Abstract: Spinal glymphatic clearance – a long term (7d) display of spinal perivascular spaces in a mouse model.

Objective
The circulation within perivascular spaces of the central nervous system (CNS), also known as the “glymphatic system” is enjoying more and more attention. Its potential to transport and clear metabolites as well as drugs into and from neuronal tissue made it an ideal candidate for research in context of neurodegenerative and neuroinflammatory diseases of the brain. However, spinal cords perivascular clearance pathways and dynamics have not yet been properly characterized. Since acute and chronic spinal cord injuries are at least partially driven by an inflammatory component, there is an intrinsic neurosurgical interest to understand spinal cords perivascular clearance dynamics.

Methods
The fluorescence marker Alexa 594 was surgically injected into the cisterna magna of 40 male C57BL/6 mice. Eight animals were euthanized after 6, 12, 24, 72 and 168h respectively and spinal cords dissected for histological analysis. Axial slices were examined via fluorescence microscopy and analyzed using ImageJ version 2.1.0/1.53c to examine tracer distribution. GraphPad Prism v9.4.1 was used for statistical evaluation.

Results
Alexa 594 was found along intramedullary vessels and in the subarachnoid compartment at any given time point. Ordinary one-way ANOVA revealed a significant decrease in stained area of the axial slices over the observed period. A relative decline in stained area comparing 6h (100% ± 63) vs. 24h (15% ± 13;$p<.05$) 6h vs. 168h (19% ± 32; $p<.01$) and 72h (80% ± 100) vs 168h ($0<.05$) as shown by Tukey-test for multiple comparisons, suggests a polynomial function of clearance. The average size of fluorescent particles found within the myelon decreases significantly between 12 and 24h ($3.6 \pm 1.2x10^{-5}$ vs. $1.5 \pm 1.1x10^{-5}mm^2$; $p<.05$) after intrathecal injection of Alexa 594 displaying a distribution along smaller-caliber vessels.

Conclusion
We demonstrate a mouse model to perform medium to long-term observation of glymphatic circulation as a basis for comparative studies in pathological conditions. To our knowledge this is the first display of perivascular influx and long term circulation from subarachnoid space into the spinal cord and vice versa. Its role in spinal cord injury can be derived from multiple studies of the brains clearance but must be investigated further.