Newest Diagnostic Methods for Inflammatory Airway Disease (IAD)

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This paper reviews the basis for bronchoalveolar lavage and lung function tests for the diagnosis of inflammatory airway disease (IAD)—defined as cough, airway mucus, and/or exercise intolerance. It is important to realize that these signs, no matter how subtle, may indicate the presence of lower airway inflammation, airway obstruction, and airway hyper-reactivity, which are inextricably linked. These diagnostic tests constitute a new early detection system and may be employed for screening at the individual horse or farm level. Author's address: Lung Function Testing Laboratory, Tufts University School of Veterinary Medicine, 200 Westboro Road, North Grafton, MA 01536. © 2002 AAEP.

1. Introduction—Why Use Bronchoalveolar Lavage and Lung Function Tests?

Inflammatory airway disease (IAD) describes a cluster of observations (cough, exercise intolerance, and/or mucus present in the airways) that we commonly recognize in adult horses of any discipline. There are many causes of these signs, including upper and lower airway inflammation, obstruction, infection, and with regard to exercise intolerance, lameness, myopathy, cardiac disease, anhidrosis, insufficient training, and others. If other factors can be largely excluded, IAD should be seriously considered as a cause of poor performance. Finally, if respiratory signs such as a cough become chronic or non-responsive to conventional treatment with antimicrobials, IAD should be investigated.

There are a variety of approaches; however, the diagnosis of IAD may be described as involving four phases: (1) determining the likelihood of IAD based on history and physical examination, (2) assessing airway inflammation, (3) measuring functional impairment, and (4) evaluating treatment response—using performance, clinical signs, and lung function tests as determinants.

It is advisable to employ a detailed rather than superficial diagnostic approach to IAD, because this condition usually does not present with clinical signs that can be detected by simple auscultation or percussion of the thorax until severe. Furthermore, the treatment can be lengthy as well as expensive, so staying on target requires a careful and knowledgeable approach. Currently, the newest methods include bronchoalveolar lavage (BAL) and lung function tests, used singly or as complimentary tests. These tests provide a wealth of information concerning the status of the airways. More superficial methods of diagnosis include the observation of mucus in the airways, combination of tracheal mucus score and evidence of tracheal inflammation, principally neutrophilia, and cough. One may conclude that tracheal mucus is highly prevalent with low specificity, and chronic cough has higher specificity for IAD. Hence, the combination of chronic cough, tracheal mucus, and tracheal inflam-
IN DEPTH: INFLAMMATORY AIRWAY DISEASE

Emphasizes the importance of newer methods of diagnosis, including BAL and lung function tests, which will be important tests in the future of equine practice.

2. Bronchoalveolar Lavage

This review emphasizes the technique of BAL and provides some guidelines for interpretation as recently reviewed by the author. In brief, the method for BAL in the field requires the following materials (Table 1 and Fig. 1): silicone tube, lidocaine (0.5% without epinephrine, diluted from 2%), large syringes (60–120 ml) filled with sterile saline (37°C) or saline bottle with dispenser pin (requires means to pressurize bottle and solution set), sterile lubricant, and sample vials (large ethylenediamine tetra-acetic acid (EDTA) tubes).

It is advisable to forewarn the owners that there is significant coughing associated with BAL. It is imperative to sedate moderately with detomidine (4–6 mg, IV) or xylazine (300–400 mg, IV) for a 500-kg horse, with or without the addition of butorphanol (3–5 mg, IV). The use of a twitch is recommended where appropriate. Alpha antagonists (xylazine and detomidine) have beneficial bronchodilator properties. However, bronchodilator treatment using a β2 agonist is a more reliable strategy to prevent excessive coughing and bronchospasm, particularly in this subset of horses suspected with IAD that are prone to exaggerated constriction. I use albuterol (450 μg) given by inhalation. The horse’s

