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Title: **Does airway hyperresponsiveness monitoring lead to improved asthma control?**

Short title: AHR for asthma control

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ABSTRACT

The current guidelines recommend an approach to asthma management based on asthma control, rather than asthma severity. Although several specific questionnaires have been developed and control criteria have been established based on clinical guidelines, the evaluation of asthma control is still not optimal. In general, these indicators provide

adequate assessment of current control, but they are more limited when estimating future
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risk. There is much evidence demonstrating the persistence of airway inflammation and airway hyperresponsiveness (AHR) in patients with total control. Therefore, the objective of this review is to analyze the possible role of AHR monitoring as an instrument for assessing asthma control. We will evaluate its capacity as an indicator for future risk, both for estimating the possibility of clinical deterioration as well as loss of lung function or exacerbations. Furthermore, its relationship with inhaled corticosteroid treatment will be analyzed, while emphasizing its capacity for predicting response and adjusting dosage, as well as information about the capability of AHR for monitoring treatment. Last of all, we will discuss the main limitations and emerging opportunities of AHR as an assessment instrument for asthma control.

KEY WORDS: Airway hyperresponsiveness; Asthma; Control; Treatment

INTRODUCTION

Over the course of recent decades, growing importance has been given to the concept of asthma control, meaning the degree to which the manifestations of the disease are either absent or minimized with therapeutic intervention. As the aim of treatment should be to achieve and maintain control for prolonged periods [1], current guidelines recommend an approach to asthma management based on asthma control, rather than asthma severity [2,3,4].

Consequently, several composite assessment instruments have been developed to measure asthma control, among which are the Asthma Control Questionnaire (ACQ) [5] and the Asthma Control Test (ACT) [6]. Although most of these questionnaires have comparable content, they present notable differences in range of values, repeatability and

responsiveness over time and to treatment, validity and practicality [7]. Moreover, there is increasing recognition of the need for the concept of asthma control to encompass not only recent clinical manifestations, but also future risk [2,3,8]. That is, the potential for experiencing adverse outcomes, such as loss of control in the near or distant future, exacerbations, accelerated decline in lung function, or treatment-related side effects (Figure 1). Therefore, in addition to assessing daytime symptoms, limitation of activities, nocturnal symptoms, need for rescue inhalers and lung function (FEV₁ or PEF), it is recommended to include the risk domain of asthma control and to assess the history of asthma exacerbations, exposure to cigarette smoke, high doses of medications, treatment adherence and potential side effects [2-4].

It is striking that, out of the four domains included in the classic definition of asthma (symptoms, variable airway obstruction, airway hyperresponsiveness and airway inflammation) [2,3], only the first two are systematically included in the evaluation of asthma control. In particular, although airway hyperresponsiveness (AHR) is an essential element in the pathophysiology of asthma, the current guidelines highlight its importance in diagnostic evaluation, but they do not consider it among the established criteria for defining asthma control [2-4]. In light of this situation, the objective of the present review is to critically evaluate the information available about the potential usefulness of bronchial hyperresponsiveness as an instrument for controlling asthma.

AIRWAY HYPERRESPONSIVENESS MONITORING

The measurement of airway responsiveness with inhaled bronchoconstrictor stimuli has become routine practice in the diagnosis and monitoring of asthmatic patients. Usually, bronchial challenge tests are divided into direct and indirect challenges depending on the

mechanism through which they induce bronchoconstriction [9] (Figure 2). Direct stimuli (methacholine, histamine, leukotrienes and prostaglandins) act on their respective receptors on bronchial smooth muscle cells, which in turn depolarize and contract, thereby constricting the bronchi [10]. In contrast, indirect stimuli act on intermediate pathways, such as via mediator release from inflammatory cells and the release of neuropeptides from sensory nerves [11].

Methacholine is the direct stimuli most widely used. It is a synthetic derivative of acetylcholine with no nicotinic action, which acts on the cholinergic receptors of the bronchial tree and is biotransformed, like acetylcholine, by acetyl cholinesterase, but at a slower rate [12]. The indirect bronchoprovocation challenges commonly available in pulmonary function laboratories include physical stimuli, such as exercise, eucapnic voluntary hyperpnea, mannitol and hypertonic saline, and the pharmacological agent adenosine monophosphate (AMP) [11,13].

