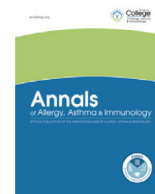




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Review

Treatment approaches for the patient with T2 low asthma

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ABSTRACT

Objective: To identify treatment approaches that can be used in the management of patients with asthma who lack significant type 2 inflammation, also called T2 low asthma.

Data Sources: Recent expert guideline updates on the management of asthma, recent journal articles and review articles, and foundational journal articles are referenced.

Study Selections: This review cites clinical cohort studies of highly characterized patients with asthma, clinical interventional trials of high impact, mechanistic studies relevant to T2 low asthma, and emerging work in this area.

Results: T2 low asthma accounts for approximately one-third to one-half of individuals with asthma. Characteristics of participants with T2 low asthma include higher body mass index, cigarette smoking/smoke exposure, relative lack of responsiveness to steroids, less bronchodilator reversibility, and often the presence of neutrophilic inflammation. Multiple available interventions target these characteristics, including standard inhalers, azithromycin, and lifestyle interventions of weight loss and smoking cessation.

Conclusion: Treatment of T2 low asthma should involve currently available approaches and will benefit from improved definition and understanding of disease pathobiology.

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Introduction

Clinicians who treat patients with asthma will readily recognize that individuals with severe or difficult-to-control asthma require treatments beyond the usual doses of inhaled corticosteroid and β -agonist. The biologic medications currently available in the United States all target pathways of type 2 inflammation which are suspected to be driving asthma disease severity and exacerbation risk. These medications are not indicated for patients without having substantial type 2 inflammation, also termed T2 low asthma. The presence of T2 low asthma has been long recognized, and subgroups are defined based on dominant inflammatory and clinical characteristics of those patients.¹ Nevertheless, specific therapies targeted at suspected inflammatory pathways in T2 low asthma have not found the same degree of benefit for asthma control as the existing biologics have for T2 asthma. In this review, we will discuss the prevalence of T2 low asthma, clinical and physiological characteristics of T2 low asthma, and current therapies available for patients with T2 low asthma.

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Defining T2 Low Asthma

Type 2 low asthma can be defined as the absence of substantial type 2 inflammation involving the lung. Type 2 inflammation is inflammation characterized by type 2 innate lymphoid cells and type 2 CD4⁺ helper T cells producing pathognomonic cytokines interleukin (IL)-4, IL-13, and IL-5. Patients with type 2 asthma therefore have clinical features downstream of these cytokines, notably the production of antigen-specific immunoglobulin E (IgE) from B cells class switched by IL-4, airway hyperreactivity, and mucus production spurred by IL-13 and eosinophils in airway, tissue, or peripheral blood supported by IL-5. Evidence of the activity of these pathways can be easily obtained through allergen skin testing, blood measurement of IgE and eosinophil counts, and measurement of the fraction of exhaled nitric oxide (FeNO).² Patients with T2 low asthma therefore do not meet these criteria. It is important to note however that T2 low does not require the complete absence of blood eosinophils or T2 inflammation, and for some of these patients, low-grade T2 inflammation may yet contribute to their disease (Table 1).

Prevalence and Characteristics of T2 Low Asthma

Determination of T2 marker levels can be challenging, as they are affected by multiple factors. For example, inhaled and systemic corticosteroid use can reduce blood eosinophils and FeNO levels.³

Key Messages

- T2 low asthma is defined by the lack of prominent T2 inflammation and can be defined by exhaled and blood biomarkers, sputum transcriptomics, or sputum cellular characteristics.
- T2 low asthma accounts for approximately one-third to one-half of individuals with asthma.
- Characteristics of participants with T2 low asthma include higher body mass index, cigarette smoking, relative lack of responsiveness to steroids, less bronchodilator reversibility, and often the presence of neutrophilic inflammation.
- Multiple interventions currently available target characteristics of T2 low asthma, including standard inhalers, azithromycin, and lifestyle interventions of weight loss and smoking cessation.
- The future of treating T2 low asthma will involve a greater understanding of the disease pathobiology and additional testing of emerging therapies in clearly defined subgroups.

