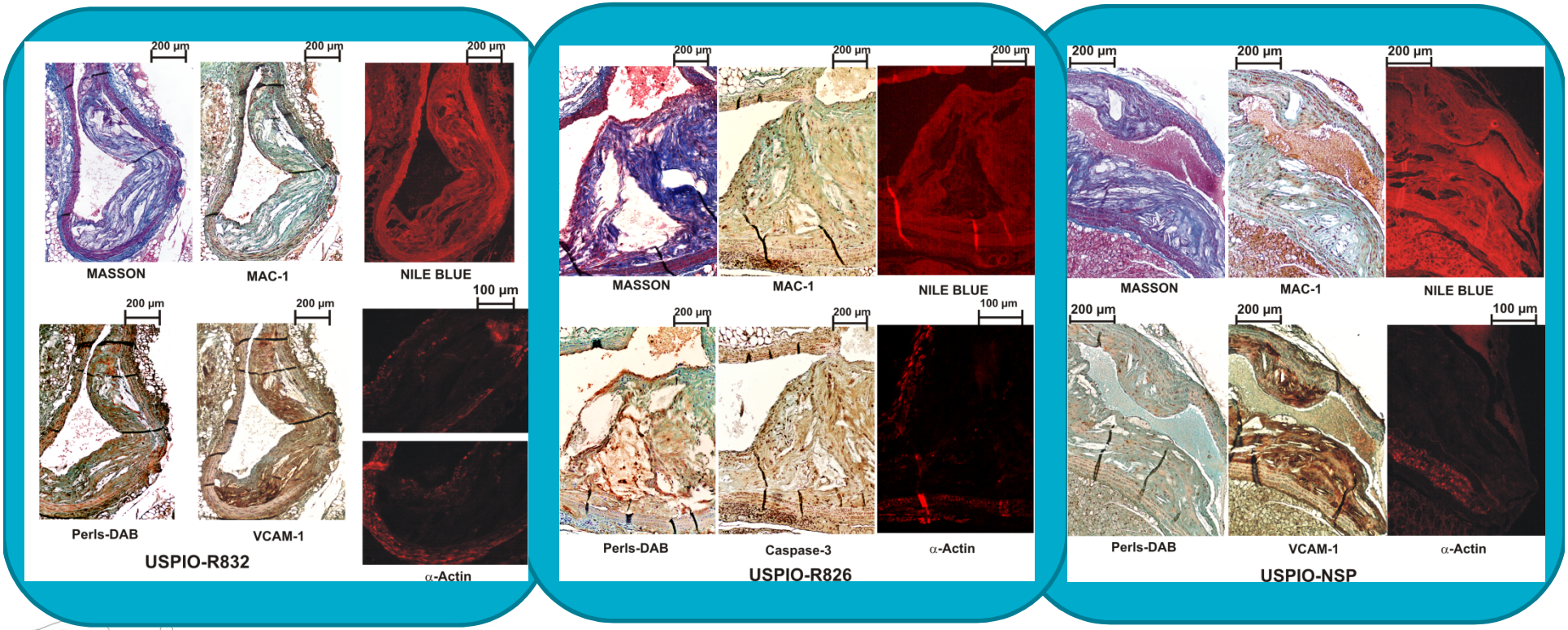

USPIO-NSP

Both USPIO-R832 and USPIO-R826 produced a maximum negative contrast 30 min after administration, being constant until the end of MRI studies (90 min). The plaque surface and black pixel distribution were measured on images (ImageJ software) and it was correlated to the level of plaque enhancement and to the histological observations.

