Allergy and Immunology

AIMS Allergy and Immunology, 8(1): 56–79.
DOI: 10.3934/Allergy.2024005
Received: 09 December 2023
Revised: 16 March 2024
Accepted: 22 March 2024
Published: 10 April 2024

http://www.aimspress.com/journal/Allergy

Review

Scoping review on severe asthma: Cytokines, cells, triggers and multi-omics approaches

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Abstract: Severe asthma (SA) is a refractory condition that does not respond well to conventional treatments. Patients with SA have heterogenetic endotypes. Endotypes for SA can be classified as type 2 cytokine-high and type 2 cytokine-low. The condition can also be classified as eosinophilic, neutrophilic, mixed, and paucigranulocytic SA. Abnormalities in T_H1 and T_H17 cytokines can be present in some SA patients. Innate lymphoid cells, airway smooth muscle cells, and lung epithelial cells have important roles in the disease. Viral infections, bacterial infections, fungal infections, smoking, allergens, and pollutants are major triggers that determine the severity of the disease. Biologics have been proven to be effective for some type 2 high patients. Multi-omics approaches, including genomics, transcriptomics, proteomics, metabolomics, and metagenomics, have identified many novel genes or molecules that could serve as biomarkers or potential therapeutic targets for SA. Investigations of the mechanisms of novel genes and molecules will help us understand the condition and find new treatment means for SA in the future.

Keywords: severe asthma; endotypes; cytokines; inflammatory cells; multi-omics approaches; therapeutic targets

1. Introduction

Asthma is the most common chronic-respiratory disease and is a highly heterogeneous condition characterised by reversible bronchial obstruction [1]. In most cases, asthma can be managed by using standard inhaled corticosteroids alone or in combination with other treatments; however, 5–10% of patients are inadequately controlled [2]. Clinically, it is not easy to define severe asthma (SA); the term includes all cases of therapy-resistant disease in all age groups. The European Respiratory Society and American Thoracic Society define SA as asthma requiring treatment with high-dose inhaled corticosteroids (ICS) plus a controller with or without systemic corticosteroids to maintain control of disease, or, despite this therapy, having sub-optimally controlled asthma [3]. SA is currently estimated to affect 3% to 10% of asthmatic adults and up to 2.5% of asthmatic children [4]. The difficulty in managing SA is due to the heterogenic phenotypes and endotypes from individual patients. Endotypes are referred to as mechanisms of disease [5] and are now widely accepted in the guidance of appropriate treatments in clinic. There are many endotypes proposed for asthma [6]. For SA, endotypes can be divided as type 2 cytokine high or type 2 cytokine low due to expression levels of cytokine clusters. Type 2 inflammation is induced by cytokines secreted by T helper 2 cells and other inflammatory cells. High type 2 cytokine expression is present in many, but not all, SA patients. Other cytokine pathways such as T_H1 and T_H17 also have important roles in SA. Endotypes of SA can also be defined as eosinophilic, neutrophilic, mixed, and paucigranulocytic due to inflammatory cells in the lungs. Innate lymphoid cells, epithelial cells, and airway smooth muscle also have roles for SA in some patients. SA can be triggered by viral infection, bacterial infection, fungi infection, allergens, smoking, and pollutants. Biologics to treat type 2 high SA have been shown to control the symptoms well. They have a great impact on the outcomes such as exacerbations, oral corticosteroids (OCS) use, and lung function improvement. For type 2 low SA, there is still a lack of effective drugs. The unmet need for managing SA promotes extensive investigations for this condition. There are many review studies available on asthma, from mechanisms to clinical managements. In this review, we searched for progress on investigations of SA, over the past ten years, and we briefly discuss the roles of major cytokines, immune-related cells, and triggers for SA. We focus on recent progress from multi-omics approaches for SA including genomics, transcriptomics, metabolics, and metagenomics and update the novel findings that could serve as biomarkers or potential therapeutic targets for SA in the future.

2. Cytokines and severe asthma

Human T lymphocytes are derived from the thymus and consist of T helper cells, T cytotoxic cells, T regulatory (Treg) cells, and T natural killer (NK) cells. T helper cells can be divided as T_H1 helper cells and T_H2 helper cells. T_H1 cells mainly release interleukin-2 (IL-2), interferon-γ (IFNγ), and lymphotoxin-α that can activate macrophage and cytotoxic T-cells. T_H2 helper cells can release cytokines IL-4, IL-5, and IL-13 [7], which activate B lymphocytes. B lymphocytes produce immunoglobulins and can undergo class switching to generate IgE due to allergen or antigen stimulation [8].

2.1. Type 2 cytokines

T_H2 cytokines are termed as type 2 cytokines and are released from T_H 2 cells but can also be released from eosinophils, basophils, mast cells, group2 innate lymphoid cells (ILC2s), and B

lymphocytes [7]. IL-4 regulates antibody production, haematopoiesis, and inflammation. IL-5 has important effects on eosinophils, promoting the cells' maturation, activation, and survival. IL-5 helps eosinophils' migrate from blood and recruit to the airways [9]. IL-13 exerts eosinophilic inflammation, mucus secretion, and airway hyperresponsiveness [10]. Clinically, T_H2 response increases fractional exhaled nitric oxide (FeNO), eosinophilia in blood, and type-2 inflammatory epithelial gene signatures [11]. Other cytokines and regulators in T_H2 response are IL-25, IL-33, and thymic stromal lymphopoietin (TSLP). They can be activated in airway epithelial cells by exposure to oxidants, viral and bacterial infections, and other pollutants, initialling T_H2 response.