Therefore, evaluation of the presence of T2 inflammation may be best done in a patient not currently using these medications. Nevertheless, for individuals with more severe asthma, withholding steroid therapy has the potential for loss of control or exacerbation, a risk that may preclude this strict assessment. Cigarette smoke can reduce FeNO⁴; blood eosinophil levels vary diurnally⁵; and total IgE can fluctuate with steroids and environmental exposures.⁶

The true prevalence of T2 low asthma is unknown and likely strongly influenced by definition. Some examples are provided herein. Early descriptions of T2 high and T2 low asthma used gene expression of pathognomonic T2 genes, such as those encoding IL-4, IL-5, and IL-13, in airway tissue to differentiate between individuals in these groups. Woodruff et al⁷ evaluated relative expression of IL-13–inducible genes (periostin, CLCA1, SerpinB2) in the airway epithelial brushings from 42 patients with mild-to-moderate asthma and an additional 28 healthy control subjects. The T2 low subjects with asthma had similar gene expression to the healthy controls and accounted for approximately half of the participants with asthma (20 of 42). This group was more of female sex, had less bronchial hyper-reactivity, and had lower serum IgE and blood eosinophil numbers. Interestingly, they also revealed less clinical improvement on inhaled corticosteroids as measured by a change in FEV1, when compared with the T2 high group. Peters et al⁸ evaluated gene expression in sputum cells from 37 patients with asthma and 15 healthy controls. In this cohort, a mean expression for IL-4, IL-5, and IL-13 was determined, for which 70% (26 of 37) of the participants with asthma had levels above 2 SD than the healthy mean, which was defined as T2 high. In addition, sputum eosinophilia greater than 2% and blood eosinophils greater than 230/μL strongly correlated with elevated T2

gene expression. In a separate unsupervised hierarchical gene expression clustering analysis of sputum from patients with asthma by Baines et al,⁹ 3 phenotypes emerged—eosinophil enriched, neutrophil enriched, and relatively paucicellular. The neutrophil-enriched group, representing 24% of the cohort, had variable sputum eosinophils, but also had higher body mass index, more smoking, and less atopy than the eosinophil-enriched group.

Sputum inflammatory cell counts and proportions can be used to differentiate those lacking T2 inflammation. McGrath et al¹⁰ analyzed sputum of 995 subjects with asthma enrolled in clinical trials of the National Heart Lung and Blood Institute's (NHLBI) Asthma Clinical Research Network to identify characteristics of those with or without sputum eosinophilia. In these individuals, only 36% of subjects not on ICS and 17% of subjects using ICS had evidence of sputum eosinophils greater than or equal to 2%. In a subgroup treated with 4 to 6 weeks of low-dose ICS, those who remained not well controlled despite therapy were more likely to be noneosinophilic than those who were well controlled. The NHLBI's Severe Asthma Research Program (SARP), which extensively characterizes cohorts of asthma with enrichment for severe disease, performed unsupervised cluster analysis of characteristics, including sputum inflammatory cells across the severity spectrum.¹¹ In 2 clusters of more severe, overweight, older participants, sputum was almost entirely neutrophil-predominant and mixed granulocytic (neutrophils and eosinophils).

Taken together, these studies of cytology or gene expression from the airway support that a T2 low asthma group exists at a prevalence of one-quarter to one-half of patients with asthma and has consistent characteristics across the cohorts.

Bronchial epithelial cells and sputum cells are relatively challenging to procure and not usually obtained during routine clinical practice in the treatment of asthma. Therefore, clinical programs are using commercially available tests as biomarkers for this inflammation, such as a composite of FeNO and blood eosinophil count, to determine the presence and to quantify T2 inflammation with the goal of characterizing T2 low and T2 high groups. Investigators of SARP recently presented combined data from the first 3 phases of the program.¹² Of the 831 participants who had available data, 327 (39%) had both blood eosinophils of less than 300/μL and FeNO less than 25 parts per billion. Compared with the T2 high groups, this T2 low group was slightly heavier and more likely to be of female sex, with better preserved lung function, less bronchodilator and corticosteroid responsiveness, and lower sputum eosinophils and total IgE. Approximately half were classified as severe, a proportion that did not differ from the T2 high groups.

A recent publication of the United Kingdom Severe Asthma Registry,¹³ which enrolls patients with uncontrolled or severe asthma who are referred to specialist centers, compared T2 low and high groups using blood eosinophils of less than 150/μL and FeNO less than 25 ppb to define low. Of the 2225 patients enrolled in this cohort, only 9.4% (210) were low on both parameters. Compared with those who were high in both parameters, the T2 low group were also more likely to be of female sex and heavier, with better lung function, lower IgE, and a higher rate of depression, anxiety, and current smoking. The difference in prevalence between this cohort and SARP may be a

Table 1
Biomarker Characteristics of T2 High and Low Asthma

Biomarker	T2 high asthma	T2 low asthma
Blood eosinophils	>150–300 cells/μL	<150–300 cells/μL
IgE	>100 IU/mL	<100 IU/mL
Specific IgE	Present, with exposure to antigen causing symptoms	Undetectable or irrelevant
Fraction of exhaled nitric oxide	>35 ppb in 5–12 years old 50 ppb in >12 years old	<20 ppb in 5–12 years old <25 ppb in >12 years old

Abbreviations: IgE, immunoglobulin E; Ppb- parts per billion.

result of the more strict eosinophil cutoff in the United Kingdom Severe Asthma Registry and the inclusion of nonsevere asthma in SARP. Nevertheless, the clinical and phenotypic similarities found across the registries suggest that the T2 low asthma phenotype may relate to obesity and factors that impair corticosteroid responsiveness, such as smoking.