# Results

## Immunohistochemistry studies

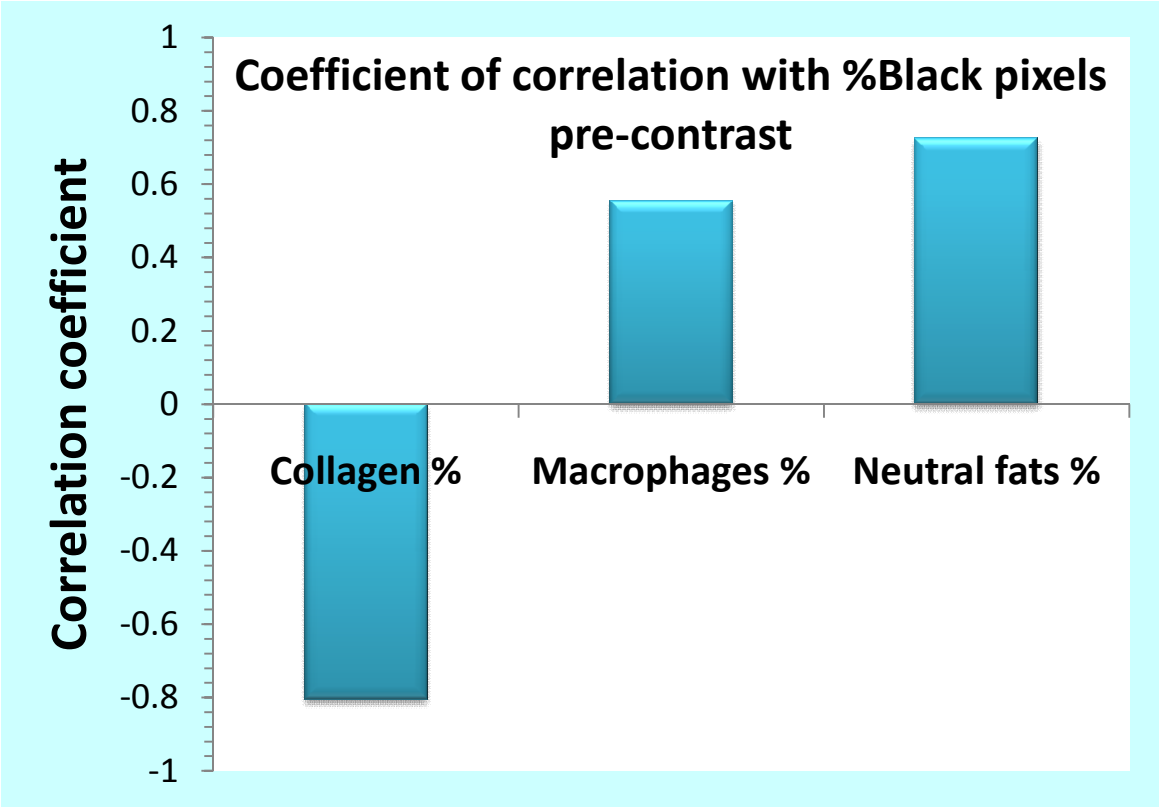
**VCAM-1** | **Apoptosis** | **Non-specific**




- Immunostaining of aorta samples collected from ApoE-KO mice after MRI studies
- Most of the plaques are type V lesions, i.e. fibroatheroma.
- Histological pictures were analyzed with ImageJ software.

