



Research Article

Clinical Features and Management of Neutrophilic Asthma

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Abstract

Asthma is a chronic inflammatory airway disease with several distinct phenotypes, characterized by different immunopathological pathways, clinical presentation, severity of the disease and response to treatment. The phenotypes of asthma include eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic asthma. Approximately 3.6-10% of patients with asthma have severe refractory disease, which is uncontrolled on high doses of inhaled corticosteroids and long-acting β 2-agonists. Some of these individuals with severe disease suffer from neutrophilic phenotype. Neutrophilic asthma is a severe and persistent disease, with frequent exacerbations and hospitalizations. It is characterized by the presence of high levels of neutrophils in the lungs and airways and fixed airflow obstruction. The T Helper 17 lymphocytes (Th17) cytokines, Interleukin-17 (IL-17) and IL-17F play an important role in the pathogenesis of neutrophilic asthma. IL-17 plays a key role in the immunophysiology of neutrophilic asthma by expressing the secretion of chemoattractant cytokines, chemokines, adhesion molecules and growth factors which lead to the recruitment and activation of neutrophils. Activated neutrophils release multiple proteinases, cytokines, chemokines and reactive oxygen species which cause airway epithelial cell injury, inflammation, hyperresponsiveness and airway remodeling. Neutrophilic asthma is unresponsive to high dose inhaled corticosteroids and to novel monoclonal antibody therapies. There is need for targeted precision biologics and other treatment modalities for patients with neutrophilic asthma, such as long-acting phosphodiesterase 4 inhibitors, macrolide antibiotics and bronchial thermoplasty.

Keywords: Airway smooth muscle; Bronchial thermoplasty; Macrolides; Neutrophilic asthma

Introduction

Asthma is a significant public health problem, affecting more than 358 million individuals globally [1] and its prevalence has been

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increasing during the last 40 years [1-3]. It is the most common chronic respiratory disease in children in the developed countries [4] and its prevalence is steadily increasing in the developing world [5].

Asthma is a chronic inflammatory airway disease with several distinct phenotypes, characterized by different immunopathological pathways, clinical presentation, severity of the disease and response to treatment [6-11]. The phenotypes of asthma include eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic asthma [7].

Patients with eosinophilic asthma have an eosinophil count $\geq 3\%$ [12-14], whereas patients with neutrophilic asthma have elevated sputum neutrophil count between $\geq 61\%$ [14] and $\geq 64\%$ [15], depending on the study. Mixed granulocytic phenotype is characterized by increase in both eosinophils ($>3\%$) and neutrophils ($>61\%$ or $>64\%$) [15]. Paucigranulocytic phenotype embraces patients with very few eosinophils ($<3\%$) and neutrophils ($<61\%$ or $<64\%$) in induced sputum [15]. Non-eosinophilic asthma is the term used to classify patients with low eosinophil numbers ($<3\%$), which include neutrophilic asthma and paucigranulocytic phenotype [7].

Approximately 3.6-10% of patients with asthma have severe refractory disease, which is uncontrolled despite treatment with high-dose Inhaled Corticosteroids (ICS) and Long-Acting β 2-Agonists (LABA) [16-19]. Neutrophilic asthma is the most common phenotype in adult patients presenting with acute severe asthma, whereas eosinophilic asthma is the most common phenotype in children with acute severe asthma [20]. However, paucigranulocytic asthma is the most common phenotype in both adults and children in patients with stable asthma [21].

The definition, evaluation and treatment of severe refractory asthma has been refined and continues to be updated [19,22-24]. The American Thoracic Society (ATS) guidelines on the definition of severe refractory asthma is based on two major and seven minor criteria for the diagnosis of severe refractory asthma [22]. The criteria for established diagnosis of refractory asthma include fulfilling one, or both major criteria and at least two minor criteria [21]. The ATS criteria for the diagnosis of severe refractory asthma are given in table 1.