IL-33: IL-33 is a member of the IL-1 cytokine family which is generated from lining and structural cells including endothelial cells, fibroblasts, and epithelial cells of the lungs when exposed to the environment. Nuclear cytokine IL-33 can be released during cell necrosis, suggesting that IL-33 may function as an alarmin that alerts the human immune system during physical stress, infection, and trauma. IL-33 activates basophils, eosinophils, macrophages, mast cells, and ILC2s through IL-33 receptor ST2 [12]. In paediatric patients who had severe steroid-resistant asthma, IL-33 promotes airway remodelling [13].

IL-25: IL-25 previously was called IL-17E. The cytokine IL-17 family has 6 members, from IL-17A to IL-17F. IL-25 has a unique structure and function. The main T_H2 cells highly express receptor of IL-25 [14]. The expression of IL-25 in airway epithelial cells is a key determinant of type 2 response activation in asthma. Plasma IL-25 level can reflect airway type 2 response and can be used as a factor to predict response to therapy [15]. Sputum IL-25 levels correlate with asthma severity [16].

TSLP: Levels of TSLP protein and mRNA were found to increase in airways of asthma patients, and the level of expression correlated with disease severity [17,18]. *TSLP* encodes a hemopoietic cytokine and signals through a heterodimeric receptor complex. The complex consists of the thymic stromal lymphopoietin receptor (TSLPR) and IL-7R alpha chain. TSLP induces the release of chemokines from monocytes and promotes the maturation of CD11c (+) dendritic cells (DCs) [19]. TSLP promotes T_H2 cell reactions in asthma patients [20,21]. The shorter isoform of TSLP has antifungal and antibacterial activities [22]. TSLP is mainly secreted by airway smooth muscle cells (ASMCs), epithelial cells, fibroblasts, keratinocytes, mast cells, stromal cells, monocytes, macrophages, granulocytes, and DCs [23,24]. TSLP underlies asthma pathophysiology through multiple pathways to influence airway inflammation: for example, as the signal transducer and activator of transcription 3 (STAT3), transcription 5 (STAT5), nuclear factor kappa light chain enhancer of activated B cells (NF-kB), and mitogen-activated protein kinases (MAPKs) [25].

2.2. Type 1 cytokines

T_H2 low asthma patients have low T_H2 cytokines in blood and are often not associated with systemic and airway eosinophils. These patients lack response to glucocorticoids and inhibitors of type 2 inflammation. One-third of SA patients are type 2 low, and this status is clinically challenging [26]. Type 1 T cells release cytokines including IL-2, IFNγ, and IL-12.

IL-2: IL-2 affects Treg cells growth, survival, and activity [27], and it is a chemoattractant for eosinophils [28]. An indirect role of IL-2 is to regulate IL-5 production [29] that is involved in SA.

IFN-γ: IFN-γ is an epithelial mediator with SA [30]. Type 1 T cells can release IFN-γ to influence cytokines CXCL9, CXCL10, and the transcription factor interferon regulatory factor 5 (IRF5) of macrophages/dendritic cells in SA [31,32].

IL-12: IL-12 normally opposes T_H2 response, but it can contribute to allergic airway disease upon allergen exposure in the post-sensitization phase, by recruiting eosinophils and CD4+ T cells with up-regulation of T_H2 chemokines, cytokines, and vascular cell adhesion molecule 1 (VCAM-1). IFN-γ-producing cells play an important role in this unexpected proinflammatory effect of IL-12 in allergic airway disease [33].

2.3. Type 17 cytokines

Naïve T cells differentiate to T_H17 cells with the presence of TGF-β, IL-6, IL-1β, and IL-23, leading to activation of STAT-3 and orphan nuclear receptor RORC2 [34]. In some SA patients, it has been found that increased neutrophils are associated with increased T_H17 cytokines including IL-17A, IL-17F, and IL-22 in the bronchoalveolar lavage fluid (BALF). T_H17 cytokines recruit neutrophils to the airway, induce mucous cell metaplasia, and have multiple effects on airway smooth muscle to result in narrowing of the airways [35].

IL-22: IL-22 is released by T_H17 cells and type 3 ILCs and exclusively functions on non-hematopoietic cells including epithelial cells. It displays a broad range of action in host protection and regeneration. IL-22 is involved in SA through airway allergic inflammation [36].

IL-23: IL-23 is an IL-12-related cytokine. It is essential for the survival and functional maturation of T_H17 cells involved in airway inflammation induced by antigens [37] in SA.

The major cytokines and their roles in SA are listed in Table 1.

Table 1. Major cytokines and mediators in severe asthma.

