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Airway hyperresponsiveness to mannitol improves in both type 2 high and type 2 low asthma after specialist management

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ABSTRACT

Objectives: Type 2 low (T2-low) asthma is reported to respond less to anti-inflammatory treatment compared with Type 2 high (T2-high) asthma. Airway hyperresponsiveness (AHR) to mannitol, a marker of airway mast cell activation, may be indicative of response to treatment in patients with T2-low disease. We investigated whether AHR to mannitol improves in patients with T2-low asthma after specialist management.

Methods: Patients with asthma or suspected asthma, referred to our specialist outpatient clinic, were enrolled consecutively and assessed with FeNO, asthma control, blood eosinophils, mannitol and methacholine tests and induced sputum. T2-low asthma was defined in patients with FeNO < 25ppb and sputum eosinophils < 3% and blood eosinophils < 300 μ l⁻¹ at inclusion. Patients with asthma and AHR to mannitol (PD15 $\leq$ 635 mg) were followed and reassessed after 12 months of specialist management.

Results: Thirty-two patients (Females: 56%, age: 22 years (15–59)) were followed. Fourteen (44%) with T2-high and 18 (56%) with T2-low asthma. Baseline AHR to mannitol was comparable: G_{mean} PD15: 150 mg (95% CI 61–368) and 214 mg (95% CI 106–432) for T2-high and T2-low asthma respectively ($P = 0.51$). Both groups improved equally: G_{mean} PD15: 488 mg (95% CI 311–767) and 507 mg (95% CI 345–746); corresponding to a doubling-dose of: 3.00 (95% CI 1.58–5.74, $P = 0.003$) and 2.28 (95% CI 1.47–3.53, $P = 0.001$) respectively. There were no concomitant improvements in AHR to methacholine.

Conclusion: Patients with asthma and AHR to mannitol improve similarly in responsiveness to mannitol after 12 months of specialist management regardless of Type 2 inflammatory biomarker levels. Mechanisms driving AHR in T2-low asthma need to be further elucidated.

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Introduction

A significant proportion of patients throughout the spectrum of asthma has, despite thorough assessment, no sign of eosinophilic inflammation and low expression of Type 2 inflammation biomarkers (1–3). Patients with Type 2 low asthma have a less well described endotype and are often difficult to manage from a clinical perspective, with a poor response to anti-inflammatory treatment and no effective target-specific treatment (4). The evidence of a poor response to inhaled corticosteroids is; however, somehow conflicting. While some studies show unresponsiveness to corticosteroids (5–9), others have shown some or similar responses compared with patients with Type 2 high asthma (10–12). This inconsistency in findings may reflect the disease heterogeneity of

patients with low Type 2 inflammatory markers, and emphasizes the need of a better characterization to improve treatment (13,14).

One key pathophysiological feature of asthma across endotypes is airway hyperresponsiveness (15). Quantification of airway hyperresponsiveness with bronchial provocation tests can be done with either direct agents such as methacholine or with indirect agents such as mannitol or exercise (16). Indirect tests increase osmolarity in the periciliary fluid due to either dry air hyperpnea or the administration of osmotic agents causing a subsequent release of mediators from the airway epithelium and inflammatory cells leading to bronchoconstriction (17). Hence, airway hyperresponsiveness to indirect challenges may better reflect the presence of ongoing inflammation, especially mast cell activation and infiltration, which

has been demonstrated specifically for the mannitol test (18–20). Direct challenges acting directly on airway smooth muscle may reflect more fixed changes in the airways and are thus less dependent on ongoing inflammation (21).

In a study by Berry et al. (6), both patients with non-eosinophilic and eosinophilic asthma were found to have increased mast cell numbers in the airway smooth muscle, suggesting that mast cells could be drivers of airway hyperresponsiveness in non-eosinophilic asthma. In this study, patients with non-eosinophilic asthma did not improve in airway hyperresponsiveness to methacholine when treated with inhaled corticosteroids. In comparison, Cowan et al. found that, when treated with inhaled corticosteroids, both patients with high and low sputum eosinophils improved in airway hyperresponsiveness to AMP – an indirect bronchoprovocation test (10). However, patients with high FeNO were not excluded from the noneosinophilic group and some of the effect may therefore reflect suppression of Type 2 inflammation.

High doses of inhaled corticosteroids have been shown to reduce airway mast cells in patients with asthma, which is why we hypothesize that airway hyperresponsiveness to mannitol may be reduced by steroid treatment – also in patients with Type 2 low asthma (22,23).

We conducted a *post hoc* analysis on airway hyperresponsiveness using data from patients with asthma obtained before and after 1 year of specialist management.