# SEMI-QUANTITATIVE ANALYSIS

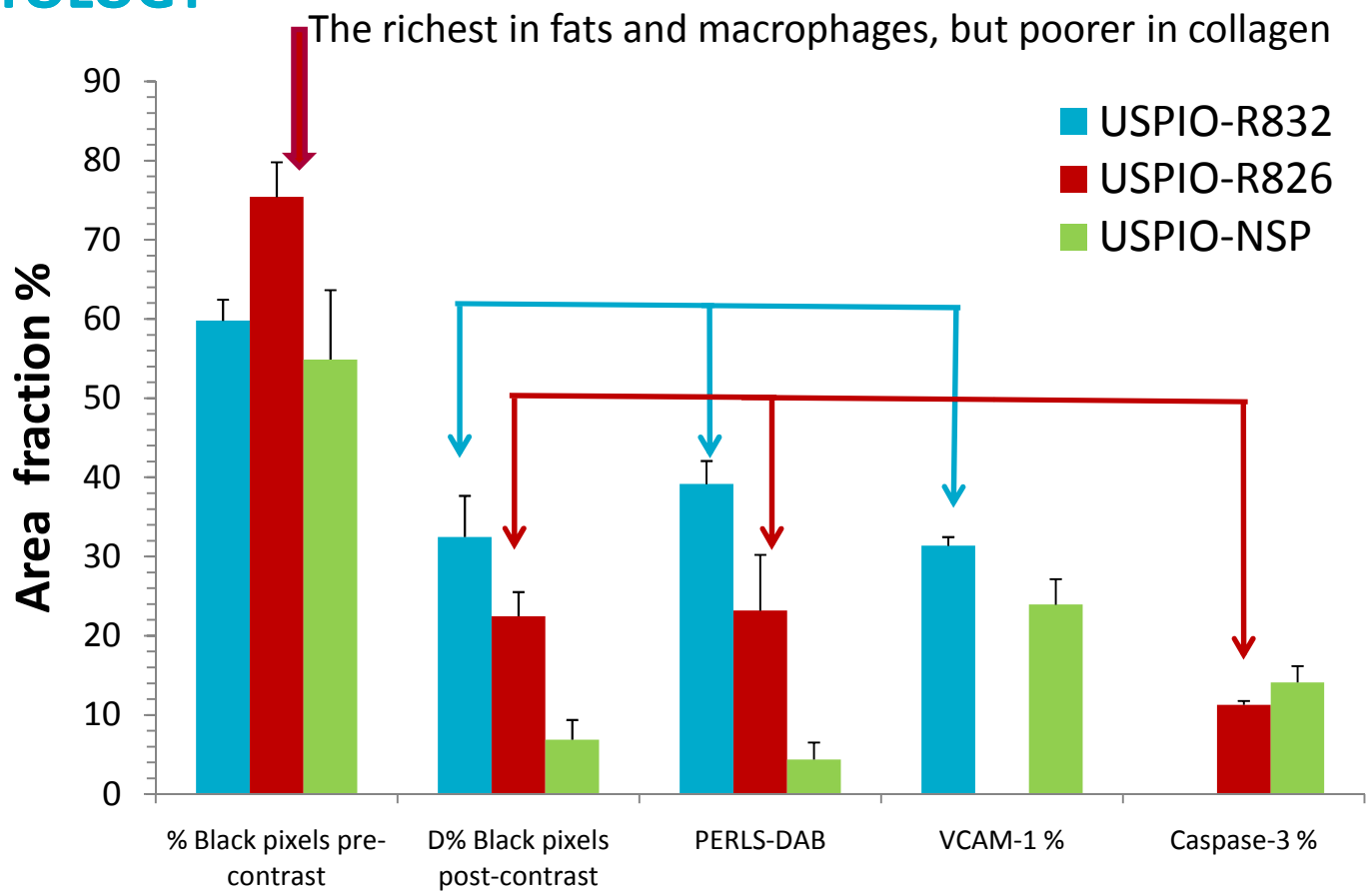
## CORRELATION PRE-CONTRAST MRI AND HISTOLOGY




The semi-quantitative analysis of MRI and histological pictures shows a correlation between the percentage of black pixels in pre-contrast MR images and the distribution of collagen, macrophages and neutral fats staining

