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Detection of neutrophilic extracellular traps in bronchoalveolar lavage fluid of healthy dogs and of dogs with bronchiectasis, bronchomalacia and bacterial bronchopneumonia

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Neutrophilic extracellular traps (NETs) are structures composed of DNA fibres coupled to citrullinated histones and antimicrobial proteins such as myeloperoxidase (MPO). They are released by neutrophils to eliminate invading pathogens. While they play a crucial role in immune responses, they might also contribute to tissue damage when overproduced or insufficiently cleared. In human medicine, in bronchiectasis (BE), NETs have been associated with disease exacerbations and increased mortality. In dogs, NETs have not yet been investigated in respiratory conditions. In chronic respiratory conditions associated with airway damage, such as bronchiectasis (BE) and/or bronchomalacia (BM), chronic neutrophilic inflammation is probably a key factor although pathogenesis remains poorly understood.

This study aimed to assess NETs in the bronchoalveolar lavage fluid (BALF) of dogs, using healthy dogs (H) and dogs with acute bacterial bronchopneumonia (BBP) as well as in dogs with BE and/or BM.

Client-owned H-dogs (n=15), dogs diagnosed with BBP (n=6) and dogs presented with chronic cough and diagnosed with BE (n=9), BM (n=11), or BEBM (n=12) were retrospectively included in this study. NETs were quantified in BALF by low specificity quantification of cell-free DNA (cfDNA) and by detection of MPO-DNA complexes via enzyme-linked immunosorbent assay (ELISA)

cfDNA concentrations were significantly higher in dogs with chronic respiratory disease (mean ± SD; 0.71±1.15) compared to H-dogs (0.1 ± 0.06) ($p=0.011$) and in BEBM dogs (1.24 ± 1.59) compared to H-dogs ($p=0.007$). ELISA analysis showed significantly more MPO-DNA complexes in dogs with chronic respiratory disease (0.87 ± 0.44) compared to H-dogs (0.36 ± 0.18) ($p<0.0001$) and in dogs with BBP (0.82 ± 0.29) compared to H-dogs ($p=0.001$). No significant difference was found between dogs with chronic respiratory diseases and BBP ($p=0.938$). Among dogs with chronic respiratory diseases, higher MPO-DNA levels were observed in BE (1.19 ± 0.43) and BEBM dogs (0.87 ± 0.36) compared to H-dogs ($p=0.000$ and $p=0.001$, respectively), with higher levels in BE compared to BM dogs (0.6 ± 0.34) ($p=0.004$).

This study shows that NETs can be assessed in the BALF in dogs, and that NETs are elevated in both acute BBP and in chronic diseases such as BE and/or BM. Whether they are overproduced by neutrophils, or only reflect the severity of neutrophilic infiltration, or whether they are insufficiently cleared remains to be determined.