Major criteria
Treatment with continuous or near continuous ($>50\%$ of the year) oral corticosteroids Need for treatment with high-dose inhaled corticosteroids.
Minor criteria
Need for additional daily treatment with controller medication (long-acting β 2-agonist, leukotriene receptor antagonist, theophylline) Asthma symptoms needing short-acting β 2-agonists use on a daily or near daily basis Persistent airway obstruction (FEV1 $<80\%$ predicted, diurnal peak flow variability $<20\%$ predicted) One or more urgent care visit for asthma Three or more oral steroid bursts per year Prompt deterioration with $>25\%$ reduction in oral or inhaled corticosteroids Near fatal asthma event in the past.
Diagnosis
One major criterion plus two minor criteria; other diseases should have been excluded, exacerbating factors treated and patient is general adherent.

Table 1: American thoracic society criteria for severe/refractory asthma.

Clinical Features of Neutrophilic Asthma

Neutrophilic asthma is an adult-onset disease which usually starts after 12 years. It is the most common phenotype in adult patients presenting with acute severe asthma compared with eosinophilic asthma [20]. However, eosinophilic asthma is the most common phenotype in children presenting with acute severe asthma [20], but paucigranulocytic phenotype is the most common phenotype in both adults and children with stable asthma [21].

Neutrophilic phenotype is characterized by severe persistent asthma [25-28], with frequent exacerbations, although the exacerbations are not as severe as those encountered in patients with eosinophilic asthma [29,30]. Patients with neutrophilic asthma have frequent urgent visits to emergency rooms, hospitalization and intubation [31]. This phenotype of asthma has been associated with sudden-onset fatal asthma in about 23% of the patients [32]. Furthermore, patients with severe bronchial neutrophilia are more likely to be admitted to hospital for noninfectious status asthmaticus [33].

Neutrophilic asthma is worse at night with frequent nocturnal attacks [34]. Martin et al. [34], found a greater than three-fold increase in the number of granulocytes in Bronchoalveolar (BAL) fluid at 04:00 hr compared with 16:00 hr. Patients with neutrophilic asthma may require chronotherapy with intensification of treatment at night or treatment with long-acting anti-asthma agents [35]. Furthermore, neutrophilic asthma is typically associated with a worse quality of life and has a poor prognosis [7,18,19,28,36].

Neutrophilic asthma is characterized by a high neutrophil count in induced sputum ranging from 40% to 76% of sputum cells [30,32-34], or a neutrophil count of $500 \times 10^4/\text{ml}$. Additionally, patients with neutrophilic asthma have less sputum eosinophil count which has been quoted to be between less than 1.9% and 3% by various authors [7,31,37,38].

Increased neutrophils in sputum has been associated with severe persistent asthma [7,23,26], fixed airway obstruction [36,39-42], with very low Forced Expired Volume in 1 second (FEV1) [39,40] and post-bronchodilator FEV1 [43]. Shaw and colleagues [43], have reported that both patients with eosinophilic asthma and neutrophilic asthma had low pre-bronchodilator FEV1, but only patients with neutrophilic asthma had lowest post-bronchodilator FEV1, indicating persistent airflow limitation. Furthermore, patients with neutrophilic asthma are less atopic [42-44] and have less responsiveness to methacholine challenges compared with patients with eosinophilic asthma [41,42,44].

Patients with neutrophilic asthma are unresponsive to LABA and high dose ICS [37,45,46] and the newly introduced targeted biologics [16,47]. There are no specific biomarkers for the diagnosis of neutrophilic asthma. Currently, there are specific biomarkers, such as exhaled Nitric Oxide (FeNO), serum periostin and dipeptidyl peptidase 4 for phenotyping asthma, which are useful for the diagnosis and targeted treatment of Th2-driven eosinophilic inflammation [8,16,48-50]. The clinical and diagnostic features of neutrophilic asthma are shown in table 2.

