

# **X-ray Micro-CT limits and possibilities for in vivo and in vitro detection of vascular calcifications**

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## **Background:**

Chronic renal failure (CRF) is associated with a 10 to 20-fold increase in cardiovascular risk. Vascular calcification (VC) is a prominent feature of cardiovascular disease in dialysis patients, and contributes to the excess mortality in this population. High resolution X-ray microtomography (micro-CT) is a suitable imaging technique to visualize calcified tissue.

## **Aim:**

In the present report, we examined the accuracy of in vivo and ex vivo micro-CT as a tool to detect and quantify vascular calcifications in living rats with chronic renal failure (CFR).

## **Methods:**

CRF was induced by feeding rats a diet containing 0.75% adenine (0.92% P and 1.0% Ca) for 4 weeks, which has been shown to induce a stable, moderate to severe renal function impairment[1].

For scanning a desktop in vivo X-ray micro-CT system was used (Skyscan 1076, Kontich, Belgium) without gating for cardiac or respiratory motion. For in vivo acquisition a Ti filter was used. Living rats were scanned repetitively at different time intervals, to detect and to follow-up calcifications in the aorta. In addition to in vivo scanning, the whole aorta was extracted and embedded in paraffin after sacrifice of the rats. These paraffin blocks were scanned again with Al filter. Both in the in vivo and the in vitro condition, voxel size was 35x35x35 µm.

## **Results:**

We could show that in vivo micro-CT was a sensitive method to detect vascular calcifications. Imaging by micro-CT allowed non-invasive discrimination between rats that developed calcifications and animals that did not. The presence of these calcifications was validated by histology and by the determination of the bulk calcium content in the tissue using Atomic Absorption Spectroscopy (AAS). Moreover, imaging by in vivo micro-CT proved to be reproducible as shown by repetitive scans. For quantification, the volume and area of the calcified tissue could be calculated after in vitro scanning of the paraffin blocks. In vivo detection limits were estimated to 5 mg of Ca per gram of wet tissue measured by AAS. Ex vivo micro-CT quantitative results had almost equal sensitivity compared to AAS.

**Conclusions:**

In vivo micro-CT scanning proved to be a sensitive method to detect vascular calcifications in rats with chronic renal failure. This non-invasive imaging technique allows to follow-up and to quantify the development, and potential reversal during treatment, of vascular calcifications in living animals.

**References:**

[1] Okada H et al., Clin Exp Immunol 3:82-88, (1999)