AMP exerts its effects through the activation of specific receptors on intermediary inflammatory cells, such as mast cells, and possibly on afferent nerve endings [14]. Although the main mechanism of AMP-induced bronchoconstriction is the stimulation of the specific adenosine receptors of the A2B type in the mast cells, which produces cellular calcium mobilization and enhances the release of histamine and other preformed mediators, such as tryptase, cysteinyl leukotrienes, prostaglandins and interleukins [15], the activation of both cholinergic and peptidergic neural pathways may also contribute to the contractile airway response to adenosine in asthma [15]. Exercise- and hyperventilation-induced bronchoconstriction is caused by respiratory water loss leading to the increased osmolarity of airway surface liquid. This, in turn, leads to the release of inflammatory mediators, such as histamine, prostanoids and leukotrienes, from the epithelial and mast cells [16]. Mannitol

acts by increasing osmolarity in the airway surface liquid, bypassing the need to cause respiratory water loss by exercise or hyperpnea.

Comparative studies have demonstrated that indirect challenge tests are more specific but less sensitive than direct challenge tests for diagnosing asthma [17-19]. Whereas a negative direct challenge test can be used to rule out the presence of active asthma, a positive direct challenge test does not necessarily confirm the presence of asthma. In contrast, due to their high specificity, indirect challenge tests function best to confirm the presence of disease [11].

Current evidence shows that AHR to indirect agents more specifically reflects airway inflammation as compared with direct stimuli, such as methacholine or histamine [17,20]. Moreover, indirect AHR also presents a more pronounced response to both ICS (Figure 3) [21,22] and the avoidance of allergens [23,24]. This has led many authors to propose that indirect challenges can be used to improve the accuracy of the diagnosis of asthma and to monitor disease activity [17,25,26].

THE LIMITATIONS OF AHR MONITORING AS AN INSTRUMENT FOR ASSESSING CONTROL

A main limitation for using AHR as an indicator of asthma control is the insufficient standardization of its measurement. Although there are several related guidelines [12,27], variability continues to be favored by the many methodological controversies: duration of the interruption of medication before the test; tidal breathing or dosimeter; number of steps used; minimum and maximum bronchoconstrictor concentrations; nebulizer type and output; timing, repetition, and selection of spirometric maneuvers; reference value from baseline or post-diluent inhalation [27]. Without a doubt, greater specificity would be desirable in the recommended procedures in order to favor the comparison of results.

Along these lines, a recent document of the American Academy of Allergy, Asthma and Immunology (AAAAI) recommends the use of a dosimeter with a 5-dose or 10-dose schedule over other provocation methods [28]. Meanwhile, the incorporation of abbreviated 5-dose protocols with dosimeters [29,30] substantially decreases the duration and technical complexity of the test, which would allow it to be more easily incorporated into routine practice in the follow-up of selected patients.

Another controversial aspect is how to measure the response to bronchial challenge testing. While FEV_1 is the universally-accepted variable for the diagnosis of AHR, other variables could be especially useful for evaluating asthma control. It has been suggested that the percentage of decrease in forced vital capacity when the FEV_1 falls 20% compared to the reference value (ΔFVC) could define asthma severity better than PC_{20} FEV_1 as it reflects not only hypersensitivity but also the involvement of the small airway and air trapping [31,32]. In fact, in children with asthma it has been observed that for a similar level of AHR, ΔFVC differentiates between those with symptoms and those who remain asymptomatic, with a more relevant role of the small airway in the development of symptoms [33]. Likewise, the determination of bronchial reactivity indices from the slope of the dose-response curve to the bronchoconstrictor also seems to provide information that complements PC_{20} . It has been reported that bronchial reactivity indices better correlate with the oxidative stress of the airways [34] and that they could be more useful than PC_{20} for discriminating between patients with AHR associated with asthma or COPD [35], in addition to maintaining a better correlation with the health-related quality of life of asthma patients [36]. It has also been demonstrated that the bronchial reactivity indices better identify uncontrolled asthma than PC_{20} [37]. Thus, it should be examined whether the interpretation of bronchial challenge

tests used to determine asthma control should be done with the same parameter as when done as a diagnostic test (PC_{20} FEV₁) or whether new variables should be incorporated.