Airway Remodeling in Neutrophilic Asthma

The pathophysiological feature of neutrophilic asthma is airway hyperresponsiveness and airway remodeling, which is associated

with persistent fixed airway obstruction. There is a strong association between neutrophilic airway inflammation and progression of airflow limitation in patients with neutrophilic asthma [51]. Airway remodeling and bronchoconstriction in asthma involves structural changes, such as airway smooth muscle hyperplasia and hypertrophy; subepithelial basement membrane thickening and fibrosis; extracellular matrix protein deposition; hypertrophy of the submucous glands, goblet cell hyperplasia; thickening and shedding of the epithelium; and neoangiogenesis (Table 3) [52-60]. Airway smooth muscle hypertrophy, hyperplasia and changes in phenotype of ASM is considered the main factor involved in airway hyperresponsiveness [61].

Adult on-set, most cases after 20 years
Less atopic compared with eosinophilic asthma
Less severe exacerbations compared with eosinophilic asthma
Sputum neutrophil count, 40%-64%; eosinophils <2%-3%
Low FeNO <30 ppb
Low serum periostin and dipeptidyl peptidase 4 levels
High hydrogen sulfide levels
Less subepithelial fibrosis compared with eosinophilic asthma
Fixed airflow limitation (low FEV1)
Less responsiveness to methacholine challenge tests
Corticosteroid unresponsiveness
Poor response to biologics

Table 2: Clinical and diagnostic characteristics of neutrophilic asthma.

Epithelial injury due to allergens, respiratory viral infections, and pollutants
Release of cytokines, chemokines, growth factors, and adhesion molecules
Airway epithelial thickening, shedding, and further release of "alarmin" cytokines
Submucous glands and goblet cell hyperplasia, and mucus hypersecretion
Airway hyperresponsiveness
Subepithelial basement membrane fibrosis, and thickening
Deposition of extracellular matrix proteins
Mast cell infiltration of airway smooth muscle cells
Activation of myofibroblasts, and fibroblasts
Airway smooth muscle hyperplasia, and hypertrophy
Neoangiogenesis, and exaggerated vasodilatation
Airway remodeling

Table 3: Pathophysiological features of airway remodeling in patients with neutrophilic asthma.

Patients with severe refractory asthma, including the neutrophilic phenotype have airway smooth muscle cell hyperplasia and hypertrophy, especially patients with fixed airflow limitation [56,59-62]. Additionally, ASM hypertrophy and hyperplasia is associated with progressive decline in lung function (low FEV1), persistent asthma and corticosteroid unresponsiveness [63-66].

Airway smooth muscle cells are productive and secretory cells, which display plasticity depending on inflammatory milieu. They display pro-inflammatory and immunomodulatory functions by synthesis and expression of a variety of surface molecules, integrins and Toll-like receptors [67,68]. They are capable of secreting a milieu

of pro-inflammatory cytokines (IL-1 β , IL-8, IL-5, IL-6, IL-8, IL-10 and IL-11); chemokines (eotaxins and Gro- α); growth factors (EGF-1, FGF, PDGF; VEGF and IGF-1); and angiogenic factors (angiogenin and angiopoietin) [58,67,68]. In addition, ASM cells from asthmatic patients have a distinct hyper reactive “primed” phenotype, which is characterized by increased release of pro-inflammatory cytokines, chemokines and growth factors [56,68]. These mediators act in an autocrine or paracrine fashion and amplify airway inflammation, AHR and remodeling [56].

Another feature of airway remodeling in asthma is deposition of Extracellular Matrix (ECM) protein in the reticular basement region, lamina propria and submucosa, which contributes to airway wall thickening and airflow obstruction. It is also accompanied by increase in the numbers of airway resident cells, such as fibroblasts, myofibroblasts and ASM cells [62,67-69]. There is an increase in ECM deposition around ASM cells and within the muscle bundle in patients with asthma [66,67]. The ECM proteins contain higher amount of collagen I, III, V, fibronectin, tenascin, glycosaminoglycans, proteoglycans and elastic fibers [66,67] and the composition of the proteins is different compared with non-asthmatic subjects [70]. Extracellular matrix proteins and mediators released by mast cells, T lymphocytes and ASM cells may modulate ASM phenotype and functions, including proliferation, migration and contraction [71,72].