Table 1. Materials for Field BAL

- Twitch
- Xylazine or detomidine
- Bronchodilator (see text)
- Sterile lubricant
- BAL tube
- Stopcock
- Syringe 1 x 5 ml
- Saline (see text)
- Bottle (500 ml)
- Syringes (4 x 60 or 120 ml)
- Solution administration set

Fig 1

Fig. 1. Example of equipment used for BAL in the field. Depicted are a BAL tube (Bivona, Gary, IN), which can be substituted with an endoscope (>2 m working length). The suction pump shown provides gentle pressure (–10 to −15 cm H2O) that avoids lung collapse, but a syringe can be used instead.
head is extended, easing the passage of the tube through the glottis, akin to intubation. Lidocaine (10–20 ml; 0.5%) can be sprayed through the tube (blindly) on the pharynx and/or glottis before tracheal insertion. Further boluses can be delivered into the trachea, but they are not particularly helpful. The tube is advanced at a rate slower than used for passing a stomach tube and stopped only to allow bouts of coughing to pass (Fig. 2a). At this point, you need to wedge the tip of the catheter into an airway. You will know that you are wedged when there is soft, not firm, resistance. The cuff on the tube is inflated (5 ml), and the position of the wedged tube is maintained by pressure at the nose (Fig. 2b). It is important that you do not let the tube move from this position, because the fluid from the tube will leak into the trachea. There is little harm to leakage other than subsequent reduced recovery. Once the tube is wedged, rapidly infuse a volume of 250 ml (4 syringes × 60 ml pre-filled with warm saline) into the lung, wait 5 s, and suction out using the syringes, or a lower pressure suction pump (−10 to −15 cm H₂O). Repeat the instillation a second time (you can reuse the syringes if you store the first aliquot recovered into a polypropylene or similar flask), and pool the two aliquots. If you have performed a good BAL, there will be evidence of surfactant (foam) on the surface of your samples or in the syringe (Fig. 3).

The pooled samples are mixed gently by swirling, and the EDTA or clot tubes (10 ml) are filled if the intent is to process within 4 h. For longer storage during the day, place the sample on ice or in a refrigerator. If the sample is going to be sent overnight to a laboratory, it is advisable to add commercial calf serum (1:10) and ship on ice. The quality of the cytology derived from your shipped fluid sample will be good if processed within a 24-h window, so plan your procedure around delivery times. Otherwise, ship air-dried unfixed slides. The preparation of slides is technically more difficult than typically necessary for transtracheal aspirate cytology (TTA) samples, which are thicker and do not require a concentration step. The BAL fluid sam-
ple needs to be concentrated substantially, so spin the 10-ml aliquots in a standard table top centrifuge (600 g, 10 min), and pour off the supernatant. The small pellet (1–2 mm) that results (Fig. 4a) is best extracted from the bottom of the tube by agitation of the drop of tenacious fluid that remains using a plastic pipette. Transfer a tiny drop (as for blood smearing) and smear to a feathered edge (Fig. 4b) by using a fan.

Fig. 4. After pouring off supernatant from BAL, there is a small pellet (a). The pellet is then agitated and mixed with the remaining saline (−0.1 ml) by flicking the tube several times. The cell-rich fluid that remains is placed on a clean glass slide (b) for smearing as done for a blood smear. The most important step is drying, which must be done very fast either by waving the slide or (card d) by using a fan.

If you wish to perform the stains in your practice, you can use the Diff Quik fixative for staining with Toluidine Blue for 30 min. Although infection is rarely a consideration when evaluating BAL, bacterial infection can be present as a secondary process, and Gram staining is worthwhile if bacteria are seen on standard stains. All the other cell types of interest (macrophages, lymphocytes, neutrophils, and eosinophils) stain readily with Diff Quik or other standard stains. Examples of typical cytologic findings are depicted in Figure 5 (a–i). The most common mistake made by cytologists not familiar with equine BAL analysis is to miscount mast cells. This occurs in part because conventional stains do not readily stain mast cell granules unless there are tight quality controls in place. Mast cells that have a kidney-shaped nucleus are mistaken for lymphocytes or macrophages when granules are poorly stained. The BAL is also a very sensitive method to diagnose “occult” exercise-induced pulmonary hemorrhage (EIPH). It is not necessary to perform special stains (e.g., Prussian blue) to count or identify hemosiderin-laden macrophages. Cytologists routinely count 400–500 cells to obtain a differential for BAL samples. Automatic cell counters do not perform accurate differentials on BAL; for instance, they grossly overestimate neutrophil percentages, so they should not be used.