The natural variability of AHR tests is another crucial aspect. A meta-analysis evaluated the reproducibility of measurements in bronchial challenge in asthmatic patients and found that the intraclass correlation coefficients (ICC) were higher than 0.9 in short-term studies. Long-term studies, however, had lower ICC, which was less than 0.5 in some cases [32]. Classically, it had been reported that natural allergen exposure or asthma exacerbations enhance methacholine or histamine AHR by, on average, 1-2 doubling doses [33]. However, as the diagnostic predictive value of AHR tests is dependent upon the pretest likelihood of asthma [39], variability is also lower in asthmatic patients or subjects with high suspicion of asthma than in normal subjects in whom AHR can be measured [40].

Assuming that AHR has two components (one persistent or structural, and another variable, more related to inflammation) [41] it could be considered that AHR variability mainly reflects fluctuations in airway inflammation. In fact, the increase in AHR with natural allergen exposure has been associated with increases in a number of markers of airway inflammation, including circulating levels of eosinophils, eosinophil cationic protein and basophils, bronchoalveolar lavage fluid levels of activated eosinophils and lymphocytes, and levels of eosinophils, neutrophils and metachromatic cells in induced sputum [42]. In any case, it should be emphasized that, although the total explained variance of improvement in AHR by changes in airway eosinophilic inflammation is greater for indirect than for direct stimuli, this only justifies 36% of its variation [21]. This reinforces the potential role of AHR as an indicator of asthma control as it reflects many other aspects of the disease other than eosinophilic inflammation.

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Lastly, it is necessary to consider the cost and resource implications of AHR monitoring in asthma care. AHR assessment requires certain resources that are not available in all healthcare settings, although in recent years simplified bronchial provocation protocols have been developed, and some agents can be administered in dry powder inhalers.

DOES ASTHMA CONTROL ALSO CONTROL AIRWAY HYPERRESPONSIVENESS?

There are several indications demonstrating that proper asthma control does not go hand in hand with a correction of AHR. It has been reported that up to 41% of children with well-controlled asthma according to the ACQ have AHR documented with an exercise provocation test in cold air [43]. This implies that their asthma is not well-controlled.

While keeping in mind that the definition of controlled asthma based on questionnaires is less strict than the criteria of guidelines [44], the results are similar. To investigate whether total control of asthma according to the GINA guidelines is accompanied by the remission of AHR, Hanxiang *et al.* [45] evaluated 76 patients with totally controlled asthma compared with 30 currently untreated asthma patients and 20 healthy subjects. Interestingly, sixty-five (85.5%) patients with totally controlled asthma showed positive methacholine challenge tests. They also identified a higher number of eosinophils as well as higher levels of eosinophil cationic protein and interleukin-5 in the induced sputum of patients with totally controlled asthma versus healthy subjects. Consequently, it seems that both airway hyperresponsiveness and airway inflammation still persist during total symptoms-based control of asthma.

The problem of persistent AHR is magnified when we consider the recent report that there are few differences in the slope of adenosine 5-monophosphate (AMP) hyperresponsiveness

and in eosinophilic inflammatory markers between patients with totally controlled and well-controlled asthma [46], which in any event are higher than expected in healthy subjects.

The persistence of AHR in spite of adequate asthma control according to current conventional criteria demonstrates that it does not correlate with other aspects of the disease. In fact, in cross-sectional studies, AHR is only weakly associated with symptoms, lung function, and airway inflammation markers [47]. But, precisely because AHR is a surrogate for neither clinical symptoms nor airway inflammation, this could confer it greater value for the control of asthma as it provides independent information that complements other parameters. This approach concurs with the proposal of characterizing the heterogeneity of asthma into five relatively independent domains labelled A to E, in which A would be airway hyperresponsiveness, B bronchitis or airway inflammation, C clinical symptoms, D damage to the airways and surrounding lung parenchyma, and E extrapulmonary factors [48]. Although these domains can interact to a certain extent, they are relatively independent, suggesting that this A to E approach coincides in positioning AHR not as a surrogate measure of just clinical asthma control or inflammation, but as an additional measure for instance for future risk assessment.

THE ROLE OF AHR ASSESSMENT IN THE RISK DOMAIN OF ASTHMA CONTROL

While current control is able to predict future poor control and health care utilization [49], there is increasing awareness that other pathologic and physiologic measurements predict future risk, regardless of the level of current clinical control. It is especially interesting to assess the ability of new measurements for estimating impairment and clinical instability, lung function decline and risk for exacerbations. Although AHR in young infants can predict the development of asthma later in life [50], and asymptomatic AHR in the general

population appears to be a significant risk factor for subsequent development of wheezing or physician-diagnosed asthma [51], it is necessary to examine its relevance in the clinical course of asthma. Several longitudinal studies have demonstrated that AHR might be useful to predict some aspects of future risk.