	Endotypes involved	**Roles in severe asthma**	**Biologic treatments**
TH2 cytokines and mediators			
IL-4	Type 2 high	Regulating B cells to produce IgE that mediates allergic severe asthma	Dupilumab
IL-5	Type2 high	Promoting eosinophil maturation, activation, survival, migration, and recruitment to airway	Mepolizumab, Reslizumab, Benralizumab
IL-13	Type 2 high	Eosinophilic inflammation, mucus secretion, and airway hyperresponsiveness	
IgE	Type2 high	Mediating allergic severe asthma	Omalizumab
IL-33	Type2 high	Acting as alarmin to activate allergic inflammation	REGN3500
IL-25	Type2 high	Regulating internal safety of adaptive immune responses	

Continued on next page

	Endotypes involved	Roles in severe asthma	Biologic treatments
TSLP	Type2 high	Activating MAPKs, NF-kB, STAT3, and STAT5 to induce inflammatory response	Tezepelumab
Th1 cytokines and mediators			
IL-2	Type 2 low	Influencing Treg cell growth, survival, and activity	
IFN-γ	Type 2low	Influencing expression of CXCL9 and CXCL10 as well as the macrophage/dendritic cell transcription factor IRF5	
IL-12	Type 2 high	Counteracting Th2 sensitization and contributing to full-blown allergic airway disease upon airway allergen exposure in the post-sensitization phase, with enhanced recruitment of CD4+ T cells and eosinophils and with up-regulation of Th2 cytokines	
Th17 cytokines			
IL-17	Type 2 low	Recruiting neutrophils to the airway by increasing secretion of epithelial-derived neutrophilic chemokines	
IL-22	Type 2 low	Acting on non-hematopoietic cells including epithelial cells of mucosal surface and exhibits a broad range of action in regeneration and host protection	
IL-23	Type 2 high	Essential for survival and functional maturation of Th17 cells and involved in antigen-induced airway inflammation	

3. Cell endotypes and severe asthma

For SA, human T and B lymphocytes are important immune cells in influencing the process. T helper cells release type 2 cytokines, and B cells produce IgE antibodies when stimulated by antigens or allergens through class switching [8]. Other cells, such as eosinophils and neutrophils, are all shown to have associations with SA. SA can then also be classified as the endotypes eosinophilic, neutrophilic, mixed, and paucigranulocytic. Mixed cell endotypes present with the characteristics of both eosinophilic and neutrophilic SA. Paucigranulocytic SA does not show any abnormalities in eosinophils or neutrophils in the patients' lungs or blood. Innate lymphoid (ILC) cells including ILC2, ILC3, airway smooth muscle cells and airway epithelial cells can also have roles in SA. Anti-inflammatory therapy may not work with patients who have paucigranulocytic disease [38]. The symptoms of these patients could be driven by airway hyper-responsiveness. These patients may benefit from the use of airway smooth muscle-directed therapies.

3.1. Eosinophils

Eosinophils are one of the major cell types in the development of asthma exacerbations. The T_H2 cytokines network in the airways can be maintained with eosinophilic inflammation. It comprises a cascade consisting of vascular cell adhesion molecule 1 (VCAM-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), CC chemokines, and eosinophil growth factors. Periostin is an extracellular matrix protein and is considered a biomarker of the T_H2 immune response. It directly activates eosinophils in vitro. Adhesion of eosinophils to intercellular adhesion molecule (ICAM-1) on airway epithelial cells can upregulate the functions of eosinophils. ICAM-1 is a receptor of rhinovirus (RVs). In virus-induced asthma, virus infection can upregulate the expressions of cysteinyl leukotrienes (cysLTs) and CXCL10. CysLTs can directly induce eosinophilic infiltration in vivo, and CXCL10 can activate eosinophils. Eosinophil activation has several mechanisms to contribute to the development of SA including (1) through T_H2 cytokines (e.g., IL-5 or GM-CSF), (2) through proteins induced by viral infection (e.g., CXCL10), and (3) through interaction with other cells (e.g., neutrophils) [39]. Eosinophils of greater than 3% in sputum can indicate a response to corticosteroids treatments or biologics therapies against type 2 cytokines [38].

3.2. Neutrophils

Neutrophils are one other major cell type in the development of SA. In some patients, asthma severity is associated with airway neutrophilia. Neutrophilia correlates with asthma refractory to corticosteroid treatment [40]. Neutrophils can release many cytokines and chemokines after stimulation [41]. An increase in neutrophil counts has been reported in the sputum of adults with persistent asthma and of children with acute asthma exacerbations [42,43]. IL-8 is a key chemoattractant for neutrophils, and has been found to be upregulated in some cases of SA. Lipopolysaccharide (LPS) can induce release of IL-8 from airway epithelial cells, and IL-8 levels were also found to be raised in the lower airways of asthma patients who were corticosteroid-resistant. Even without chemo-attractants for eosinophils, IL-8 or LPS can stimulate neutrophils to increase the trans-basement membrane migration of eosinophils. Neutrophil SA may represent a non-T2-driven condition, and it can be a predictor of response to antibiotics and as well as possibly a predictor of treatment targeted at pathways that lead to neutrophil recruitment [38].