Materials and methods

Study overview

The MAPOut II study was a prospective observational study performed on all patients referred to the respiratory outpatient clinic at Bispebjerg Hospital in Copenhagen, Denmark either suspected for or with known asthma during a 12-month period from March 2011 through April 2012. Exclusion criteria were age <15 years, respiratory disease other than asthma, individuals older than 40 years with a smoking history of more than 10 pack years, respiratory infections within past 6 weeks of inclusion and pregnancy. All patients gave written informed consent and the study was approved by the local ethics committee (H-3-2011-121).

Patient assessments were performed at baseline and at follow-up after 12 months and has been described in detail elsewhere (24). In short: Lung function testing was done in all subjects according to ERS/ATS guidelines (25). To assess airway hyperresponsiveness

to both indirect and direct agents, bronchial challenge tests with inhalation of dry-powder mannitol (Pharmaxis, Sydney, Australia) and methacholine were performed (26,27). For Mannitol, the test was performed according to the recommendations of the manufacturer. Inhalation of dry powder mannitol leads to an increase in the osmolarity of the periciliary fluid in the airways causing a subsequent release of mediator. A positive test was defined as a fall in FEV₁ of $\geq 15\%$ with a cumulative dose of mannitol of ≤ 635 mg. Sensitivity to mannitol was calculated as the dose resulting in a 15% decrease in FEV₁ (PD15) and the degree of responsiveness was calculated as the response–dose ratio (RDR): percent reduction in FEV₁/mg mannitol administered. For patients who had a negative test at follow-up, a PD15 of 635 mg was assigned (26). For methacholine, the test was performed according to ATS guidelines (27). A maximum dose of 7.8 μ mol methacholine was administered. If a decrease in FEV₁ of 20% was observed the test was considered positive. The dose causing a 20% decrease in FEV₁ (PD20) was calculated. For negative tests, a PD20 of 7.8 μ mol was assigned. The fraction of exhaled nitric oxide (FeNO) was measured at the rate 0.05 L/s with the Nitric Oxide Analyzer (NIOX; Aerocrine AB, Solna, Sweden) or the Ecomedics CLD88sp. (Ecomedics, Duernten, Switzerland). Sputum induction was performed in accordance with ERS recommendations (28). Sputum plugs were selected and standard cytokines were prepared. Finally, a differential cell count was performed (29). Skin prick test was performed according to European standards with a standard 10-panel of allergen extracts including: grass, birch, mugwort, dog, cat, horse, *Dermatophagoides pteronyssinus* and farinae, *Alternaria*, and *Cladosporium herbarum*. Atopy was defined by a positive response with a cutoff value of 3 mm (30). Eosinophils were measured in blood and information on medicine, smoking status, age of onset, demographics and ACQ-5 was collected.

Study material

In this post hoc study, eligible subjects were all patients with a valid asthma diagnose and a positive mannitol test at baseline who participated in the 12 months follow-up.

Definition of asthma endotype

Patients with FeNO < 25 ppb, no sputum eosinophilia, defined as <3% eosinophils in sputum and low blood eosinophils <300 μ l⁻¹ at baseline, were categorized as

Table 1. Baseline characteristics of patients with Type 2 high and Type 2 low (FeNO $\leq$ 25 pp, sputum eosinophils $<3\%$ and blood eosinophils <0.3 109/l) asthma.

	Type 2 high asthma (n = 14)	Type 2 low asthma (n = 18)	P
Age, years	27 (30)	19 (17)	0.12
Females (%)	9 (64)	9 (50)	0.42
BMI	22.1 (5.2)	22.1 (2.7)	0.78
Smokers (%)	0	1 (6)	0.37
Atopy (%)	9 (64)	10 (56)	0.62
ACQ score	1.30 (1.80)	1.20 (1.20)	0.58
PD15 to mannitol, mg	165 (95% CI 71–386)	226 (95% CI 115–442)	0.54
PD20 to methacholine, μmol	0.92 (95% CI 0.24–3.47)	2.13 (95% CI 1.30–3.48)	0.16
Positive methacholine (%)	10 (71)	14 (78)	0.71
FEV1, L	3.37 (0.74)	3.75 (1.11)	0.67
FEV1 % predicted	93 (17)	92 (31)	0.81
FVC, L	4.29 (0.74)	4.65 (1.01)	0.28
FVC % predicted	95 (21)	98 (20)	0.38
FEV1/FVC	0.83 (0.11)	0.82 (0.14)	0.54
Reversibility	1 (7)	1 (6)	0.92
Blood IgE, U/ml	213 (1037)	44 (248)	0.08
FeNO, ppb	44 (66)	15 (9)	<0.001
Sputum eosinophils, %	11.4 (14)	0.25 (0.72)	0.001
Sputum eosinophils $>3\%$	8 (57)	0	<0.001
Blood eosinophils, μl^{-1}	300 (460)	130 ($\pm$ 100)	<0.001

Presented as medians with Interquartile range and numbers with percentages. Test for difference between Type 2 high and Type 2 low asthma were performed with Mann–Whitney *U* and Pearson's χ^2 .

having Type 2 low asthma. All patients with either FeNO > 25 or blood eosinophilia or sputum eosinophilia at baseline were categorized as having Type 2 high asthma. A combination of three biomarkers was used to increase accuracy in the identification of patients with type 2 inflammation (31).

