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Abstract

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Superficial dorsal horn volume loss in dogs with neuropathic pain and syringomyelia – a quantitative and qualitative histological characterisation of cervical spinal cord lesions

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Background and aim: Syringomyelia give rise to central neuropathic pain (CNeP) in humans as well as dogs of the breed Cavalier King Charles Spaniel (CKCS). A correlation between symptoms and magnetic resonance imaging (MRI) findings (syrinx dimension, syrinx / spinal cord ratio and degree of syrinx asymmetry) has been established in both humans and dogs. However, descriptions of histomorphological characteristics and their association with symptoms of central neuropathic pain in humans and dogs with syringomyelia are sparse. We hypothesise that CKCS with syringomyelia-related signs of CNeP represent a spontaneous model of CNeP. In order to characterise this translational model, we aimed to quantify and describe the neurohistopathological lesions in the cervical spinal cord. In this study we investigated (1) a possible relation between symptoms and volume loss of a specific anatomical entity involved in nociception and (2) if syringomyelia affected specific functional spinal cord units of nociception.

Methods: Private owned CKCS with a well-characterised pain phenotype and MRI-confirmed syringomyelia (n = 8) and asymptomatic controls (n = 4) were included. The dogs were donated by their owners after euthanasia. Spinal cord segments C1–C8 were sampled, formalin-fixed and paraffin-embedded. Serial 30 µm sections were stained with haematoxylin-eosin, luxol fast-blue (myelin) and thionine (nuclei) for neurohistopathological characterisation. 10 µm sections were stained with a neurofilament triplet H protein-specific primary antibody (SMI-32) to delineate the dorsal horns' laminae I-III as a representation of spinothalamic

neurons after systematic random sampling. The block sampling fraction was 1:2. Volumes of specific anatomical entities (the central canal, syrinx – if present, left and right dorsal horns' laminae I-III, the remaining left and right grey matter and dorsal, lateral and ventral white matter columns) were estimated using the 2D nucleator and the Cavalieri estimator.

Results: Paired comparison of total volumes of the specific anatomical entities between the affected and non-affected halves of the spinal cord in cases with lateralised clinical signs of CNeP ($n = 7$) was undertaken. A significant volume loss of the dorsal horn laminae I-III was found on the affected side to which the clinical signs were ascribed: 34 μm^3 (range 20–48) compared to the non-affected side, 42 μm^3 (range 27–54, $P = 0.034$). In the spinal cord segment most significantly affected by syringomyelia, the mean reduction in dorsal horn area on the affected side was 56% (range 8%–95%) compared to the contralateral, non-affected dorsal horn area. The remaining comparisons revealed non-significant differences between affected and non-affected sides. Unpaired comparisons of the total mean volumes of the spinal cord and sub-volumes revealed no significant differences between cases and controls. No histopathological abnormalities were found in the asymptomatic controls' spinal cords. A clear pattern of ipsilateral changes in the dorsal root entry zone characterised by deafferentation and reorganization of first-order axons into deeper laminae was found in cases with lateralised clinical signs.

Conclusions: Syringomyelia in CKCS with clinical signs of CNeP primarily affects the spinal cord grey matter. The loss of dorsal horn grey matter and dorsal root entry zone pathology are neurohistopathological characteristics shared with human syringomyelia patients. The neurohistopathological findings confirm the back- and forward translational potential of this spontaneous model to fill the gap between induced rodent models and human patients. Establishing the causal relation between syringomyelia and CNeP in the CKCS will further strengthen this spontaneous model's translational potential.