T2 Low Inflammation in Asthma

What inflammatory characteristics characterize the T2 low asthma, if not T2 pathways? Neutrophils have been implicated as not only present but potentially pathobiologically contributing to disease. In response to bacterial and fungal infections, T_H17 cells can produce multiple proinflammatory cytokines, including interferon gamma, IL-6, and IL-17, which contribute to neutrophil recruitment.^{14,15} Neutrophils release proteases, contribute to oxidative stress, and release neutrophil extracellular traps that can induce local inflammasome activation.¹⁶ Nevertheless, the presence of neutrophils in T2 low asthma is inconsistent and may represent a subphenotype of T2 low asthma or reveal the concurrent presence of type 2 inflammation.^{17–19} For example, in the aforementioned SARP cohort, sputum neutrophil counts were not the highest in this group, instead highest in those with low FeNO but high blood eosinophils.¹² In asthma, the presence of systemic inflammation, as measured by plasma IL-6, is significantly related to blood neutrophils and severity of asthma²⁰ but is not related to eosinophils or other T2 markers, such as FeNO or IgE. This also suggests a metabolic and neutrophilic inflammatory milieu of severe asthma that may coexist with other forms of inflammation in asthma.

On stimulation by a variety of airway exposures, such as irritants, infection, smoke, or allergen, airway epithelial cells release a set of alarmins, cytokines that drive local immune responses. One such alarmin, thymic stromal lymphopoeitin (TSLP), which is known to drive production of type 2 immune responses in the lung of patients with asthma, may also influence non-T2 mechanisms of asthma. For example, TSLP may induce the differentiation of naive CD4⁺ T cells into T_H17 cells.²¹ TSLP may also contribute directly and indirectly to airway remodeling in chronic asthma through pathways affecting

airway smooth muscle cells—proinflammatory gene transcription²² and migration.²³

Despite emerging literature detailing non-T2 mechanisms of asthma, this group still lacks a clear definition and will likely emerge as a collection of subgroups, each with its own pathobiological characteristics and best course of treatment.²⁴

Treating T2 Low Asthma

Evaluation of T2 biomarkers seems most useful for patients with difficult-to-treat or severe asthma,^{4,25} because high T2 biomarkers may indicate appropriateness of therapy or possible benefit from a biologic directed at components of type 2 inflammation, such as IgE, eosinophils, or the IL-4 signaling pathway. Although all available biologic therapies for asthma are targeted at T2 high asthma, some available biologic or nonbiologic interventions or pipeline therapies are agnostic to T2 inflammation or are targeted at the characteristics of T2 low asthma (Fig 1). It is important to recognize that some or many of these characteristics may also be present in and contributing to disease in T2 high asthma.

Inhaled Corticosteroids/Long-Acting Beta Agonist

Despite the variability and heterogeneity of inflammation in asthma, national and international asthma guidelines still encourage the use of standard asthma medications for all individuals.^{2,4} That is, patients should receive treatment with inhaled corticosteroid (ICS), alone or in combination with long-acting β -agonist (LABA), with reassessment of clinical response and disease control at periodic intervals and adjustment of dosage or addition of alternative therapies as indicated. The 2020 Focused Update to the Asthma Management Guidelines: A Report from the National Asthma Education and Prevention Program Coordinating Committee Expert Panel Working Group (2020 NAEPP Update) did provide updates related to the use of FeNO in the management of asthma. This report acknowledges that FeNO may be used as an adjunct test for asthma diagnosis, wherein other tests are inconclusive, but warns on factors that

