Review

Beyond the Usual Suspects: Unmasking Low-T2 Asthma in Children

Iva Mrkić Kobal , Marta Navratil , Helena Munivrana Škvorc , Andrija Miculinić , Davor Plavec *

Posted Date: 16 December 2025

doi: 10.20944/preprints202512.1436.v1

Keywords: T2 low asthma; biomarkers; children; endotype; neutrophils; therapy

 doi:10.20944/preprints202512.1436.v1

Review

Beyond the Usual Suspects: Unmasking Low-T2 Asthma in Children

Iva Mrkić Kobal [1,2], Marta Navratil [2,3], Helena Munivrana Škvorc [1, 4,] Andrija Miculinić [1] and Davor Plavec [5,2,*]

[1] Children's Hospital Srebrnjak, Zagreb Croatia; imrkic@bolnica-srebrnjak.hr
[2] Medical Faculty University of Osijek, Osijek, Croatia
[3] Children's Hospital Zagreb, Zagreb, Croatia
[4] University North, Varaždin, Croatia
[5] Prima Nova, Zagreb, Croatia
* Correspondence: davor.plavec@primanova.hr

Abstract

Type -2 (T2) low asthma in children represents a clinically important yet still insufficiently recognized endotype. Current data suggest that it is more prevalent than previously thought and is defined by low type 2 biomarkers, non-allergic clinical profiles, and a high burden of comorbidities such as obesity and passive smoke exposure, while remaining largely understudied phenotype in terms of validated biomarkers and specific targeted therapies. This review aims to highlight and clarify T2-low asthma in children by summarizing emerging evidence on risk factors, pathophysiological mechanisms, biomarkers, and treatment strategies, with the goal of informing and improving future care for affected children.

Keywords: T2 low asthma; biomarkers; children; endotype; neutrophils; therapy

1. Introduction – Asthma: More Than Eosinophils

Asthma has long been recognized as a chronic airway inflammatory disease, with a prominent role attributed to eosinophils and the type-2 (T2) immune response [1]. This paradigm, focused on eosinophil subtypes, has led to therapeutic breakthroughs and the development of biological agents (anti- immunoglobulin (Ig)E, anti- interleukin (IL)5, anti-IL4 receptor (R)) [2]. These innovations have significantly improved outcomes for patients with severe T2-high asthma. Clinical practice and large cohort studies have shown that eosinophil-driven asthma responds best to corticosteroid and biologic therapy [3,4]. However, a growing body of research and clinical experience highlights the heterogeneity of asthma, which extends well beyond the traditional focus on eosinophils [5]. Asthma is not a single disease, but rather an umbrella term encompassing a group of clinical syndromes with multiple pathogenic pathways (endotypes), phenotypic variations, and distinct immune characteristics [6]. The new "omics era"—characterized by the systematic use of transcriptomics, proteomics, and other advanced technologies—has revealed a wide range of inflammatory profiles. Among these, particularly in children, rarely identified or previously "masked" forms such as TT2-low) asthma are beginning to emerge [7]. T2-low asthma (non-eosinophilic, often neutrophilic or paucigranulocytic asthma) is receiving increasing attention for several reasons. In large population analyses and among schoolchildren, T2-low asthma accounts for a significant proportion of asthma patients—according to some authors, up to 50% [8,9]. These patients lack a response to standard therapy and often have comorbidities such as obesity or metabolic syndrome or are exposed to environmental influences especially in children. Existing diagnostic strategies and treat-to-target approaches largely fail to identify these patients due to lack of practical and reliable biomarkers - making timely and personalized treatment more difficult [7,10,11]. Recent research indicates that the

 doi:10.20944/preprints202512.1436.v1

presence of mixed or overlapping inflammatory profiles (T2 and non T2 pathways), along with complex mechanisms at the levels of epithelium, microbiota, systemic inflammation, and other low-grade processes, calls for a redefinition of diagnostic, prognostic and therapeutic paradigms in asthma [12]. Additionally, biomarker-guided approaches, important for T2-high asthma still haven't found a clear position in recognition and treatment of T2-low and untypical forms of asthma. Complexity of these non-classic phenotypes emphasizes the need for development of new diagnostic tools regarding the whole spectrum of immunological mechanism, epithelial barrier, microbiome dysregulation and metabolic abnormalities [10,13]. Therefore, the aim of this narrative review is to present clinical and pathophysiological diversity of asthma with focus on T2-low asthma and "masks" of non-eosinophilic inflammation, diagnostic challenges in T2-low asthma, the latest insights into the mechanism, clinical significance, and treatment possibilities of "beyond eosinophils" particularly in children and adolescents.

2. Materials and Methods

To achieve our aim of investigating the characteristics, biomarkers, pathophysiology, and therapeutic approaches for T2-low asthma in children, we conducted a literature search in the PubMed and Web of Science databases. Papers published between January 2020, and November 2025 were included, identified using the following keywords in various combinations: "children", "pediatric", "asthma", "T2-low", "type 2 low", "non-type 2", "therapy", "treatment", "biomarkers", "pathophysiology", and "endotype". Only peer-reviewed articles written in English were considered. We screened titles and abstracts for relevance, prioritizing studies with pediatric populations (age <18 years), explicit T2-low asthma definitions (e.g., low blood eosinophils <300 cells/μL and/or FeNO <25 ppb), and primary data on biomarkers, mechanisms, or interventions. Full texts were then assessed for eligibility, excluding reviews, case reports, and non-English publications. Disagreements were resolved by consensus between the authors to ensure high relevance and methodological rigor.

3. Results

3.1. Shifting Paradigms: From T2 High to T2 Low

Asthma phenotyping began in the late 1940s, when Rackemann [14] identified two distinct types of the disease: "extrinsic" asthma, primarily linked to atopy, and "intrinsic" asthma, which occurs without atopic features. These early efforts to phenotype asthma relied solely on patient's clinical and functional features, with no attention given to underlying molecular profiles [15]. At the end of 1990s Wezel et al. have defined two different asthma phenotypes: eosinophilic and neutrophilic [16]. In 2008, Anderson introduced the concept of asthma endotypes, to understand different forms of the disease. The concept of endotype emerged as an idea of a single pathway underlying all clinical features in a single phenotype. The goal of endotyping according to Anderson was to link biological mechanisms to clinical manifestations to develop more precise diagnostics and targeted therapeutic approaches. Rather than viewing asthma as a single condition, he pointed out that there are different pathophysiological mechanisms (e.g., eosinophil inflammation, neutrophils, epithelial dysfunction, airway hyperresponsiveness) that lead to similar clinical symptoms [17]. One well-defined asthma endotype, known as classical T2)-high asthma, has been the focus of extensive research, particularly in pediatric and adolescent populations. This endotype is distinguished by elevated activity of type 2 cytokines (IL-4, IL-5, and IL-13) which drive airway eosinophilia and allergic inflammation. Clinically, T2-high asthma is often identified through increased serum total immunoglobulin E (IgE), elevated blood eosinophil counts, higher fractional exhaled nitric oxide (FeNO) levels and good response on corticosteroids treatment [18]. T2-low asthma is a biologically distinct endotype that differs from the classical T2-high inflammatory pattern. It is characterized by reduced activation of type 2 immune pathways and consequently low levels of biomarkers such as interleukins IL-4, IL-5, and IL-13, total IgE, blood and airway eosinophils, and FeNO [19]. Based on inflammatory activity and cellular response we differentiate several subtypes of T-2 low asthma: neutrophilic, paucigranulocyte, mixed granulocyte asthma and asthma related to obesity[20]. An endotype may

 doi:10.20944/preprints202512.1436.v1

be conceptualized as a "hidden subtype" of asthma, one whose biological identity often remains obscured amidst clinical complexity. The additional challenge remains the fact that these biological subtypes are not fixed; they can shift or overlap over time in response to various internal and external influences. For instance, a child's underlying endotype may remain "masked" until a change in therapy, a shift in environmental exposure, or the development of a new immune response unmasks a different inflammatory mechanism [21].

3.2. Spotlight on T2 Low Asthma in Children

The reported prevalence of T2-low asthma differs widely across studies due to variations in its definition, the populations examined, and the diagnostic criteria applied, making direct comparison of findings challenging [22] A study conducted in the United States analyzed data from the National Health and Nutrition Examination Survey (NHANES) from 2007 to 2012, including 4,284 children aged 6 to 17 years. According to the results, 45.7% of children with asthma had T2-low asthma, defined as a blood eosinophil count of less than 300 cells/μL and a FeNO level of less than 25 ppb. These data suggest that T2-low asthma is a significant endotype among children with asthma in the United States [23]. The ALLIANCE cohort study represents one of the most comprehensive studies to analyze the distribution of T2-inflammatory asthma phenotypes across different age groups, including children, adolescents, and adults. It is a multicenter study conducted by the German Lung Research Center (DZL), with a total of 1,125 participants, of whom 776 had asthma and 349 were healthy controls without a diagnosis of asthma. The following phenotypes were defined: T2-high (elevated eosinophils and allergic sensitization), eosinophilic without atopy, atopic without eosinophilia and T2-low (no eosinophilia and no sensitization). The threshold for eosinophilia in children was set at ≥ 470 cells/μL, while for adults it was ≥ 360 cells/μL. The results of the ALLIANCE study indicate that T2-inflammatory phenotypes dominate in childhood asthma, while T2-low asthma represents an important clinical entity that requires a specific therapeutic approach. The prevalence of T2-low asthma in the study ranged from 11.9% to 64.8%, depending on the age group of the children. The prevalence rates were higher in infants and preschool children. These findings further emphasize the need for a phenotypic and endotypic approach to the diagnosis and treatment of children with asthma [24]. A retrospective study conducted in China analyzed a data of children and adolescents with asthma who were hospitalized for asthma exacerbations in period from January 2016 to December 2021. The children were classified into four asthma phenotypes: Only-atopy, Only-EOS, T2-high, and T2-low groups based on their blood EOS count and sIgE results before or after 3 days of admission. Different eosinophil thresholds have been proposed to stratify asthma severity and T2 inflammation, with 150 cells/μL, 300 cells/μL, and 470 cells/μL. The prevalence rates of T2-low asthma were: 19.4%, 25.6% and 28.2%, according to EOS count. [25] Overall, current evidence suggests that T2-low asthma represents approximately 30–50% of asthma cases, underscoring its clinical relevance and the need for tailored therapeutic approaches (Table 1).