Asthma management

Between the baseline visits and the follow-up visits, all patients were followed at the specialist outpatient clinic at Bispebjerg University Hospital for 12 months. All patients were given standard care, where the management of each patient's asthma was carried forward at the discretion of the clinic specialists.

Statistics

Standard descriptive statistics were performed. Results are presented as means with standard deviations and numbers with percentages for normal distributed data and medians with interquartile range for data that are not normally distributed. *T*-test or Mann–Whitney *U* test for continuous data and Pearson χ^2 test for categorical data were used to test for differences. Data on airway hyperresponsiveness (RDR, PD15 and PD20) were logarithmically transformed to avoid distribution skewness (32). PD15 and PD20 were transformed using the logarithm to base two scale. Data are presented as geometric means with 95% CI. Differences are presented as fold change corresponding to doubling doses and *t*-tests were used for comparison.

All analyses were performed using IBM SPSS statistics version 25 (IBM Software, IL, USA).

Results

A total of 95 patients with asthma, 45 (47%) with Type 2 high and 50 (53%) with Type 2 low asthma were followed for 1 year. At baseline, 14 patients (31%) with Type 2 high asthma and 18 patients (34%) with Type 2 low asthma had a positive mannitol test, giving a total of 32 patients included for analysis. Patients with Type 2 high and Type 2 low asthma had comparable demographic and clinical characteristics at baseline, except for FeNO and eosinophilic counts related to the predefined stratification. Airway hyperresponsiveness to mannitol at baseline was comparable in the two groups: geometric mean PD15: 150 mg (95% CI 61–368) and 214 mg (95% CI 106–432) for Type 2 high and Type 2 low asthma respectively ($P = 0.51$) (Table 1).

After one year of specialist management, the proportion of patients on inhaled corticosteroids had increased from 49% to 85% of patients with Type 2 high asthma and from 44% to 72% of patients with Type 2 low asthma. The average dose of inhaled corticosteroids increased significantly from 600 μg (95% CI 249–951) to 1100 μg (95% CI 849–1351) and from 571 μg (95% CI 300–842) to 1117 μg (95% CI 813–1421) budesonide equivalents in the Type 2 high and Type 2 low asthma group respectively. No significant differences doses between the two groups were detected ($P = 0.93$) (Table 2).

Table 2. Medication at baseline and at follow-up.

	Type 2 high asthma (n = 14)		Type 2 low asthma (n = 18)		P
	Baseline	Follow-up	Baseline	Follow-up	
SABA (%)	11 (79)	10 (71)	10 (56)	13 (72)	0.23
ICS (%)	4 (28)	8 (57)	4 (22)	9 (50)	0.91
ICS/LABA (%)	3 (21)	4 (28)	4 (22)	4 (22)	0.87
LTRA (%)	3 (21)	2 (14)	1 (6)	1 (6)	0.33
Budesonide equivalent (μ g)	600 (249–951)	1100 (849–1351)*	571 (300–842)	1117 (813–1421)*	0.93

SABA: short acting beta2 agonist; ICS: inhaled corticosteroids; LABA: long acting beta2 agonists; LTRA: leukotriene receptor antagonist.

* $P < 0.001$ for difference between baseline and follow-up.

P values for difference in change in medication from baseline to follow-up between patients with Type 2 high and Type 2 low asthma.

