# Materials and Methods e-1

## Human subjects

The study was approved by our local ethical review board (approval number EA1/071/17), and carried out in accordance with the Declaration of Helsinki.

## Histological studies

The formalin fixed paraffin embedded tissues used for the diagnostic autopsy of the cerebellum were reanalyzed regarding the quality of the tissue and the evidence of the dentate nucleus as region of special interest in this study using standard H&E stained 3 µm thin sections **(Figure e-1 and e-2)**. If tissue integrity was preserved, areas of the dentate nucleus were visible and no structural changes could be detected, a serial section of the same tissue blocks were cut in 10 to 15 µm thin sections for LA-ICP-MS measurements and tissue mineralization. No deparaffinization was performed for LA-ICP-MS measurements, as deparaffinization would lead to additional delocalization of tissue lipids and other components and potential further loss of analytes leading to loss of spatial resolution.

## Quantification

For the determination of the total amount of gadolinium within the tissue, parallel sections of the brain tissues were digested with nitric acid and hydrogen peroxide (tissue mineralization) for 2 hours at 95 °C, dried and finally analyzed with ICP-MS.

## Printing of internal standard for LA-ICP-MS

Holmium (Ho) was used as internal standard printed as a thin layer on top of each tissue sample mounted on a microscopic slide as described in our previous work [1](#_ENREF_1), [2](#_ENREF_2). Briefly, Ho-spiked ink was produced on request by Proteome Factory AG (Berlin, Germany). Laser ablation-holmium ink (4 mg Ho L-1) consisted of a yellow ink matrix with Ho as an internal standard. A commercial ink jet printer (Pixma iP4950, Canon), equipped with a CD holder, was used for printing the ink onto the top of the tissue samples. For this purpose, the microscopic slide was loaded into a holder made from a conventional CD and the CD printing holder was used. The commercial empty ink cartridge was filled with the spiked ink using a syringe. The printing system was equilibrated afterwards by printing 10 DIN A4-pages. Prior to sample printing, all tissue slides were 3x coated with 5% gelatin. The gelatin-coated slides were printed 3x with 50% color density in high resolution mode.

## LA-ICP-MS analysis

LA-ICP-MS analysis was performed on serial slices of the same subject (n=5) on a commercial LA system (UP-213, ESI, Portland, USA) coupled to a sector field ICP-MS (Element XR, Thermo Fisher Scientific, Bremen, Germany). The ICP-MS was synchronized using the LA unit in an external trigger mode. The operating conditions are shown in **Table e-1**. The ICP-MS operating conditions were tuned daily for maximum intensity and low oxide ratio ((ThO/Th) < 0.7%) using a reference glass slide (SRM 612, NIST, Maryland USA). The coated tissue sections were ablated continuously in line scans. Overlapping line scans and high laser shot repetition rates were applied to ensure complete ablation of the tissue sample.

Mass traces of 31P, 34S, 57Fe, 63Cu, 64Zn, 158Gd, 153Eu and 165Ho were recorded at low resolution (R=300). Ho was used as internal standard whereas Eu was monitored to assess the general accumulation of lanthanides due to environmental exposure. For S and Fe monitoring, the more abundant isotopes 32S and 56Fe suffer severe interferences from 16O16O+ and 40Ar16O+, respectively in the low resolution mode. Although these spectral interferences can be overcome by operating the ICP-MS at medium resolution (R=4000), this comes at the expense of sensitivity. Therefore, the less abundant isotopes, 34S and 57Fe, were monitored so that all elements are measured simultaneously at low resolution. The analysis of tissue sections (with 20×20 mm average dimensions) required about 14 h. Serial sections of the tissue was analyzed and representative images are shown. Data was exported to Origin 2016 (OriginLab Corporation, Northampton, MA) where data normalization and generation of color-coded images were performed. The analytical optimization was performed on a control sample, Co2, taken from an infant who died one month old with no history of receiving GBCA.

Table e-1. Instrumental parameters of the LA-ICP-MS measurements

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| **ICP-MS** | | **LA system** | |
| RF plasma power | 1074 W | Wavelength | 213 nm |
| Plasma gas flow (Ar) | 15 L min-1 | Helium gas flow | 0.25 L min-1 |
| Sample gas flow (Ar) | 1.3 L min-1 | Laser energy | 5.2 J cm-2 |
| Auxiliary gas flow (Ar) | 1.0 L min-1 | Laser spot size | 100 µm |
| Mass resolution (*m/∆m*) | 300 (LR) | Scan speed | 100 µm s-1 |
| Sample time | 2 ms | Repetition rate | 20 Hz |
| Scanning mode | B & E scan | Line overlapping | 20% |
| Detected isotopes | 31P, 34S, 57Fe, 63Cu, 64Zn, 158Gd, 153Eu, 165Ho |  |  |

## Magnetic resonance imaging

Two weeks before death, the head of patient Gd1 was imaged with the following sequences: an axial fat saturated T2-weighted turbo spin echo sequence (TSE); a diffusion and a T2\*-weighted imaging sequence; a time of flight-angiography; a T1-weighted TSE and a coronary T2 turbo-inversion recovery-magnitude (TIRM). After i.v. GBCA application (7 ml Gadovist®), a sagittal T1-weighted MPRAGE (fast 3D gradient echo pulse sequence using a magnetization preparation pulse) that is shown in **Figure e-1** was performed. The following parameters were used: repetition time 2000 ms, echo time 2.67 ms, inversion time 900 ms, flip angle 8°, imaging matrix 246 x 256, field of view 230 mm x 230 mm, slice thickness 1 mm, field strength 1,5 Tesla. The last of the four recorded MRI examinations with GBCA applications of patient Gd2 was done 4 weeks before death. The imaging sequences were a T2- and a T1-weighted TSE sequence axial; TIRM axial and coronary and a diffusion weighted imaging sequence. After i.v. GBCA application, an axial T1-weighted TSE was done that is shown in **Figure e-2** which had the following parameters: repetition time 500 ms, echo time 7.8 ms, imaging matrix 448 x 512, field of view 201 mm x 230 mm, slice thickness 6 mm. Additionally a MPRAGE (field strength 1.5 Tesla) was done.

References

1. Hoesl S, Neumann B, Techritz S, et al. Internal standardization of LA-ICP-MS immuno imaging via printing of universal metal spiked inks onto tissue sections. Journal of Analytical Atomic Spectrometry 2016;31:801-808.

2. Moraleja I, Esteban-Fernandez D, Lazaro A, et al. Printing metal-spiked inks for LA-ICP-MS bioimaging internal standardization: comparison of the different nephrotoxic behavior of cisplatin, carboplatin, and oxaliplatin. Anal Bioanal Chem 2016;408:2309-2318.