3.3. Beneath the Surface: Mechanism and Biomarkers

1. Risk Factors and Pathophysiological Modulators of T2-Low Asthma in Children and Adolescents

a. Environmental Exposures and Epithelial Injury as Early Drivers of T2-Low Asthma

Environmental exposures appear to play a predominant role in the early development and priming of T2-low asthma in children, establishing a foundation for non-eosinophilic, neutrophil-dominant airway inflammation [26]. Environmental risk factors for T2-low asthma include older age, female gender, obesity, exposure to tobacco smoke, poorer lung function, and frequent respiratory infections in early life [27,28]. Chronic exposure to air pollutants, including nitrogen dioxide (NO_2), ozone (O_3), and fine particulate matter ($PM_{2.5}$), have been shown to directly injure airway epithelial cells and compromise barrier integrity. This dysfunction not only facilitates the penetration of environmental agents and microbial products but also triggers the release of epithelial-derived "alarmins" and pro-inflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor – α (TNF-

 doi:10.20944/preprints202512.1436.v1

α) [29]. These mediators subsequently activate Th1 and Th17 immune pathways and promote neutrophil recruitment and activation, hallmarks of T2-low asthma endotypes. In parallel, recurrent viral infections during early life - particularly with rhinovirus, respiratory syncytial virus (RSV), and influenza - can skew immune maturation toward type 1 and type 3 responses, suppressing Th2 polarization and driving non-eosinophilic inflammation [30,31]. This deviation may be further amplified by alterations in the microbiome due to antibiotic exposure, urban living, or reduced microbial diversity, all of which have been linked to a loss of immune tolerance and the emergence of T2-independent airway disease [32–34].

b. Vitamin D deficiency

The association with vitamin D3 is controversial. Vitamin D deficiency is associated with increased inflammation, worse lung function, and more severe asthma exacerbations in children, including those with T2-low asthma [35]. Low vitamin D levels correlate particularly with neutrophilic inflammation and Th17-mediated cytokines in T2-low asthma, which is characterized by more steroid resistance and distinct immune pathways compared to T2-high asthma [36]. Vitamin D supplementation may help reduce inflammation and improve lung function and asthma control in children, though most strong evidence relates to the broader pediatric asthma population [37]. However, the role of vitamin D in T2-low asthma specifically appears crucial, given its stronger correlation with lung function and airway obstruction compared to T2-high asthma, highlighting potential targeted therapeutic benefits [36].

c. Hormonal and Pubertal Modulation of the T2-Low Asthma Phenotype

While environmental exposures initiate T2-low inflammatory pathways early in life, pubertal and hormonal changes act as key modulators that influence the persistence, phenotype, and treatment response of asthma during adolescence. Puberty represents a transitional period characterized by profound endocrine, metabolic, and structural changes, all of which interacts with the immune system and airway physiology. The sex shift in asthma prevalence—from a male predominance in childhood to a female predominance after puberty—strongly suggests that sex hormones exert active immunomodulatory effects [38,39]. Estrogens and progesterone jointly upregulate IL-17A expression in Th17 cells via a Let-7f/IL-23R-dependent pathway [40]. Elevated IL-17A levels correlate with neutrophilic inflammation, a hallmark of corticosteroid-resistant asthma, indicating that estrogen's role extends beyond classical type 2 inflammation [41]. Conversely, studies in allergic mouse models demonstrated that testosterone treatment reduces neutrophilic inflammation [42]. Beyond immunological effects, estrogen also modulates airway epithelial function [43] and glucocorticoid receptor signaling [44]. Notably, sex differences in airway dimensions appear to result primarily from hormonal changes during puberty and occur independently of height, supporting a mechanistic link between pubertal development and asthma susceptibility [45]. Collectively, these findings suggest that pubertal hormonal changes function as amplifiers rather than initiators of T2-low inflammation, reinforcing the neutrophilic, non-eosinophilic phenotype established by earlier environmental exposures. The combined impact of environmental priming and hormonal modulation likely underlies the persistence, severity, and steroid insensitivity characteristic of T2-low asthma in adolescent females.

2. Potential Mechanisms of T2-Low Asthma

Several distinct mechanisms have been proposed to underlie T2-low asthma, reflecting its complex pathophysiology and poor responsiveness to conventional corticosteroid therapy [46].

a. Non-T2 Inflammation in the Lung: neutrophilic asthma

One of the primary mechanisms involves non-T2 inflammation within the airways, typically marked by neutrophilic infiltration. This inflammatory pattern is often associated with type 1 (T1) immune responses mediated by interferons (IFNs), or type 3 (T3) immune pathways driven by IL-17. As previously described, these immune responses can be triggered by infections, environmental pollutants, or microbiome alterations, contributing to persistent airway inflammation and remodeling even in the absence of eosinophilic activity [47].

b. Role of Neutrophils and neutrophil extracellular traps (NETs)in T2-Low Asthma

 doi:10.20944/preprints202512.1436.v1

Neutrophils, the most abundant cells of the innate immune system, play a central role in host defense through mechanisms such as phagocytosis, degranulation, and the formation of NETs. NETs are web-like structures composed of DNA and granule proteins that trap and kill pathogens, including bacteria and viruses, thereby preventing their dissemination [48]. Airway neutrophilia has been frequently observed in patients with more severe and chronic forms of asthma and is a hallmark feature of the T2-low phenotype. Although neutrophilic inflammation can occur independently of eosinophilia, the presence of both (mixed granulocytic inflammation) is often associated with particularly severe disease. Neutrophilia is also commonly seen during asthma exacerbations and is believed to contribute to epithelial injury through several mechanisms: release of proteolytic enzymes, induction of oxidative stress, stimulation of goblet cell degranulation, and NET formation [49]. NETs can disrupt the integrity of the bronchial epithelium by damaging tight junctions, leading to the leakage of intracellular components. Through direct interaction with bronchial epithelial cells (BECs), NETs stimulate the secretion of inflammatory mediators that enhance airway inflammation and contribute to respiratory symptoms [50–52]. Specific components of NETs, such as high-mobility group box 1 protein (HMGB1), can stimulate BECs to produce mediators implicated in asthma pathogenesis, including TSLP, TNF-α, MMP-9, and VEGF. Consequently, NETs may play a role in asthma development and exacerbations by compromising bronchial epithelial barrier integrity and inducing the release of upstream cytokines, or alarmins, such as TSLP and IL-33 [53]. Moreover, various bacterial components (e.g., formyl-methionyl-leucyl-phenylalanine, fMLP) [54] as well as viral pathogens such as rhinovirus and influenza virus [55] have been shown to induce the formation of NETs. Recent studies further indicate that rhinoviral infections trigger NETs release, which may paradoxically amplify type 2 (T2) T cell responses. This enhanced T2 inflammation can contribute to airway hyperresponsiveness and disease exacerbations, even in patients with pre-existing allergic asthma [56]. The NET/IL-17A axis plays an important pathogenic role in asthma exacerbation, linking airway inflammation to fibroblast dysfunction and fibrosis [57].

c. Neutrophilic asthma

Neutrophilic airway inflammation is commonly associated with environmental exposures, such as air pollutants, as well as with infections and bacterial colonization, both of which can exacerbate inflammation and contribute to increased disease severity [58]. Chronic bacterial colonization and recurrent infections constitute an additional important factor driving to neutrophilic airway inflammation in T2-low asthma. Patients with T2-low asthma frequently exhibit reduced airway bacterial diversity and an increased presence of respiratory pathogens, particularly *Moraxella* and *Haemophilus* species. In a bronchoscopy study of 126 children with severe asthma, 15.9% showed isolated neutrophilia in BAL, of whom 65% had detectable respiratory pathogens [59]. Lung microbiome dysbiosis, including persistent colonization by non-typeable *Haemophilus influenza* [60], has been associated with the neutrophilic phenotype, suggesting a potential therapeutic role for antibiotics such as azithromycin. Patients experiencing non-eosinophilic asthma exacerbations, characterized by low FeNO levels (≤20 ppb) and higher bacterial load, may derive greater benefit from antimicrobial therapy compared with those with eosinophilic exacerbations, who typically exhibit elevated FeNO levels (≥50 ppb) and a lower bacterial burden [61].

d. Non-T2 Immune Pathways in T2-Low Asthma: Type 3 and Type 1 Immunity

Epithelial injury contributes to non-eosinophilic, T2-low asthma by compromising the airway barrier and facilitating the entry of environmental agents. This epithelial dysfunction triggers the release of alarmins and pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), which activate TH1 and TH17 cells, as well as ILC1 and ILC3. The resulting type 1 (IFN-mediated) and type 3 (IL-17–mediated) immune responses drive neutrophilic inflammation, steroid resistance, and more severe asthma phenotypes [29].

e. Type 3 (IL-17–Mediated) Immunity

Type 3 immunity is driven by the IL-17 cytokine family, particularly IL-17A, which is secreted by a variety of immune cells, including Th17 cells, γδ T cells, $CD8^+$ T cells, natural killer T cells, and

 doi:10.20944/preprints202512.1436.v1

subsets of innate lymphoid cells (ILCs), such as ILC3s. IL-17A has several pathogenic roles in the asthmatic airway:

- Promotion of neutrophilic inflammation via induction of granulocyte colony-stimulating factor (G-CSF) and neutrophil-attracting chemokines,
- Contribution to corticosteroid resistance [62],
- Enhancement of airway hyperresponsiveness [63,64].

Animal models have shown that allergic sensitization through the airway or skin can induce bronchial Th17 responses and airway hyper-reactivity [65]. In humans, elevated levels of IL-17A or IL-17–producing cells have been observed in blood, sputum, and bronchial biopsies of patients with severe asthma [66]. Despite promising observational data, clinical trials targeting IL-17 have not yet yielded clear benefits. A phase 2 trial of brodalumab, an anti–IL-17 receptor antibody, did not improve asthma control across unselected patients [67]. These results highlight the need for more precise patient stratification, possibly using IL-17–related biomarkers. Interestingly, there is emerging evidence that suppression of T2 pathways may unmask or even promote IL-17–driven inflammation, suggesting that dual targeting of T2 and IL-17 pathways could be a rational approach for certain patients [68].

f. Type 1 (IFN–Mediated) Immunity

Type 1 immunity, primarily directed against intracellular pathogens, involves IFN-γ–producing cells such as Th1 $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and ILC1s [69]. Additionally, airway epithelial cells contribute to type 1 responses through the secretion of type I (IFN-α, IFN-β) and type III (IFN-λ) interferons, especially in response to viral infections and some bacteria [70,71]. The role of IFNs in asthma is complex and appears to be context-dependent:

- Deficient IFN signaling, particularly in the airway epithelium, has been reported in some patients with asthma [72], potentially increasing susceptibility to viral infections and asthma exacerbations [73],
- However, increased IFN-γ expression has also been observed in patients with severe asthma, especially in those with steroid-resistant disease. Elevated IFN-γ levels in bronchoalveolar lavage fluid correlate with markers of airway inflammation and mast cell activation, such as increased CXCL10 expression [74] and IFN regulatory factor 5 (IRF5) [75].

To date, randomized trials of inhaled interferon therapy have failed to demonstrate significant improvements of asthma symptoms or reductions in virus-induced exacerbations [76]. In children with severe, treatment-refractory asthma, airway inflammation is dominated by memory $CCR5^+$ Th1 and Th17-type responses, with $CD8^+$ T cells primarily producing IFN-γ. Despite low Th2 cell numbers, Th2 cytokines were detectable and correlated with total IgE, particularly in multi-sensitized children. Innate type 2 cells (ILC2s) and basophils were scarce in BAL fluid, while plasmacytoid and $IgE^+Fc\varepsilon RI^+$ myeloid dendritic cells were consistently present. Cytokine profiles, including IL-5, IL-33, and IL-28A/IFN-λ2, were associated with allergen sensitization patterns, age, and eosinophil counts, highlighting a complex interplay of Th1, Th17, and selective Th2 responses in the airway immune environment of pediatric severe asthma [77].