3.3. ILCs

ILCs are derived from common lymphoid progenitors (CLPs). The ILCs can be classified into three groups based on the differences in their transcription factor and cytokine profiles [44]. ILC2 cells are a novel family of haematopoietic effector cells. ILC2 cells specifically produce type 2 cytokines and depend on GATA3 and retinoic acid-related orphan receptor alpha (RORα) for their functions [7]. ILC2 cells in SA demonstrate an increase in proliferative capacity, expression of TSLP receptor (TSLPR), GATA3, and NFATc1 protein, enhancing IL-5 and IL-13 release. ILC2 cells can release IL-6 after stimulation. ILC2 cells have active phenotypes in severe allergic and eosinophilic asthma, which include increased proliferation; TSLPR, GATA3 and NFATc1 expression; and increased IL-5, IL-13 and IL-6 levels. Mepolizumab for anti-IL-5 can decrease markers of ILC2 cell activation [45].

ILC3 cells are constitutively presented at mucosal barrier sites in many organs such as the lungs, gut, spleen, liver, and skin [46]. In an asthma model from obese mice, ILC3 cells have been found to be involved in the development of airway hyperreactivity (AHR) induced by IL-1β. Blockade of IL-1β reduces ILC3 cell numbers and abolishes the AHR [47]. In lungs from non-allergic neutrophilic asthma mice, ILC3 cell numbers are found to be increased [48]. IL-17+ ILC cells are also found in bronchoalveolar lavage fluid (BALF) from human SA patients [47]. ILC3 cells are elevated in patients with non-eosinophilic asthma and associated with neutrophilic inflammation from the release of neutrophil chemo-attractants. They are also resistant to steroid treatment [49].

3.4. ASMCs

ASMCs are effector cells for bronchoconstriction in asthma and are responsible in secreting chemokines and cytokines in inflammatory responses. Small G proteins of the Rho family, including RhoA, Rac1, and Cdc42, are major functional regulators of ASMCs. In patients with SA, Rac1 is overactive and is essential for ASMC proliferation [50]. Drugs directed at ion channels on the smooth muscle membrane have been found to be ineffective in SA [51]. ASMCs are major targets for both β2-agonist and ICS treatments, but ASMCs derived from SA patients have similar levels to mild asthma patients in fixed airflow obstruction (FAO) responding to β2-agonists and corticosteroids [52]. This suggests ASMCs may have other mechanisms of ASMCs except airflow obstruction in SA patients.

3.5. Airway epithelial cells

Table 2. Major cell endotypes in severe asthma.

Cells	Endotypes involved	Roles in severe asthma
Eosinophils	Eosinophilic	T_H2 network, which comprises a cascade of vascular cell adhesion molecule-1/CC chemokines/eosinophil growth factors, including granulocyte-macrophage colony-stimulating factor (GM-CSF), involving virus induced severe asthma
Neutrophils	Neutrophilic	Can release many cytokines and chemokines after stimulation, helping recruit cytokines TNF, IL-1, IL-6, IL-8, IL-23, and IL-17
ILC2	Eosinophilic	Producing type 2 cytokines and depends on GATA-binding protein 3 and retinoic acid-receptor-related orphan receptor-a for their development and proliferative capacity, TSLP receptor (TSLPR), and NFATc1 protein expressions and increasing IL-5 and IL-13 release
ILC3	Neutrophilic	Influencing non-allergic neutrophilic asthma and associating with neutrophil inflammation by release of neutrophil chemo-attractants and were glucocorticoid resistant
Airway smooth muscle cells	Paucigranulocytic	Major effector cells of bronchoconstriction in asthma and contribute to the inflammatory process by secreting pro-inflammatory cytokines
Airway epithelial cells	Paucigranulocytic	Recognizing microbes, parasites, and allergens as well as alarmins/damage-associated molecular patterns; promoting release of pro-inflammatory cytokines/chemokines, including IL-6, IL-8, CCL20, CCL17, TSLP, IL25, IL-33, and GM-CSF

The airway epithelial cells (AECs) constitute an efficient physical barrier in human lungs. The cells are the first line of defence against allergens, airborne irritants, and microorganisms. The most prominent character of AECs is that the cells express various important receptors in order to recognise

dangers and foreign invading proteins. These receptors include toll-like receptors (TLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), pattern recognition receptors (PRRs), nucleotide-binding oligomerisation domain (NOD)-like receptors (NLRs), C-type lectin receptors (CLRs), protease activated receptors (PAR)-2, and purinergic receptors [53]. These receptors recognise pathogen-associated molecular patterns (PAMPs) from inhaled allergens, microbe parasites, alarmins, and damage-associated molecular patterns (DAMPs) from death cells. After recognition, AECs can activate downstream signalling to release inflammatory chemokines and cytokines including IL-6, IL-8, IL-25, IL-33, CCL17, CCL20, TSLP, IL25, and GM-CSF [54]. Genome wide association studies (GWASs) have identified a few genes that express in human airway epithelium and associated with SA such as *ORMDL3*, *GSDMB*, and *TSLP* [55]. ORMDL3 mediates sphingolipid synthesis, cell stress, glycolysis, and viral infections. One of the functional roles of GSDMB in asthma path-physiology is to regulate cell pyroptosis, a specific type of cell death through GSDMB's N terminals to form pores on the epithelial cell membrane. TLSP works as an important driver of type 2 cytokine response in SA [56,57].

The major endotypes of cells in SA are listed in Table 2.

4. The triggers of severe asthma

The triggers of SA come from viral, bacterial, and fungal infections, allergens, smoking, and pollutants.

