

EDITORIAL

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Bronchial provocation testing and collection of sputum with inhaled mannitol

This editorial discusses the findings of the two papers in this issue by Miedinger et al. [5] and Wood et al. [6]

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The problem of diagnosing asthma for purposes of entry into the military service has been discussed for many years [1]. Asthma is often diagnosed on the basis of a past history and response to a bronchodilator. However, not all those with asthma in childhood will have asthma in adulthood and many young adults have normal spirometry rendering bronchodilator reversibility tests of little clinical utility. Furthermore, 'healthy' people active in sport can have moderate to severe exercise-induced bronchoconstriction (EIB) with or without significant symptoms or a diagnosis of asthma [2]. EIB is a potential problem for the military as the ability to exercise is necessary for most personnel. In recognition that asthma severity ranges from very mild to severe with most people being mild and untroubled by the disease, some defence forces are requesting bronchial provocation tests (BPTs) with the view to being more lenient on those with asthma wishing to serve.

One technique used to measure BHR is the inhalation of increasing concentrations of methacholine chloride or histamine diphosphate. However, the military is aware that a negative test to these agents does not exclude EIB and it is now common to use a surrogate for the exercise stimulus. The best known surrogate is the eucapnic voluntary hyperpnoea (EVH) test developed and standardized at the Walter Reed Army Hospital in Washington, DC [3].

The stimulus for exercise and hyperpnoea of dry air to provoke airway narrowing and EIB is water loss by evaporation from the airway surface as a result of the need to humidify large volumes of air in a short time. Dehydration of the airway surface and a transient increase

in osmolarity has been proposed as a stimulus for EIB. The hyperosmolar saline BPT was developed to investigate this hypothesis and responses to 4.5% saline compared favourably with dry air hyperpnoea and exercise. Using the 4.5% saline protocol for BPT combined with the protocol for inducing sputum, Gibson et al., in an elegant series of studies, linked the inflammation of asthma (eosinophils and mast cells) with BHR to hyperosmolar saline [4].

The mannitol test, reported in this issue of the journal by Miedinger et al. [5] and by Wood et al. [6], is a new osmotic challenge that has been simplified for use as a point of need test. The mannitol test comes in a kit of capsules containing different doses that are delivered using a dry powder inhaler in nine dose steps (0, 5, 10, 20, 40, 80, 160, 160 and 160 mg) (Pharmaxis Ltd, Frenchs Forest, Australia). Forced expiratory volume in 1 s (FEV₁) is measured 1 min after each dose using a spirometer. A positive test result is a 15% fall in FEV₁ at ≤ 635 mg cumulative dose or a 10% fall in FEV₁ between consecutive doses of mannitol. In this issue of the journal Drs Wood, Powell and Gibson report the use of the mannitol test to induce sputum to analyse for cell types and inflammatory markers at the same time as measuring BHR to mannitol. Their data with mannitol compared favourably with the hyperosmolar saline [6]. This finding confirms the earlier work of Leuppi [7] who successfully collected sputum during mannitol challenge and advanced the topic by confirming equivalence with hyperosmolar saline. This means that a simple method is now available to identify two important hallmarks of asthma – BHR and airway inflammation – in one patient investigation. Eosinophils were found more frequently when mannitol was used even though the total cell count was lower.

It is well recognized that the identification of cell type in sputum is important in making decisions about treatment with inhaled corticosteroids. The technique most commonly in use for sputum induction at present takes about 30 min to perform as it requires the administration

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of a bronchodilator with a 10 min wait to allow the onset of action followed by 7 min exposure of up to four different concentrations of saline (e.g. 0.9%, 3%, 4% and 5%) delivered from an ultrasonic nebulizer. The time to collect and process the sputum, the need for technical expertise in processing the sample that includes staining and identifying the cells means this important procedure is not widely used and the result is not available at the time of consultation. Wood et al. have simplified the procedure whereby both sputum collection and a measure of bronchial responsiveness can be carried out at the same time, with the responsiveness result being immediately available.