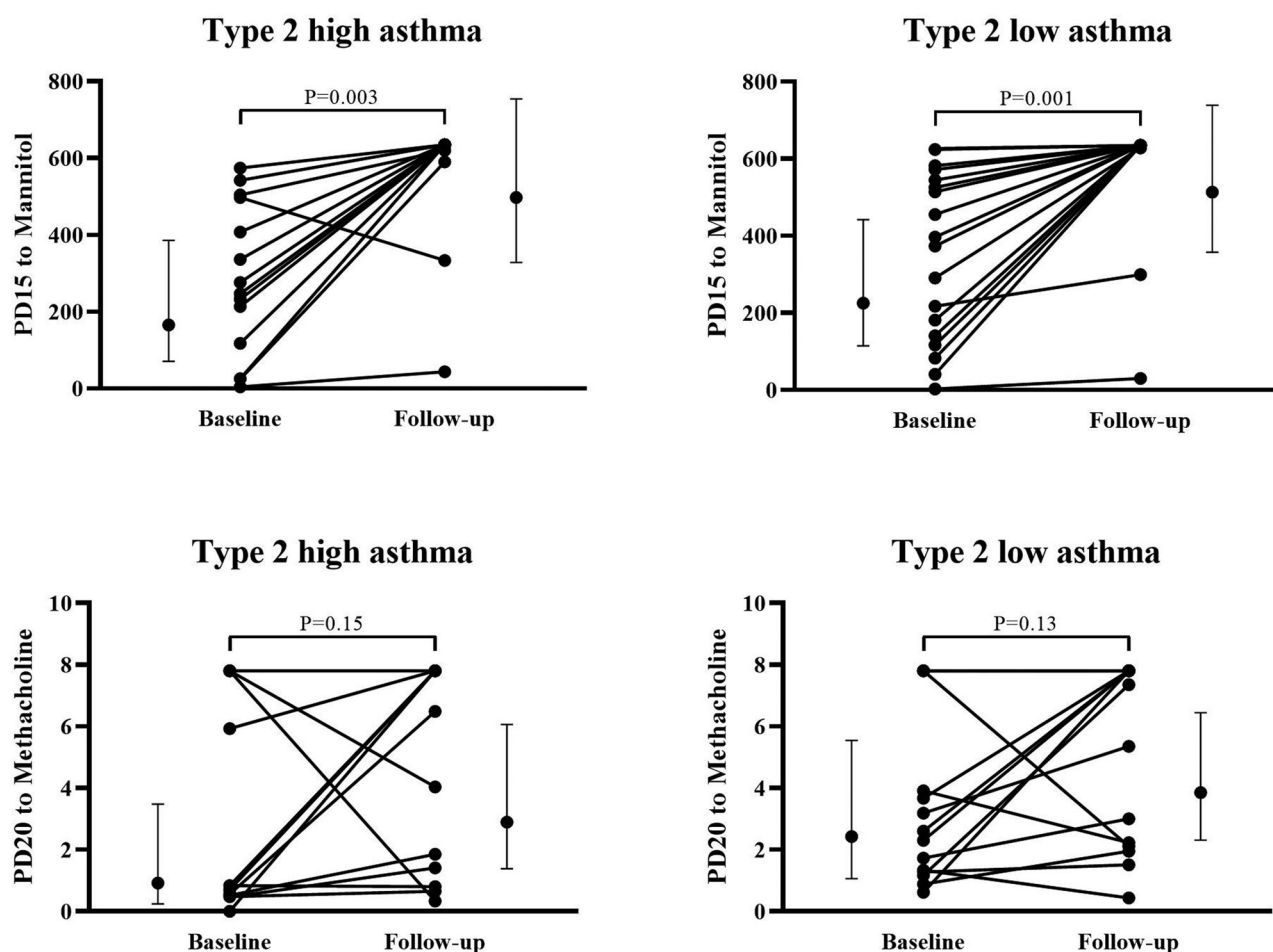


Figure 1. Airway hyperresponsiveness to mannitol (top row) and methacholine (bottom row) before and after 1 year of specialist management in patients with Type 2 high and Type 2 low asthma. Presented as absolute measures of PD15 (mg) to mannitol and PD20 (μ mol) to methacholine and geometric means with 95% CI. P values for difference between baseline and follow-up. For patients with a negative test, a PD15 of 635 mg for mannitol and a PD20 of 7.8 μ mol for methacholine has been assigned.

Patients with Type 2 high and Type 2 low asthma had improved in airway hyperresponsiveness to mannitol to a similar degree after one year of specialist management. In the Type 2 high asthma group, 10 patients (71%) had a negative test at follow-up versus 15 patients (83%) in the Type 2 low asthma group. Among patients who still had a positive mannitol test (PD15 < 635 mg) at follow-up, two patients (14%) with Type 2 high asthma and two patients (11%) with

Type 2 low asthma had not improved clinically significant by at least one doubling dose. Geometric mean PD15 at follow-up was: 488 mg (95% CI 311–767) and 507 mg (95% CI 345–746) corresponding to an increase by a factor (doubling dose) of 3.00 (95% CI 1.58–5.74, $P=0.003$) and 2.28 (95% CI 1.47–3.53, $P=0.001$) for Type 2 high and Type 2 low asthma respectively ($P=0.40$) (Figure 1). Improvements in airway hyperresponsiveness to mannitol expressed as fold decrease

in RDR was likewise comparable in the two groups: 7.80 (95% CI 4.08–14.92, $P < 0.001$) and 4.80 (95% CI 2.64–8.72, $P < 0.001$) in Type 2 high and Type 2 low asthma respectively ($P = 0.25$).

At follow-up, 8 patients with Type 2 high asthma and 9 patients with Type 2 low asthma had a positive methacholine test. Geometric mean PD₂₀ at follow-up were: 2.58 μ mol (95% CI 1.26–5.29) and 3.37 μ mol (95% CI 1.93–5.88), corresponding to an improvement in doubling dose of 3.16 (95% CI 0.62–15.95, $P = 0.15$) and 1.59 (95% CI 0.86–2.92, $P = 0.13$) for Type 2 high and Type 2 low asthma respectively (Figure 1).