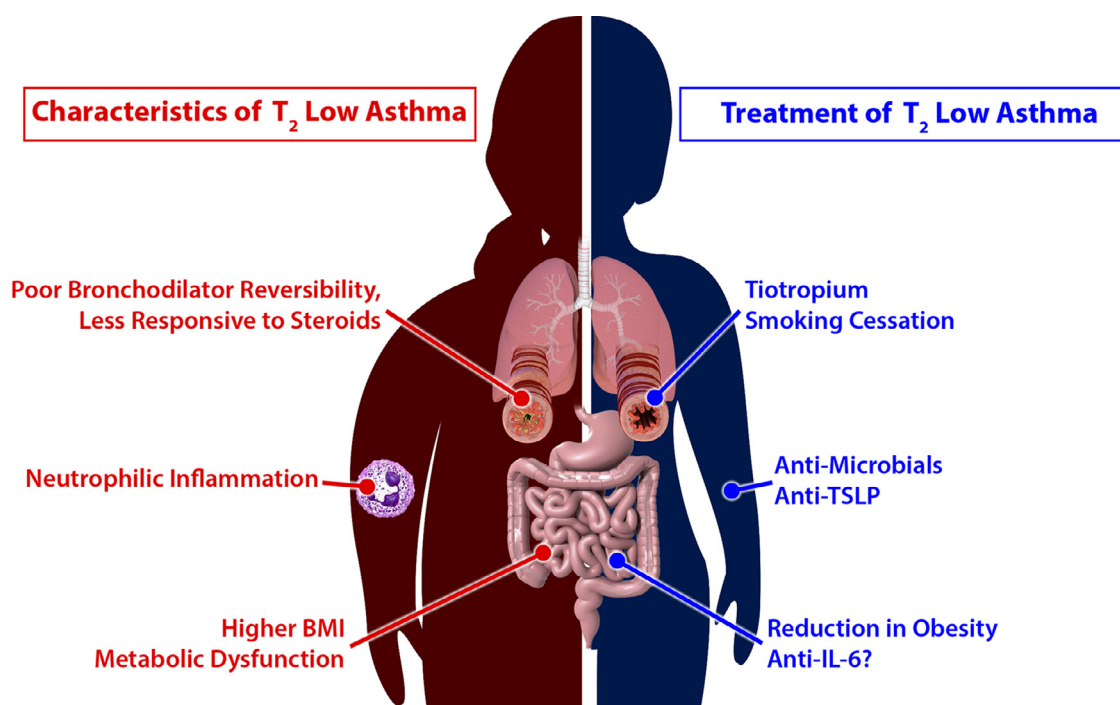


Figure 1. Clinical characteristics and focused interventions for T2 low asthma.

influence this result. Importantly, although high FeNO may indicate individuals who are more responsive to corticosteroid therapy, there are no recommendations to withhold or reduce steroid dose for individuals with low FeNO.

Long-Acting Muscarinic Antagonists

Long-acting muscarinic antagonist (LAMAs) are long-acting bronchodilators with a mechanism of action distinct from β -agonists. Two LAMAs are approved by the FDA for treatment of asthma: tiotropium bromide and umeclidinium in combination with fluticasone furoate and vilanterol. Multiple randomized controlled trials studied the efficacy of adding LAMA vs LABA to ICS for uncontrolled asthma, with similar exacerbation rates, quality of life, and use of rescue medication between the 2 bronchodilators.² In general, reported adverse events and safety outcomes were similar. Nevertheless, a real-world comparative effectiveness study, the Blacks and Exacerbations on LABA vs Tiotropium (BELT) study, revealed a higher rate of asthma-related hospitalizations in the tiotropium plus ICS group²⁶ compared with the LABA-ICS arm. Therefore, potential side effects or harms should be considered before initiating therapy with a LAMA, perhaps especially in Black patients.

LAMA may also be used as add-on therapy for adolescents and adults who are not well controlled on combination ICS-LABA therapy. The addition of LAMA may improve asthma control and quality of life but has not been clearly found to improve exacerbation risk.

The NHLBI's AsthmaNet designed and implemented a randomized, placebo-controlled, double-blinded, triple crossover study comparing placebo, ICS, and LAMA in patients at least 12 years old with mild-to-moderate asthma and was stratified by sputum eosinophils greater than 2%.²⁷ In the low eosinophil group, which accounted for 75% of participants, a similar percentage of participants had a better response to mometasone than placebo and tiotropium than placebo, suggesting tiotropium is a reasonable option for monotherapy in this T2 low inflammation group. Casale et al²⁸ reported exploratory analyses of clinical trials of tiotropium for asthma, comparing primary end points in those designated as T2 low or T2 high by IgE and blood eosinophils. Clinical benefit of tiotropium for asthma was independent of T2 status.

