

Report of the proposal : MD-870

Proposal Title **XRF and XANES analyses to resolve the presence and composition of microcalcifications in the jugular vein wall of Multiple Sclerosis patients**

It has been recently demonstrated that the internal jugular vein of patients with Multiple Sclerosis may exhibit abnormalities classified as truncular venous malformations (TVMs). The investigation of possible morphological and biochemical anomalies at jugular tissue level could help to better understand the link between brain venous drainage and neurodegenerative disorders, recently found associated with jugular TVMs.

To proposal had this aim and we performed sequential X-ray Fluorescence (XRF) analyses on jugular tissue samples from two TVM patients and two control subjects, using complementary energies. This investigation, coupled with conventional histological analyses, revealed anomalous micro-formations in the pathological tissues and allowed the determination of their elemental composition.

The measures at ID21 were performed at two energies : 7.2 and 4 kev.

The XRF analyses at 7.2 allowed to reveal an increased Ca presence in the pathological samples, mainly localized in tunica adventitia microvessels. Investigations at 4 kev demonstrated that the high Ca level corresponded to micro-calcifications, also containing P.

In figure 1 an example of the results showing P and Ca colocalization and high Ca content in some hot spots onto the wall of microvessels in the diseased samples.

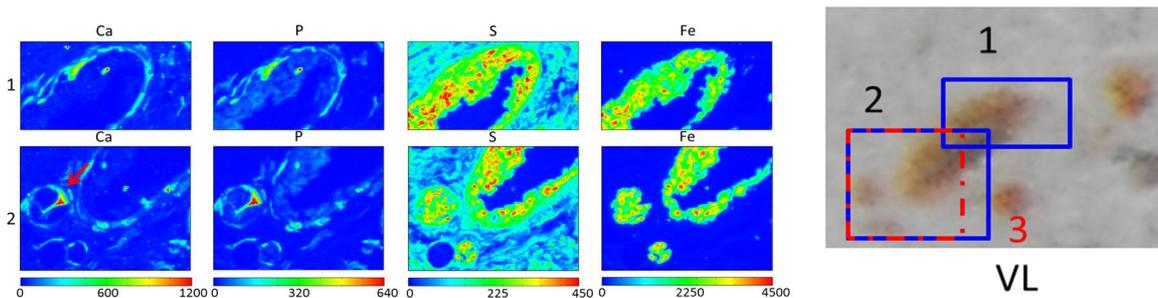


Figure 1. XRF elemental maps obtained at 4.12 keV. Upper row (a), (25 μ m x 30 μ m) elemental maps of S, P, Fe and Ca on a sub-region of the sample MS2 indicated with the red arrow in Figure 2 (region 1);

Interestingly, on hot spots the Ca/P XRF intensity suggested calcifications.

Micro XANES analyses were also performed to resolve the nature of calcifications. The X-ray beam energy was scanned between 4.02 and 4.14 keV with 0.2 eV energy steps. Each XANES spectrum was acquired as the sum.

From XANES analyses, while in control tissues, the major contributions in the XANES spectra seem to come from organic Ca salts, in the MS tissues the XANES results are in line with a substantial presence of

hydroxyapatite (and other inorganic calcium salts) in clear connection with the vasa venorum. However, the complex nature of these calcium precipitates requires further analyses (possibly in conjunction with other techniques) to definitely resolve the different Ca phases.

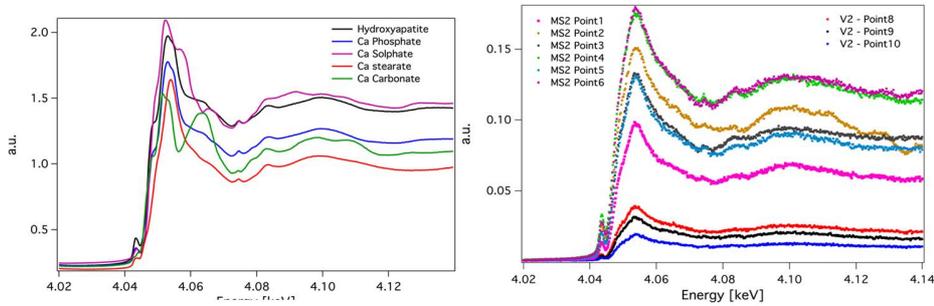


Figure 2: XANES analyses on standards (left) and microvessels from MS and control tissues (right).

Results are now published in :

Pascolo L, Gianoncelli A, Rizzardi C, Tisato V, Salomé M, Sali F, Paterson D, Zamboni P. "Calcium micro-depositions in jugular tissues of multiple sclerosis patients revealed by Synchrotron-based XRF imaging". (2014) *Sci Rep*, 7;4:6540.