# SEMI-QUANTITATIVE ANALYSIS

## MRI AND HISTOLOGY

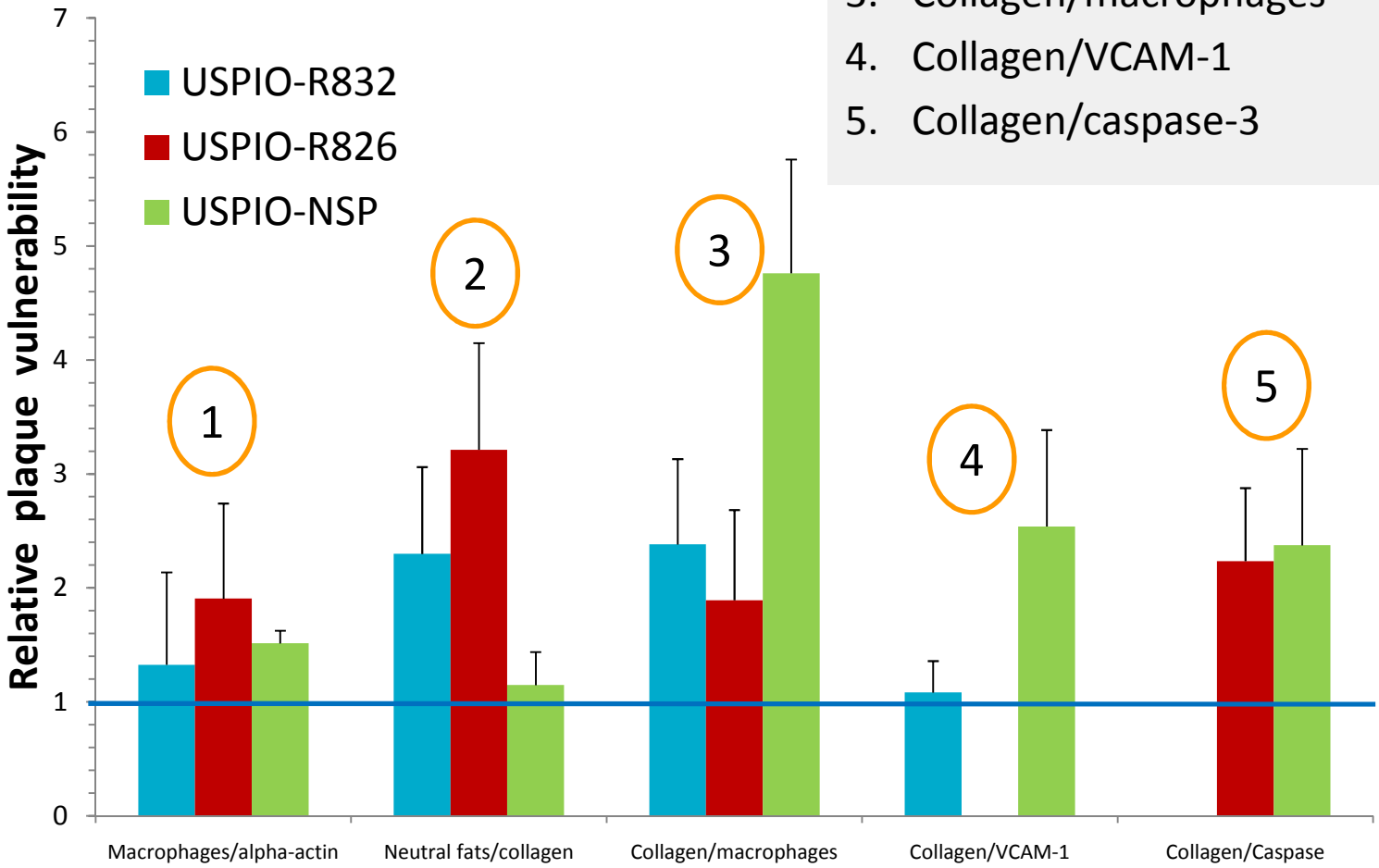



Correlation between the percentage of black pixels in post-contrast MR images and the distribution of Perl's-DAB and VCAM-1 and caspase-3 staining