Azithromycin

Macrolide therapy has been of longstanding interest as a potential therapy for individuals with severe asthma, both for its anti-inflammatory effects and potential to reduce bacterial burden, including of atypical infections such as *Mycoplasma pneumoniae*.²⁹ The AMAZES study was a randomized, double-blinded, placebo-controlled trial of oral azithromycin 500 mg 3 times per week for adults with uncontrolled asthma despite the use of inhaled corticosteroid and long-acting bronchodilator.³⁰ The azithromycin group had fewer asthma exacerbations than placebo. In this trial, 57% of participants had low sputum eosinophils. A secondary analysis revealed similar reduction in asthma exacerbations among those with high and low sputum eosinophils. A predefined substudy of AMAZES reported that treatment with azithromycin reduced airway burden of *Haemophilus influenzae* in the noneosinophilic group and may have a mild effect on inflammatory cytokines.³¹ Although azithromycin was generally well tolerated except for increased rate of diarrhea, this trial excluded individuals with prolonged QT interval and hearing impairment, and increase in antibiotic resistance was noted.³² These findings suggest that azithromycin is a treatment option for select individuals with uncontrolled moderate-to-severe T2 low asthma and may function through reducing airway bacterial burden. Patients should be educated on risks and adverse effects, and electrocardiographic monitoring of QT interval is indicated.

Obesity and Weight Loss

The relationship of asthma and obesity is very complex. Epidemiologic data support the possibility of multiple pathways relating these 2 conditions. Obesity may increase the risk of developing asthma through influencing type 1 and type 2 inflammation.^{33,34} In a cross-sectional analysis of the National Health and Nutrition Examination Study,³⁵ the relationship between obesity and asthma was strongest in those with high insulin levels, supporting a metabolic or insulin-resistance mechanism as well. Asthma may also increase the risk of developing obesity, which may be attributed at least in part to use of systemic corticosteroids and reduced physical activity.³⁶ Comorbid obesity in asthma may contribute to severity through corticosteroid resistance, less response to β -agonists,³⁷ and influencing lung mechanics.³⁸

Although weight loss has not been effective for improving asthma in all trials, it is likely that, in addition to metabolic and cardiovascular benefits, overweight or obese patients will experience less dyspnea in addition to better asthma control.³⁹ Some symptom improvement will be attributable to improvements in chronic deconditioning, mechanical restriction, and potentially comorbid sleep apnea.⁴ With weight loss, some patients with asthma may also experience improved lung function, reduction in exacerbations, and improvement of systemic inflammatory biomarkers, such as IL-6 and leptin.⁴⁰ A recent review of lifestyle interventions summarized the conflicting data for obesity-related diet and exercise modifications and called for rigorous study on the physiology, immunology, and metabolic influences on outcomes.⁴¹

Smoking Cessation

Multiple inflammatory pathways are also implicated in the role of cigarette smoke on the development and severity of asthma.⁴² Some of these pathways, including type 1 inflammation, neutrophil activity, and downstream effects of IL-6 and IL-17, are implicated in T2 low asthma irrespective of smoking status, suggesting primary or secondhand cigarette smoke exposure is likely to profoundly worsen, if not cause, T2 low disease for some patients with asthma. Indeed, cigarette smoke exposure is known to increase the risk of asthma exacerbations, even for patients who seem otherwise well controlled.^{4,43}

Patients with asthma who are currently smoking, or those who have a smoking history of more than 10 pack years, are usually excluded from clinical trials studying interventions for asthma, to reduce the likelihood of comorbid chronic obstructive pulmonary disease. This has, therefore, required that for these patients, use of biologic and nonbiologic medications is extrapolated based on responsiveness of nonsmokers. Active and previous smoking was found to impair the therapeutic response of both oral and inhaled corticosteroids for asthma, abrogating improvement in lung function and symptom control.^{44,45} Higher doses of inhaled corticosteroids may therefore be necessary to gain adequate control of uncontrolled asthma symptoms. Individuals who smoke should be strongly encouraged to stop smoking and provided resources and advice to this end.⁴ After smoking cessation or avoidance of passive smoke exposure, individuals with asthma can expect a reduction in exacerbations, improvement in asthma control, and improvement in lung function.^{46,47} Electronic cigarette (e-cig, vaping) use may induce airway inflammation similar to that induced by tobacco smoke, is associated with asthma exacerbations, and may result in the development of lung diseases, including asthma.^{48,49} Cessation of electronic smoking may therefore be indicated for improved control of T2 low asthma; however, robust clinical data are not yet available.

Alternative Biologics and Novel Interventions Under Investigation

Thymic Stromal Lymphopoietin

Because TSLP influences both T₂ and non-T₂ pathways as discussed previously, inhibition of this molecule may present an option for treatment of T₂ low asthma. Corren et al⁵⁰ presented phase 2 data of a randomized, double-blinded, placebo-controlled dose ranging trial of tezepelumab, an anti-TSLP antibody, in adult patients with moderate-to-severe uncontrolled asthma. A subpopulation analysis stratified the primary outcome, annualized exacerbation rate, by blood eosinophil count (<250/ μ L), FeNO (<24 ppb), and T_H2 low vs high status (low defined as IgE < 100 IU/mL or eosinophils < 140/ μ L). In each of these analyses, the exacerbation rate was similar in the high and low groups, suggesting an anti-TSLP antibody may be effective for T₂ low asthma. The phase 3 study results were not yet published at the time of this publication.