For patients with Type 2 high asthma the median ACQ score at baseline was 1.30 (IQR: 1.80) and decreased to 0.80 (IQR: 1.70) whereas for patients with Type 2 low asthma the median baseline ACQ-score was 1.20 (IQR: 1.20) and decreased to 0.40 (IQR: 1.10). This corresponds to a median decrease in ACQ of 0.30 (IQR: 0.90) and 0.40 (IQR: 0.85).

Discussion

In this real-life study on patients with mild to moderate asthma, we found significant and comparable improvements in airway hyperresponsiveness to mannitol but not to methacholine in patients with both Type 2 high and Type 2 low asthma after 1 year of specialist management. Inhaled corticosteroid use was increased, whereas the use of second controllers did not increase significantly. These findings suggest that patients with asthma and airway hyperresponsiveness to mannitol may respond to inhaled corticosteroids despite having low Type 2 markers.

The effect of anti-asthmatic treatment mainly inhaled corticosteroids on airway hyperresponsiveness to mannitol in asthma has been demonstrated in several controlled studies earlier. The magnitude of decrease in responsiveness found in the current study is higher or comparable to what has been observed in controlled studies directly investigating the effect of inhaled corticosteroids (33). In the current study, the RDR was reduced by a factor of 7.85 and 5.00 after 12 months in patients with Type 2 high and Type 2 low asthma respectively. In comparison the RDR was reduced by a factor of 4.2 after 6–9 weeks of treatment with budesonide in a study by Brannan et al. (33). Other studies have investigated responsiveness to indirect bronchial challenges with saline, AMP, eucapnic hyperventilation and exercise and have likewise showed decreased response following treatment with inhaled corticosteroids (34,35). Cowan et al. also

showed an effect of inhaled corticosteroids on airway hyperresponsiveness to AMP in patients with noneosinophilic asthma. The noneosinophilic group, however, also included patients with elevated FeNO, which also turned out to be a predictor of effect in this study. Some of the effect demonstrated, could therefore be driven by a reduction in Type 2 inflammation (10). The current study is, to our knowledge, the first to support the hypothesis of an effect of inhaled corticosteroids on airway hyperresponsiveness to mannitol in patients with asthma and low levels of Type 2 markers.

We hypothesize that the reduction seen in airway hyperresponsiveness to mannitol in patients with low Type 2 inflammation is mediated through effect of inhaled corticosteroids on mast cells in the airway epithelium and smooth muscle (6,10,22,36). However, this study was observational without obtainment of bronchial biopsies to verify this assumption and other mechanisms of inhaled corticosteroids could plausibly contribute. Corticosteroids have *in vitro* been shown to modulate gene expression in airway smooth muscle cells related to both cytokine production, hypertrophy and hyperplasia, and contractility (37–40). Further, an *in vivo* study by Yick et al. showed that treatment with oral corticosteroids in steroid free patients with asthma, altered gene expression in micro dissected airway smooth muscle (41).

In this study, we show a comparable improvement in response to the indirect bronchial challenge with mannitol after 1 year of real-life specialist management in patients with both Type 2 low and Type 2 high asthma. The effect was observed without a significant concomitant improvement in airway hyperresponsiveness to the direct agent methacholine. This discrepancy may well be an illustration of the different mode of actions of the two tests. While indirect bronchial challenges assess airway hyperresponsiveness related to ongoing airway inflammation, the direct tests assess airway hyperresponsiveness that, at least to some degree, is caused by more fixed changes in the airways. Changes that may not be suppressed by treatment with inhaled corticosteroids (6,34,42).

While earlier studies have demonstrated an association between reduction in airway hyperresponsiveness to mannitol and asthma symptoms (33), the reduction in asthma symptoms observed in this study did not reach statistical or clinical significance. Most likely due to a combination of small sample size and relatively low baseline ACQ.

During the year of specialist management, an increased number of patients initiated inhaled corticosteroids and the average dose increased. No changes

in second controllers either long acting β_2 -agonists or leukotriene receptor antagonists were observed, suggesting that real-life treatment with inhaled corticosteroids improves airway hyperresponsiveness in patients with both Type 2 high and Type 2 low asthma and airway hyperresponsiveness to mannitol. Clinical parameters of asthma, however, can over time, especially when observed in an uncontrolled setting, improve simply due to fluctuations of asthma severity. Because patients are most likely to be referred when symptoms are uncontrolled, any measured improvement at a succeeding point in time may be, at least partly, explained simply by spontaneous improvement. In an observational study like the current, this introduces risk of regression toward the mean in measured outcomes. Likewise, due to its observational character, this study cannot be used to directly assess efficacy of treatment in patients with Type 2 high versus Type 2 low asthma. Further, it cannot either directly assess the efficacy of treatment with inhaled corticosteroids in patients with asthma. It can, however, elucidate on the response in patients from a clinical representative population in a real-life setting and add to the discussion on which patients respond to anti-asthmatic treatment – including inhaled corticosteroids – and which do not.

