Science-in-brief: Equine asthma diagnosis: Beyond bronchoalveolar lavage cytology

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Equine asthma diagnosis: beyond bronchoalveolar lavage cytology

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Abstract

The diagnosis of equine asthma is currently based on the presence of clinical signs indicative of a lower airway disease combined with abnormal bronchoalveolar lavage fluid (BALF) cytology results. However, the type and degree of lower airway inflammation is not correlated with the severity of the clinical signs, and it does not prognosticate on the progression of the disease. We summarize here the results of recent work published in non-veterinary journals or presented at veterinary congresses reporting on the structural alterations (remodeling) occurring in the equine asthmatic airways and their relationship with lung function and inflammation. Using archived tissues, endobronchial and thoracoscopic peripheral lung biopsies, remodeling was found to occur in both central and peripheral airways. The observed changes included increased in the smooth muscle mass, fibrosis, and deposition of elastic fibers, which were correlated with the lung function. Endobronchial biopsies and endobronchial ultrasound are new methods that have been validated for the non-invasive assessment of remodeling and inflammation in the central airways of clinical equine cases, which reflect peripheral airway lesions. The future implementation of these methods could change the clinical approach to equine asthma in favor of an early recognition of the condition for its prevention, and hopefully, for the development of targeted therapies to prevent, or even reverse, established tissue remodeling and inflammation.

It has long been known that the domestication of horses led to the development of chronic non-infectious small airway diseases. The terminology used to describe these conditions has evolved in parallel with findings related to their clinical, anatomical, histologic, or functional features. Heaves, equine emphysema, chronic bronchiolitis, equine chronic obstructive pulmonary disease (COPD), summer pasture associated obstructive pulmonary disease (SPAOPD), lower airway diseases, recurrent airway obstruction (RAO), and inflammatory airway disease (IAD) have been used either interchangeably or to describe specific manifestations or characteristic of the disease. As new features (functional, anatomical, and pathobiological) of these conditions are emerging, the term «equine asthma» has been proposed [1], given its many similarities with human asthma [2].

Driven by the incurable nature of the condition in some horses, and the progression of clinical signs often leading to premature retirement or even euthanasia in its severe form, our research group has focused its attention on the alterations (remodeling) affecting the lower airways and their response to current therapies. We will summarize here recent findings that may change the paradigm dictating the clinical approach to equine asthma in favor of an early recognition of the condition for its prevention, and hopefully, the development of targeted therapies to prevent, or even reverse, established tissue remodeling. Although BAL cytology remains the cornerstone of the diagnosis of equine asthma, novel diagnostic methods have been developed to assess the remodeling affecting the equine airways and to eventually identify horses predisposed to progress to the severe phenotype.

Equine asthma: definitions and diagnostic challenges

Based on the severity of clinical signs, equine asthma may be divided into three main phenotypes, namely mild, moderate, and severe. Mild equine asthma comprises horses with lower airway inflammation identified by bronchoalveolar lavage fluid (BALF) cytology or abnormal lung

function, presenting clinical signs of airway diseases that may be limited to a decreased performance. The clinical signs of horses with moderate equine asthma are more obvious and usually include coughs and possibly tachypnea at rest. These conditions were previously known as IAD. Severe equine asthma (heaves, RAO, SPAOPD, equine emphysema and COPD) comprises all cases characterized by recurrent episodes of increased respiratory effort at rest (labored breathing), which is at least partially reversible with therapy [3].

The clinical diagnosis of equine asthma should ideally be based on the documentation of lower airway obstruction; however, the lack of sensitive and portable lung function tests currently prevents this approach in clinical practice. For these reasons, the diagnosis currently relies on history, clinical signs, and in the presence of lower airway inflammation as detected by BALF cytology. The rationale behind this approach comes from the groundbreaking work of Dr. Viel who reported that the neutrophilia in BALF cytology correlated with the severity of the pulmonary lesions [4]. Another descriptive study also speculated on a possible correlation between the small airway lesions and the clinical severity [5]. However, the clinical significance of BALF inflammation deserves further investigations as the cut-off normal values for the different cell types in BALF have recently been questioned, and little is known on the relationship between the degree and type of BALF inflammation, the severity of disease, and the response to therapy.