Interventions targeting neutrophilic, T_H17, and T_H1 inflammation have thus far failed to reveal benefit for patients with severe asthma. Brodalumab, an anti-IL17 monoclonal antibody, failed to reveal improvement in asthma control in an undifferentiated population of patients with moderate-to-severe asthma.⁵¹ Tocilizumab, an anti-IL-6 antibody, failed to protect against allergen-induced bronchoconstriction in a small clinical trial of patients with mild intermittent asthma allergic to house dust mite.⁵² Inhaled IFN- β did not meet the primary end point of preventing viral-induced asthma symptoms in participants using inhaled corticosteroids.⁵³ Despite these disappointing initial results, targeting one or more of these pathways may reveal benefit in subpopulations of asthma with prominent neutrophilic inflammation or elevated cytokine levels.

The NHLBI's PreCISE Network is using an adaptive platform clinical trial design to evaluate novel therapies for severe asthma with the development of predictive biomarkers, and most of the planned interventions may potentially benefit those with T₂ low asthma.⁵⁴ Imatinib, a kit receptor inhibitor, will be evaluated in T₂ low population; an anti-IL-6 clazakizumab is predicted to target those with elevated IL-6, such as in obesity and metabolic dysfunction; medium-chain triglyceride supplementation may influence high arginine metabolic asthma; the bacterial extract BronchoVaxom may influence airway immune responses; the s-nitrosogluthathione reductase cavanostat may benefit those with increased enzyme activity by genotype.

Evaluation of Comorbidities or Alternative Diagnoses

Lastly, for a patient with T₂ low asthma who has difficult-to-treat or severe disease, careful evaluation for mimickers and comorbidities can reveal alternative diseases that can be addressed separately. For example, patients should have asthma diagnosis supported by bronchodilator reversibility or airway hyperreactivity through pulmonary function testing. Lung volumes and carbon monoxide diffusion will help to rule out emphysema and interstitial disease. High-resolution chest computed tomography can detect parenchymal and airway lesions—including bronchiectasis—and may eventually be used as a biomarker for phenotype in asthma. Patients with recurrent bronchitis or pneumonia should be evaluated for immunodeficiency and aspiration. Bronchoscopy may be very helpful to evaluate for tracheo-bronchomalacia and subglottic stenosis and for culture of infectious agents. Functional laryngoscopy can help to evaluate for vocal cord dysfunction.

Conclusion

T₂ low asthma is present in one-quarter to one-half of patients with asthma. Clinical characteristics of T₂ low asthma are consistent across cohorts and include higher body mass index, relative lack of

response to steroids, higher presence of smoking, and frequent presence of neutrophil-predominant inflammation in the airway. Although T₂ low asthma is less well understood than asthma with T₂ inflammation, a variety of interventions are currently available to treat these characteristics and contributing factors of T₂ low asthma. Novel interventions under investigation may provide additional therapeutic options for patients with T₂ low asthma that is not controlled by the available treatments. Studies focused on defining T₂ low asthma and identifying pathobiologic mechanisms of T₂ low asthma will likely guide the development of new interventions or identify subpopulations who may benefit from targeted therapies.

