ESRF	**Experiment title:** Vascular calcification in patientswith end stage renal disease. Gathering knowledge on the identity andultra-structural heterogeneity of the mineral phase	**Experiment number**: MD-264
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Background.

An estimated 4.5 Million Europeans suffer from renal disorders. The elderly are disproportionally affected, but renal disease is also a condition that severely affects children. The annual death rate in patients with end stage renal disease (ESRD) is 20% of which 50% is related to cardiovascular events. In these patients, vascular disease is often accompanied by arterial calcifications (1). Several studies have demonstrated that calcifications of the coronary and the large arteries are already present in young ESRD patients (2;3) and show rapid progression (4). In a prospective study in hemodialysis patients, the presence and extent of such ectopic calcifications was associated with diminished survival and the risk of death was increased with the number of vascular sites involved by calcifications (5;6).

A trans-differentiation of vascular smooth muscle cells towards cells with an osteogenic phenotype, expressing the major bone specific proteins, is a key event in the process of media calcification. Based on these observations it is generally assumed that the mineral deposited in the vascular wall has the physicochemical properties of hydroxyapatite, the main mineral compound of bone. To solidify this assumption vascular mineral deposits in two different frequently used rat models of uremia-related vascular calcification were investigated by applying µ-X-ray diffraction in the setting of a previous experiment (SC-1605) performed at the ESRF beamline ID22/ID18F (7). Here, we were able to demonstrate at the ultra-structural level, that in phosphate induced vascular calcification central calcified apatitic regions were accompanied by deposition of amorphous calcium-phosphate, at the outer edge of the calcified deposits allowing us to hypothesize this to be an early calcium-phosphate precipitate which matures over time to apatite. Interestingly, in addition to the apatite and amorphous calcium phosphate phases we also found whitlockite in the aortic wall of rats treated with vitamin D. Whitlockite is a calcium orthophosphate that especially in biology has been related to the presence of magnesium [$(Ca,Mg)_3(PO_4)_2$]. Moreover, it has been shown that vitamin D supplementation in addition to calcium and phosphorus can stimulate intestinal magnesium uptake (8;9).

Since the treatment with vitamin D in ESRD patients is indispensable to control secondary hyperparathyroidism, magnesium accumulation might further fuel the process of vascular calcification. To test this hypothesis that is based on our previous rodent studies, human calcified artery samples either from ESRD patients or subjects with a normal renal function were investigated.

Methods.

Renal or iliacal arteries isolated from chronic renal failure patients during renal transplantation (N=11), aorta samples from transplant donor tissue showing macroscopic abnormalities (N=5) and intima specimens collected during carotidectomy (also in absence of uremia; N=4) were fixed in methacarn fixative and embedded in paraffin. To ascertain the presence of calcification, only Von Kossa-positive samples were included in the current study. Unstained 4µm thick sections sequential to those used for the Von Kossa staining were used for the synchrotron X-ray µ-analysis. By the application of a focussed synchrotron beam with 2x7µm dimensions at an energy of 14.4 keV, X-ray fluorescence for calcium guided us through the sample and indicated regions of interest to be further investigated by X-ray µ-diffraction. In total 56 calcified regions of interest were investigated on the 20 human artery samples. Crystallinity was estimated by Scherrer analysis of the diffraction peak widths.

Results.

Figure 1 shows a the results of a line scan through a calcified region of a human artery sample. X-ray fluorescence and diffraction were recorded in a simultanous way. In this way the spatial heterogeneity of the mineral phase along the line scan path was investigated. By the application of such an approach for all samples we found in 8 sample regions poorly crystalline apatite as the only mineral phase. Interestingly, in 33 of the sample regions only whitlockite was found and 14 regions showed a mixture of whitlockite and apatite whilst in only one sample an amorphous calcium phosphate was found. No significant correlation was found between the occurrence of one or more mineral phases and the presence/absence of a uremic environment.