4.1. Viral infection

Most asthma exacerbations are associated with viral respiratory infections. Viral infections are closely associated with wheezing illnesses in children. The major viruses that trigger SA are human rhinovirus (HRV), respiratory syncytial virus (RSV), influenza virus, and SARS-CoV-2 virus.

HRV: HRVs are positive-sense, single-stranded-RNA (ssRNA) viruses that belong to the family Picornaviridae and the genus Enterovirus. Based on phylogenetic sequence, HRVs are classified as three species: HRV-A, HRV-B, and HRV-C. HRV-A and HRV-B species can be cultured in normal culture. HRV-C strains cannot be cultured in standard culture medium, although HRV-C strains have a genomic organisation like that of HRV-A and HRV-B [57]. Approximately 60% of asthma exacerbations have been linked with HRV infections [58]. HRV-C infections are responsible for the majority of asthma attacks in children that result in hospitalisation [59]. Viral upper respiratory tract infections with HRV in adults are the most common cause of asthma exacerbation [60]. Most of its serotypes enter epithelial cells in the respiratory tract via binding to ICAM-1 receptor [61–63] while a minority of them use the low-density lipoprotein (LDL) receptor for virus entry. It normally infects the upper respiratory tract, causing the common cold. However, in patients with asthma, their asthma symptoms get worse, and it could also infect the lower respiratory tract [64]. In fact, the genetic variants of *ORMDL3* are linked with significant increases of wheezing episodes induced by HRV infection in children. In asthma patients, elevated levels of ICAM-1 expression increase their susceptibility to HRV infection as ICAM-1 promotes binding of HRV and entry of viral components into the host cells.

RSV: Human RSV is an enveloped, non-segmented negative-stranded RNA virus of the family Paramyxoviridae. RSV can induce wheezing illnesses during infancy. The effect of the infection can

influence respiratory health for years [65]. RSV infections can damage human airways and cause obstruction and recurrent wheezing. Palivizumab is an anti-RSV monoclonal antibody that can decrease the risk of severe illness and wheezing induced by RSV [66]. Investigating the mechanisms of severe viral illnesses caused by RSV might lead to new treatments for viral wheezing illnesses and may reduce the risk for SA [67].

Influenza virus: Influenza viruses are members of the family Orthomyxoviridae. This family represents enveloped viruses consisting of negative-sense single-strand RNA segments in the genome [68]. In 2009, an influenza virus pandemic was highly transmissible and caused severe respiratory disease. The influenza pandemic was co-morbid with asthma, and this resulted in hospitalisations. Animal studies showed pandemic H1N1 infection had characteristics of allergic airway disease and had signs of asthma exacerbation. It was thought that although the immune response caused exacerbations, it occurred actually to protect the host from severe outcomes caused by influenza [69].

SARS-CoV-2 virus: SARS-CoV-2 is a positive-sense single-stranded RNA virus [70]. SARS-CoV-2 virus invades lung cells through the angiotensin-converting enzyme 2 (ACE2) receptor. As IL-13 can decrease ACE2 expression, asthma would not be a significant risk factor for severe COVID-19. However, several SA-associated risk factors might occur in both SA and COVID-19. Although the respiratory symptoms of asthma related to COVID-19 were mild to moderate, some patients had chronic worsening of their asthma symptoms and required an increment in their medications to control the conditions [71,72].

4.2. Bacterial infection

In clinical practice, bacterial organisms are also contributors to asthma exacerbations, but they have received much less attention than viruses for SA. Asthma exacerbations can be caused by Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Tropheryma whipplei [73,74].

Streptococcus pneumoniae: S. pneumoniae is commonly found in the nasopharynx [75] in what is usually the initial step of infection. Carriage of S. pneumoniae is most prevalent in children [76], and it declines with increasing age [77]. This may be due to acquired immunity from exposure. A Prospective Study on Asthma in Childhood (PSAC) found that the infants colonised with *S. pneumoniae* had an increased risk of a first wheezy episode. These infants can develop persistent wheeze or asthma during follow-up. Early nasopharyngeal carriage of S. pneumoniae was also linked to increased eosinophil counts in blood, higher total IgE in serum, airway reversibility, and subsequent asthma [78–81].

Haemophilus influenzae: Nontypeable Haemophilus influenzae (NTHi) is a respiratory tract pathobiont that can chronically colonise the airways of patients. It is often associated with neutrophilic SA [82]. In SA patients, NTHi persistence with pulmonary macrophages can induce T_H17 response and cause chronic airway inflammation, leading to decreased lung function and reduced effectiveness of steroid treatments. Reducing the burden of NTHi could improve the outcomes of managing SA patients in clinic [83].

Moraxella catarrhalis: Moraxella catarrhalis is also more abundant in patients with severe neutrophilic asthma. It has been found that specific genes have been linked to inflammasome and neutrophil activation in SA patients with *Moraxella catarrhalis* infections [84].

Tropheryma whipplei: Tropheryma whipplei belongs to Actinobacteria but has reduced size in genome. The abundance of *Tropheryma whipplei* is associated with gene expression of IL-13 type 2 and ILC2 signatures and correlate positively with sputum eosinophils in SA. How the bacterial species drive the inflammatory response in SA needs further evaluation [84].