A normal BAL sample derived from an initial infusion of 500 ml saline includes alveolar macrophages (40–60%), lymphocytes (40–60%), neutrophils (<5%), mast cells (<2%), and eosinophils (<1%). These reference values from my laboratory were derived from horses considered normal on the basis of physical examination, endoscopy, radiography, and lung function tests. The greatest variation between reference laboratories is the percentage of mast cells, with higher percentages recovered when lower volumes of BAL instillate are used. If your wedge fails, you will pick up tracheal (epithelial) cells. If the horse coughs excessively, there is a possibility that the sample will be contaminated with oro-pharyngeal bacteria and squamous epithelial cells. Sites within the lung periphery do not differ significantly in terms of BAL cytology. In fact, the tube usually lodges in the same (right dorsal diaphragmatic) lobe, so the site is generally consistent.

3. Lung Function Testing

Measurement of lung function for clinical purposes constitutes one of the latest advances in equine medicine. These tests are complementary to clinical observations and ancillary tests such as BAL. They verify the presence or absence of mechanical disturbances that contribute to cough and exercise intolerance. The underlying mechanical problem is airway narrowing, the nature of which varies from horse to horse. Constriction results in airway hyper-reactivity (tendency to bronchoconstrict on exposure to an allergen or agonist), uneven ventilation, and consequently reduced oxygenation during

IN DEPTH: INFLAMMATORY AIRWAY DISEASE

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exercise. The rider observes coughing, reduced speed ("fading at the ¾ pole"), lethargy, lack of endurance, poor or exaggerated respiratory recovery, and vague signs of sport refusal. Perhaps the most important message regarding lung dysfunction is its silent nature. As for inflammation, there can be no physically detectable clinical signs, analogous to low-grade asthma in humans.

Measuring Airway Obstruction
The basis for lung function testing in IAD is the examination of the airways for the mechanical prop-
property of flow limitation. There are many other aspects of “lung function” per se that can be evaluated, for example, ventilation, lung volumes, gas diffusion, alveolar clearance, ventilation-perfusion matching, and shunt. These other facets of lung function, while interesting and measurable, are not particularly relevant to clinical practice at present. Tests performed during maximal exercise add a new dimension to lung function testing, but these can not be performed on all patients; therefore, the following discussion is restricted to outpatient lung function testing for airway properties at rest. This is an area that has rarely been revisited in veterinary medicine.

Airflow limitation can be detected directly by one of four means: (1) comparison between pleural pressure, the driving force of respiration, and flow or volume (outputs) during natural breathing (conventional testing), (2) measurement of flow during compressive maneuvers that provoke flow limitation in normal as well as abnormal patients (forced maneuvers), (3) oscillation of the respiratory system with an external source of flow and measurement of the pressure response (forced oscillometry), or (4) plethysmographic measurement of volume shifts at the body surface in comparison with volume shifts at the nose (flowmetrics). A practical comparison of methods (Table 2) reveals that most of these methods are performed on a referral basis because of their technical difficulties, necessity for stable laboratory conditions, lack of portability, and cost.

