SUPPLEMENTARY INFORMATION

Calcium micro-depositions in jugular truncular venous malformations revealed by Synchrotron-based XRF imaging

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Supplementary Material and Methods

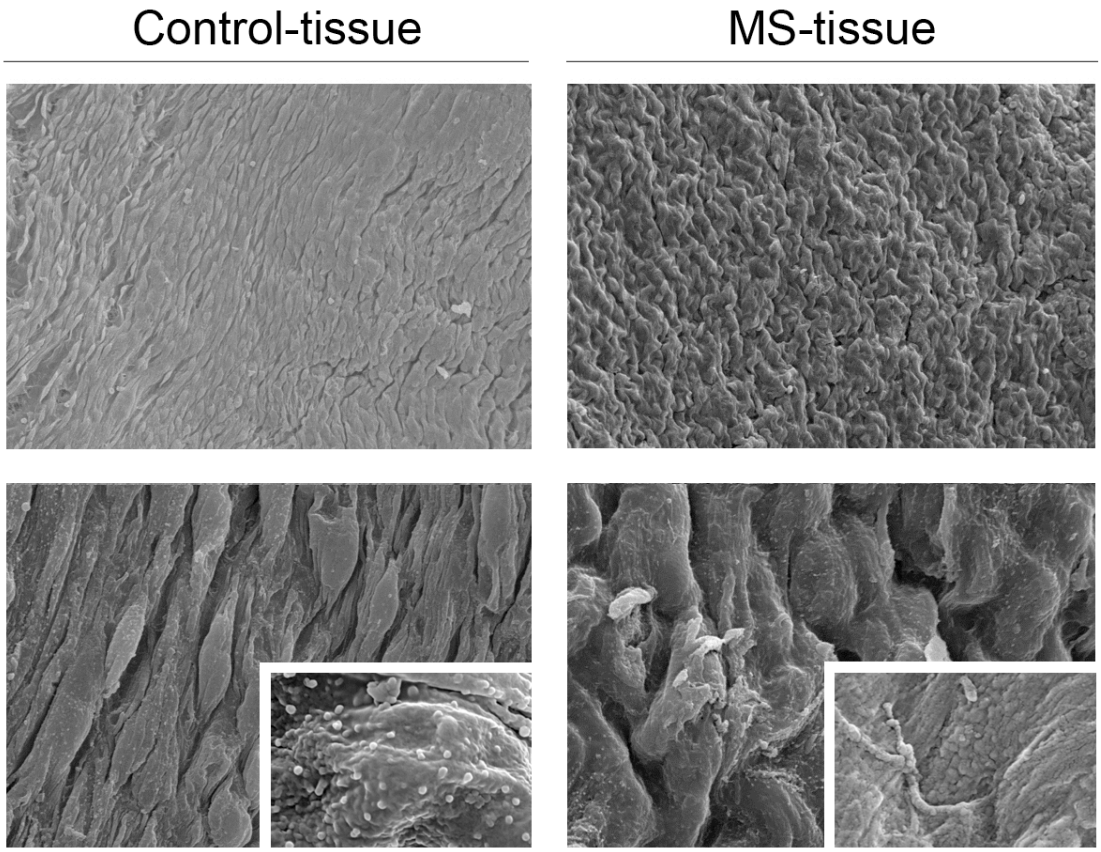
Scanning Electron Microscopy (SEM)

After collection, venous specimens intended for scanning electron microscopy (SEM) analysis were rapidly cut longitudinally, washed and placed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (ph 7.4) for 24 hours at 4°C and post-fixed in 2% osmium tetroxide in the same buffer at room temperature. The samples were then treated with decreasing concentration of ethanol ending with a passage on propylene oxide. Finally, the samples were mounted on a metal stub and coated with a gold sputter coating (S 150 Sputter Coater Edwards, England) and examined under a Scanning electron microscope Zeiss EVO 40 (Cambridge, England).