3. Systemic Inflammation

Several factors, including obesity, metabolic syndrome, aging, and inflammaging, contribute to systemic inflammation in adult patients with T2-low asthma [78]. These systemic factors can exacerbate airway hyperresponsiveness and influence the immune milieu of the lungs, even in the absence of overt local inflammation. In children, systemic inflammation appears to be **less pronounced and differently regulated** compared with adults, and thus likely contributes differently to the development of T2-low asthma. In **adults,** obesity is strongly associated with **chronic low-grade systemic inflammation**, which contributes to T2-low asthma via **M1 macrophage–mediated secretion of IL-1β, IL-6, and TNF-α in adipose tissue**, promoting airway neutrophilia and more severe disease. Elevated CRP, IL-6, and leptin in adults are also linked to alterations in both airway

 doi:10.20944/preprints202512.1436.v1

and systemic immune profiles, correlating with worse asthma outcomes [79]. In **children**, while obese asthmatic patients may exhibit low-grade systemic inflammation (e.g., elevated leptin, IL-6, and TNF-α), the **magnitude and impact of this inflammatory burden are generally lower** than in adults. In adolescents, obesity and asthma are independently and synergistically associated with elevated high sensitivity (hs)-CRP, indicating some contribution of systemic inflammation, but it remains **less robust than in adults [80,81]**. Nevertheless, in children, T2-low asthma is often associated with obesity and female sex, distinguishing it from T2-high phenotypes. In a **cross-sectional study** of **4,845 children aged 6 to 17 years** who participated in the **2007–2012 NHANES study,** children with **T2-low asthma** were found to be **1.7 to 2.1 times more likely to be female** and to have a **body mass index (BMI) z-score at or above the 85th percentile,** compared with children with T2-high asthma [82]. During adolescence, female sex is associated with a distinct asthma risk profile: whereas asthma is more prevalent in boys during childhood, the pattern reverses around puberty, with girls showing equal or higher incidence [83]. Although most research focuses on T2-high (eosinophilic) asthma, emerging evidence suggests that female sex may also predispose to non-eosinophilic, low-T2 asthma phenotypes. For example, adult-onset female asthma clusters have been characterized by obesity, neutrophilic inflammation and minimal atopy—features consistent with T2-low asthma [27]. Mechanistically, sex-hormone effects (especially estradiol and progesterone) can modulate airway immune responses, alter barrier/epithelial function and shift immune balance away from classic T2 pathways—potentially favoring alternative (T1/T3) routes of inflammation [27,38]. In adolescents, lower lung function, increased airway responsiveness and altered airway development in females may further contribute to a phenotype prone to non-eosinophilic inflammation [84,85]. Thus, being female during adolescence may represent a risk modifier for low-T2 asthma via hormonal and structural-immunologic mechanisms, warranting focused investigation. **Aging** may further amplify systemic inflammation in adults through a process called **inflammaging,** characterized by elevated pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, CRP) due to accumulation of senescent cells and chronic innate immune activation. Factors such as genetics, gut microbiome, oxidative stress, and obesity further drive inflammaging. In asthma, aging is associated with elevated sputum IL-6, IL-17A, and neutrophils, while reduced macrophage and neutrophil phagocytic function may impair pathogen clearance and promote airway colonization [79]. These **age-dependent differences** suggest that while systemic inflammation may modulate airway responsiveness and non-T2 immune pathways across all ages, its **role in driving T2-low asthma is far more prominent in adults than in children,** where local airway or developmental mechanisms may play a larger role. In children, developmental mechanisms refer to non-inflammatory processes governing early lung and airway maturation that may predispose to asthma independent of classic T2 inflammation. During early life, structural airway changes—such as increased reticular basement membrane thickness, airway smooth muscle hyperplasia, and vascular remodeling—are observable even in the absence of marked eosinophilic inflammation [86]. These alterations suggest that airway remodeling can precede overt inflammation and thus may represent a distinct mechanistic pathway in childhood asthma [87]. Moreover, immune ontogeny in early childhood appears to differ from that in adults—neonatal and preschool-aged subjects show a preferential reliance on IL-13$^+$ CD4$^+$ T cells for the initiation of airway hyperresponsiveness, rather than innate ILC2-driven responses typical of older individuals [88]. In a cohort of 105 children aged 1–5 years with recurrent severe wheeze [89], four distinct clusters were identified: atopic (23.1%), non-atopic with low infection and high ICS use (36.5%), non-atopic with high infection and highest BAL neutrophils (21.2%), and non-atopic with low infection and low ICS use (19.2%). Notably, most children (76.9%) fell into the non-atopic clusters, highlighting the potential for early-life, T2-low or non-eosinophilic phenotypes to contribute to persistent asthma later in childhood. These observations underscore that mechanisms driving severe asthma and its risk factors differ between adults and children, with early-life non-T2 phenotypes potentially representing a fundamentally different pathophysiology compared with adults. Obesity and asthma are chronic conditions characterized by increased arginase activity, reduced L-arginine levels, and elevated asymmetric dimethylarginine, which accumulates under oxidative stress [90]. In obese asthma

 doi:10.20944/preprints202512.1436.v1

patients, a lower L-arginine/asymmetric dimethylarginine ratio disrupts nitric oxide synthase 2, favoring superoxide over nitric oxide production, reducing airway nitric oxide bioavailability, impairing bronchodilation, and increasing oxidative stress. This also enhances mitochondrial respiration, generating reactive oxygen species and activating inflammatory pathways. Compared to lean individuals, airway epithelium in obese asthma shows higher mitochondrial respiration and glycolysis, though its impact on asthma morbidity remains unclear. L-arginine dysregulation in obesity promotes inflammation and bronchoconstriction [91,92].

4. Non-Inflammatory (Paucigranulocytic) Mechanisms

A subset of T2-low asthma cases exhibits a paucigranulocytic phenotype, defined by the absence of both eosinophilic and neutrophilic inflammation. In such cases, non-inflammatory mechanisms—including airway smooth muscle dysfunction, neural dysregulation, and altered epithelial barrier function—may contribute to clinical symptoms [93]. Airway efferent nerves, controlled by parasympathetic cholinergic neurons, mediate airway smooth muscle (ASM) contraction and contribute to airway hyperresponsiveness (AHR). In mice, nerve growth factor (NGF) administration induces AHR comparable to allergen exposure without airway inflammation [93,94]. Human studies show increased cholinergic nerve density and TrkB expression in asthmatic airways, independent of eosinophil levels [95]. Genetic factors can also dissociate AHR from inflammation. Polymorphisms in 17q21 leading to ORMDL3 overexpression reduces sphingolipid synthesis, increase AHR, and promote airway remodeling without inflammation [96]. T2-low asthmatic children show lower serum sphingolipids than T2-high or non-asthmatic children [97]. GPCR signaling regulates ASM contraction via Gαq-mediated calcium influx, while RGS proteins terminate signaling. Reduced RGS2/5 expression in asthma or knockout mice leads to AHR independently of inflammation [98]. Ongoing studies aim to clarify the role of RGS dysregulation in paucigranulocytic asthma. In adult patients, paucigranulocytic asthma (PGA) is typically associated with well-controlled disease and preserved lung function under optimal therapy, including low-dose inhaled corticosteroids (ICS). In this population, PGA may predominantly represent the therapeutic resolution of previously eosinophilic asthma rather than a distinct inflammatory endotype. It is generally characterized by lower levels of inflammatory biomarkers and a reduced requirement for high-dose ICS [99]. In contrast, data from a large bronchoscopy study of 126 children with severe asthma showed that 52% exhibited a paucigranulocytic pattern, characterized by less post-bronchodilator airflow limitation, lower blood eosinophilia, and less frequent pathogen detection [59]. This high prevalence suggests that, in children, paucigranulocytic asthma may reflect a distinct pathophysiological mechanism, potentially differing from the largely treatment-responsive phenotype observed in adults.

5. Biomakers

T2-low asthma is a heterogeneous subtype of asthma defined primarily by the absence of type 2 (T2)-mediated inflammation and its associated biomarkers, including eosinophils and T2 cytokines such as IL-4, IL-5, and IL-13, rather than by the presence of any specific low T2 marker. In clinical trials, it is often characterized by a blood eosinophil count (BEC) of less than 150 cells/μL and FeNO levels below 20–25 ppb. However, these biomarkers can be highly variable and are susceptible to suppression by corticosteroid therapy, which complicates accurate classification. As a result, a reliable diagnosis may require a multidimensional approach that includes multiple biomarkers and repeated longitudinal assessments. Despite these efforts, the identification of T2-low asthma using clinically accessible and validated biomarkers remains a significant unmet need. Immunologically, T2-low asthma is associated with alternative inflammatory pathways involving Th1 and Th17 cells, neutrophilic airway inflammation, and increased expression of pro-inflammatory cytokines, including IL-1β, IL-6, IL-8, IL-17A/F, and IFN-γ [46,79]. Recent studies have shown the heterogeneity of T2-low asthma, identifying nine molecular clusters distinguished by combinations of enrichment scores (ES) across different pathways, including T2-high and T2-low pathways, which were associated with variations in clinical and inflammatory characteristics. T2-low asthma was found to be heterogeneous, comprising eight distinct clusters, whereas T2-high asthma appeared relatively

 doi:10.20944/preprints202512.1436.v1

homogeneous. The inclusion of two proteomic platforms in the analysis allowed for greater granularity within the T2-low clusters. The presence of multiple T2-low phenotypes poses challenges for the identification of specific biomarkers and the development of targeted therapies, in contrast to the single T2-high cluster. By integrating sputum transcriptomic and serum proteomic analyses, these studies identified distinct molecular subgroups within the T2-low asthma population. The cohort consisted of adult patients with clinically characterized T2-low asthma, defined by low Type 2 inflammatory biomarkers and absence of eosinophilic airway inflammation. This integrative multi-omics approach demonstrated that T2-low asthma is not a homogeneous phenotype but comprises several biologically distinct endotypes, each characterized by unique gene-expression and protein-biomarker signatures. These findings underscore the value of combining sputum and serum molecular profiling to improve disease stratification and inform the development of more targeted therapeutic strategies for non–Type 2 asthma [100].

Potential T2-low asthma biomarkers in children are presented in Table 2.