**Lung Function Tests Performed in Referral Clinics**

This section will only briefly review referral methods and restricts detail to one field method developed in our laboratory. Conventional testing has been the mainstay of lung function testing in horses for research purposes since its introduction by Gillespie et al. and is the best understood method. In this method, pleural pressure (driving force) is compared with the outcome of that effort, flow or tidal volume. Useful indices of lung function are obtained including resistance (pressure-flow relationship) and dynamic compliance (volume-pressure relationship). This method requires the insertion of an esophageal balloon catheter, a procedure much like passing a stomach tube. The catheter is left in place during the measurements, which adds to the invasiveness and reduced acceptance of the method by horses. The system has not been used to any extent in clinical patients and has low sensitivity for detection of airway obstruction. As for the other methods, however, the system has greater sensitivity when coupled with provocation tests, which aim to bronchoconstrict the horse.

Forced expiratory maneuvers, first attempted in anesthetized horses by Gillespie, was later modified for clinical use by Couetil et al. and is most familiar to any person that has undergone their own pulmonary function testing for asthma or emphysema (COPD). Because of the fact that the small airways are compressible and lack cartilage support, they narrow somewhat under the load of forceful exhalation. The small airways thus become flow-limiting during maximal exercise, for example, when thoracic pressures during expiration are extremely high. Flow-limitation by the small airways has a maximum, i.e., further force of exhalation does not produce greater flow limitation. Hence, the analogy of flow limitation to a shower. No matter how hard you turn on the shower at the head, the emptying rate at the drain stays the same. Couetil et al. at Purdue University, developed a procedure to induce forced exhalation in horses. This is done by gaining access to the airways through nasotracheal intubation under sedation. The horse’s lung is filled and then rapidly emptied using controlled suction. This procedure replicates the maximum expiratory flow achieved voluntarily in humans and compresses the small airways sufficiently to observe the limits of flow. The results of this test have demonstrated that horses with IAD have reduced maximal flows towards the latter part of emptying; this is irrefutable evidence of small airway obstruction. Because increased ventilation and cardiac output maintain aerobic capacity, flow-limitation can be seen as a major constraint to exercise tolerance. In conclusion, the forced maneuver not only closely replicates the system used in humans, but it demonstrates that even in the earliest stages of lower airway disease, there is lung dysfunction, long before there are clinical signs present, such as heaves.

Oscillometry is an ideal lung function test for outpatients because it is non-invasive, allows the horse to breathe freely without interruption, is moderately sensitive, is adaptable to challenge testing, and provides additional information concerning airway function that is unavailable by other methods.
It is a system that is highly sophisticated but can be readily established in a referral practice. The basis for oscillimetry is similar to conventional testing: flow-limitation can be measured as “resistance” to airflow. Resistance is measured as the driving pressure that produces some increment of flow. While the conventional system measures pleural pressure against flow, oscillimetry ignores those natural signals and introduces its own. An oscillatory flow is input to the respiratory system by a facemask, and the pressure response (amplitude, phase) is measured. This external oscillator is simply a valve that is open and closed under control by a computer. The reason we oscillate the respiratory system is to measure pressure-flow data at selected frequencies, which is impossible during natural breathing where only one frequency (the breathing frequency) is measured. Measurement at varying frequencies probes the branching respiratory system for heterogeneity in airway diameters. In the last century, Otis et al. and Dubois et al. recognized that the branching design of the respiratory tract has significant implications with regard to the behavior of flow. The behavior of flow in a branching system, when compared with a simple tube, is much more affected by the frequency of respiration, or in the case of oscillimetry, by oscillation. For example, the resistance is much higher at slower frequencies in this case, and unchanged at higher frequencies. This frequency dependence is exaggerated in the presence of small airway disease, because certain areas of the lung are emptying at a slower rate than others. Picture a group of Olympic 1000-m swimmers leaving their mark. After 5 laps, the spread between the leaders and laggards is increasing, and after 20 laps, they may be going in opposite directions. Such is the characteristic of flow in the cornices of the lung, where the filling and emptying rates can differ in adjacent segments. It is precisely this “heterogeneity” of airway function that is detected up by oscillimetry. Horses with low-grade small airway disease or bronchoconstriction by an agonist, show frequency dependence of resistance. In horses with IAD, we have observed several permutations of airway constriction patterns, including elevation of resistance at all frequencies (homogeneous or central airway constriction), elevation at the lowest frequencies only (heterogeneous peripheral airway constriction), or both, suggesting that the pathophysiology of lower airway disease is not uniform in horses. These constriction patterns are directly relevant to the clinical signs, including cough and exercise intolerance. Frequency dependence suggests heterogeneous constriction of small airways, uneven ventilation, and poor gas exchange. Elevation of the baseline of resistance across frequencies suggests constriction of larger airways. Normal oscillimetry may indicate that (1) the horse is normal or (2) the abnormality involves small airways or tissue to such a small extent it is undetectable. Because normal tests do not completely exclude IAD at this functionally early stage, it is recommended to perform a challenge test in every case, and interpret the findings in light of the BAL cytology. The oscillimetry system has been automated for horses. Some training is necessary to operate the computer, but testing is ultimately manageable by a trained technician.