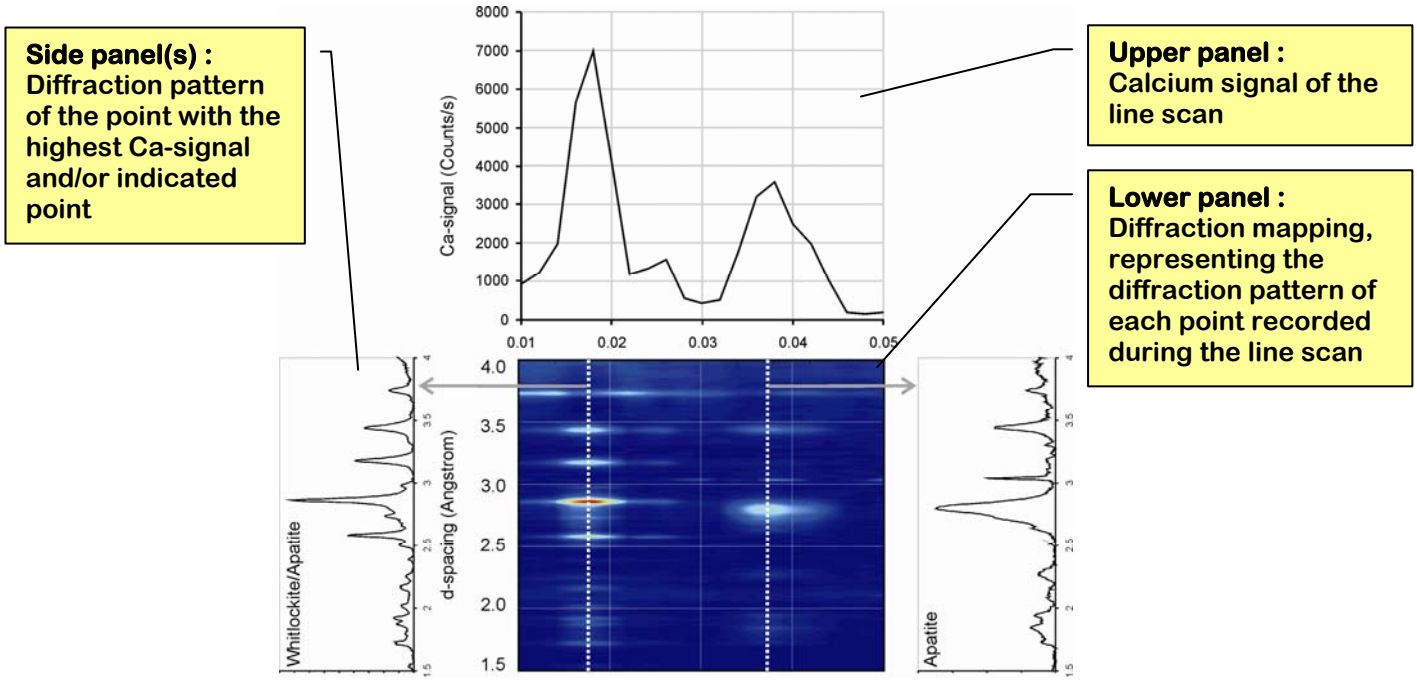

Figure 1: Upperpanel indicates the Ca-fluorescence profile of the line scan with the corresponding diffraction pattern in the lower panel. Here a uniform mineral phase is found that could be identified as whitlockite. Two different mineral compositions were identified as whitlockite/apatite (left panel) and apatite alone (right panel).

By the application of the Scherrer derivation on the azimutal integrated Debye diffraction patterns the crystallinity of the mineral in the samples was estimated. A significant difference ($p<0.05$;

Finure 2) was found between the crystal size of calcium apatite vs. whitlockite in samples originating from both uremic patients and subjects with normal renal function. Moreover both the whitlockite and apatite phase showed a more crystalline nature in samples of subjects with normal renal function vs those of uremic patients.

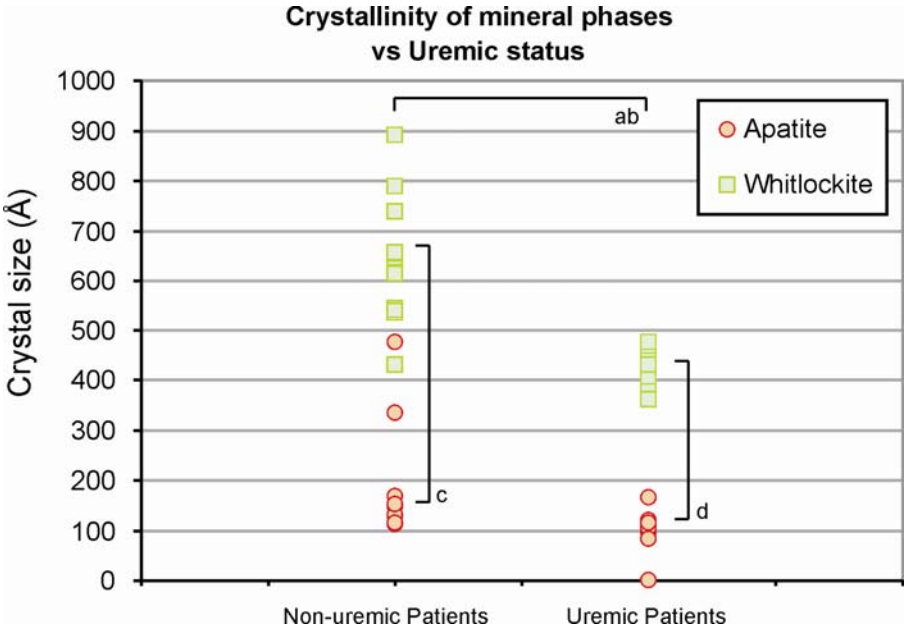

Figure 2. Crystal size estimated by the Debye-Scherrer function calculated from the X-ray diffraction data. A significant higher crystal size was found for both mineral phases apatite[a] and whitlockite[b] in samples originating from subjects with a normal renal function vs those from uremic patients. Within a sample group of non-uremic or uremic patients whitlockite was the m crystalline mineral phase present[cd]. ($p<0.05$; a: Non-uremic vs uremic apatite; b: Non-uremic vs. uremic whitlockite; c: apatite vs whitlockite in samples of non-uremic origin; d: apatite vs whitlockite in samples of uremic origin)

To which extent the presence of whitlockite can be related to an altered magnesium metabolism, like we observed in the remnant kidney rat is not clear. Since mineral crystallinity increases in a slow precipitation and further matures over time the discrepancy between poorly crystalline apatite and whitlockite can be the result of a much slower deposition rate and/or increased mineral age of the whitlockite phase. The same is true for the difference between the ectopic precipitates in uremic vs. non-uremic samples. Here the physicochemical results suggest a much faster calcification in the uremic vs a non-uremic environment, an observation which underpins the rapid progression of vascular calcification in uremic patients.

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