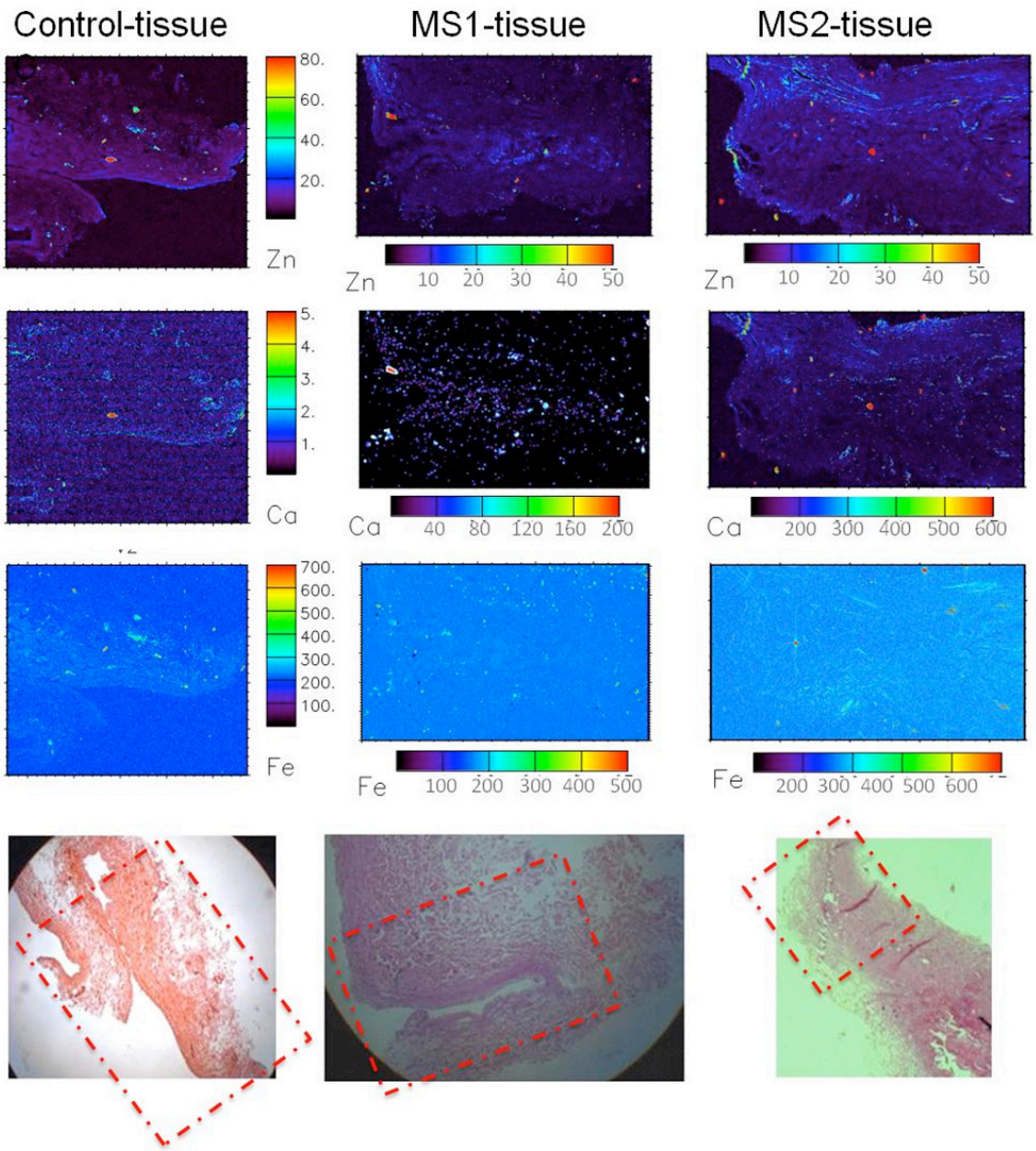
Supplementary Description

XRF analyses in Figure S2

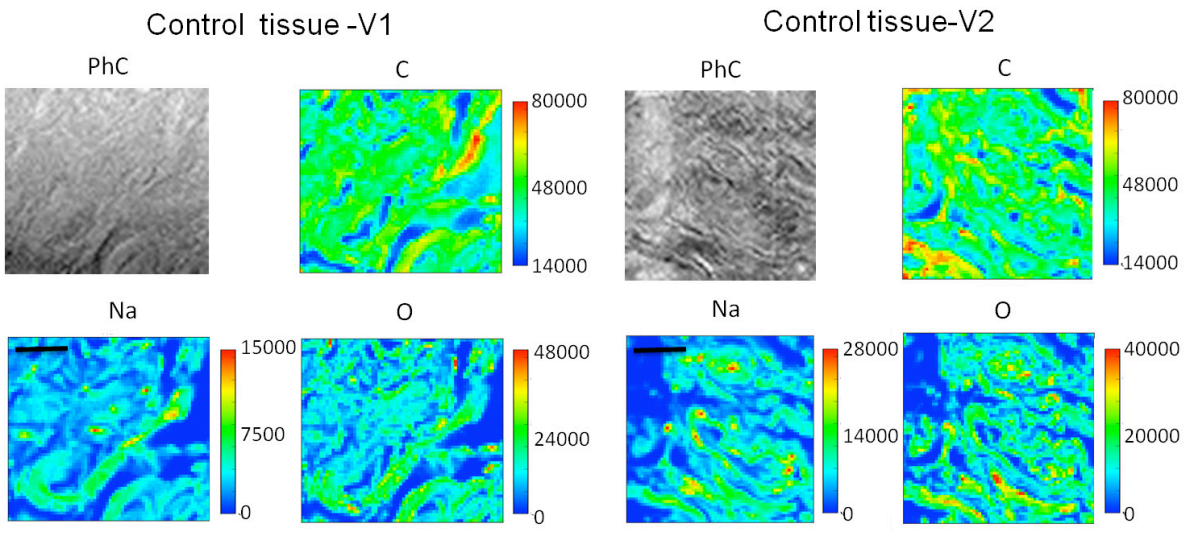
Figure 2S shows the elemental maps of Zn, Ca and Fe collected on representative slices of the control vein (V2) coming from a healthy patient and jugular vein sections from the two MS patients, with the corresponding calculated concentrations reported in ppm in the scale bars. Comparable results in terms of distribution and average elemental concentration were found in different and consecutive sections from each tissue sample. Generally, iron has overall concentrations that at this low spatial resolution appear in the same order of magnitude in all samples, co-localised with Zn in some hotspots. A very precise quantification of iron is quite prevented by the background iron signal, for instance outside the tissue, possibly due to iron-containing materials in the set-up of the beamline. While Zn concentration appears to be similar in all tissues (of the order of 10-100 ppm), Ca presence appears to be increased in MS samples compared to control tissues, reaching values from 10 to 100 times higher in some subregions.