Lung Function Test Used in the Field
Flowimetry is a simple, non-invasive method employed in the field that is based on the concepts of plethysmography (from “plethysmos” derived from Greek “increase”), which is the measurement of volume displacement at the body surface. This system models the respiratory system like a hand bellows, with the body surface (chest and abdomen) analogous to the body of the bellows, and the movement of air in and out at the tip, equivalent to actual flow at the nose. The measurement of volume displacement of the bellows and the volume of air moved at the tip should be equal. However, filling of the bellows requires a slight negative pressure (generated by work at the handles) and emptying is driven by a slight positive pressure. Such is the respiratory system, with alveolar pressure representing this pressure gradient generated by the chest wall and diaphragm. A minor difference between the bellows and the respiratory system is that the latter heats and humidifies the air, and there is a slight resistance to airflow in the normal respiratory tract, contributing to discrepancies between effort and flow at the nose. The flowmetric system compares flow at the body surface with flow at the nose (hence the term “flowmetrics”). Again, these should be nearly equal in the absence of lung pathology. In the presence of airway obstruction, a marked difference develops because of the compression (during exhalation) or expansion (during inhalation) of gas required for mobilization of flow. In horses with heaves, there are enormous differences between flow measured at the chest/abdomen and flow at the nose, which represents the wasted work in heaves and correlates with the exaggerated abdominal movement. The flowmetric (plethysmographic) method uses sensors to measure the change in the circumference of the chest and abdomen, obtain their sum as a measurement of body volume displacement, and compare this signal with actual (uncompressed) flow at the nose that is collected using a facemask and pneumotachometer. The changes in circumference are measured using respiratory inductance bands that are light and elastic and readily tolerated. The circumferential changes are corrected to the horse’s tidal volume measured during inspiration, so that the measurements are referenced to body size and the level of ventilation. The baseline measurement is recorded. If the baseline measurement is within normal limits, a histamine challenge may be performed to assess airway reactivity. Flow measured at the chest/ab-
Domen level exceeds actual flow measured at the nose (N)—pneumotachometer, and at the chest/abdomen surface (C/A)—respiratory inductance plethysmography (Respirtrace) bands. This reaction can be quantified in a dose-response fashion to obtain an index of the response. Airway reactivity is a highly repeatable trait and has given inference to the idea that airway reactivity is a phenotype. Airway reactivity is thought to be a sensitive measure of inflammation and structural changes in the airways.

In one study, airway reactivity correlated with mast cell percentages in BAL fluid. This supports the notion that the mast cell, which mediates allergic reactions, is integral to remodeling of the airways and promotes airway reactivity.