Conclusion

In a real-life population where patients with asthma were followed for 1 year in a specialist setting, airway hyperresponsiveness to mannitol but not to methacholine was reduced in both patients with Type 2 high and Type 2 low asthma. The results suggest that patients with Type 2 low asthma and airway hyperresponsiveness to mannitol benefits from anti-asthmatic treatment through mechanisms beyond the well-known anti-Type2-inflammatory property of inhaled corticosteroids. The clinical effect of inhaled corticosteroids in patients with Type 2 low asthma and airway hyperresponsiveness to mannitol needs to be further explored, as well as the mechanisms underpinning this effect.

Disclosure statement

No conflicts exist for Morten Hvidtfeldt, Asger Sverrild, Vibeke Backer or Celeste Porsbjerg.

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References

- Samitas K, Zervas E, Gaga M. T2-low asthma: current approach to diagnosis and therapy. *Curr Opin Pulm Med* 2017;23(1):48–55. doi:10.1097/MCP.0000000000000342.
- Thomson NC. Novel approaches to the management of noneosinophilic asthma. *Ther Adv Respir Dis* 2016; 10(3):211–234. doi:10.1177/1753465816632638.
- Carr TF, Zeki AA, Kraft M. Eosinophilic and noneosinophilic asthma. *Am J Respir Crit Care Med* 2018; 197(1):22–37. doi:10.1164/rccm.201611-2232PP.
- Busse WW, Holgate S, Kerwin E, Chon Y, Feng J, Lin J, Lin S-L. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. *Am J Respir Crit Care Med* 2013;188(11): 1294–1302. doi:10.1164/rccm.201212-2318OC.
- Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. *Lancet* 1999;353(9171):2213–2214. doi:10.1016/S0140-6736(99)01813-9.
- Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, Bradding P, Wardlaw AJ, Pavord ID. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax* 2007;62(12):1043–1049. doi:10.1136/thx.2006.073429.
- Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002;57(10):875–879. doi:10.1136/thorax.57.10.875.
- Bacci E, Cianchetti S, Bartoli ML, Dente FL, Di Franco A, Vagaggini B, Paggiaro P. Low sputum eosinophils predict the lack of response to beclomethasone in symptomatic asthmatic patients. *Chest* 2006; 129(3):565–572. doi:10.1378/chest.129.3.565.
- Demarche SF, Schleich FN, Henket MA, Paulus VA, Van Hees TJ, Louis RE. Effectiveness of inhaled corticosteroids in real life on clinical outcomes, sputum cells and systemic inflammation in asthmatics: a retrospective cohort study in a secondary care centre. *BMJ Open* 2017;7(11):e018186. doi:10.1136/bmjopen-2017-018186.
- Cowan DC, Cowan JO, Palmay R, Williamson A, Taylor DR. Effects of steroid therapy on inflammatory cell subtypes in asthma. *Thorax* 2010;65(5):384–390. doi:10.1136/thx.2009.126722.
- Godon P, Boulet LP, Malo JL, Cartier A, Lemièrre C. Assessment and evaluation of symptomatic steroid-naïve asthmatics without sputum eosinophilia and their response to inhaled corticosteroids. *Eur Respir J* 2002; 20(6):1364–1369. doi:10.1183/09031936.02.00017502.
- Lemièrre C, Tremblay C, FitzGerald M, Aaron SD, Leigh R, Boulet LP, Martin JG, Nair P, Olivenstein R, Chaboillez S. Effects of a short course of inhaled corticosteroids in noneosinophilic asthmatic subjects. *Can Respir J* 2011;18(5):278–282. doi:10.1155/2011/108079.
- Demarche S, Schleich F, Henket M, Paulus V, Louis R, Van Hees T. Step-down of inhaled corticosteroids in non-eosinophilic asthma: a prospective trial in real