# SEMI-QUANTITATIVE ANALYSIS

## HISTOLOGY – Relative plaque vulnerability

1. Macrophages/alpha-actin
2. Neutral fats/collagen
3. Collagen/macrophages
4. Collagen/VCAM-1
5. Collagen/caspase-3

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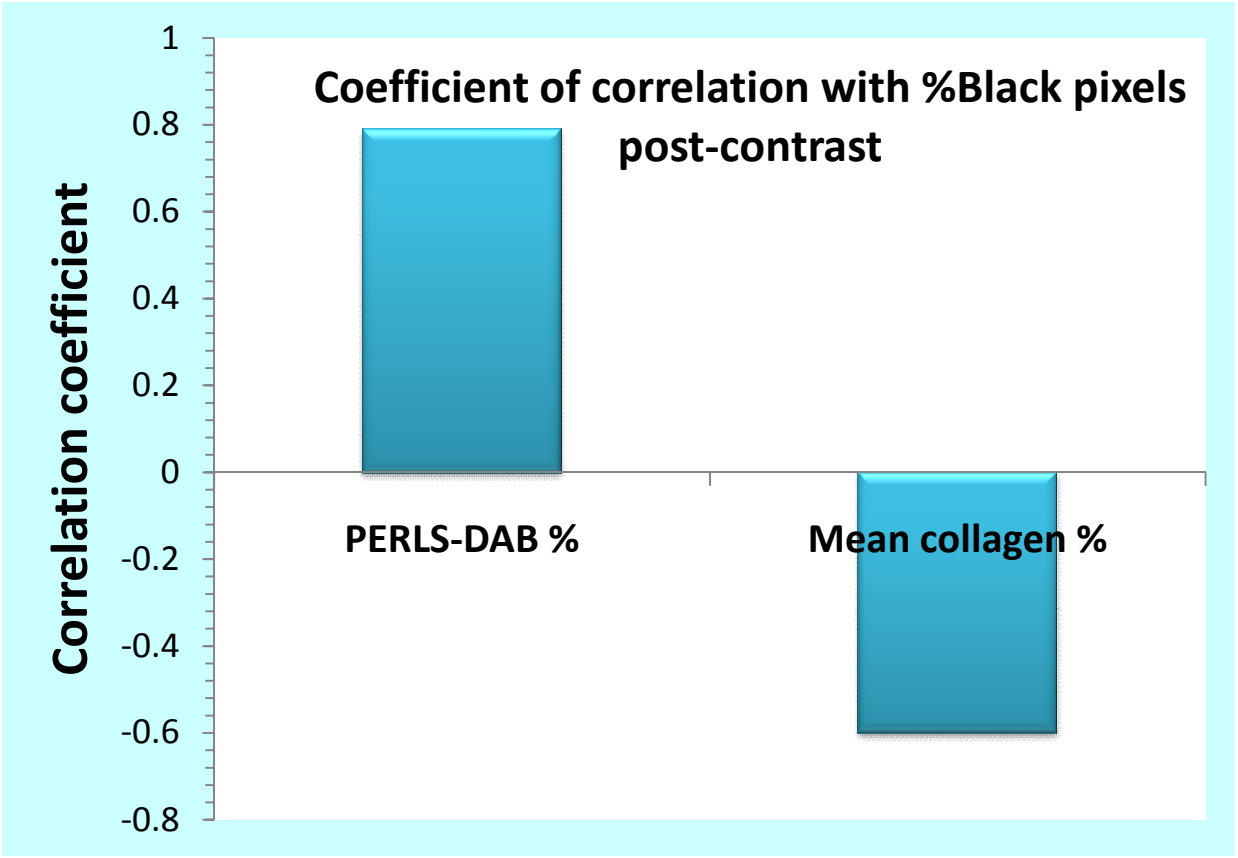
The ratio between various biomarkers of plaque vulnerability suggests the following level of vulnerability: USPIO-R826 > USPIO-R832 > USPIO-NSP