Pseudomonas aeruginosa (P. aeruginosa) is a ubiquitous and opportunistic microorganism and is one of the most significant pathogens that produce chronic colonisation and infection of the lower respiratory tract, especially in people with chronic inflammatory airway diseases such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), and bronchiectasis. The role of *P. aeruginosa* in SA also requires further investigation [85].

4.3. Fungal infection

Many airborne fungi can also trigger SA. These fungi include species of Alternaria, Aspergillus, Cladosporium, and Penicillium [86]. Some mould allergens can produce more severe airway disease than other common allergens. In daily human life, airway exposure to airborne fungal spores is almost constant. C. herbarum, A. alternata, and A. fumigatus are the most airborne fungi. Comparing to allergens such as house dust mites, grass pollen, and cat dander, fungi cause disease not only as sources of allergenic proteins but also by having the additional ability to colonise the respiratory tract and on the host skin. Therefore, fungi have a much greater impact on human SA for triggering host defences and producing nonallergenic toxins and enzymes. Asthma patients with severe disease who are sensitized to one or more fungi can be defined as having SA with fungal sensitization (SAFS) [87]. SAFS is a relatively new classification of allergic asthmatic subjects, but it is gradually becoming more recognised [88]. Identifying fungal cases of SA is important, and treatment with imidazole antifungals can provide significant benefit. Antifungal treatment is a key part of the successful management of SAFS [89].

4.4. Allergens

Allergens can induce SA through IgE mediated allergic response. Inhalant allergens can cause chronic SA. Recent studies found that high titre IgE allergic response, particularly to dust mites, was associated with asthma exacerbations with rhinovirus infection. As we discussed above, it is now well recognised that the fungus Aspergillus can colonise the human airway and cause SA in some patients [89].

4.5. Smoking

Cigarette smoking can be a predictor of poor asthma control and asthma severity [67].Cigarette smoking exposure can impact asthma development in children [90]. Cigarette smoking may induce a neutrophil-predominant inflammation of the airways [91], which may make patients resistant to asthma treatment [92]. Persistent cigarette smoking induces both allergic T_H2-driven inflammation [93] and T_H1-mediated inflammatory responses in SA patients [94]. E-cigarette use can have significant associations with asthma in adolescents [95]. However, how vaping and e-cigarette use influence SA needs to be investigated further.

4.6. Pollutants

In a French epidemiological study on the relation between SA and air pollutants, the relationships between air nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and ozone (O_3) concentrations and SA were investigated. Asthma severity was significantly related to O_3 concentrations, while it was unrelated to NO_2. SO_2 was associated with asthma severity only for the model-based assignment of exposure [96]. Inhaled O_3 could play a part for both acute and chronic inflammation in the lungs. O_3 could induce T_H2 pattern response [97] and eosinophilic airway inflammation [98]. Volatile organic compounds (VOCs) are ubiquitous domestic pollutants, but their role in asthma exacerbations is uncertain. In a systematic analysis of 11 databases and three repositories for the relationships between VOC exposure and exacerbations of asthma, the results were inconsistent [99].

The triggers and their possible roles in SA are listed in Table 3.

Table 3. Most common triggers for severe asthma.

Triggers	Roles in severe asthma
Virus	
Human rhino virus	Regulating ICAM-1 expression in airway epithelial cells in asthma patients increasing their susceptibility to HRV infection as ICAM-1 promotes binding of HRV and entry of viral components into the host cells
Respiratory syncytial virus	Inducing bronchiolitis to damage the airways, promoting airway obstruction, and recurrent wheezing
Influenzas virus	Governing immune responses that cause exacerbations
SARS-CoV-2 virus	Subgroup of these asthmatic patients evolved with a chronic worsening of their asthma requiring an increment in asthma medication to control the disease when having COVID-19
Bacteria	
Streptococcus pneumoniae:	Increasing blood eosinophil count, higher total serum IgE, and airway reversibility
Hemophilus influenzae:	Contributing to chronic airway inflammation and T_H17 responses in severe asthma, which can lead to decreased lung function and reduced steroid responsiveness.
Moraxella catarrhalis:	Abundant in severe neutrophilic asthma and genes linked to inflammasome and neutrophil activation
Tropheryma whipplei	Associating with T_H2 response in SA
Fungi	
Alternaria, Aspergillus, Cladosporium and Penicillium	Acting as allergens, also having the additional ability to actively germinate and infect the host skin or attempt to colonise the respiratory tract to induce SA
Allergens	IgE mediates severe asthma
Smoking	Induce a neutrophil-predominant inflammation of the airways
Pollutants	O_3 induces a T_H2 pattern response and eosinophilic airway inflammation

5. Biological therapies for SA

A majority of biological therapies for SA are antibody treatments against IgE and type 2 airway inflammation. These biologics include omalizumab against IgE, dupilumab against IL-4Rα, mepolizumab and reslizumab against IL-5, benralizumab against IL-5Rα, tezepelumab against TSLP, REGN3500 against IL-33, and fevipiprant against PGD2 [100]. Biologics have some interesting results

and could be the potential treatment for uncontrolled asthma. Optimizing the standard therapies of biologics is currently ongoing and the appropriate using biologics can decrease asthma-related morbidity [101]. Although current biologics have effects in the control of SA through improving FEV1 to decrease eosinophil counts, SA patients with type 2 low or paucigranulocytic disease are still very hard to control.