6. Clinical Manifestations: When Typical/Usual Signs Go Missing

While T2-high asthma is predominant among patients with severe asthma, T2-low phenotypes appear to be more commonly observed in individuals with mild to moderate disease. Notably, in patients with severe T2-low asthma, disease exacerbations may be associated with a temporary shift toward a T2-high inflammatory profile, indicating a dynamic and potentially fluctuating immunological response [79]. It is also important to note that oral corticosteroids significantly reduce blood eosinophil counts and moderately suppress FeNO levels, which may contribute to the overdiagnosis of T2-low asthma following corticosteroid rescue treatment during exacerbations [79,101]. It is well established that corticosteroid treatment during asthma exacerbations can induce a shift from a T2-high to a T2-low inflammatory profile. This corticosteroid-driven modulation of inflammation partly explains the relatively low prevalence of T2-low asthma observed in patients with severe asthma, as many present with T2-high features during exacerbations but may appear T2-low under corticosteroid influence. Notably, a study showed that only 5% of patients with severe asthma maintained a T2-low profile after tapering oral or inhaled corticosteroids [102]. In contrast, among patients with mild to moderate asthma who were corticosteroid-naive, approximately 40.4% exhibited a T2-low phenotype defined by the absence of elevated sputum eosinophils [103]. These findings highlight that T2-low asthma is more predominant in mild to moderate asthma compared to severe asthma, where corticosteroid use and disease severity mask the true immunological phenotype. This underscores the dynamic and fluctuating nature of inflammatory pathways in asthma and the need for careful phenotyping, particularly in the context of corticosteroid exposure. Comorbidities frequently associated with T2-low asthma—such as obesity, depression, and anxiety—may, in many cases, arise because of inadequate disease control and prolonged exposure to ineffective therapies. Due to their reduced responsiveness to corticosteroids and short-acting β_2-agonists (SABAs) [46,104], patients with T2-low asthma often receive stepwise treatment intensification according to current guidelines, which primarily rely on escalating corticosteroid doses. In T2-low asthma, this approach may lead to overtreatment with corticosteroids, offering limited therapeutic benefit while increasing the risk of treatment-related adverse effects. Notably, corticosteroid overuse has been linked to the development or worsening of obesity, depression, and anxiety [100], suggesting that these comorbidities may not only contribute to poor asthma outcomes but also reflect the consequences of suboptimal and non-targeted management strategies in this asthma endotype.

7. Therapy Reimagined: Management Strategies for Low-T2 Asthma

As it was already mentioned before, asthma is not a single disease, but a syndrome consisting of specific similar characteristics, namely airway inflammation, airway hyperresponsiveness and episodes of bronchoconstriction which all lead to the usual clinical manifestations of asthma (caugh, wheezing, shortness of breath...) [105]. Treatment options up until a few years ago were primarily focused on the most common type of asthma, with Th2 inflammation (eosinophillic asthma). In T2-low asthma, however, most medications targeting the Th2 inflammation pathway show little to no

 doi:10.20944/preprints202512.1436.v1

effect, and most patients with this type of asthma were supposed as having severe or difficult to treat asthma, although recent studies show that this might not be true [106]. Due to the fact that the diagnostic tools available today help clinicians to better define the underlying pathophysiologic mechanisms, the search for new, or rather targeted therapies may be promising. In the next few paragraphs, we will try to shed light on current and potential treatment options and their mechanisms of action.

a. Corticosteroids

Corticosteroids are among the most potent anti-inflammatory drugs. Because airway inflammation is the hallmark of asthma, corticoids should in theory always be useful. However, in T2-low asthma, corticosteroids, either inhaled or systemic, have shown poor asthma control. Additionally, prescribing higher doses (according to GINA step-up therapy and NICE guidelines) did not achieve any benefit, only the risk for more side-effects [107,108]. Oral corticosteroids have shown to even be able to worsen T2-low asthma by means of promoting neutrophil accumulation and reducing their natural apoptosis, prolonging the inflammatory process of the lungs [109]. Therefore, corticosteroid therapy should not be considered as the main controlling medication, if mild to moderate doses do not achieve reduction of exacerbations.

b. Bronchodilators

Bronchodilators, particularly short-acting beta-agonists (SABAs), remain a central component for rapid symptom relief and management of acute exacerbations across asthma inflammatory phenotypes, and are generally administered alongside controller medications. Long-acting beta-agonists (LABAs) are consistently prescribed only in fixed combination with inhaled corticosteroids (ICS) [110]. More recently, long-acting muscarinic antagonists (LAMAs) such as tiotropium bromide, glycopyrronium, and umeclidinium have emerged as additional bronchodilator options, and contemporary Global Initiative for Asthma (GINA) reports now include LAMAs as an add-on treatment for severe asthma in school-aged children and adolescents [107,111]. Clinical trial data indicate that tiotropium bromide, when added to existing controller therapy, improves lung function and is associated with reduced use of rescue medication in both school-aged children and adolescents with moderate-to-severe or severe asthma [112].

c. Biologic therapy

Most biologic agents are monoclonal antibodies (or colloquially called MABs) synthesized to selectively target and bind to a cell type or signal molecule thus blocking the downwards path of their cascade of actions. This concept has revolutionized modern medicine without which many immunologic, hematologic, endocrine and other fields would be almost powerless [113,114]. As our focus is on asthma, we can mention a multitude of biologics, for instance omalizumab, dupilumab, mepolizumab, tezepelumab... Omalizumab has surprisingly, shown to reduce asthma exacerbations in both T2-high and T2-low asthma, although patients with eosinophillic asthma tend to benefit more (it lowers the biomarkers an also effector cells) [115]. Mepolizumab (an IL-5 inhibitor) and dupilumab (an IL-4/IL-13Ra inhibitor) on the other hand, have not shown any benefit, as they target specific molecules on the T2 inflammation pathway [116,117]. Tezepelumab has already shown its efficacy in treating, by other means difficult to treat asthma, not only in T2-high, but also in the T2-low endotype. The explanation is simple – the target of this biologic drug is the thymic stromal lymphopoietin (TSLP) an inflammatory mediator above the T2-high and -low levels, thus leading to better control in both endotypes [118]. It is also approved for use in some countries for children with severe asthma above the age of 12, but there are ongoing trials examining the efficacy and safety in preschool and school children between the ages of 5 to 12 years [119]. Clazakinumab, an anti-IL6 biologic agent, could be a promising candidate for some subgroups of patients with T2-low asthma, because some of them, especially older patients with metabolic syndrome (central obesity and diabetes) have been shown to have an increase in circulating IL-6 [120,121].

d. Antibiotics

Of all the antibiotics, one specifically stands out - azithromycin. Some use it almost as a kind of “panacea” because it has shown promising effects on several diseases where inflammation plays a

 doi:10.20944/preprints202512.1436.v1

key pathophysiologic mechanism. Immunomodulation is the reason why this antibiotic drug shows more than only an antimicrobial function. It has shown beneficial effects in children with asthma, but without a direct effect on inflammatory markers [122,123]. Studies have shown that a long-term use of azithromycin three times a week has achieved symptom remission in adult patients with both T2-high and T2-low asthma [124]. However, the underlying mechanism is not well understood and certainly does not affect the reduction of T2-high asthma biomarkers. More studies are needed to define the optimal long-term low-dose regimen [125].

e. Nonpharmacologic treatment (targeting preventable causes/traits)

As T2-low asthma shares some characteristics with comorbidities, after extensive research has been done, a connection between environmental factors and T2-low asthma has been established. The main causes of non-eosinophilic inflammation seem to be air pollution, smoking (active or passive) and obesity [126,127]. This is another proof that a healthy diet and microclimate have a huge impact on overall health, not only in patients with asthma, but many other diseases. A cross-sectional study established a connection between T2-low asthma prevalence and obesity and cigarette smoke exposure [27,128]. Also, the same asthma endotype was more prevalent in countries with significant airway pollution. This is supposed to be the result of neutrophil inflammation activated by pathogen-associated molecular patterns [128]. In conclusion, therapy for T2-low asthma should be carefully adjusted according to several individual factors. Primarily following guidelines may be beneficial in mild to moderate asthma, but in severe cases or unresponsiveness to standard treatment biologic agents like tezepelumab or long-term azithromycin may be considered. Finally, especially in high-risk children (like a positive family history of asthma, atopy, recurrent wheezing episodes in early childhood), leading a healthy and unburdened lifestyle may be the best way to prevent later asthma development.

4. Discussion - Toward Personalized Medicine: Future Directions

To achieve genuinely personalized care for pediatric asthma, future research must move beyond the simple T2-high/T2-low dichotomy and adopt multidimensional, longitudinal assessments at the level of each individual patient [129]. Evidence from pediatric studies indicates that inflammatory patterns are frequently heterogeneous and evolving and are not fully reflected by existing biomarkers. This highlights the need for integrative approaches that bring together clinical, physiological, molecular, and environmental data. High-throughput analyses across genomics, transcriptomics, proteomics, metabolomics, and the microbiome offer an unprecedented opportunity to more precisely define pediatric asthma endotypes, including rare or “hidden” T2-low phenotypes. These technologies can uncover upstream regulatory circuits, airway epithelial signaling, and non-T2 inflammatory mechanisms that are not detected by conventional biomarkers such as blood eosinophils, FeNO, and IgE [130]. In doing so, they open the door to novel therapeutic targets for children who do not conform to classic T2-high profiles. Longitudinal omics studies, ideally using minimally invasive specimens such as nasal brushings, exhaled breath condensate, and stool, will be essential for monitoring endotype stability over time and in response to therapy, and for distinguishing short-term biomarker variability from stable disease trajectories [131]. To achieve personalized care in future we’ll have to integrate systemic inflammation, airway biology, and clinical status into composite risk scores relying on biomarker panels rather than single analyte cut offs. Creating and validating practical, age-specific biomarker algorithms that are feasible for routine clinical use – ideally based on blood testing and simple measures of lung function or symptoms should be a major research priority, especially for low T-2 asthma endotypes. Biological therapy targeting epithelial alarmins represent a key step toward endotype-agnostic therapy, with tezepelumab already showing efficacy across both high and low T2 biomarker group [132]. Future directions include refining which pediatric subgroups benefit most from such agents, investigating combinations or sequencing with existing T2 biologics, and evaluating emerging drugs targeting IL-33 and related pathways that influence both T2 and non T2 inflammation. At the same time, there is a need to revisit non-biologic strategies, such as macrolides, weight management and treatment of

 doi:10.20944/preprints202512.1436.v1

metabolic and environmental "drivers" and to define their true disease modifying potential in children. The concept of treatable traits offers a pragmatic structure for implementing precision medicine by shifting the focus from diagnostic categories to specific, measurable targets such as airflow limitation, chronic infection, obesity, psychological burden, an environmental exposure. Evidence from severe asthma indicates that the systematic identification and management of these traits can improve disease control and reduce exacerbations [133,134]. Future efforts should adapt and validate these approaches for pediatric population across the entire severity spectrum, not solely within tertiary care settings. Incorporating traits associated with T2 low asthma will be especially important for children who currently lack targeted biologic therapies. In the end, we must emphasize the need to rethink how studies are designed and which children are enrolled. Future studies must deliberately include younger age groups, under-represented endotypes (especially T2 low and mixed inflammation), and diverse ethnic and environmental backgrounds. This will help translate mechanistic insights into actionable, individualized care pathways that can be implemented into routine pediatric practice.

5. Conclusions

T2-low asthma in children is clinically relevant but still under-recognized. Evidence indicates that T2-low asthma in children is more common than previously assumed, characterized by low Th2 biomarkers, non-allergic features, and frequent comorbidities such as obesity and exposure to tobacco smoke, yet remains largely an "orphan" phenotype with respect to validated biomarkers and targeted therapeutic options. To move beyond this imbalance, future research must integrate omics-based endotyping, composite biomarker development, and treatable traits-oriented care. Upstream biologics targeting epithelial alarmins and non-T2 pathways, alongside refined use of existing therapies and non-pharmacological interventions, offer a realistic path toward genuinely personalized medicine, in which each child's inflammatory pattern, comorbidities, and environment inform tailored management rather than a one-size-fits-all approach centered on eosinophils alone.