Severe refractory asthma is histopathologically characterized by thickening and fibrosis of the Subepithelial Reticular Membrane (SRM) in both adults and children with asthma [69,73-77]. Payne et al. [77] have demonstrated that SRM thickening is already present in children with difficult asthma and to a similar extent to that seen in adults with asthma. In addition they found that SRM thickening is not associated with age, symptom duration, lung function, or concurrent eosinophilic airway inflammation [77]. Thus, SRM thickening and fibrosis can occur in different phenotypes of severe refractory asthma. Thickening of the subepithelial reticular basement membrane is due to increased deposition of collagen I and III, tenascin and fibronectin, versican, laminin and lumican [73,74], produced mainly by fibroblasts [75] and to a less extent myofibroblasts [76]. The increase in subepithelial basement membrane thickness has been shown to correlate with ASM hypertrophy [76] and the severity of airflow limitation [78]. The thickening in SRM may contribute to unresponsiveness to corticosteroids [79].

The airway epithelium provide a physical protective barrier against inhaled micro-organisms, allergens and pollutants. Severe asthma, including the neutrophilic asthma is characterized by epithelial thickening, shedding, destruction of ciliated cells, goblet cell, exposure of neuronal terminals, secretion of ‘alarmin’ cytokines (IL-25, IL-33, TSLP) and upregulation of growth factors [80-82]. The extent of epithelial injury parallels with increasing AHR and severity of the disease [83,84].

There is an increase in numbers and branching of blood vessels in the airways of patients with asthma due to neoangiogenesis [58,85-87], mediated by overexpression of Vascular Endothelial Growth Factor (VEGF), angiopoietin and angiogenin [88,89]. Increased release of pro-inflammatory mediators in the airways may lead to vasodilatation and edema which may perpetuate airway narrowing. Neoascularization has been shown to correlate with airflow limitation and bronchial hyperresponsiveness [89-91], hence contributing to severity of asthma. The pathophysiological features of airway remodeling in neutrophilic asthma are summarized in table 3.

Treatment of Severe Neutrophilic Asthma

Severe refractory asthma has classically been treated using stepwise guidelines of increasing drugs and dosages depending on the severity of the disease, such as the Global Initiative for Asthma (GINA) guidelines [1]. The GINA strategy recommends intensification of the treatment according to the severity of the disease, based on the treatment required to control and reduce symptoms and exacerbations. The GINA classic guidelines has five levels of treatments constituting increasing treatment according to severity. Steps 1 to 3 are classified as mild-moderate asthma and steps 4 and 5 include patients with moderate-severe disease. The current paradigm recommends use of relief SABA and ICS/LABA as needed at step 1. The step-wise guidelines recommend treatment with ICS at step 2, followed by increasing the dosage of ICS up to 800 μ g/day and adding a long-acting β 2-agonist to achieve control at step 3. In patients with severe asthma, steps 4 and 5, the dosage of ICS is increased up to 2000 μ g/day and therapeutic alternatives, such as Leukotriene Receptor Antagonists (LTRA), slow-release theophyllines, or Long-Acting Muscarinic Antagonist (LAMA) are added to the regimen [1]. Table 4 lists the drugs used to treat asthma.

Despite treatment according to guidelines, monitoring adherence and adequate inhaler technique, a significant proportion of asthma patients do not achieve adequate control of asthma symptoms with the standard care treatment [1,92]. Between 49% and 53% of adults receiving treatment adequately have poorly controlled asthma [93,94] and up to 64% of adolescent patients have asthma that is inadequately controlled by the currently available therapies [95]. Table 4 shows the list of drugs available for the treatment of asthma.