# SEMI-QUANTITATIVE ANALYSIS

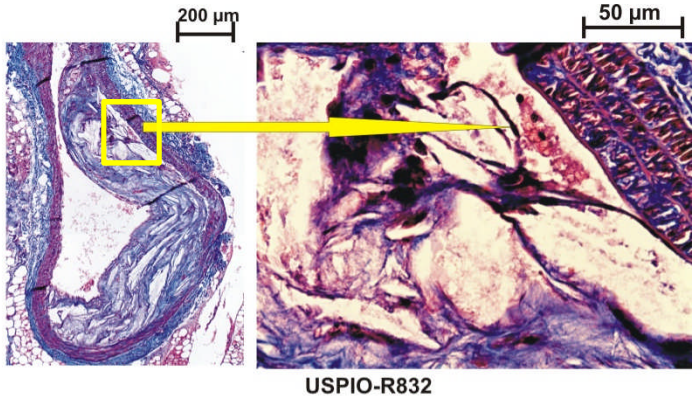
## CORRELATION POST-CONTRAST MRI AND HISTOLOGY



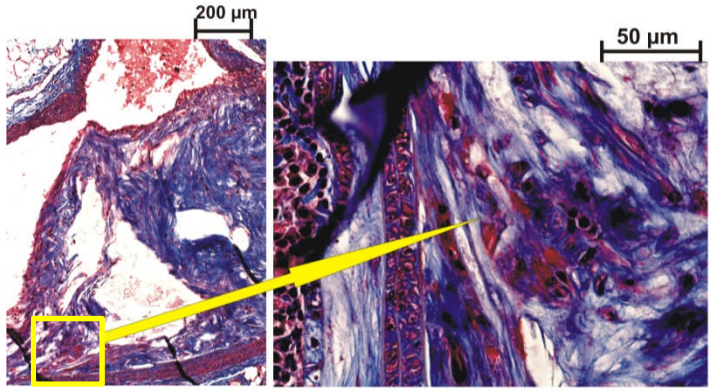

The semi-quantitative analysis of MRI and histological pictures shows a correlation between the percentage of black pixels in post-contrast MR images and the Perls-DAB staining and the distribution of collagen.

# SEMI-QUANTITATIVE ANALYSIS

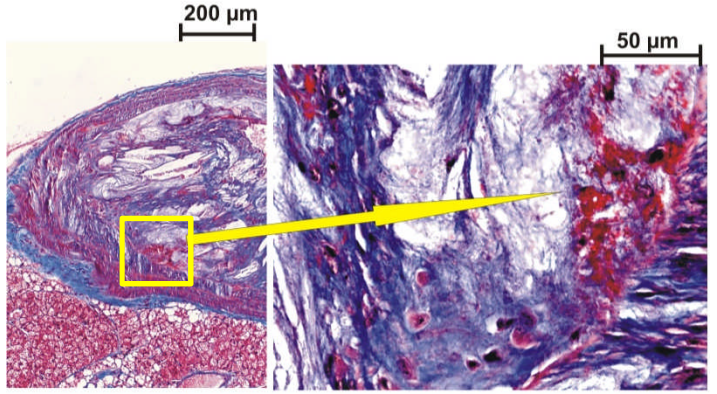
## HISTOLOGY – Plaque hemorrhage




USPIO-R832




USPIO-R826




USPIO-NSP

MASSON'S TRICHROME STAIN:
plaque hemorrhage in red, collagen in blue

Some phenomena of plaque hemorrhage were observed in each mouse, but no real thrombus could be found.