6. Multi-omics approaches for SA

Multi-omics refers to applying modern technologies such as next generation sequencing, systemic protein profiling, and high output measurements of small molecules to carry out cutting-edge approaches that combine data from different biomolecular levels, such as DNA, RNA, proteins, metabolites, and epigenetic markers, in order to obtain a holistic view of how living systems work and interact [102]. In the review, we only report the progresses in genomics approaches, transcriptomics approaches, proteomics approaches, metabolomics approaches, and metagenomics approaches for SA.

6.1. Genomics approaches

Genomics approaches to SA have been achieved by GWASs. More than ten genes were identified to have association with SA worldwide. The loci include *THSD4*, *IL33* [103], *MUC5AC* [104], *FLJ22447* [105], *ORMDL3/GSDMB*, and *IL1RL1/IL18R1* [106]. THSD4 (thrombospondin type 1 domain containing 4) is an extracellular matrix protein and has roles in microfibril formation to maintain the structural integrity of human lungs [107]. MUC5AC has pathogenic roles in airway hyper-responsiveness and mucus plugging during asthma exacerbation in human lungs [108]. *IL-33, ORMDL3, GSDMB*, and *IL1RL1/IL18R1* are also reported to have associations with asthma through previous GWAS studies [109,110]. The most potential therapeutic targets are ORMDL3 and GSDMB. ORMDL3 medicates HRV infection through regulating ICAM-1 receptor to induce SA [57]. GSDMB works as a key molecule in pyroptosis to regulate cell inflammation [111]. Ongoing study into the function and inhibition for products of these genes would play a valuable role in discovering new therapeutic targets in the pathways that are involved in the pathophysiology of SA.

6.2. Transcriptomic approaches

The transcriptomic approaches for SA include sequencing RNA samples from blood, sputum, and brushed cells from the lungs.

Circulation: Expression of RAR-related orphan receptor A (RORA) is significantly higher in patients with SA. SA patients have decreased glucocorticoid receptor signalling and increased activity of mitogen-activated protein kinase (MAPK) and Jun kinase [112]. SA is found to be linked with the activation of circulating CD8+ T cells. The response is correlated with the downregulation of miR-146a/b and miR-28-5p [113]. T_H2-biomarker transcriptomic signature was found in SA patients, but with no signature in association with T_H2-biomarker-low patients [114].

Sputum: T_H2-driven eosinophilic and non-T_H2 phenotypes of both neutrophilia or non-inflammatory paucigranulocytic patterns have been identified in sputum transcriptomics [115]. A signature of six-gene expression biomarkers (*CLC*, *CPA3*, *DNASE1L3*, *IL1B*, *ALPL* and *CXCR2*) in sputum can predict response to corticosteroid managements, and the six gene expression levels predict

SA better than sputum and blood eosinophils [116]. In a separate cohort of SA patients, an enrichment of genes was shown to be involved in the p38 signalling pathway in SA. Phosphorylation of p38 was increased in some patients and can associate with neutrophilic airway inflammation. p38 activation may have roles in steroid-resistant inflammation, and it can continue recruitment of neutrophils to the airways in SA [117].

Bronchial brush samples: High baseline expression levels of calcium-activated family member 1 (CLCA1), periostin, and serpinB2 are found to be linked with a better clinical response to corticosteroids. Meanwhile, high expression levels of FK506-binding protein (FKBP51) are linked with a poor response to corticosteroids. IL-13 increases expressions of CLCA1, periostin, and serpinB2. Corticosteroids induce expression of FKBP51 [118]. Transcript analysis from bronchial brush samples of SA patients identified that the dysregulation of neural signals, apoptosis, REDOX, and O-glycan process are linked to disease severity. In non-T_H2 subtype SA patients, the neural signals and IL-26-related co-expression module are significantly dysregulated. These genes encode proteins related to transmembrane transport, the apoptotic process, O-glycan processing, and amino acid biosynthetic process [119]. Genes associated with SA were enriched in co-expression modules for NK cell-mediated cytotoxicity and interleukin production. In an integrated analysis of systemic and local airway transcriptomes, NKG7 and perforin (PRF1) are identified as key drivers in SA. Nasal genes are enriched in the tricarboxylic acid (TCA) cycle module in SA patients. Network analyses identified G3BP stress granule assembly factor 1 (G3BP1) and InaD-like protein (INADL) are key nasal drivers for SA [120].

6.3. Proteomic approaches

Proteomic analysis involves systemically investigating the identification and quantification of the complete compositions of proteins in a biological system (e.g., cell, tissue, and organ). Most samples for proteomic approaches in SA are from plasma and sputum.

Plasma: SA patients are found to be distinguished from each other by 365 different protein abundancies and the plasma proteome defines distinct patients' endotypes within SA. These proteins included HSP90A, PTPN11, CCL5, IL4, CD86, FGF9, IDG15, NME2, IFNG, and C3. Some groups of proteins are significantly enriched in the exosomal markers of immune and inflammatory cells [121].