Supplementary Figure S1. Scanning Electron Microscopy images of internal jugular vein lumen. Morphological investigation of the endothelium was performed by SEM in jugular samples. A representative panel of images from a control subject (left panels: original magnification 800x top, 5000x bottom, 50000x insert) and a MS-patient (right panels: original magnification 800x top, 5400x bottom, 50000x insert) is shown. The luminal pathological vessel surface was characterized by the loss of the integrity of the endothelial monolayer, evidenced by a discontinuous surface of the endothelium with craters and cavities.



Supplementary Figure S2. Elemental maps of Zn, Ca and Fe collected at 12.74 keV on three different samples: control jugular vein V2 (column a) from a healthy patient, and two jugular veins (MS1 and MS2) from two MS patients (columns b and c respectively). The last row in the image shows the corresponding maps in the stained tissues (Vi, MS1, MS2 respectively). The concentrations reported on the scale bars are in ppm. Panels a: 1.75m x 1.85mm; Panels b 1.7mm x 1.1mm; Panels c: 1.7mm x 0.9mm.



Supplementary Figure S3. Low energy (1.5 keV) elemental maps of control tissue. Phase contrast (PhC) and C, O and Na elemental maps of the two areas of V1 and V2 control samples shown in Figure 5. Both analysed area are 80μm x 80μm in size and were analysed with a spatial resolution and step size of 0.5μm and 10s/pixel.