References

- Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med*. 2012;18(5):716–725.
- 2020 Focused Updates to the Asthma Management Guidelines: A Report From the National Asthma Education and Prevention Program Coordinating Committee Expert Panel Working Group. National Heart, Lung, and Blood Institute; 2020. Available at: <https://www.nhlbi.nih.gov/health-topics/asthma-management-guidelines-2020-updates>. Accessed June 14, 2021.
- Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FeNO) for clinical applications. *Am J Respir Crit Care Med*. 2011;184(5):602–615.
- Global Initiative for Asthma. *Global strategy for asthma management and prevention*. 2019. Available at: www.ginasthma.org. Accessed June 14, 2021.
- Winkel P, Statland BE, Saunders AM, Osborn H, Kupperman H. Within-day physiologic variation of leukocyte types in healthy subjects as assayed by two automated leukocyte differential analyzers. *Am J Clin Pathol*. 1981;75(5):693–700.
- Ballow M, Akdis CA, Casale TB, et al. Immune response modifiers in the treatment of asthma: a PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology. *J Allergy Clin Immunol*. 2012;130(2):311–324.
- Woodruff PG, Modrek B, Choy DF, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med*. 2009;180(5):388–395.
- Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin Immunol*. 2014;133(2):388–394.
- Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. *J Allergy Clin Immunol*. 2011;127(1):153–160.
- McGrath KW, Icitovic N, Boushey HA, et al. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. *Am J Respir Crit Care Med*. 2012;185(6):612–619.
- Moore WC, Hastie AT, Li X, et al. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *J Allergy Clin Immunol*. 2014;133(6):1557–1563. e5.
- Li X, Hastie AT, Peters MC, et al. Investigation of the relationship between IL-6 and type 2 biomarkers in patients with severe asthma. *J Allergy Clin Immunol*. 2020;145(1):430–433.
- Jackson DJ, Busby J, Pfeffer PE, et al. Characterisation of patients with severe asthma in the UK Severe Asthma Registry in the biologic era. *Thorax*. 2021;76(3):220–227.
- Acosta-Rodriguez EV, Rivino L, Geginat J, et al. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat Immunol*. 2007;8(6):639–646.
- Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T(H)17 cells. *Nature*. 2008;453(7198):1051–1057.
- Wright TK, Gibson PG, Simpson JL, McDonald VM, Wood LG, Baines KJ. Neutrophil extracellular traps are associated with inflammation in chronic airway disease. *Respirology*. 2016;21(3):467–475.
- Irvin C, Zafar I, Good J, et al. Increased frequency of dual-positive TH2/TH17 cells in bronchoalveolar lavage fluid characterizes a population of patients with severe asthma. *J Allergy Clin Immunol*. 2014;134(5):1175–1186. e7.
- Liu W, Liu S, Verma M, et al. Mechanism of T_H2/T_H17-predominant and neutrophilic T_H2/T_H17 low subtypes of asthma. *J Allergy Clin Immunol*. 2017;139(5):1548–1558. e4.
- Choy DF, Hart KM, Borthwick LA, et al. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. *Sci Transl Med*. 2015;7(301):301ra129.
- Peters MC, McGrath KW, Hawkins GA, et al. Plasma interleukin-6 concentrations, metabolic dysfunction, and asthma severity: a cross-sectional analysis of two cohorts. *Lancet Respir Med*. 2016;4(7):574–584.
- Liang Y, Yu B, Chen J, et al. Thymic stromal lymphopoietin epigenetically upregulates Fc receptor γ subunit-related receptors on antigen-presenting cells and induces T_H2/T_H17 polarization through dectin-2. *J Allergy Clin Immunol*. 2019;144(4):1025–1035. e7.
- Shan L, Redhu NS, Saleh A, Halayko AJ, Chakir J, Gounni AS. Thymic stromal lymphopoietin receptor-mediated IL-6 and CC/CXC chemokines expression in human airway smooth muscle cells: role of MAPKs (ERK1/2, p38, and JNK) and STAT3 pathways. *J Immunol*. 2010;184(12):7134–7143.