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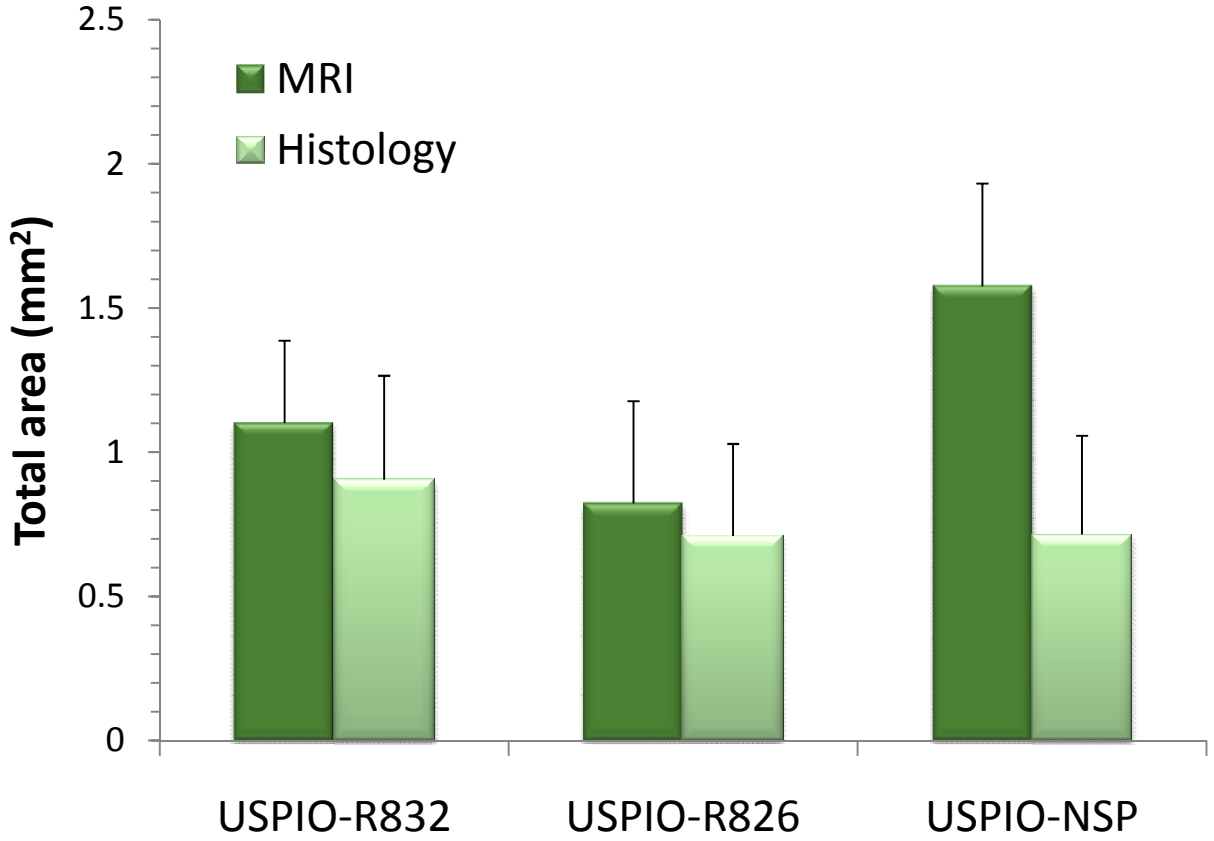
# SEMI-QUANTITATIVE ANALYSIS

## MRI and HISTOLOGY – plaque size

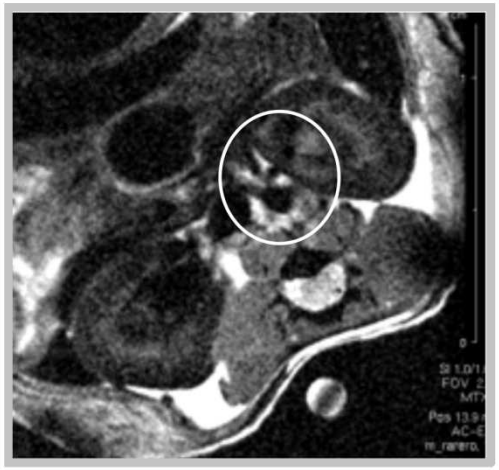



The total plaque areas measured by MRI and histology are almost equivalent.