Author Contributions: Conceptualization, investigation, methodology, writing - original draft preparation I.M.K., Conceptualization, investigation, methodology, writing - original draft preparation - M.N., Investigation, methodology, writing - original draft preparation – H.M.Š., Investigation, methodology, writing - original draft preparation – A.M., Supervision, writing—review and editing – D.P.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

T2 – type 2
Ig – immunoglobulin
IL- interleukin
FeNO - Fraction of exhaled nitric oxide
NHANES - National Health and Nutrition Examination Survey
NO - nitrogen dioxide
O_3 - ozone
$PM_{2\text{-}5}$ - fine particular matter
TNF-α - tumor necrosis factor – α
RSV – respiratory syncicial virus
NETs - neutrophil extracellular traps
TSLP - thymic stromal lymphopoietin
HMGB1 - high-mobility group box 1 protein
MMP-9 - Matrix Metalloproteinase-9

 doi:10.20944/preprints202512.1436.v1

VEGF - Vascular Endothelial Growth Factor
fMLP - formyl-methionyl-leucyl-phenylalanine
ILCs - innate lymphoid cells
GCSF - granulocyte colony-stimulating factor
IRF 5 - regulatory factor 5
ICS - inhaled corticosteroids
SABA - short-acting β_2-agonists
GINA – Global Initiative for Asthma
NICE - National Institute for Health and Care Exellence
LAMAs - long-acting muscarinic antagonists
LABAs - long-acting β_2-agonists
MABs - monoclonal antibodies

Appendix A

Table 1. Prevalence of T2-low asthma in different studies.

Study	Population / Setting	How T2-low or Non-T2 Defined	Reported Prevalence or Proportion of Th2-low asthma	Key Findings Relevant for Children with Th2-low asthma
NHANES 2007-2012 (USA, school-aged children 6-17 yrs)	505 children with asthma	T2-low defined by AEC < 300 cells/μL *and* FeNO < 25 ppb (secondary thresholds also used)	45.7%	female, older, overweight/obese
ALLIANCE cohort ("T2-high asthma phenotypes across lifespan", 2022)	Mixed ages: preschool, school-age children, and adults with asthma (children total 473)	Phenotypes defined by blood eosinophils and allergen-specific IgE; "T2-low" = neither eosinophilia nor atopy; "T2-high" = eosinophilia + atopy; plus other categories like eosinophilia-only or atopy-only.	0-2 yr – 64.5% 3-5 yr – 36.9% 6-8 yr – 20.5% 9-11 yr – 11.9% 12-14 yr – 18.5% 15-17 yr – 11.1%	With increasing age, T2-low tends to decrease; atopy or eosinophilia tends to become more common.

 doi:10.20944/preprints202512.1436.v1

"Pediatric Asthma in Hospitalized Children - Exploring airway inflammation" (2024-25)	Hospitalized children with asthma, total 351 children included which could be classified as the known type of airway inflammation.	Classification using blood eosinophil counts, specific IgE (sIgE), and age stratification; thresholds like EOS 150 (Standard 1), 300 (Standard 2), 470 (Standard 3) cells/μL and sIgE ≥0.7 kU/L; defining groups: "Only-atopy", "Only-EOS", "T2-high", "T2-low" (neither eosinophilia nor atopy)	Standard 1 – 19.4% Standard 2 – 25.6% Standard 3- 28.2%	With increasing age, T2-low tends to decrease; atopy or eosinophilia tends to become more common. Findings indicated that patients with T2-low airway inflammation could have a longer time from symptoms onset to admission, a longer time for hospitalization, a lower proportion of atopic dermatitis, and a higher proportion of siblings.

Table 2. Potential T2-low Asthma Biomarkers in Children.

Category	Biomarker	Comments/Phenotype	References
Non eosinophilic/neutrophilic marker	Sputum neutrophils IL-8 (CXCL8)	High neutrophil proportion associated with the T2-low phenotype Elevated sputum levels in more severe disease	1,2 3
Systemic inflammatory markers	IL-6 Leptin	Sporadically elevated in children; reflects low-grade systemic inflammation Leptin modulates Th1/Th2 balance and Th17-driven non-T2 inflammation in obese children with asthma	4, 5 6
Exhaled breath markers	FeNO	Low values (<25 ppb) suggest absence of T2 inflammation	7
Molecular/cytokine markers	IL-17	Th17 cytokine; associated with neutrophilic infiltration	2
Infectious/microbiome markers	Bacterial load in sputum	Elevated in T2-low exacerbations	2

 doi:10.20944/preprints202512.1436.v1

	BAL neutrophils+ proteomics	Associated with infection and the T2-low phenotype	8,9

References

1. A. Lindsley, N. Lugogo, K. Reeh, J. Spahn, and J. Parnes, "Asthma Biologics Across the T2 Spectrum of Inflammation in Severe Asthma: Biomarkers and Mechanism of Action," *J Asthma Allergy*, vol. Volume 18, pp. 33–57, Jan. 2025, doi: 10.2147/JAA.S496630.
2. B. Gyawali, S. N. Georas, and S. Khurana, "Biologics in severe asthma: a state-of-the-art review.," *Eur Respir Rev*, vol. 34, no. 175, Jan. 2025, doi: 10.1183/16000617.0088-2024.
3. M. Lampalo, A. Štajduhar, D. Rnjak, N. Ferara, H. S. Stanić, and S. Popović-Grle, "Effectiveness of biological therapy in severe asthma: a retrospective real-world study," *Croat Med J*, vol. 66, no. 1, pp. 3–12, Feb. 2025, doi: 10.3325/cmj.2025.66.3.
4. Y. Hamada, D. Thomas, V. M. McDonald, M. Fricker, L. G. Heaney, and P. G. Gibson, "Clinical remission in severe asthma treated with biologics and macrolides: Definition, prevalence, associated factors, and future perspectives," *Allergology International*, Oct. 2025, doi: 10.1016/j.alit.2025.10.001.
5. W. Zhang, Y. Zhang, L. Li, R. Chen, and F. Shi, "Unraveling heterogeneity and treatment of asthma through integrating multi-omics data," *Frontiers in Allergy*, vol. 5, Nov. 2024, doi: 10.3389/falgy.2024.1496392.
6. M. E. Kuruvilla, F. E.-H. Lee, and G. B. Lee, "Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease," *Clin Rev Allergy Immunol*, vol. 56, no. 2, pp. 219–233, Apr. 2019, doi: 10.1007/s12016-018-8712-1.
7. M. Yue *et al.*, "Transcriptomic Profiles in Nasal Epithelium and Asthma Endotypes in Youth," *JAMA*, vol. 333, no. 4, p. 307, Jan. 2025, doi: 10.1001/jama.2024.22684.
8. Y.-S. Hahn and S.-Y. Eom, "Reply to 'Small airway dysfunction is common even in mild asthma, suggests increased exacerbation risk,'" *J Allergy Clin Immunol Pract*, vol. 12, no. 7, pp. 1947–1948, Jul. 2024, doi: 10.1016/j.jaip.2024.04.025.
9. I. Esteban-Gorgojo, D. Antolín-Amérigo, J. Domínguez-Ortega, and S. Quirce, "Non-eosinophilic asthma: current perspectives," *J Asthma Allergy*, vol. Volume 11, pp. 267–281, Oct. 2018, doi: 10.2147/JAA.S153097.
10. D. Thomas, Y. Hamada, P. Gibson, C. E. Brightling, M. Castro, and L. G. Heaney, "Diagnosis and Treatment Options for T2-Low Asthma.," *J Allergy Clin Immunol Pract*, vol. 13, no. 7, pp. 1527–1539, Jul. 2025, doi: 10.1016/j.jaip.2025.04.055.
11. H. Shailesh, A. A. Bhat, and I. A. Janahi, "Obesity-Associated Non-T2 Mechanisms in Obese Asthmatic Individuals," *Biomedicines*, vol. 11, no. 10, p. 2797, Oct. 2023, doi: 10.3390/biomedicines11102797.
12. X. Chu, B. Zhang, V. A. C. M. Koeken, M. K. Gupta, and Y. Li, "Multi-Omics Approaches in Immunological Research," *Front Immunol*, vol. 12, Jun. 2021, doi: 10.3389/fimmu.2021.668045.
13. N. Muzaffar, M. Baber, and H. Iqra Malik, "Role of precision medicine on different endotypes of asthma," *Exploration of Asthma & Allergy*, vol. 3, Jun. 2025, doi: 10.37349/eaa.2025.100984.
14. F. M. Rackemann, "A working classification of asthma," *Am J Med*, vol. 3, no. 5, pp. 601–606, Nov. 1947, doi: 10.1016/0002-9343(47)90204-0.
15. F. L. M. Ricciardolo, G. Guida, F. Bertolini, A. Di Stefano, and V. Carriero, "Phenotype overlap in the natural history of asthma," *European Respiratory Review*, vol. 32, no. 168, p. 220201, Jun. 2023, doi: 10.1183/16000617.0201-2022.
16. S. E. WENZEL *et al.*, "Evidence That Severe Asthma Can Be Divided Pathologically into Two Inflammatory Subtypes with Distinct Physiologic and Clinical Characteristics," *Am J Respir Crit Care Med*, vol. 160, no. 3, pp. 1001–1008, Sep. 1999, doi: 10.1164/ajrccm.160.3.9812110.
17. G. P. Anderson, "Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease," *The Lancet*, vol. 372, no. 9643, pp. 1107–1119, Sep. 2008, doi: 10.1016/S0140-6736(08)61452-X.
18. N. G. Papadopoulos *et al.*, "Type 2 Inflammation and Asthma in Children: A Narrative Review," *J Allergy Clin Immunol Pract*, vol. 12, no. 9, pp. 2310–2324, Sep. 2024, doi: 10.1016/j.jaip.2024.06.010.