Impairment and clinical instability

The reduction and later suppression of inhaled corticosteroid treatment (ICS) is probably the most effective model for evaluating mid-term clinical deterioration in patients with asthma. In recent years, evidence has been compiled to show that increased AHR predicts loss of asthma control in adult patients when the ICS are withdrawn.

Leuppi *et al.* [52] evaluated predictors for failed ICS reduction due to clinical impairment in 50 subjects with well-controlled asthma taking a median dose of 1,000 µg ICS/d, in which the ICS doses were halved every two months. The significant predictors of ICS reduction failure were hyperresponsiveness to both histamine and mannitol at baseline, as well as hyperresponsiveness to mannitol during the dose-reduction phase (Figure 4). In contrast, symptoms, spirometry and exhaled nitric oxide (NO) failed to discriminate patients with ICS reduction failure. Thus, the assessment of AHR might be useful as a guideline for the reduction of ICS doses.

With a similar design but another bronchoconstrictor agent, Prieto *et al.* [53] investigated the utility of the determination of airway responsiveness to inhaled AMP and exhaled NO as markers for safely reducing the dose of ICS in well-controlled asthma patients with moderately high ICS doses. In this case, the significant predictors for ICS reduction failure were having both bronchoconstriction in response to AMP and higher exhaled NO levels at baseline. Moreover, the change in the provocative concentration of AMP, causing a 20% fall

in FEV₁ (PC₂₀) during the dose-reduction phase as predictor for failure of ICS reduction, was near statistical significance (p=0.062).

These observations suggest that AHR, both to direct and indirect agents, might be useful to identify clinical impairment in well-controlled asthmatic patients.

Lung function decline

In the general population, several studies have shown that AHR is an independent risk factor for the development of irreversible loss of lung function [54,55]. A prospective, longitudinal study of 912 middle-aged and senior men participating in the Veterans Administration's Normative Aging Study (NAS) examined the relationship of methacholine responsiveness to the subsequent rate of change in lung function over a 3-year follow-up period [55]. After adjusting for age, height, smoking status, and initial level of lung function, the AHR to methacholine, expressed as the dose-response slope, was a significant predictor of the rate of decline of FEV₁ and FEV₁/FVC, and a borderline significant predictor of FVC. Although the AHR to methacholine was a significant predictor of accelerated lung function decline, this cohort included very few subjects with a current or past history of asthma (3.8%), so their results should be extrapolated with care to the overall asthma patient population.

With a study centered exclusively on asthma, Grol *et al.* [56] examined childhood factors associated with lung function decline during adulthood in asthma patients. The authors found that a low FEV₁ and AHR to histamine (assessed by the provocative concentration causing a 10% decline in FEV₁ or PC₁₀ <16 mg/ml) between the ages of 5 and 14 were significantly associated with a lower level of FEV₁ at ages 22 to 32. Thus, in asthma patients, AHR in childhood is an independent risk factor for accelerated lung function decline in early adulthood.

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In adults with a history of moderate-to-severe allergic asthma, AHR to methacholine is also associated with a loss of lung function [57]. In addition to the duration of asthma, abnormal spirometric results were associated with increased childhood methacholine sensitivity as assessed by the PC₂₀ [57]. In short, the information available to date in patients with asthma shows that AHR predicts lung function decline in childhood; meanwhile, data in adults are not as consistent. This underlines the importance of lung development in lung function decline, as AHR and other factors that adversely affect lung development during childhood predispose to reduced lung function and increased respiratory morbidity throughout life [58]. In any event, it is possible that AHR also contributes to the decline in lung function in adults, although the size of the studies done in asthma patients and the follow-up times were not sufficient to detect this. In fact, the Lung Health Study has demonstrated that AHR is an important predictor for the progression of airway obstruction in continuing smokers with early chronic obstructive pulmonary disease, regardless of the baseline level of airflow limitation [59].