In contrast, if there is a significant abnormality detected at baseline, it is preferable (and safer) to examine the response to a bronchodilator (e.g., albuterol, 450 µg by inhalation) rather than pursue a measure of airway reactivity. The effect of bronchodilation can provide insight into the reversibility of the response, give further credence to a diagnosis of lower airway obstructive disease, and

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**Fig. 6.** (a) Flowmetric (plethysmographic) measurement of airway obstruction in the horse. Shown are flow sensors at nose (N)—pneumotachometer, and at the chest/abdomen surface (C/A)—respiratory inductance plethysmography (Respirtrace) bands. (b) Flowmetric (plethysmographic) system depicted in entirety, including sensors on the horse (see Fig. 6a), interface, laptop computer, and nebulizer-compressor for histamine challenge or bronchodilator test. (c and d) Flow waveforms derived from flowmetric (plethysmographic) sensors, including nasal (N) and chest/abdomen (C/A) signals before (c) and after (d) bronchoconstriction with histamine aerosol during a challenge test to measure airway reactivity. (e and f) Flow waveforms derived from flowmetric (plethysmographic) nasal (N) and chest/abdomen (C/A) sensors in a horse with heaves, before (e) and after (f) bronchodilation with albuterol aerosol.
verify that specific bronchodilators are effective. In some horses, for example, a better response is obtained with anti-cholinergic agents (e.g., ipratropium bromide) than with \( \beta_2 \) adrenoreceptor agonists (e.g., albuterol).

4. Clinical Application of BAL and Lung Function Tests

The information from BAL and lung function tests is complimentary in the diagnosis of IAD. In IAD, there is inflammation detected by BAL that correlates with lung dysfunction.\(^{4,13,17,20}\) Alternatively, it may be possible to perform lung function tests to screen horses for airway disease, and those that are clearly negative (normal baseline, normal airway reactivity) can be excluded from the more invasive procedure of BAL. However, BAL should not be excluded if the results are equivocal or the owner wants absolute confirmation, because the sensitivity and specificity of each of the lung function testing procedures (in comparison with airway morphometry) has not been established. When there is a positive test (airway hyper-reactivity, for example) we give our clients a choice of further diagnostics.

5. Case Studies

Case Example 1

- **Signalment:** 4-yr-old Thoroughbred gelding racehorse
- **CC:** decline in performance; “fading at the ¾ mile mark”
- **Physical examination:** within normal limits
- **Endoscopy:** small globs of mucus in trachea, particularly after exercise
- **BAL** (Fig. 7): macrophages, 52.8%; lymphocytes, 32.8%; neutrophils, 10.4%; mast cells, 2%; eosinophils, 0%; exercise induced pulmonary hemorrhage moderate
- **Oscillometry:** Resistance at 1, 2, and 3 Hz was 0.41, 0.24, and 0.33 cm H\(_2\)O/l/s, respectively
- **Histamine challenge:** dose of histamine that doubled resistance (1 Hz) 5.7 mg/ml (normal > 8 mg/ml)
- **Interpretations:** Neutrophilic inflammation, frequency dependence of resistance, and mild airway hyperreactivity
- **Diagnosis:** IAD; decline in performance is related to airway inflammation causing low-grade airway inflammation as reflected in airway obstruction and airway hyper-reactivity

Case Example 2

- **Signalment:** 12-yr-old Hanovarian gelding
- **Chief complaints:** chronic cough, unresponsive to hay removal and treatments with Tri-Hist, Azium
- **Physical examination:** cough induced with rebreathing
- **BAL** (Fig. 8): macrophages, 36.8%; lymphocytes, 56.2%; neutrophils, 2.4%; mast cells, 3.8%; eosinophils, 0.8%
- **Oscillometry:** Resistance at 1, 2, and 3 Hz was 0.43, 0.41, and 0.47 cm H\(_2\)O/l/s, respectively
- **Histamine challenge:** dose of histamine that doubled resistance (1 Hz) 3.7 mg/ml
- **Interpretations:** Mast cell inflammation and moderate airway hyper-reactivity
- **Diagnosis:** IAD; cough and airway hyper-reactivity is result of chronic inflammation

References

IN DEPTH: INFLAMMATORY AIRWAY DISEASE


a BAL tube: Bivona, Gary, IN.
b Diff Quik: American Scientific Products, McGaw, IL.
c On The Nose: Scientific Solutions, Loughborough, UK.
d IOS Unit, MasterScreen, Jaeger, Germany.