Aims. Atherosclerosis is a chronic progressive, inflammatory, systemic disease with remarkable regional differences in disease manifestation, including a prominent involvement of coronary arteries in contrast to peripheral vasculature. Vasa vasorum (VV) are microvessels, forming a complex network in the adventitial layer of arteries. Arterial VV supply the vessel wall with oxygen and nutrients, venous VV drain the arterial wall into a concomitant vein. VV, providing a considerable endothelial exchange surface for potential beneficial as well as harmful circulating substances and cells to the vessel wall, have been implicated to contribute to inflammatory infiltration and intraplaque hemorrhage in atherosclerotic plaques, thus playing an important role in the pathogenesis of atherosclerosis.

The current study was designed to test the hypothesis that VV density differs between human coronary, renal and femoral arteries, mirroring the known heterogeneity in the propensity of these vessels to develop atherosclerosis. Additionally, coronary arteries were investigated to describe the relation between VV and different stages of atherosclerosis in humans.

Method. 42 human arteries of 32 patients were harvested after autopsy or explantation, and investigated by three-dimensional microscopic computed tomography (micro-CT). VV data as density (VV number / vessel wall area), endothelial surface fraction (VV circumference / vessel wall volume) and vascular area fraction (VV area / vessel wall area) were calculated for coronary, renal and femoral arteries. In addition, the analysis of coronary VV was specialized, regarding the association of VV and different stages of atherosclerosis. Therefore coronary arteries were subdivided into 50 segments and classified according to their plaque burden:

- Control (Plaque-free, patients with no documented atherosclerosis)
- At risk (Plaque-free, patients at high cardiovascular risk and/or documented atherosclerosis in other vessel segments)
- Non-calcified plaque (Severe lumen compromising, non-calcified plaque)
- Calcified plaque (Severe vascular calcification)

Micro-CT analysis was supplemented by histology, focusing on inflammation (CD68), fibrosis (collagen) and angiogenesis (vascular endothelial growth factor (VEGF)) as well as on intraplaque hemorrhage (CD235a, iron) and calcification.

Results. VV density is three-times higher in coronary than in renal and femoral arteries (2.12±0.26 n/mm² vs. 0.61±0.06 n/mm² and 0.66±0.11 n/mm², respectively, p< 0.05 for both), mirroring the reported, enhanced susceptibility for atherosclerosis of coronary arteries compared to peripheral vasculatures.
Figure 1: 3D-Micro-CT images, representative for each group, showing the lumen in red and perfused VV in blue. VV density is significantly higher in coronary arteries, compared to renal and femoral arteries.
* $p<0.05$ vs. Coronary artery
(Modified from Hildebrandt et al., Atherosclerosis, 2008, 199:47-54)

VV density varies temporarily throughout the atherosclerotic process; coronary segment analysis demonstrates a VV increase in segments at risk and plaque segments compared to control segments ($2.50\pm0.24$ n/mm$^2$ and $2.72\pm0.56$ n/mm$^2$ vs. $0.91\pm0.11$ n/mm$^2$, respectively, $p<0.05$ for both). Interestingly, segments with calcified plaque demonstrate a lower VV density compared with segments at risk and segments showing lumen compromising non-calcified plaque ($0.82\pm0.04$ n/mm$^2$ vs. $2.50\pm0.24$ n/mm$^2$ and $2.72\pm0.56$ n/mm$^2$, respectively, $p<0.05$ for both).
Figure 2: 3D-Micro-CT images, representative for each group, showing the lumen in red, perfused VV in blue and calcification in yellow. The VV density is significantly higher in coronary artery segments at risk or non-calcified plaque-segments, compared to control-segments and calcified plaque-segments.

* $p<0.05$ vs. Control; + $p<0.05$ vs. Calcified plaque

Histological review shows a positive correlation between VV density and vascular macrophage density as well as VEGF immunoreactivity. Likewise there is a significant negative correlation between VV density and collagen I content. Iron deposits are localized around calcifications, glycophorin A accumulates directly inside the calcified areas.

**Conclusion.** The current study concludes that VV are involved in several phases of the pathogenesis, playing an important role in the distribution, initiation, progression and complication of atherosclerosis in humans. Histologic results suggest VV as conduits for inflammatory cells and demonstrate an association between intraplaque hemorrhage - very likely due to VV rupture - and vascular calcification.

**References:**

2 Moulton et al., Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. Proc Natl Acad Sci USA, 2003, 100: 4736-41
5 Hildebrandt et al., Differential distribution of vasa vasorum in different vascular beds in humans. Atherosclerosis, 2008, 199:47-54