What is the clinical significance of bronchoalveolar lavage inflammation in equine asthma?

There is no data in the literature clearly linking the degree and type of bronchoalveolar inflammation to specific pulmonary lesions in asthmatic horses. That is, how should a clinician interpret an increase in neutrophil vs. mast cell or eosinophil differential cell count in equine asthma? What is the clinical implication of a mild vs. a moderate increase in either of these cell populations? Normal cut-off values for inflammatory cell percentages in BALF have been

established, but they provide practitioners no clues concerning the severity of the pulmonary lesions associated with the disease or to the prognosis in terms of progression and reversibility of the disease. Lastly, several studies have shown that BALF neutrophilia does not normalize after effective treatment with inhaled or systemic corticosteroids when horses are kept in the offending environment [6; 7]. These findings add further complexity to our ability to interpret the clinical meaning of BALF inflammation in everyday practice, when horses are under different management conditions, and receiving different asthma medications.

In this perspective, our group has recently studied in well-characterized mild and severely asthmatic horses the relationship between the severity of specific pulmonary lesions and BALF neutrophilia [8]. The results show that in mild equine asthma, luminal neutrophilia (>5% in BALF) is associated with the presence of acute (neutrophilic) bronchiolitis. However, no association was found with the structural alterations present in the small airways and in the pulmonary parenchyma. Conversely, in severe equine asthma, two different cytological phenotypes were observed: a classical neutrophilic phenotype (>20%) and a paucigranulocytic phenotype, characterized by a moderate (5-20%) or by a lack of increase in neutrophils at BALF cytology. Unexpectedly, in neutrophilic severe equine asthma, the peripheral airway lesions were milder than those observed in paucigranulocytic cases. Paucigranulocytemia was also associated with peripheral mucostasis, which was not detected in horses with the neutrophilic phenotype. The presence of mucostasis could explain the reduced BALF neutrophilia in this group, as mucus plugs could prevent the most peripheral bronchioles and alveoli to be reached and sampled during the BAL procedure. However, if this was the case, a decrease in macrophages rather than neutrophil differential cell count would have been expected due to the typically more distal location of these cells into the bronchial tree. Overall, these findings suggest a complex role for airway neutrophils in equine asthma pathophysiology, and the possibility that pulmonary neutrophils may sustain phenotypic switching as recently hypothesized in equine and human asthma [9; 10].

What's beyond bronchoalveolar lavage cytology?

Due to the absence of a significant association between lower airway inflammation detected with BALF cytology and the degree pulmonary dysfunction in equine asthma, the study of structural airway alterations (remodeling) affecting both the small and the large airways has recently gained interest in equine respiratory medicine and research.

Early studies have described milder histological lesions in the central airways of asthmatic horses when compared to those observed in the peripheral airways [4; 11]. This was recently confirmed by our group using histomorphometric methods. On average, severe asthmatic horses have 300% more (i.e. 3 fold increase) airway smooth muscle in their peripheral airways when compared to those of age-matched healthy controls, while only a 50% increase is observed in central airways [12; 13]. This is important, as recent findings from a cohort of severely asthmatic horses (n=12) suggested that the clinical severity of the disease is correlated with the amount of peripheral smooth muscle [14]. The peripheral airway myocytes of severely asthmatic horses are also biochemically and functionally different from those isolated more cranially in the bronchial tree and trachea. Indeed, a specific myosine isoform ((+)insert), which is known to promote a faster contraction of the smooth muscle, is overexpressed in peripheral airways of asthmatic horses [15; 16].The changes are not limited to the smooth muscle layer, as there is also a deposition of collagen in the peripheral airway wall in asthmatic horses that is positively correlated with pulmonary resistance [17]. Also, the positive association between elastic fiber content and pulmonary elastance observed in controls is lost in asthmatic animals. Collectively, these findings indicate that there is a strong structure and function relationship in equine asthma, and that the remodeling of the airways represents a novel therapeutic target.