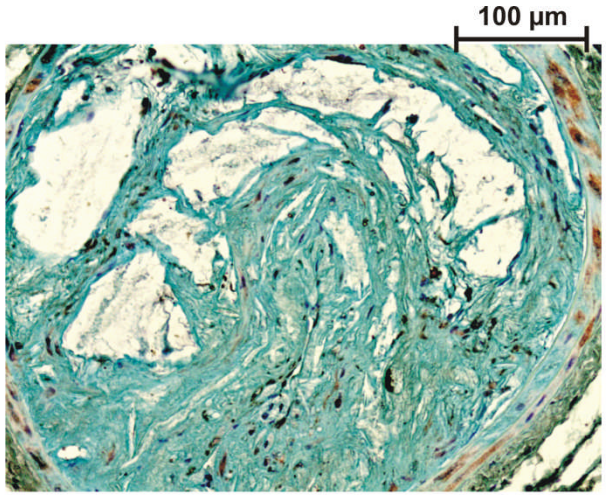
# PLAQUE NEGATIVE FOR APOPTOSIS TARGETING

## MRI - HISTOLOGY

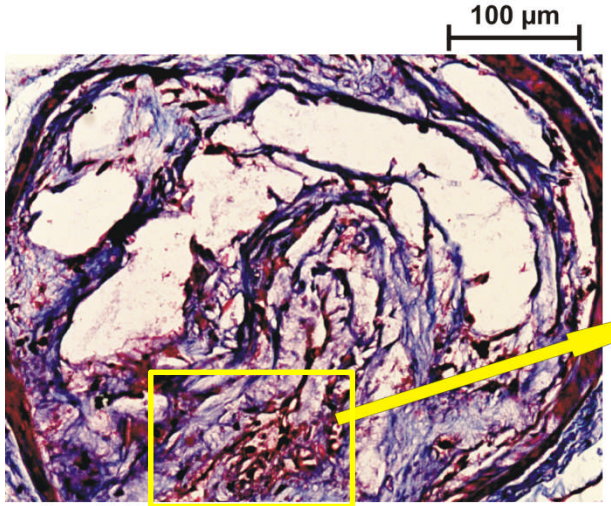
The expression of caspase-3 is almost negative and plaque hemorrhage was observed.

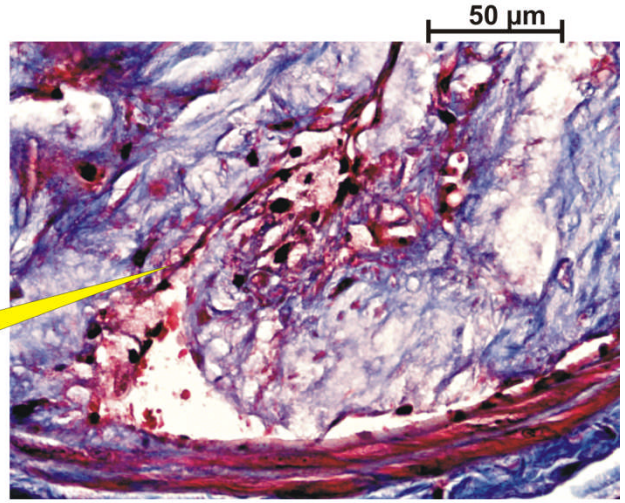


ApoE-E5: 52' post USPIO-R826




ApoE-E5: CASPASE-3




ApoE-E5: MASSON

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# Conclusions

- Most of the histological parameters suggest stable plaques, which are characteristic for this mouse model of atherosclerosis.
- Our VCAM-1 and apoptotic cell targeted USPIO derivatives seem to be highly promising tools for atherosclerosis imaging contributing to the detection of vulnerable plaques.
- They are able to attain their target in low doses and as fast as 30 min after administration.
- An important progress in comparison with previously developed superparamagnetic agents designed for the same purpose.
- The lower immunogenic potential and the cost-effectiveness when compared with antibody-conjugated contrast agents represent supplementary arguments for a possible implementation in the clinical practice.

# Acknowledgements


- Anne Dencausse (Guerbet) is gratefully acknowledged for the preparation of ApoE-KO mice
- Mrs Annik Maes and Mrs Morgane Delanoy (UMons) are gratefully acknowledged for the preparation of the histological material


**Thank you for your attention**