 doi:10.20944/preprints202512.1436.v1

19. C. Kyriakopoulos, A. Gogali, K. Bartziokas, and K. Kostikas, "Identification and treatment of T2-low asthma in the era of biologics," *ERJ Open Res*, vol. 7, no. 2, pp. 00309–02020, Apr. 2021, doi: 10.1183/23120541.00309-2020.
20. F. Peri, A. Amaddeo, L. Badina, M. Maschio, E. Barbi, and S. Ghirardo, "T2-Low Asthma: A Discussed but Still Orphan Disease," *Biomedicines*, vol. 11, no. 4, p. 1226, Apr. 2023, doi: 10.3390/biomedicines11041226.
21. P. R. Polu and V. K. Bikki, "Asthma endotypes in flux: integrating type 1 and type 2 inflammation for biological therapy advancement," *Journal of Asthma*, pp. 1–21, Sep. 2025, doi: 10.1080/02770903.2025.2555300.
22. K. Porpodis *et al.*, "T2-low severe asthma clinical spectrum and impact: The Greek PHOLLOW cross-sectional study," *Clin Transl Allergy*, vol. 15, no. 2, Feb. 2025, doi: 10.1002/clt2.70035.
23. Y.-Y. Han, K. Gaietto, M. Yue, F. J. Rosser, W. Chen, and J. C. Celedón, "Prevalence and Potential Risk Factors for T2-Low Asthma Among School-Aged Children in the National Health and Nutrition Examination Survey, 2007–2012," *J Allergy Clin Immunol Pract*, vol. 13, no. 8, pp. 2075-2082.e2, Aug. 2025, doi: 10.1016/j.jaip.2025.04.040.
24. N. Maison *et al.*, "T2-high asthma phenotypes across lifespan," *European Respiratory Journal*, vol. 60, no. 3, p. 2102288, Sep. 2022, doi: 10.1183/13993003.02288-2021.
25. P. Han, J. Yin, H. Zou, A. Jiao, Y. Liu, and K. Shen, "Exploring the types of airway inflammation in hospitalized children with asthma," *BMC Pediatr*, vol. 25, no. 1, p. 359, May 2025, doi: 10.1186/s12887-025-05596-7.
26. L. Pecoraro *et al.*, "The Role of Environmental Exposures in Pediatric Asthma Pathogenesis: A Contemporary Narrative Review," *Children*, vol. 12, no. 10, p. 1327, Oct. 2025, doi: 10.3390/children12101327.
27. Y.-Y. Han, K. Gaietto, M. Yue, F. J. Rosser, W. Chen, and J. C. Celedón, "Prevalence and Potential Risk Factors for T2-Low Asthma Among School-Aged Children in the National Health and Nutrition Examination Survey, 2007–2012," *J Allergy Clin Immunol Pract*, vol. 13, no. 8, pp. 2075-2082.e2, Aug. 2025, doi: 10.1016/j.jaip.2025.04.040.
28. P. E. Mishra, E. Melén, G. H. Koppelman, and J. C. Celedón, "T2-low asthma in school-aged children: unacknowledged and understudied," *Lancet Respir Med*, vol. 11, no. 12, pp. 1044–1045, Dec. 2023, doi: 10.1016/S2213-2600(23)00369-7.
29. M. Aghapour *et al.*, "Role of air pollutants in airway epithelial barrier dysfunction in asthma and COPD," *European Respiratory Review*, vol. 31, no. 163, p. 210112, Mar. 2022, doi: 10.1183/16000617.0112-2021.
30. N. Mthembu, P. Ikwegbue, F. Brombacher, and S. Hadebe, "Respiratory Viral and Bacterial Factors That Influence Early Childhood Asthma," *Frontiers in Allergy*, vol. 2, Jul. 2021, doi: 10.3389/falgy.2021.692841.
31. X. Wu, F. Huang, W. Yao, and Z. Xue, "The role of innate immune system in respiratory viral infection related asthma," *Front Cell Infect Microbiol*, vol. 15, Jun. 2025, doi: 10.3389/fcimb.2025.1604831.
32. C. Liu *et al.*, "Microbial dysbiosis and childhood asthma development: Integrated role of the airway and gut microbiome, environmental exposures, and host metabolic and immune response," *Front Immunol*, vol. 13, Sep. 2022, doi: 10.3389/fimmu.2022.1028209.
33. M. S. Kelly, S. Bunyavanich, W. Phipatanakul, and P. S. Lai, "The Environmental Microbiome, Allergic Disease, and Asthma," *J Allergy Clin Immunol Pract*, vol. 10, no. 9, pp. 2206-2217.e1, Sep. 2022, doi: 10.1016/j.jaip.2022.06.006.
34. R. Aslam, L. Herrles, R. Aoun, A. Pioskowik, and A. Pietrzyk, "Link between gut microbiota dysbiosis and childhood asthma: Insights from a systematic review," *Journal of Allergy and Clinical Immunology: Global*, vol. 3, no. 3, p. 100289, Aug. 2024, doi: 10.1016/j.jacig.2024.100289.
35. M. Sung, "Trends of vitamin D in asthma in the pediatric population for two decades: a systematic review," *Clin Exp Pediatr*, vol. 66, no. 8, pp. 339–347, Aug. 2023, doi: 10.3345/cep.2022.01109.
36. Y. Zhou *et al.*, "Evaluating the effects of vitamin D Level on airway obstruction in two asthma endotypes in humans and in two mouse models with different intake of vitamin D during early-life," *Front Immunol*, vol. 14, Jan. 2023, doi: 10.3389/fimmu.2023.1107031.
37. M. Liu, J. Wang, and X. Sun, "A Meta-Analysis on Vitamin D Supplementation and Asthma Treatment," *Front Nutr*, vol. 9, Jul. 2022, doi: 10.3389/fnut.2022.860628.

 doi:10.20944/preprints202512.1436.v1

38. M. Fröhlich *et al.*, "Is there a sex-shift in prevalence of allergic rhinitis and comorbid asthma from childhood to adulthood? A meta-analysis," *Clin Transl Allergy*, vol. 7, no. 1, p. 44, Dec. 2017, doi: 10.1186/s13601-017-0176-5.
39. A. Keselman and N. Heller, "Estrogen Signaling Modulates Allergic Inflammation and Contributes to Sex Differences in Asthma," *Front Immunol*, vol. 6, Nov. 2015, doi: 10.3389/fimmu.2015.00568.
40. D. C. Newcomb *et al.*, "Estrogen and progesterone decrease let-7f microRNA expression and increase IL-23/IL-23 receptor signaling and IL-17A production in patients with severe asthma," *Journal of Allergy and Clinical Immunology*, vol. 136, no. 4, pp. 1025-1034.e11, Oct. 2015, doi: 10.1016/j.jaci.2015.05.046.
41. R. Borrelli *et al.*, "Sex-Based Differences in Asthma: Pathophysiology, Hormonal Influence, and Genetic Mechanisms," *Int J Mol Sci*, vol. 26, no. 11, p. 5288, May 2025, doi: 10.3390/ijms26115288.
42. J.-J. Lai, K.-P. Lai, W. Zeng, K.-H. Chuang, S. Altuwaijri, and C. Chang, "Androgen Receptor Influences on Body Defense System via Modulation of Innate and Adaptive Immune Systems," *Am J Pathol*, vol. 181, no. 5, pp. 1504–1512, Nov. 2012, doi: 10.1016/j.ajpath.2012.07.008.
43. M. Vijeyakumaran *et al.*, "Dual activation of estrogen receptor alpha and glucocorticoid receptor upregulate CRTh2-mediated type 2 inflammation; mechanism driving asthma severity in women?," *Allergy*, vol. 78, no. 3, pp. 767–779, Mar. 2023, doi: 10.1111/all.15543.
44. Z. Zhou *et al.*, "Estrogen decreases tight junction protein ZO-1 expression in human primary gut tissues," *Clinical Immunology*, vol. 183, pp. 174–180, Oct. 2017, doi: 10.1016/j.clim.2017.08.019.
45. J. G. Ripoll *et al.*, "Sex differences in paediatric airway anatomy," *Exp Physiol*, vol. 105, no. 4, pp. 721–731, Apr. 2020, doi: 10.1113/EP088370.
46. A. M. Fitzpatrick, B. E. Chipps, F. Holguin, and P. G. Woodruff, "T2-'Low' Asthma: Overview and Management Strategies," *J Allergy Clin Immunol Pract*, vol. 8, no. 2, pp. 452–463, Feb. 2020, doi: 10.1016/j.jaip.2019.11.006.
47. S. N. Hudey, D. K. Ledford, and J. C. Cardet, "Mechanisms of non-type 2 asthma," *Curr Opin Immunol*, vol. 66, pp. 123–128, Oct. 2020, doi: 10.1016/j.coi.2020.10.002.
48. T. Chen *et al.*, "Receptor-Mediated NETosis on Neutrophils," *Front Immunol*, vol. 12, Nov. 2021, doi: 10.3389/fimmu.2021.775267.
49. I. Iwaszko, K. Specjalski, M. Chełmińska, and M. Niedoszytko, "Neutrophilic Asthma—From Mechanisms to New Perspectives of Therapy," *J Clin Med*, vol. 14, no. 20, p. 7137, Oct. 2025, doi: 10.3390/jcm14207137.
50. Y. Li *et al.*, "Extracellular RNAs from lung cancer cells activate epithelial cells and induce neutrophil extracellular traps," *Int J Oncol*, May 2019, doi: 10.3892/ijo.2019.4808.
51. M. E. Lachowicz-Scroggins *et al.*, "Extracellular DNA, Neutrophil Extracellular Traps, and Inflammasome Activation in Severe Asthma," *Am J Respir Crit Care Med*, vol. 199, no. 9, pp. 1076–1085, May 2019, doi: 10.1164/rccm.201810-1869OC.
52. D. L. Pham *et al.*, "Neutrophil autophagy and extracellular <scp>DNA</scp> traps contribute to airway inflammation in severe asthma," *Clinical & Experimental Allergy*, vol. 47, no. 1, pp. 57–70, Jan. 2017, doi: 10.1111/cea.12859.
53. Y. Liang *et al.*, "HMGB1 binding to receptor for advanced glycation end products enhances inflammatory responses of human bronchial epithelial cells by activating p38 MAPK and ERK1/2," *Mol Cell Biochem*, vol. 405, no. 1–2, pp. 63–71, Jul. 2015, doi: 10.1007/s11010-015-2396-0.
54. Á. Teijeira *et al.*, "CXCR1 and CXCR2 Chemokine Receptor Agonists Produced by Tumors Induce Neutrophil Extracellular Traps that Interfere with Immune Cytotoxicity," *Immunity*, vol. 52, no. 5, pp. 856-871.e8, May 2020, doi: 10.1016/j.immuni.2020.03.001.
55. S. Tcherniuk *et al.*, "Formyl Peptide Receptor 2 Plays a Deleterious Role During Influenza A Virus Infections," *Journal of Infectious Diseases*, vol. 214, no. 2, pp. 237–247, Jul. 2016, doi: 10.1093/infdis/jiw127.
56. M. Toussaint *et al.*, "Host DNA released by NETosis promotes rhinovirus-induced type-2 allergic asthma exacerbation," *Nat Med*, vol. 23, no. 6, pp. 681–691, Jun. 2017, doi: 10.1038/nm.4332.
57. M. Ntinopoulou *et al.*, "Interleukin-17A-Enriched Neutrophil Extracellular Traps Promote Immunofibrotic Aspects of Childhood Asthma Exacerbation," *Biomedicines*, vol. 11, no. 8, p. 2104, Jul. 2023, doi: 10.3390/biomedicines11082104.