- life. *Clin Exp Allergy* 2018;48(5):525–535. doi:[10.1111/cea.13106](https://doi.org/10.1111/cea.13106).
14. Lazarus SC, Krishnan JA, King TS, Lang JE, Blake KV, Covar R, Lugogo N, Wenzel S, Chinchilli VM, Mauger DT, et al. Mometasone or tiotropium in mild asthma with a low sputum eosinophil level. *N Engl J Med* 2019;380(21):2009–2019. doi:[10.1056/NEJMoa1814917](https://doi.org/10.1056/NEJMoa1814917).
15. Chapman DG, Irvin CG. Mechanisms of airway hyper-responsiveness in asthma: the past, present and yet to come. *Clin Exp Allergy* 2015;45(4):706–719. doi:[10.1111/cea.12506](https://doi.org/10.1111/cea.12506).
16. Nair P, Martin JG, Cockcroft DC, Dolovich M, Lemiere C, Boulet L-P, O'Byrne PM. Airway hyper-responsiveness in asthma: measurement and clinical relevance. *J Allergy Clin Immunol Pract* 2017;5(3):649–659.e2. doi:[10.1016/j.jaip.2016.11.030](https://doi.org/10.1016/j.jaip.2016.11.030).
17. Anderson SD. 'Indirect' challenges from science to clinical practice. *Eur Clin Respir J* 2016 ;3(1):31096. doi:[10.3402/ecrj.v3.31096](https://doi.org/10.3402/ecrj.v3.31096).
18. Gulliksson M, Palmberg L, Nilsson G, Ahlstedt S, Kumlin M. Release of prostaglandin D2 and leukotriene C4 in response to hyperosmolar stimulation of mast cells. *Allergy Eur J Allergy Clin Immunol* 2006; 61(12):1473–1479. doi:[10.1111/j.1398-9995.2006.01213.x](https://doi.org/10.1111/j.1398-9995.2006.01213.x).
19. Brannan JD, Gulliksson M, Anderson SD, Chew N, Kumlin M. Evidence of mast cell activation and leukotriene release after mannitol inhalation. *Eur Respir J* 2003;22(3):491–496. doi:[10.1183/09031936.03.00113403](https://doi.org/10.1183/09031936.03.00113403).
20. Brannan JD, Gulliksson M, Anderson SD, Chew N, Seale JP, Kumlin M. Inhibition of mast cell PGD2 release protects against mannitol-induced airway narrowing. *Eur Respir J* 2006;27(5):944–950. doi:[10.1183/09031936.06.00078205](https://doi.org/10.1183/09031936.06.00078205).
21. Brannan JD, Loughheed MD. Airway hyperresponsiveness in asthma: mechanisms, clinical significance, and treatment. *Front Physiol* 2012;3:460. doi:[10.3389/fphys.2012.00460](https://doi.org/10.3389/fphys.2012.00460).
22. Pavord ID, Jeffery PK, Qiu Y, Zhu J, Parker D, Carlsheimer Å, Naya I, Barnes NC. Airway inflammation in patients with asthma with high-fixed or low-fixed plus as-needed budesonide/formoterol. *J Allergy Clin Immunol* 2009;123(5):1083–1089.e7. doi:[10.1016/j.jaci.2009.02.034](https://doi.org/10.1016/j.jaci.2009.02.034).
23. James A, Gyllfors P, Henriksson E, Dahlén SE, Adner M, Nilsson G, Dahlén B. Corticosteroid treatment selectively decreases mast cells in the smooth muscle and epithelium of asthmatic bronchi. *Allergy Eur J Allergy Clin Immunol* 2012;67(7):958–961. doi:[10.1111/j.1398-9995.2012.02836.x](https://doi.org/10.1111/j.1398-9995.2012.02836.x).
24. Backer V, Sverrild A, Ulrik CS, Bødtger U, Seersholm N, Porsbjerg C. Diagnostic work-up in patients with possible asthma referred to a university hospital. *Eur Clin Respir J* 2015;2(1):27768. doi:[10.3402/ecrj.v2.27768](https://doi.org/10.3402/ecrj.v2.27768).
25. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CPM, Gustafsson P, Jensen R, ATS/ERS Task Force Standardisation of Spirometry. *Eur Respir J* 2005;26(2):319–338. doi:[10.1183/09031936.05.00034805](https://doi.org/10.1183/09031936.05.00034805).
26. Anderson SD, Brannan J, Spring J, Spalding N, Rodwell Leanne T, Chan K, Gonda I, Walsh A, Clark AR. A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. *Am J Respir Crit Care Med* 1997;156(3):758–765. doi:[10.1164/ajrccm.156.3.9701113](https://doi.org/10.1164/ajrccm.156.3.9701113).
27. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, et al. Guidelines for methacholine and exercise challenge testing 1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000;161(1):309–329. doi:[10.1164/ajrccm.161.1.ats11-99](https://doi.org/10.1164/ajrccm.161.1.ats11-99).
28. Paggiaro PL, Chanez P, Holz O, Ind PW, Djukanović R, Maestrelli P, Sterk PJ, Sterk PJ. Sputum induction. *Eur Respir J Suppl* 2002;37(37 suppl):3s–8s. doi:[10.1183/09031936.02.00000302](https://doi.org/10.1183/09031936.02.00000302).
29. Efthimiadis A, Spanevello A, Hamid Q, Kelly MM, Linden M, Louis R, Pizzichini MMM, Pizzichini E, Ronchi C, Van Overvel F, et al. Methods of sputum processing for cell counts, immunocytochemistry and in situ hybridisation. *Eur Respir J Suppl* 2002;37(37 suppl):19s–23s. doi:[10.1183/09031936.02.00001902](https://doi.org/10.1183/09031936.02.00001902).
30. Heinzerling L, Mari A, Bergmann K-C, Bresciani M, Burbach G, Darsow U, Durham S, Fokkens W, Gjomarkaj M, Haahtela T, et al. The skin prick test – European standards. *Clin Transl Allergy* 2013;3(1):3. doi:[10.1186/2045-7022-3-3](https://doi.org/10.1186/2045-7022-3-3).
31. Silkoff PE, Laviolette M, Singh D, FitzGerald J, Mark Kelsen S, Backer V, Porsbjerg CM, Girodet PO, Berger P, Kline JN, Chupp G, Susulic VS, Barnathan ES, Baribaud F, Loza MJ, Strambu I, Lam S, Eich A, Ludwig-Sengpiel A. Airways Disease Endotyping for Personalized Therapeutics (ADEPT) Study Investigators. Identification of airway mucosal type 2 inflammation by using clinical biomarkers in asthmatic patients. *J Allergy Clin Immunol* 2017;140(3):710–719. doi:[10.1016/j.jaci.2016.11.038](https://doi.org/10.1016/j.jaci.2016.11.038).
32. Peat JK, Unger WR, Combe D. Measuring changes in logarithmic data, with special reference to bronchial responsiveness. *J Clin Epidemiol* 1994;47(10):1099–1108. doi:[10.1016/0895-4356\(94\)90096-5](https://doi.org/10.1016/0895-4356(94)90096-5).
33. Brannan John D, Koskela H, Anderson SD, Chan HK. Budesonide reduces sensitivity and reactivity to inhaled mannitol in asthmatic subjects. *Respirology* 2002;7(1):37–44. doi:[10.1046/j.1440-1843.2002.00357.x](https://doi.org/10.1046/j.1440-1843.2002.00357.x).
34. Du Toit JI, Anderson SD, Jenkins CR, Woolcock AJ, Rodwell LT. Airway responsiveness in asthma: bronchial challenge with histamine and 4.5% sodium chloride before and after budesonide. *Allergy Asthma Proc* 1997;18(1):7–14. doi:[10.2500/108854197778612817](https://doi.org/10.2500/108854197778612817).
35. Vathenen AS, Knox AJ, Wisniewski A, Tattersfield AE. Effect of inhaled budesonide on bronchial reactivity to histamine, exercise, and eucapnic dry air hyper-ventilation in patients with asthma. *Thorax* 1991; 46(11):811–816. doi:[10.1136/thx.46.11.811](https://doi.org/10.1136/thx.46.11.811).
36. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002; 346(22):1699–1705. doi:[10.1056/NEJMoa012705](https://doi.org/10.1056/NEJMoa012705).
37. Patel JK, Clifford RL, Deacon K, Knox AJ. Ciclesonide inhibits TNF α - and IL-1 β -induced monocyte chemotactic protein-1 (MCP-1/CCL2) secretion from human airway smooth muscle cells. *Am J*