Neutrophilic asthma is a difficult to treat phenotype, because it is unresponsive to high-dose ICS and LABA [37,45,46] and to the current anti-interleukin antagonists targeted against eosinophilic asthma [96-100].

Treatment of neutrophilic asthma requires novel anti-inflammatory agents and therapeutic strategies targeted against airway smooth muscle hypertrophy and airway remodeling, such as phosphodiesterase 4 inhibitors, macrolide antibiotics and bronchial thermoplasty.

Biologics for Neutrophilic Asthma

Interleukin-17 and its associated cytokines, IL-17F, IL-23, IL-22 and IL-8 play a key role in the pathogenesis of neutrophilic asthma [101]. However, most clinical trials using biologics targeted against IL-17, IL-8 and IL-23 have not been very encouraging. Busse et al. [102] in a randomized, placebo-controlled phase IIa trial of brodalumab, a monoclonal antibody against IL-17 receptor, in patients with moderate-to-severe asthma, reported that, brodalumab did not result in any statistically significant benefit in terms of ACO scores, FEV1, or use of rescue Short-Acting β -Agonists (SABA).

Blockade of IL-8 receptor CXCR2 also produced temperate results. Nair et al. [103] in a randomized, double-blind, placebo-controlled trial of SCH52713, a selective CXCR2 antagonist in asthmatic patients, noted significant reduction in sputum and blood eosinophil counts. SCH527123 was associated with significantly less exacerbations, but no improvement in lung function (FEV1). In a larger, multicenter, dose finding trial, O’Byrne et al. [104] investigated AZD5069, a selective CXCR2 antagonist, in patients

with uncontrolled asthma receiving medium-to-high dose ICS and LABA. In their clinical trial, they did not find significant benefits in the rates of exacerbations, ACQ-5 and FEV1. Thus, combined blockade of CXCR2 and CXCR1 may be more effective approach in targeting the neutrophil chemoattractant IL-8 [105]. Risankizumab a humanised IgG monoclonal antibody that target the p19 subunit of IL-23 is in phase 2 clinical trials [106]. Brodalumab (Siliq), secukinumab (Cosentyx) and risankizumab (Skyrizi) have been approved in several countries and are excellent drugs for the treatment of plaque psoriasis. Table 5 list the interleukin antagonists for the treatment of eosinophil and neutrophilic asthma.

Inhaled β2-agonist
Short acting (salbutamol, levalbuterol, terbutaline, pirbuterol)
Long-acting (salmeterol, formoterol)
New long-acting (indacaterol, olodaterol, vilanterol)
Combination of LABA and inhaled corticosteroids
Salmeterol and fluticasone (Advair Diskus)
Formoterol and budesonide (Symbicort)
Vilanterol and fluticasone
Triple combo (Vilanterol, fluticasone and umeclidium)
Cromones
Cromlyn sodium, nedocromil sodium
Inhaled anti-cholinergics
Short-acting (ipratropium bromide)
Long-acting (oxitropium bromide, tiotropium bromide)
New long-acting (umeclidium bromide, glycopyrrolate)
Corticosteroids
Betamethasone dipropionate
Budesonide, fluticasone, flunisolone
Ciclesonide, mometasone
Oral methylxanthines
Rapid release theophyllines
Sustained release theophyllines (Theo-24, Theocron, Uniphyll)
Phosphodiesterase (PDE)-4 inhibitor (roflumilast)
Leukotriene receptor antagonists
Montelukast, pranlukast
Cinalukast, zafirlukast
5-lipoxygenase inhibitors
Zileuton
Novel therapies
Anti-TNF therapy, e.g., infliximab, etanercept
Prostaglandin D2 receptor antagonists, e.g., fevipiprant, setipiprant
Protein kinase c-kit, Lyn, and Fyn inhibitors, e.g., mastinib, imatinib

Table 4: Standard drugs used for the treatment of asthma.