Exacerbation risk

Despite its undeniable usefulness, the model for withdrawing ICS poses some difficulties for analyzing the impact of AHR on the rate of exacerbations since most studies have used selected subpopulations and assess a short to mid-term response that is induced (at least partially) by an intervention. In contrast, a population-based model evaluating long-term exacerbations could be more illustrative. An approach of this type was done by Rassmussen *et al.* [60] when, with a population-based, non-selected birth cohort of 1037 New Zealanders, they researched the risk factors for asthma-related hospital admissions during a 26-year follow-up period. After adjusting for confounding factors, AHR was an independent

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risk factor for hospital admissions during the overall study period, with an adjusted odds ratio of 4.0 (CI95%: 1.6-10.2) for a PC₂₀ of methacholine $\leq$ 20 mg/ml. Finally, AHR might also be useful to identify patients at high risk for hospitalization for asthma, particularly with regards to multiple admissions [60].

These data are concordant with previous studies based on register- and hospital-based data that reported that AHR was a risk factor for hospitalization due to asthma, together with being male, frequent respiratory symptoms, low lung function or atopy [61,62].

AHR AS A GUIDE TO TREATMENT MONITORING

In spite of the utility of AHR for adjusting ICS doses and its capacity for predicting a response to these drugs [63], what has not been clarified is whether treatment strategies aimed at reducing AHR are more effective at controlling asthma. Some data are available, although they are not definitive.

In a small study, Sont *et al.* [64] showed that individualized treatment additionally guided by AHR to methacholine leads to fewer uncontrolled episodes in adults with asthma. Using a prospective, randomized, simple-blind study in parallel groups that had been monitored for two years, they compared the evolution of a group of patients in whom the anti-inflammatory treatment was adjusted in accordance with standard recommendations with another group in whom a strategy was added with the aim to reduce AHR. Every three months, treatment with ICS was adjusted in accordance with an algorithm based on clinical guidelines (reference strategy) or with the same algorithm but an increased dosage depending on the degree of AHR (AHR strategy). A total of 67 patients completed the two years of follow-up, and in 49 bronchoscopy was done at the start and end of the study. Although the dose of ICS was reduced in the two groups, due to the study design it was

obviously higher in the patients who followed the AHR strategy. These patients had 1.8 times fewer mild exacerbations than those of the reference strategy (0.23 and 0.43 exacerbations/patient/year, respectively) and the FEV₁ also improved to a significantly greater extent in the AHR strategy group. Moreover, a greater reduction in the thickness of the subepithelial reticular layer was noted in the AHR strategy group compared to the reference strategy group. Therefore, a treatment strategy based on AHR could potentially diminish the progression of asthma and airway remodeling.

Negative results, however, have also been published. Koenig *et al.* [65] assessed whether adding a LABA to an ICS would control AHR at an overall lower dose of ICS when titration of medication was based upon the assessment of routine clinical measures with or without the measurement of AHR. After a 40-week follow-up, they observed that the two AHR-guided therapy groups used higher doses of ICS than the reference group. Nonetheless, no differences were identified in peak-expiratory-flow, pre-dose FEV₁, percentage of symptom-free days, nighttime awakenings or rescue medication use among the three study groups. The conflicting results could be justified by some differences over the Sont *et al.* study [64], such as the shorter interval for dose adjustment (every 8 weeks instead of every 12 weeks, as recommended by the GINA) [2] and the shorter treatment duration (40 weeks vs. 2 years). Mention should also be made of the high number of patients lost (~30%) to the Koenig *et al.* study [65] and that in the AHR-controlled groups on less than 40% of occasions the dose adjustment was done by AHR. But above all, unlike placebo-controlled efficacy trials, asthma treatment algorithm studies pose methodological requirements related with the algorithms used (algorithm agreement, discordance:concordance ratio and decision points derived from populations with the disease of interest) [66], which require stricter control in order to avoid errors in the interpretation of the results.

Nuijsink *et al.* [67] have provided interesting complementary information by demonstrating that AHR-guided therapy can prevent lung function decline in allergic asthmatic children, especially in those characterized by low symptom scores despite AHR. In a multicenter, double-blind, randomized, 2-yr intervention trial with parallel groups, they studied 210 children with moderate atopic asthma. After 2 years, no difference was found in the percentage of symptom-free days between treatment strategies, whereas baseline FEV₁ was higher in the AHR strategy [67]. However, this effect appeared to be entirely explained by a gradual worsening of FEV₁ in a subgroup of children with AHR and low symptom scores allocated to the reference group. In contrast, in the group in which treatment was adjusted according to AHR assessment, FEV₁ remained stable, suggesting that treatment adjustment according to AHR causes lung function preservation instead of decline.