 doi:10.20944/preprints202512.1436.v1

58. H. S. Chang *et al.*, "Neutrophilic inflammation in asthma: mechanisms and therapeutic considerations," *Expert Rev Respir Med*, vol. 11, no. 1, pp. 29–40, Jan. 2017, doi: 10.1080/17476348.2017.1268919.
59. W. G. Teague *et al.*, "Lung Lavage Granulocyte Patterns and Clinical Phenotypes in Children with Severe, Therapy-Resistant Asthma," *J Allergy Clin Immunol Pract*, vol. 7, no. 6, pp. 1803-1812.e10, Jul. 2019, doi: 10.1016/j.jaip.2018.12.027.
60. J. L. Simpson *et al.*, "Airway dysbiosis: *Haemophilus influenzae* and *Tropheryma* in poorly controlled asthma," *European Respiratory Journal*, vol. 47, no. 3, pp. 792–800, Mar. 2016, doi: 10.1183/13993003.00405-2015.
61. J. Ackland *et al.*, "<scp> Nontypeable *Haemophilus influenzae* </scp> infection of pulmonary macrophages drives neutrophilic inflammation in severe asthma," *Allergy*, vol. 77, no. 10, pp. 2961–2973, Oct. 2022, doi: 10.1111/all.15375.
62. S. Diver *et al.*, "Relationship between inflammatory status and microbial composition in severe asthma and during exacerbation," *Allergy*, vol. 77, no. 11, pp. 3362–3376, Nov. 2022, doi: 10.1111/all.15425.
63. S. Ouyang, C. Liu, J. Xiao, X. Chen, A. C. Lui, and X. Li, "Targeting IL-17A/glucocorticoid synergy to CSF3 expression in neutrophilic airway diseases," *JCI Insight*, vol. 5, no. 3, Feb. 2020, doi: 10.1172/jci.insight.132836.
64. N. Mizutani, T. Nabe, and S. Yoshino, "IL-17A Promotes the Exacerbation of IL-33–Induced Airway Hyperresponsiveness by Enhancing Neutrophilic Inflammation via CXCR2 Signaling in Mice," *The Journal of Immunology*, vol. 192, no. 4, pp. 1372–1384, Feb. 2014, doi: 10.4049/jimmunol.1301538.
65. M. Kudo *et al.*, "IL-17A produced by αβ T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction," *Nat Med*, vol. 18, no. 4, pp. 547–554, Apr. 2012, doi: 10.1038/nm.2684.
66. R. H. Wilson, G. S. Whitehead, H. Nakano, M. E. Free, J. K. Kolls, and D. N. Cook, "Allergic Sensitization through the Airway Primes Th17-dependent Neutrophilia and Airway Hyperresponsiveness," *Am J Respir Crit Care Med*, vol. 180, no. 8, pp. 720–730, Oct. 2009, doi: 10.1164/rccm.200904-0573OC.
67. S. F. Rahmawati, M. te Velde, H. A. M. Kerstjens, A. S. S. Dömling, M. R. Groves, and R. Gosens, "Pharmacological Rationale for Targeting IL-17 in Asthma," *Frontiers in Allergy*, vol. 2, Aug. 2021, doi: 10.3389/falgy.2021.694514.
68. W. W. Busse *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*, vol. 188, no. 11, pp. 1294–1302, Dec. 2013, doi: 10.1164/rccm.201212-2318OC.
69. D. F. Choy *et al.*, "T H 2 and T H 17 inflammatory pathways are reciprocally regulated in asthma," *Sci Transl Med*, vol. 7, no. 301, Aug. 2015, doi: 10.1126/scitranslmed.aab3142.
70. D. Fang, A. Healy, and J. Zhu, "Differential regulation of lineage-determining transcription factor expression in innate lymphoid cell and adaptive T helper cell subsets," *Front Immunol*, vol. 13, Jan. 2023, doi: 10.3389/fimmu.2022.1081153.
71. V. Fensterl and G. C. Sen, "Interferons and viral infections," *BioFactors*, vol. 35, no. 1, pp. 14–20, Jan. 2009, doi: 10.1002/biof.6.
72. D. Parker and A. Prince, "Type I interferon response to extracellular bacteria in the airway epithelium," *Trends Immunol*, vol. 32, no. 12, pp. 582–588, Dec. 2011, doi: 10.1016/j.it.2011.09.003.
73. P. A. B. Wark *et al.*, "Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus," *J Exp Med*, vol. 201, no. 6, pp. 937–947, Mar. 2005, doi: 10.1084/jem.20041901.
74. M. R. Edwards *et al.*, "Impaired innate interferon induction in severe therapy resistant atopic asthmatic children," *Mucosal Immunol*, vol. 6, no. 4, pp. 797–806, Jul. 2013, doi: 10.1038/mi.2012.118.
75. M. Gauthier *et al.*, "Severe asthma in humans and mouse model suggests a CXCL10 signature underlies corticosteroid-resistant Th1 bias," *JCI Insight*, vol. 2, no. 13, Jul. 2017, doi: 10.1172/jci.insight.94580.
76. T. B. Oriss *et al.*, "IRF5 distinguishes severe asthma in humans and drives Th1 phenotype and airway hyperreactivity in mice," *JCI Insight*, vol. 2, no. 10, May 2017, doi: 10.1172/jci.insight.91019.
77. C. McCrae *et al.*, "INEXAS: A Phase 2 Randomized Trial of On-demand Inhaled Interferon Beta-1a in Severe Asthmatics," *Clinical & Experimental Allergy*, vol. 51, no. 2, pp. 273–283, Feb. 2021, doi: 10.1111/cea.13765.

 doi:10.20944/preprints202512.1436.v1

78. J. A. Wisniewski *et al.*, "TH1 signatures are present in the lower airways of children with severe asthma, regardless of allergic status," *Journal of Allergy and Clinical Immunology*, vol. 141, no. 6, pp. 2048-2060.e13, Jun. 2018, doi: 10.1016/j.jaci.2017.08.020.
79. D. Thomas, Y. Hamada, P. Gibson, C. E. Brightling, M. Castro, and L. G. Heaney, "Diagnosis and Treatment Options for T2-Low Asthma.," *J Allergy Clin Immunol Pract*, vol. 13, no. 7, pp. 1527–1539, Jul. 2025, doi: 10.1016/j.jaip.2025.04.055.
80. M. D. Althoff, K. Gaietto, F. Holguin, and E. Forno, "Obesity-related Asthma: A Pathobiology-based Overview of Existing and Emerging Treatment Approaches," *Am J Respir Crit Care Med*, vol. 210, no. 10, pp. 1186–1200, Nov. 2024, doi: 10.1164/rccm.202406-1166SO.
81. S. Miethe, A. Karsonova, A. Karaulov, and H. Renz, "Obesity and asthma," *Journal of Allergy and Clinical Immunology*, vol. 146, no. 4, pp. 685–693, Oct. 2020, doi: 10.1016/j.jaci.2020.08.011.
82. D. Rastogi and F. Holguin, "Metabolic Dysregulation, Systemic Inflammation, and Pediatric Obesity-related Asthma," *Ann Am Thorac Soc*, vol. 14, no. Supplement_5, pp. S363–S367, Nov. 2017, doi: 10.1513/AnnalsATS.201703-231AW.
83. U. I. Khan, D. Rastogi, C. R. Isasi, and S. M. Coupey, "Independent and Synergistic Associations of Asthma and Obesity with Systemic Inflammation in Adolescents," *Journal of Asthma*, vol. 49, no. 10, pp. 1044–1050, Dec. 2012, doi: 10.3109/02770903.2012.728271.
84. T. Miyasaka, K. Dobashi-Okuyama, K. Kawakami, C. Masuda-Suzuki, M. Takayanagi, and I. Ohno, "Sex Plays a Multifaceted Role in Asthma Pathogenesis," *Biomolecules*, vol. 12, no. 5, p. 650, Apr. 2022, doi: 10.3390/biom12050650.
85. C. Secco Rosário, C. Alves Cardozo, H. J. Chong Neto, and N. A. Rosário Filho, "Do gender and puberty influence allergic diseases?," *Allergol Immunopathol (Madr)*, vol. 49, no. 2, pp. 122–125, Mar. 2021, doi: 10.15586/aei.v49i2.49.
86. M. Trivedi and E. Denton, "Asthma in Children and Adults—What Are the Differences and What Can They Tell us About Asthma?," *Front Pediatr*, vol. 7, Jun. 2019, doi: 10.3389/fped.2019.00256.
87. I. S. Choi, "Gender-Specific Asthma Treatment," *Allergy Asthma Immunol Res*, vol. 3, no. 2, p. 74, 2011, doi: 10.4168/aair.2011.3.2.74.
88. G. Lezmi *et al.*, "Airway Remodeling in Preschool Children with Severe Recurrent Wheeze," *Am J Respir Crit Care Med*, vol. 192, no. 2, pp. 164–171, Jul. 2015, doi: 10.1164/rccm.201411-1958OC.
89. P. F. M. Robinson *et al.*, "Recurrent Severe Preschool Wheeze: From Prespecified Diagnostic Labels to Underlying Endotypes," *Am J Respir Crit Care Med*, vol. 204, no. 5, pp. 523–535, Sep. 2021, doi: 10.1164/rccm.202009-3696OC.
90. F. Holguin *et al.*, "L-Citrulline increases nitric oxide and improves control in obese asthmatics," *JCI Insight*, vol. 4, no. 24, Dec. 2019, doi: 10.1172/jci.insight.131733.
91. O. Tliba and R. A. Panettieri, "Paucigranulocytic asthma: Uncoupling of airway obstruction from inflammation," *Journal of Allergy and Clinical Immunology*, vol. 143, no. 4, pp. 1287–1294, Apr. 2019, doi: 10.1016/j.jaci.2018.06.008.
92. D.-V. Nguyen, A. Linderholm, A. Haczku, and N. Kenyon, "Glucagon-like peptide 1: A potential anti-inflammatory pathway in obesity-related asthma," *Pharmacol Ther*, vol. 180, pp. 139–143, Dec. 2017, doi: 10.1016/j.pharmthera.2017.06.012.
93. G. Dragunas *et al.*, "Cholinergic neuroplasticity in asthma driven by TrkB signaling," *The FASEB Journal*, vol. 34, no. 6, pp. 7703–7717, Jun. 2020, doi: 10.1096/fj.202000170R.
94. A. Braun, D. Quarcoo, O. Schulte-Herbrüggen, M. Lommatzsch, G. Hoyle, and H. Renz, "Nerve Growth Factor Induces Airway Hyperresponsiveness in Mice," *Int Arch Allergy Immunol*, vol. 124, no. 1–3, pp. 205–207, 2001, doi: 10.1159/000053711.
95. J. Chen, M. Miller, H. Unno, P. Rosenthal, M. J. Sanderson, and D. H. Broide, "Orosomucoid-like 3 (ORMDL3) upregulates airway smooth muscle proliferation, contraction, and Ca2+ oscillations in asthma," *Journal of Allergy and Clinical Immunology*, vol. 142, no. 1, pp. 207-218.e6, Jul. 2018, doi: 10.1016/j.jaci.2017.08.015.
96. J. G. Ono *et al.*, "Decreased sphingolipid synthesis in children with 17q21 asthma–risk genotypes," *Journal of Clinical Investigation*, vol. 130, no. 2, pp. 921–926, Jan. 2020, doi: 10.1172/JCI130860.