Although the changes in the peripheral airways are more severe, the assessment of large airway smooth muscle remodeling by means of endobronchial ultrasound (EBUS) predicts the histological alterations occurring more distally within the bronchial tree [14]. EBUS is an imaging technique producing transversal scans of the bronchial wall using a miniature radial ultrasound probe inserted through the working channel of a videoendoscope. Also, when the histological alterations of the central airways are assessed using a newly developed score, a correlation with the severity of airway obstruction measured with the impulse oscillometry technique is observed [18]. This score evaluates the inflammation and remodeling affecting all the structures comprised within the bronchial wall. These two techniques (endobronchial biopsy and EBUS) have their own advantages and limitations [12; 18; 19]. Endobronchial biopsies are easy to obtain, and when processed for standard histology, they allow the assessment of airway epithelium, lamina propria, and smooth muscle. However, they do not permit a precise quantification of the deepest bronchial structures (smooth muscle, cartilage, and peribronchial tissues) [19]. Moreover, they are limited to the bronchial bifurcations (carinas) of the most proximal airways (up to 15 bronchial generations) and cannot be repeated over time at the same site, which limits their usefulness to monitor disease progression or treatment response. On the other hand, EBUS, a non-invasive imaging method performed during bronchoscopy that also evaluates the central airways, allows the cross-sectional study of the bronchial wall at all levels of the bronchial tree, and it can be repeated over time on the same airways. However, it differentiates the several bronchial structures less precisely than histology, it does not provide histological-grade detail, and it currently requires expensive equipment and technical expertise [12].

Using these techniques, we have also studied how peripheral and central remodeling and inflammation respond to treatment in severely asthmatic horses. While inhaled corticosteroid monotherapy cannot reverse BALF neutrophilia unless concomitant antigen avoidance strategies are adopted, it is able to partially reverse peripheral smooth muscle remodeling after only 3 months of treatment [14]. Interestingly, a decrease in the fast contracting (+)isoform of myosine was observed

after 3 months of inhaled corticosteroid monotherapy [15]. Also, a correlation was found between the maximal velocity of shortening of peripheral airway smooth muscle of asthmatic horses and the time elapsed since the end of their corticosteroid treatment [16]. The effects of corticotherapy on the peripheral airways are not potentiated by the administration of β_2-agonist bronchodilators, at least in the first 3 months of treatment, while it better controls the airway neutrophilia and accelerates the reversal of collagen deposition in central airways [14]. Treatment duration must be prolonged up to 12 months in order to significantly reduce peripheral collagen deposition, however [6]. Based on these results, the reversal of BALF neutrophilia observed in the horses treated with a combination of inhaled corticosteroids and β_2-agonist bronchodilators was not associated with any specific parameter of central or peripheral remodeling and inflammation, again indicating the limited usefulness of BALF cytology when assessing response to therapy.

Future directions

Recent evidence suggests that although equine asthma is a disorder mainly affecting to the peripheral airways, alterations are present at all levels of the bronchial tree. Studying remodeling and inflammation occurring in the central and peripheral airways of horses with asthma of different severity could reveal important diagnostic or prognostic information. Indeed, there are currently no means to predict horses with mild asthma that would progress towards the severe form of the disease. Identifying early remodeling or inflammatory markers may fulfill this goal. In the field of equine respiratory research, an effort should be made to develop minimally invasive techniques for the study of both central and peripheral airway structures and function. Our results suggest that central airway remodeling could reflect structural changes occurring in the peripheral airways, at least for the smooth muscle compartment [14], but more studies are needed to confirm this theory and to investigate whether this is true for the remaining structures that make up the bronchial wall.

Finally, the existence of an open equine respiratory tissue bank (www.brte.ca) represents an invaluable tool to further foster research collaboration in this field. Through this virtual platform, it is possible to gather information and have access to tissues harvested from well characterized asthmatic or healthy horses originating from different specialized centers all around the world. The routine collection of endobronchial biopsies in clinical practice after a BAL is performed would also accelerate the discovery of prognostic factors for the progression of the disease using real-life cases.

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