- Physiol Lung Cell Mol Physiol 2012;302(8): L785–L792. doi:[10.1152/ajplung.00257.2011](https://doi.org/10.1152/ajplung.00257.2011).
38. Kang BN, Jude JA, Panettieri RA, Walseth TF, Kannan MS. Glucocorticoid regulation of CD38 expression in human airway smooth muscle cells: role of dual specificity phosphatase 1. *Am J Physiol Lung Cell Mol Physiol* 2008;295(1):L186–L193. doi:[10.1152/ajplung.00352.2007](https://doi.org/10.1152/ajplung.00352.2007).
39. Goldsmith AM, Hershenson MB, Wolbert MP, Bentley JK. Regulation of airway smooth muscle α -actin expression by glucocorticoids. *Am J Physiol Lung Cell Mol Physiol* 2007;292(1):L99–L106. doi:[10.1152/ajplung.00269.2006](https://doi.org/10.1152/ajplung.00269.2006).
40. Xie S, Sukkar MB, Issa R, Khorasani NM, Chung KF. Mechanisms of induction of airway smooth muscle hyperplasia by transforming growth factor- β . *Am J Physiol Lung Cell Mol Physiol* 2007;293(1): L245–L253. doi:[10.1152/ajplung.00068.2007](https://doi.org/10.1152/ajplung.00068.2007).
41. Yick CY, Zwinderman AH, Kunst PW, Grünberg K, Mauad T, Fluiter K, Bel EH, Lutter R, Baas F, Sterk PJ. Glucocorticoid-induced changes in gene expression of airway smooth muscle in patients with asthma. *Am J Respir Crit Care Med*. 2013;187(10):1076–1084. doi:[10.1164/rccm.201210-1886OC](https://doi.org/10.1164/rccm.201210-1886OC).
42. Lim SAM, Jatakanon A, John M, Gilbey T, O'Connor B J, Chung KF, Barnes PJ. Effect of inhaled budesonide on lung function and airway inflammation assessment by various inflammatory markers in mild asthma. *Am J Respir Crit Care Med*. 1999;159(1): 22–30. doi:[10.1164/ajrccm.159.1.9706006](https://doi.org/10.1164/ajrccm.159.1.9706006).