23. Redhu NS, Shan L, Movassagh H, Gounni AS. Thymic stromal lymphopoietin induces migration in human airway smooth muscle cells. *Sci Rep*. 2013;3:2301.
24. Zounemat Kermani N, Sagi M, Agapow P, et al. Type 2-low asthma phenotypes by integration of sputum transcriptomics and serum proteomics. *Allergy*. 2021;76(1):380–383.
25. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J*. 2014;43(2):343–373.
26. Wechsler ME, Yawn BP, Fuhlbrigge AL, et al. Anticholinergic vs long-acting β -agonist in combination with inhaled corticosteroids in Black adults with asthma: the BELT Randomized Clinical Trial. *JAMA*. 2015;314(16):1720–1730.
27. Lazarus SC, Krishnan JA, King TS, et al. Mometasone or tiotropium in mild asthma with a low sputum eosinophil level. *N Engl J Med*. 2019;380(21):2009–2019.
28. Casale TB, Bateman ED, Vandewalker M, et al. Tiotropium Respimat add-on is efficacious in symptomatic asthma, independent of T2 phenotype. *J Allergy Clin Immunol Pract*. 2018;6(3):923–935. e9.
29. Carr TF, Kraft M. Chronic infection and severe asthma. *Immunol Allergy Clin North Am*. 2016;36(3):483–502.
30. Gibson PG, Yang IA, Upham JW, et al. Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2017;390(10095):659–668.
31. Shukla SD, Taylor SL, Gibson PG, et al. Add-on azithromycin reduces sputum cytokines in non-eosinophilic asthma: an AMAZES substudy. *Thorax*. doi:10.1136/thoraxjnl-2020-216331.
32. Taylor SL, Leong LEX, Mobegi FM, et al. Long-term azithromycin reduces Haemophilus influenzae and increases antibiotic resistance in severe asthma. *Am J Respir Crit Care Med*. 2019;200(3):309–317.
33. Marijse GS, Seys SF, Schelpe AS, et al. Obese individuals with asthma preferentially have a high IL-5/IL-17A/IL-25 sputum inflammatory pattern. *Am J Respir Crit Care Med*. 2014;189(10):1284–1285.
34. Rastogi D. Pediatric obesity-related asthma: a prototype of pediatric severe non-T2 asthma. *Pediatr Pulmonol*. 2020;55(3):809–817.
35. Cardet JC, Ash S, Kusa T, Camargo CA, Israel E. Insulin resistance modifies the association between obesity and current asthma in adults. *Eur Respir J*. 2016;48(2):403–410.
36. Chen YC, Tu YK, Huang KC, Chen PC, Chu DC, Lee YL. Pathway from central obesity to childhood asthma. Physical fitness and sedentary time are leading factors. *Am J Respir Crit Care Med*. 2014;189(10):1194–1203.
37. McGarry ME, Castellanos E, Thakur N, et al. Obesity and bronchodilator response in Black and Hispanic children and adolescents with asthma. *Chest*. 2015;147(6):1591–1598.
38. Al-Alwan A, Bates JH, Chapman DG, et al. The nonallergic asthma of obesity. A matter of distal lung compliance. *Am J Respir Crit Care Med*. 2014;189(12):1494–1502.
39. Haase CL, Lopes S, Olsen AH, Satylganova A, Schnecke V, McEwan P. Weight loss and risk reduction of obesity-related outcomes in 0.5 million people: evidence from a UK primary care database. *Int J Obes (Lond)*. 2021;45(6):1249–1258.
40. Peters U, Dixon AE, Forno E. Obesity and asthma. *J Allergy Clin Immunol*. 2018;141(4):1169–1179.
41. Nyenhuis SM, Dixon AE, Ma J. Impact of lifestyle interventions targeting healthy diet, physical activity, and weight loss on asthma in adults: what is the evidence? *J Allergy Clin Immunol Pract*. 2018;6(3):751–763.
42. Polosa R, Thomson NC. Smoking and asthma: dangerous liaisons. *Eur Respir J*. 2013;41(3):716–726.
43. Osborne ML, Pedula KL, O'Hollaren M, et al. Assessing future need for acute care in adult asthmatics: the Profile of Asthma Risk Study: a prospective health maintenance organization-based study. *Chest*. 2007;132(4):1151–1161.
44. Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC. Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J Respir Crit Care Med*. 2003;168(11):1308–1311.
45. Lazarus SC, Chinchilli VM, Rollings NJ, et al. Smoking affects response to inhaled corticosteroids or leukotriene receptor antagonists in asthma. *Am J Respir Crit Care Med*. 2007;175(8):783–790.
46. Chaudhuri R, Livingston E, McMahon AD, et al. Effects of smoking cessation on lung function and airway inflammation in smokers with asthma. *Am J Respir Crit Care Med*. 2006;174(2):127–133.
47. Rayens MK, Burkhart PV, Zhang M, et al. Reduction in asthma-related emergency department visits after implementation of a smoke-free law. *J Allergy Clin Immunol*. 2008;122(3):537–541. e3.
48. Kaur G, Pinkston R, McLemore B, Dorsey WC, Batra S. Immunological and toxicological risk assessment of e-cigarettes. *Eur Respir Rev*. 2018;27:(147) 170119.
49. Bozier J, Chivers EK, Chapman DG, et al. The evolving landscape of e-cigarettes: a systematic review of recent evidence. *Chest*. 2020;157(5):1362–1390.
50. Corren J, Parnes JR, Wang L, et al. Tezepelumab in adults with uncontrolled asthma. *N Engl J Med*. 2017;377(10):936–946.
51. Busse WW, Holgate S, Kerwin E, et al. 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.
52. Revez JA, Bain LM, Watson RM, et al. Effects of interleukin-6 receptor blockade on allergen-induced airway responses in mild asthmatics. *Clin Transl Immunology*. 2019;8(6):e1044.
53. Djukanovic R, Harrison T, Johnston SL, et al. The effect of inhaled IFN- β on worsening of asthma symptoms caused by viral infections. A randomized trial. *Am J Respir Crit Care Med*. 2014;190(2):145–154.
54. Israel E, Denlinger LC, Bacharier LB, et al. PrecISE: precision medicine in severe asthma: an adaptive platform trial with biomarker ascertainment. *J Allergy Clin Immunol*. 2021;147(5):1594–1601.