97. N. A. Balenga, W. Jester, M. Jiang, R. A. Panettieri, and K. M. Druey, "Loss of regulator of G protein signaling 5 promotes airway hyperresponsiveness in the absence of allergic inflammation," *Journal of Allergy and Clinical Immunology*, vol. 134, no. 2, pp. 451-459.e11, Aug. 2014, doi: 10.1016/j.jaci.2014.01.019.
98. P. Ntontsi *et al.*, "Clinical, functional and inflammatory characteristics in patients with paucigranulocytic stable asthma: Comparison with different sputum phenotypes," *Allergy*, vol. 72, no. 11, pp. 1761–1767, Nov. 2017, doi: 10.1111/all.13184.
99. N. Zounemat Kermani *et al.*, "Type 2-low asthma phenotypes by integration of sputum transcriptomics and serum proteomics," *Allergy*, vol. 76, no. 1, pp. 380–383, Jan. 2021, doi: 10.1111/all.14573.
100. J. Sweeney *et al.*, "Comorbidity in severe asthma requiring systemic corticosteroid therapy: cross-sectional data from the Optimum Patient Care Research Database and the British Thoracic Difficult Asthma Registry," *Thorax*, vol. 71, no. 4, pp. 339–346, Apr. 2016, doi: 10.1136/thoraxjnl-2015-207630.
101. J. Busby, E. Khoo, P. E. Pfeffer, A. H. Mansur, and L. G. Heaney, "The effects of oral corticosteroids on lung function, type-2 biomarkers and patient-reported outcomes in stable asthma: A systematic review and meta-analysis," *Respir Med*, vol. 173, p. 106156, Nov. 2020, doi: 10.1016/j.rmed.2020.106156.
102. L. G. Heaney *et al.*, "Composite type-2 biomarker strategy versus a symptom–risk-based algorithm to adjust corticosteroid dose in patients with severe asthma: a multicentre, single-blind, parallel group, randomised controlled trial," *Lancet Respir Med*, vol. 9, no. 1, pp. 57–68, Jan. 2021, doi: 10.1016/S2213-2600(20)30397-0.
103. C. Wongsa *et al.*, "Subtype prevalence and treatment implication in adolescents and adults with mild-to-moderate asthma: Systematic review and meta-analysis," *Journal of Allergy and Clinical Immunology: Global*, vol. 4, no. 1, p. 100366, Feb. 2025, doi: 10.1016/j.jacig.2024.100366.
104. P. G. Woodruff *et al.*, "Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids," *Proceedings of the National Academy of Sciences*, vol. 104, no. 40, pp. 15858–15863, Oct. 2007, doi: 10.1073/pnas.0707413104.
105. M. D. Gans and T. Gavrilova, "Understanding the immunology of asthma: Pathophysiology, biomarkers, and treatments for asthma endotypes," *Paediatr Respir Rev*, vol. 36, pp. 118–127, Nov. 2020, doi: 10.1016/j.prrv.2019.08.002.
106. H. Rupani *et al.*, "Comprehensive Characterization of Difficult-to-Treat Asthma Reveals Near Absence of T2-Low Status," *J Allergy Clin Immunol Pract*, vol. 11, no. 9, pp. 2812-2821.e4, Sep. 2023, doi: 10.1016/j.jaip.2023.05.028.
107. P. Venkatesan, "2025 GINA report for asthma," *Lancet Respir Med*, vol. 13, no. 8, pp. e41–e42, Aug. 2025, doi: 10.1016/S2213-2600(25)00242-5.
108. K. Gruffydd-Jones, "BTS/NICE/SIGN guideline for asthma 2024: Diagnosis, monitoring and chronic asthma management. How does this compare to GINA 2024?," *NPJ Prim Care Respir Med*, vol. 35, no. 1, p. 22, Apr. 2025, doi: 10.1038/s41533-025-00425-x.
109. P. J. McDowell, J. Busby, and L. G. Heaney, "Asthma Exacerbations in Severe Asthma: Why Systemic Corticosteroids May not Always Be the Best Treatment Option," *Curr Treat Options Allergy*, vol. 10, no. 1, pp. 53–63, Feb. 2023, doi: 10.1007/s40521-023-00330-z.
110. F. L. M. Ricciardolo, V. Carriero, and F. Bertolini, "Which Therapy for Non-Type(T)2/T2-Low Asthma," *J Pers Med*, vol. 12, no. 1, p. 10, Dec. 2021, doi: 10.3390/jpm12010010.
111. G. Bolner *et al.*, "Long-acting muscarinic antagonists as add-on treatment for asthma in children under age 12: a systematic review and meta-analysis," *Paediatr Respir Rev*, May 2025, doi: 10.1016/j.prrv.2025.04.003.
112. F. Santamaria, C. Ziello, P. Lorello, C. Bouchè, and M. Borrelli, "Update on Long-Acting Anticholinergics in Children and Adolescents With Difficult and Severe Asthma," *Front Pediatr*, vol. 10, Jul. 2022, doi: 10.3389/fped.2022.896865.
113. T. W. Guilbert and W. Busse, "How Has the Biologic Revolution Improved Patient Care?," *J Allergy Clin Immunol Pract*, vol. 11, no. 9, pp. 2683–2685, Sep. 2023, doi: 10.1016/j.jaip.2023.07.029.
114. G. Shouse, "Bispecific antibodies for the treatment of hematologic malignancies: The magic is T-cell redirection," *Blood Rev*, vol. 69, p. 101251, Jan. 2025, doi: 10.1016/j.blre.2024.101251.
115. L. Melscoet, N. Khayath, N. Migueres, M.-A. Goltzene, N. Meyer, and F. de Blay, "Severe non-atopic asthma: omalizumab can reduce severe asthma exacerbations," *Journal of Asthma*, vol. 60, no. 5, pp. 881–889, May 2023, doi: 10.1080/02770903.2022.2103427.

 doi:10.20944/preprints202512.1436.v1

116. P. Flood-Page *et al.*, "A Study to Evaluate Safety and Efficacy of Mepolizumab in Patients with Moderate Persistent Asthma," *Am J Respir Crit Care Med*, vol. 176, no. 11, pp. 1062–1071, Dec. 2007, doi: 10.1164/rccm.200701-085OC.
117. J. Delgado, I. Dávila, and J. Domínguez-Ortega, "Clinical Recommendations for the Management of Biological Treatments in Severe Asthma Patients: A Consensus Statement," *J Investig Allergol Clin Immunol*, vol. 31, no. 1, pp. 36–43, Feb. 2021, doi: 10.18176/jiaci.0638.
118. M. Caminati, A. Vatrella, P. Rogliani, E. Carpagnano, A. Spanevello, and G. Senna, "Tezepelumab for severe asthma: elevating current practice to recognize epithelial driven profiles," *Respir Res*, vol. 25, no. 1, p. 367, Oct. 2024, doi: 10.1186/s12931-024-02998-6.
119. "ClinicalTrials.gov. A Study to Investigate the Efficacy and Safety of Tezepelumab Compared With Placebo in Children 5 to <12 Years Old With Severe Asthma (HORIZON). Identifier: NCT06023589. Updated 2025. Available at: https://clinicaltrials.gov/study/NCT06023589.".
120. G. Kardas, M. Panek, P. Kuna, P. Damiański, and M. Kupczyk, "Monoclonal antibodies in the management of asthma: Dead ends, current status and future perspectives," *Front Immunol*, vol. 13, Dec. 2022, doi: 10.3389/fimmu.2022.983852.
121. C. A. Dinarello, A. Simon, and J. W. M. van der Meer, "Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases," *Nat Rev Drug Discov*, vol. 11, no. 8, pp. 633–652, Aug. 2012, doi: 10.1038/nrd3800.
122. J. J. Ghimire *et al.*, "Azithromycin for Poorly Controlled Asthma in Children," *Chest*, vol. 161, no. 6, pp. 1456–1464, Jun. 2022, doi: 10.1016/j.chest.2022.02.025.
123. X. Pan *et al.*, "The efficacy and safety of azithromycin in treatment for childhood asthma: A systematic review and meta-analysis," *Pediatr Pulmonol*, vol. 57, no. 3, pp. 631–639, Mar. 2022, doi: 10.1002/ppul.25783.
124. J. Sun and Y. Li, "Long-term, low-dose macrolide antibiotic treatment in pediatric chronic airway diseases," *Pediatr Res*, vol. 91, no. 5, pp. 1036–1042, Apr. 2022, doi: 10.1038/s41390-021-01613-4.
125. D. Thomas and P. G. Gibson, "Long-Term, Low-Dose Azithromycin for Uncontrolled Asthma in Children," *Chest*, vol. 162, no. 1, pp. 27–29, Jul. 2022, doi: 10.1016/j.chest.2022.03.035.
126. I. Esteban-Gorgojo, D. Antolín-Amérigo, J. Domínguez-Ortega, and S. Quirce, "Non-eosinophilic asthma: current perspectives," *J Asthma Allergy*, vol. Volume 11, pp. 267–281, Oct. 2018, doi: 10.2147/JAA.S153097.
127. F. Peri, A. Amaddeo, L. Badina, M. Maschio, E. Barbi, and S. Ghirardo, "T2-Low Asthma: A Discussed but Still Orphan Disease," *Biomedicines*, vol. 11, no. 4, p. 1226, Apr. 2023, doi: 10.3390/biomedicines11041226.
128. N. E. Alexis and C. Carlsten, "Interplay of air pollution and asthma immunopathogenesis: A focused review of diesel exhaust and ozone," *Int Immunopharmacol*, vol. 23, no. 1, pp. 347–355, Nov. 2014, doi: 10.1016/j.intimp.2014.08.009.
129. E. Scotney, L. Fleming, S. Saglani, S. Sonnappa, and A. Bush, "Advances in the pathogenesis and personalised treatment of paediatric asthma," *BMJ Medicine*, vol. 2, no. 1, p. e000367, Jun. 2023, doi: 10.1136/bmjmed-2022-000367.
130. X.-W. Wang *et al.*, "Benchmarking omics-based prediction of asthma development in children," *Respir Res*, vol. 24, no. 1, p. 63, Feb. 2023, doi: 10.1186/s12931-023-02368-8.
131. M. Yue, S. Tao, K. Gaietto, and W. Chen, "Omics approaches in asthma research: Challenges and opportunities," *Chinese Medical Journal Pulmonary and Critical Care Medicine*, vol. 2, no. 1, pp. 1–9, Mar. 2024, doi: 10.1016/j.pccm.2024.02.002.
132. V. Plaza, C. Cañete, C. Domingo, C. Martínez Rivera, and X. Muñoz, "Efficacy and Potential Positioning of Tezepelumab in the Treatment of Severe Asthma," *Open Respiratory Archives*, vol. 5, no. 2, p. 100231, Apr. 2023, doi: 10.1016/j.opresp.2022.100231.
133. P. E. Pfeffer, H. Rupani, and A. De Simoni, "Bringing the treatable traits approach to primary care asthma management," *Frontiers in Allergy*, vol. 4, Sep. 2023, doi: 10.3389/falgy.2023.1240375.
134. I. Farinha, P. G. Gibson, V. M. McDonald, and L. G. Heaney, "Treatable Traits as a Pathway to Remission in Asthma," *J Allergy Clin Immunol Pract*, vol. 13, no. 7, pp. 1542–1552, Jul. 2025, doi: 10.1016/j.jaip.2025.05.002.

 doi:10.20944/preprints202512.1436.v1