With a slightly different approach, Lipworth *et al.* [68] have recently evaluated the utility of monitoring treatment with just AHR versus the standard strategy. In this study, middle-aged patients with mild-to-moderate persistent asthma were randomized into either having their treatment adjusted by AHR to mannitol or by a combination of symptoms, use of relievers and/or changes in PEF or FEV₁. After 12 months, the AHR group had fewer mild exacerbations (Figure 5) as well as a reduction in exhale NO, symptoms and reliever use, but the mean dose of ICS was also higher, although with no indication of adrenal suppression.

Indeed, the current data, although limited, seem to suggest that AHR monitoring could be an indirect but meaningful marker of asthma control and particularly useful to guide treatment monitoring and tailoring treatment for specific patients/phenotypes. In any event, this level of evidence from randomized studies is rather unique for AHR, since no such trials have been done for monitoring other parameters in asthma management, except sputum eosinophils and FeNO.

OPPORTUNITIES FOR AHR AS A MONITORING TOOL

As we have commented, the relationship between AHR and different eosinophilic inflammation markers is modest. Therefore, unlike exhaled NO, AHR could be useful for assessing other types of non-eosinophilic inflammation, particularly neutrophilic inflammation [69]. It has recently been reported that adults with well-controlled asthma and persistent bronchial neutrophilic inflammation have a greater AHR to methacholine than asthmatic patients with paucigranulocytic inflammation [70].

Another aspect of growing interest is the relationship of the airway microbiota with AHR and asthma control. For years, it has been proposed that bacterial colonization or infection of the bronchial mucosa could have a potential role in the pathogenesis of asthma [71]. However, recent advances in the identification of the human microbiome have demonstrated that the composition of the bacterial communities that colonize the surface of mucous membranes is extensive and varied [72]. In other approaches, the phylogenetic analysis of the sequence polymorphism of the 16S rRNA gene, which is present in all bacteria, is able to detect the community composition and relative abundance of specific bacterial families in the airways [73]. Along these lines, the study by Huang *et al.* [74] is particularly interesting, as it collected bronchial epithelial brushings from adults with suboptimally controlled asthma participating in a multicenter study of the effects of clarithromycin on asthma control. Their results showed that airway microbiota composition and diversity significantly correlated with AHR to methacholine. Specifically, the relative abundance of particular phylotypes, including members of the *Comamonadaceae*, *Sphingomonadaceae*, *Oxalobacteraceae* and other bacterial families, highly correlated with the degree of bronchial hyperresponsiveness. Furthermore, these authors verified that the patients with greater baseline airway microbiota diversity experienced a more pronounced

improvement of AHR after treatment with clarithromycin and better asthma control [79]. More information is still needed to know whether AHR monitoring could assess interventions on neutrophilic inflammation or airway microbiota in order to achieve better asthma control.

CONCLUSIONS

Given the need to incorporate new control indicators for better monitoring of asthma patients, AHR is an alternative to be considered. Until now, its application in asthma has been centered on the diagnostic evaluation process and, although to a lesser degree, the evaluation of severity. Nonetheless, some evidence suggests that AHR can be useful for estimating future risk (clinical impairment, lung function decline and exacerbations, mainly) in patients with asthma. In addition, it is sensitive to the effect of ICS and seems able to predict the functional response to these corticosteroids to be able to better adjust doses. For these reasons, it could be useful for the monitoring of treatment in certain asthma patient subgroups, especially those who show poor control with few symptoms. Its relationship with bronchial neutrophilic inflammation and airway microbiota gives it a potential complementary utility over other control indicators, although more information is required to better evaluate its usefulness as an instrument for the control of asthma.

AUTHOR CONTRIBUTIONS

FGR served as the primary author, reviewing all data, and wrote the article. RG, RC, EMC and DR developed the manuscript conception, interpreted study data, contributed to and reviewed drafts of the manuscript, and approved the final version of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

Raúl Galera, Raquel Casitas, Elisabet Martínez-Cerón, David Romero, and Francisco García-Río declare that they have no conflict of interest.