Mannitol, hyperosmolar saline, EVH and exercise are known as indirect challenges. 'Indirect challenges act by causing the release of endogenous mediators that cause the airway smooth muscle to contract with or without inducing microvascular leakage' [8]. The mediators released in response to these stimuli include prostaglandin D₂, leukotriene E₄ and histamine [9]. This mode of action renders indirect challenges distinctly different from methacholine or histamine, where the administered agonist acts directly on the smooth muscle. For example, a positive response to mannitol, has an advantage over methacholine or histamine because, in addition to identifying a smooth muscle that is hyperresponsive to locally released mediators, it also identifies airway inflammation that is currently active. Currently, active in this respect means that the number of inflammatory cells (eosinophils and/or mast cells) [4] and the concentration of mediators released from them are sufficient to make the smooth muscle contract and the airways to narrow [9].

Another advantage of using stimuli that act indirectly is that the BHR to these stimuli is significantly reduced or even completely resolved in response to inhaled corticosteroids taken in clinically recommended doses over 8–12 weeks [10]. This is likely due to a reduction in numbers of mast cells and eosinophils and a consequent reduction in the concentration of the mediator available for the muscle to contract. Thus, measurement of the response to indirect stimuli [11] offers a clear starting point and a clear end-point for treatment with inhaled corticosteroids, e.g. documentation of EIB indicates the need for treatment with ICS and resolution of EIB indicates an opportunity to back titrate the dose of ICS. This means that current defence personnel with EIB may be successfully treated with minimal interruption and potential recruits may be given the opportunity to serve. A policy is currently under trial in Australia whereby, potential recruits who have demonstrable BHR to mannitol or 4.5% saline may be offered the opportunity of reassessment after 2–3 months treatment with inhaled corticosteroids as monotherapy.

In terms of physician diagnosis in well-defined populations of asthmatic and healthy subjects, mannitol has been shown to have a specificity of 95% [12]. This high specificity was recently confirmed by others who investi-

gated asthmatics, not considering ICS, and healthy non-asthmatic subjects [13]. Sverrild found the overall accuracy of the diagnosis of asthma described by the area under the receiver-operating curve for response-dose ratio (RDR) was 89.1%. The RDR, sometimes referred to as the dose-response slope, is a measure of airway reactivity and represents the final % fall in FEV₁ divided by the dose of mannitol required to induce that fall. The RDR in the asthmatics was 0.033% per milligram, which is close to 20 times greater than the RDR in the healthy subjects, which was 0.0017% per milligram [13]. The value in their healthy subjects is similar to the Geomean of 0.0029% per milligram (95% CI 0.002, 0.004) reported in the initial Phase 3 trial of mannitol [12].

In another Phase 3 trial investigating subjects with mild symptoms of asthma but without a definite diagnosis, mannitol and methacholine had similar sensitivity and specificity to identify EIB and a physician diagnosis of asthma [14]. The sensitivity and specificity values were, however, lower compared with the well-defined groups of asthmatic and healthy subjects described above [14]. This finding was not unexpected given that this group of subjects would not have qualified for a clinical trial as either asthmatic or healthy subjects [14]. The concordance between the mannitol and methacholine test results was 69%. The same variability of the airway response is reported in the Miedinger conscripts and the concordance between the mannitol and methacholine test results was only 42%, with 14 being positive to both tests.

Thus, what useful information did these tests provide? Putting aside the physician diagnosis and reviewing the test results in Table 4 [5], we find that in the subjects negative to both challenge tests, the RDR was 0.009% fall in FEV₁/mg to mannitol with a representative example being a fall of 5.6% at 635 mg. In the subjects positive only to methacholine, the RDR to mannitol was 0.015% per milligram equivalent to a 9.5% fall in FEV₁ at 635 mg of mannitol, still a negative test result. By contrast, those conscripts positive to mannitol had an RDR of 0.05% per milligram with a representative example being a fall of 15% within 300 mg. We can conclude that in those conscripts with no definite diagnosis of asthma and mild sensitivity to mannitol (PD₁₅ of 300 mg) would have been associated with the subject having a 62% chance of having EIB [14]. For the conscripts with known asthma and an RDR of 0.05% per milligram, the chance of having EIB would have been > 90% [15, 16].