Sputum: In SA patients, the protein levels of colony-stimulating factor (CSF) 2 are higher in current smoker sputum supernatants; the levels of azurocidin 1, neutrophil elastase, and CXCL8 are found to be higher in ex-smokers [122]. In another experiment in proteomic screening, a significant increase of 23 proteins is observed in the sputum in SA patients. These proteins include A2M, APOA2, ELANE, GPI, S100A8, S100A9, and S100A12. These proteins have roles in multiple biological processes, including immunity, inflammatory, transport, protease activity, protease inhibition, metabolism, hydrolase activity, and vasculogenesis [123].

6.4. Metabolomic

Metabolomics systematically studies low molecular weight bio-chemicals in a given biological system [124]. In SA, metabolites can play an important role in disease and homeostasis from redox balance, oxidative stress, signalling, apoptosis, and inflammation [125]. All cell types involved in SA can produce and secrete exosomes that contain proteins, nucleic acids, lipids, cytokines, growth factors,

and co-stimulatory molecules [126]. Amino acids such as glycine, glutamine, and glutamate may have protective effects for SA, whereas phenylalanine can have adverse effects [127]. High FENO is demonstrated in most of the asthma population, and it is associated with greater arginine metabolism in severe and reactive asthma patients [128]. In animal models with iNOS deletion, eosinophilic inflammation is regulated by arginine metabolism. Arginine inhibits inflammation, while iNOS metabolism promotes airway inflammation [128]. Systemic IL-6 inflammation is demonstrated in obese asthma patients and also in a small subset of non-obese patients. These patents were found to have more severe asthma. IL-6 inhibitors may reduce metabolic dysfunction in some SA patients [129].

6.5. Metagenomics

Table 4. The potential biologic targets identified by omics for severe asthma.

	Genes or molecules	**Roles in severe asthma**
Genomics	*THSD4*, *IL33*, *MUC5AC*, *FLJ22447*, *ORMDL3/GSDMB*, and *IL1RL1/IL18R1*	Extracellular matrix protein that is involved in microfibril formation, Cytokine alarm, airway hyper-responsiveness and mucus plugging, HRV infection, and pyroptosis
Transcriptomics		
Circulation	*RORA: CD8+ T2* signature	Glucocorticoid receptor signalling activity of the mitogen-activated protein kinase and Jun kinase cascades; T2 response
Sputum	A six-gene expression biomarker signature (*CLC, CPA3, DNASE1L3, IL1B, ALPL* and *CXCR2)*	Predicts response to oral corticosteroids in SA
Bronchial brush	*FKBP51, CLCA1,* periostin, serpinB2, and IL-26	Neural signal, REDOX, apoptosis, and O-glycan process in SA
Proteomic		
Plasma	365 proteins including HSP90A, PTPN11, CCL5, IL4, CD86, FGF9, IDG15, NME2, and IFNG	Immune-inflammatory; exosomal markers
Sputum	23 proteins including A2M, APOA2, ELANE, GPI, S100A8, S100A9, and S100A12	Immune-inflammatory: immunity, inflammatory, chemokines, protease, protease inhibitor, metabolism, transport
Metabolomic	Glycine, glutamine, glutamine	Protective effects in SA
	Phenylalanine	Adverse effects in SA
	Arginine	Regulating FENO
	IL-6	Obese SA
Metagenomics	Actinobacteria	Indicator of steroid responsiveness
	Proteobacteria	Regulating expression of T_H17-related genes
	Klebsiella	Indicating asthma severity

Next-generation sequencing (NGS) techniques perform a more accurate microbial characterization based on genetic contents. Targeted sequencing of the 16S ribosomal RNA (16S rRNA) gene has been routed for the application of investigating the bacterial abundance and diversity of the microbiota [130]. A change in the nasopharyngeal or the salivary microbiome has been found to be associated with different phenotypes of SA [131]. As we discussed above, respiratory pathogens can be triggers for asthma exacerbations due to airway inflammation [132,133].

In patients with SA, the composition of bronchial bacteria is associated with several disease-related features. These features include body mass index, bronchial biopsy eosinophil values and sputum total leukocyte count. Actinobacteria is associated with improving asthma scores and bronchial epithelial gene expression of FK506 binding protein (FKBP5), an indicator of steroid treatment. Proteobacteria has negative correlations with biopsy eosinophil values. It is also associated with expression of the T_H17-related genes. Specific microbiotas are associated with and may regulate inflammatory processes in SA patients and associated phenotypes [134].

The novel genes and molecules identified by transcriptomic, proteomic, metabolomic, and metagenomic approaches for SA are listed in Table 4.

7. Conclusions

Management of severe asthma, clinically, is a challenge due to the heterogeneity of the conditions. Cytokines, inflammatory cells, and various triggers can interact together in disease developments. The current biologics available show great advantages for treating the condition, although there is still a necessity to optimise the criteria of how and when they should be applied in clinic. The multi-omics approaches for SA identify many novel molecules and genes that could be biomarkers and therapeutic targets for the condition. The next stage for research on SA will be investigating the mechanisms for these novel genes and molecules, integrating the data from multi-omics approaches, and validating the value of biomarkers and treatment targets. Investigations into the pathways of novel genes and molecules in SA would be of great help in developing personal medicine to treat SA cases in the future.

Acknowledgements

Hongchun Du was awarded a scholarship from China Scholarship Council (201906385024). Jinming Gao was supported by a grant from the National Natural Sciences Foundation of China (No. 81970025).

Conflict of interest

All authors declare no conflicts of interest in this paper.

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