**Title**: Histological characterisation of staged lesion topography in the MS spinal cord

**Short Title**: MS spinal cord staged lesion topography

**Authors and Affiliations**:
Alex D. Waldman[1,2,3,5] (main author), Cecilia Catania[3], Mark Jenkinson[4], Gabriele C. De Luca[3]

[1]National Institutes of Health MD/PhD Partnership and Oxford-Cambridge Scholars Program, Bethesda, MD, USA
[2]Emory University MD/PhD Program, Atlanta, GA, USA
[3]Neurodegeneration and Inflammation Research Group, Academic Unit of Neuropathology, Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, Oxford, UK
[4]Wellcome Center for Integrative Neuroimaging, Nuffield Department of Clinical Neurosciences, Oxford Centre for Functional MRI of the Brain, John Radcliffe Hospital, Oxford, UK
[5]Molecular Development of the Immune System Section, Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

**Abstract**:
Spinal cord pathology is a major determinant of irreversible disability in progressive multiple sclerosis (MS). The demyelinated lesion is a cardinal feature. Given the well-characterised anatomy of the spinal cord, the study of lesion topography and its extent of inflammatory activity may provide important clues about disease pathogenesis. The vascular and meningeal compartments have been posited to play primary roles by previous neuropathologic and radiographic studies. However, these studies have been limited by statistical power and resolution, respectively. To address these limitations, we studied the largest cohort of cervical, thoracic, and lumbar spinal cord tissue derived from 119 pathologically confirmed multiple sclerosis cases. Immunohistochemistry was used to detect demyelination (proteolipid protein) and classify its inflammatory activity (CD68). Lesions were standardised onto anatomical templates and lesion proportional area was calculated. Permutation-based cluster analysis and mixed models were used to identify patterns in the distribution and extent of lesional pathology. In our cohort, spinal cord lesions were observed in 76.5% of cases. Cases were more likely to harbor lesions at the cervical level compared to the thoracic and lumbar regions. When stratified by histological classification, inflammatory activity (active or mixed active/inactive) was a salient feature observed in 67.2% of cases. The distribution of lesions consistently mirrored the vascular network with relative subpial sparing, arguing against an outside-in gradient of spinal cord demyelination in MS. Lesional pathology also exhibited a strong relationship with clinical disease milestones. Taken together, our findings suggest that 1) the upper regions of the spinal cord are more susceptible to severe lesional pathology, 2) spinal cord lesional pathology is a substrate underpinning a more aggressive disease course, 3) inflammatory spinal cord lesions are an unappreciated feature of progressive MS pathology, and 4) the vasculature is likely the primary site involved in the pathogenesis of spinal cord lesions.