For methacholine, a number of protocols and techniques for delivery have developed and different cut points are used to define abnormality. Most respiratory physicians would agree that a PC₂₀ < 1 mg/mL or a PD₂₀ < 0.4 mg would be consistent with asthma and a PC₂₀ > 16 mg/mL or PD₂₀ > 2 mg would rule out asthma. The values for PC₂₀ between 4 and 16 mg/mL (equivalent to PD₂₀ of 0.4 and 1.6 mg) are acknowledged as borderline

[17]. This uncertainty represents a challenge in itself for any physician to diagnose asthma confidently. The problem is that a positive methacholine test result is not synonymous with current asthma as many factors including smoking, family presence of asthma or hayfever, recent infection, exposure to allergens, past history of asthma, etc. contribute to BHR to pharmacological agents. The clinical utility of methacholine is considered to be in a negative test result giving the confidence to rule out asthma. An acknowledged caveat, however, is that a negative methacholine test does not rule out EIB [18].

It is well recognized that there is no gold standard for a clinical diagnosis of asthma. The physician's diagnosis in 42 conscripts in the Miedinger study was aided by a prior diagnosis of asthma in 25 subjects and by a bronchial challenge test in 25 subjects at the time of the study. It may be considered unusual that four subjects (9.5%) with BHR to both mannitol and methacholine, three with atopy and allergic rhinitis or hayfever and one with atopy alone went without a diagnosis of asthma. Further, 15 people with a positive response to mannitol who would, on the basis of published studies, be considered as having airway inflammation and the potential for EIB went without a diagnosis of asthma and presumably an opportunity for treatment.

Further, it is possible that some conscripts over-reported symptoms and a physician diagnosis of asthma to avoid military service while others looking for a job opportunity under-reported symptoms and withheld information about their current asthma treatment. The recruitment process required conscripts to be evaluated and this provided the opportunity for using BPTs. Such a practice if continued would overcome the current problem that less than half the Swiss Armed Forces conscripts with 'asthma' have available, at conscription, the results of objective testing such as spirometry, bronchial challenge testing or a referral letter by a treating physician [19].

Hence, how can the current knowledge on mannitol help the military physician and others to interpret the findings? It is evident that spirometry alone and symptoms in this particular group of 'healthy' conscripts, many of whom have smoked, was not adequate to help with the diagnosis. The data of Sverrild *et al.* [13] and Brannan *et al.* [15] would help to make a decision based on a PD_{15} having a high specificity (98.4%) and an RDR of $> 0.008\%$ on having a high sensitivity (90.2%). The data from Wood *et al.* [6] in subjects taking inhaled corticosteroids, a positive response to mannitol was more common in those with eosinophilic (72%) vs. non-eosinophilic asthma (36%). In another study, in 62 asthmatics not taking inhaled steroids, 27 had eosinophilic asthma and 83% of these had a positive mannitol test [20]. Further, 65% of those positive to mannitol ($n = 43$) had eosinophilic or mixed granulocyte sputum profile [20]. Both these studies show that a positive response to mannitol is more likely to

be associated with an eosinophilic than a neutrophilic cell profile [20]. Given normal lung function, a negative mannitol test indicates that the chance of eosinophilia being present is low and mast cells are not in sufficient numbers to provide an adequate concentration of a mediator for the muscle to contract. This would be consistent with no currently active airway inflammation. Even if EIB was present, it would likely be mild and rapidly respond to treatment with inhaled steroids.

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