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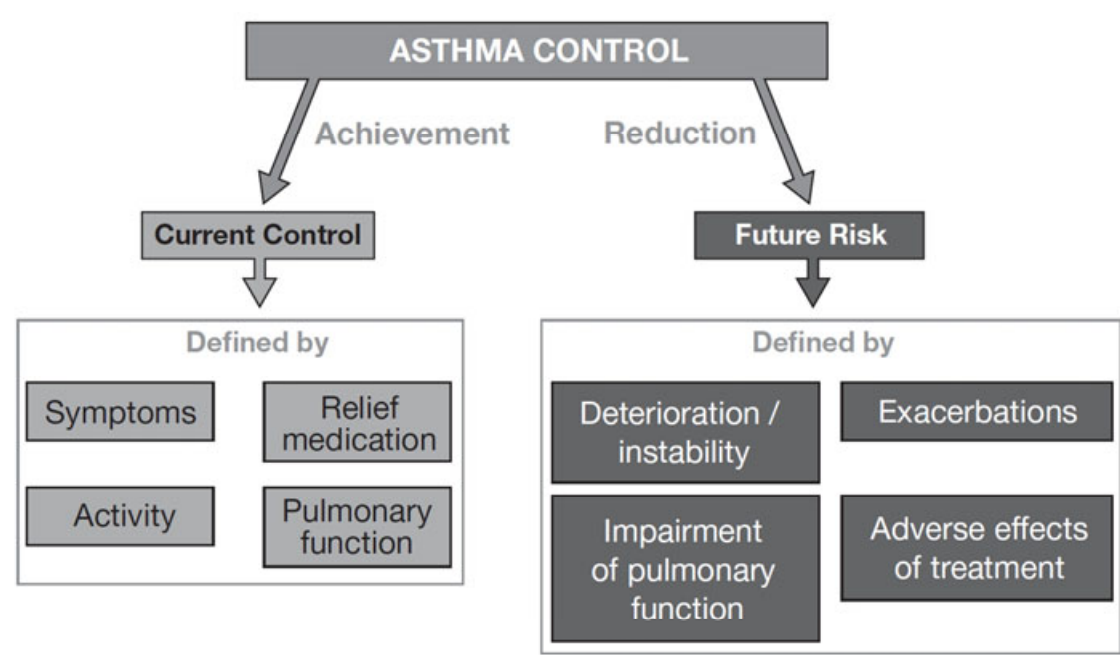
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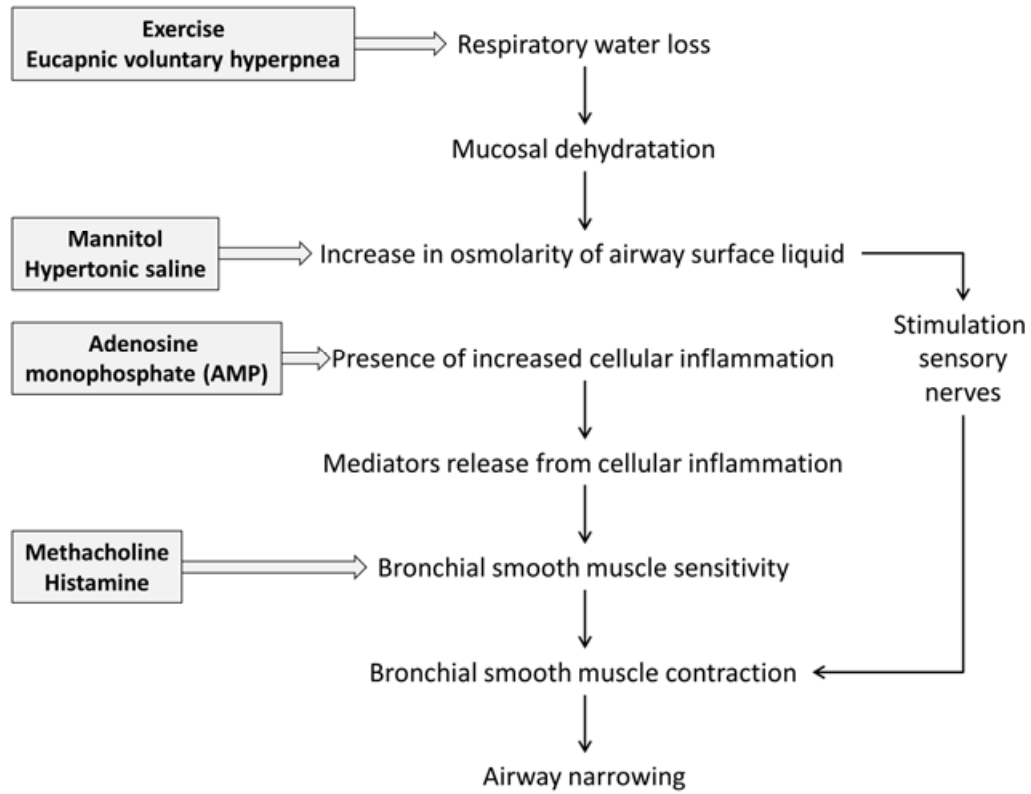
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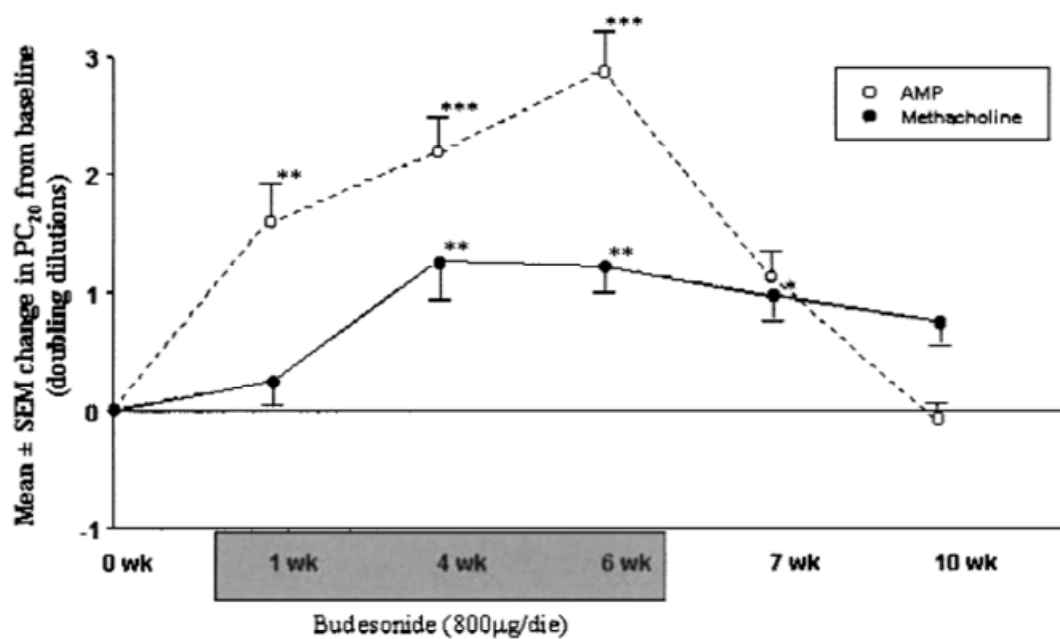
FIGURE LEGENDS

- Figure 1. Current and future risk domains that define and determine level of asthma control (published with permission from the author and publisher) [4]
- Figure 2. Mechanism leading to bronchoconstriction and the point of action of indirect and direct bronchial challenges.
- Figure 3. Comparative changes in PC₂₀ methacholine (filled circles) and PC₂₀ AMP (open circles) values expressed as mean $\pm$ SEM doubling dilutions before, during and after discontinuation of budesonide treatment; *p < 0.05, **p < 0.01, and ***p < 0.001, after treatment vs. baseline (published with permission from Prosperini *et al.*) [22].
- Figure 4. Survival curve using AHR to histamine and mannitol at baseline (A) and AHR to mannitol measured at the visit prior to an ICS reduction (B). The dashed line represents the AHR-negative subjects and the continuous line represents the AHR-positive subjects (to both challenge tests in A) (modified with permission from Leuppi *et al.*) [52].

Figure 5. Total number of mild exacerbations occurring in a 12-month period in asthmatic patients with titrating inhaled corticosteroid (ICS) against mannitol airway hyperresponsiveness (AHR) or a reference strategy (control) based on symptoms, reliever use and lung function in primary care (published with permission from Lipworth *et al.*) [68].







ICS dose (μg)	520.2	322.2	168.8	0
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