Long-Acting Phosphodiesterase 4 Inhibitors

Non-selective Phosphodiesterase (PDE) inhibitors, such as theophylline have been used for the treatment of asthma and COPD for several decades. PDE enzymes metabolize the second intracellular messengers, including Adenosine Monophosphate (cAMP) and cyclic Guanosine Phosphate (cGMP), which play important roles in intracellular signaling in the regulation of multiple cellular metabolisms [107,108]. The PDE superfamily of enzymes contains 11 gene families (PDE1 to PDE11), most of which contain several

PDE genes [107]. The PDE4 isoenzymes are encoded by a family of four different genes (PDE4A-4D), which specifically hydrolyze the 3'5' phosphodiester bond of cAMP to yield 5'Adenosine Monophosphate (5'-AMP) [10,109]. PDE4B isoenzyme is associated with bronchodilatation and anti-inflammatory effects, while PDE4D is associated with gastrointestinal side effects, such as nausea and vomiting, due to its high presence in the vomiting center in the brain [110,111].

Agent	Target	Indication	Stage of Development
Omalizumab	IgE	EA	Marketed 2003
Mepolizumab	IL-5	EA	Marketed 2015
Reslizumab	IL-5	EA	Marketed 2016
Benralizumab	IL-5R	EA	Marketed 2017
Dupilumab	IL-4?/IL-13	EA	Marketed 2018
Tezepelumab	TSLP	EA	Marketed 2018
Pitrakinra	IL-4? /IL-13	EA	II
Lebrikizumab	IL-13	EA	III
Tralokinumab	IL-13	EA	III
Fezakinumab	IL-22	EA	II
Brodalumab	IL-17RA	NA	II
Secukinumab	IL-17A	NA	II
Risankizumab	IL-23	NA	II

Table 5: Monoclonal antibodies, and interleukin antagonists and their target.

Abbreviations: EA: Eosinophilic Asthma, NA: Neutrophilic Asthma; IL: Interleukin; TSLP: Thymic Stromal Lymphopoietin. Brodalumab, secukinumab and risankizumab are approved for the treatment of plaque psoriasis.

Inhibition of PDE-4 leads to increase in the intracellular levels of cAMP and subsequent modulation of inflammatory responses and maintenance of the immune balance [112]. Therefore, PDE4 inhibitors are effective therapeutic strategy for the treatment of inflammatory respiratory diseases, characterized by airway remodeling and bronchoconstriction. Increase in the levels of cAMP can activate downstream phosphorylation pathways [113], which leads to relaxation of airway smooth muscle cells, bronchodilatation; and suppression of airway inflammation.

PDE4 is the most studied subfamily and pharmacological research has yielded several pharmacological agents for the treatment of many chronic inflammatory diseases, such as asthma, Chronic Obstructive Pulmonary Disease (COPD), [113-115] psoriasis [116], atopic dermatitis [117], inflammatory bowel disease [118] and neuropsychiatric disorders [119].

Patient with inflammatory diseases have higher expression of PDE4 than healthy individuals [120]. Inhibition of PDE4 results in increase in intracellular cAMP and subsequent activation of PKA, cyclic nucleotide-gated ion channels and Epac1/2. These signaling pathways are involved in the regulation of pro-inflammatory and anti-inflammatory cytokine synthesis [121]. *In vitro* inhibition of PDE4 has been shown to decrease expression of cell surface markers in many inflammatory cells, such as T cells and decreased release of cytokines, such as TNF- α , IL-1 β and IL-10 in many types of cells [122,123]. *In vivo* inhibition of PDE4 also leads to a broad spectrum of effects, such as inhibition of cell trafficking and cytokine and chemokine release from inflammatory cells, such as neutrophils, eosinophils,

macrophages and T cells [124]. In addition, PDE inhibitors promote apoptosis of these cells [122,125]. Animal studies have shown that roflumilast reduced accumulation of neutrophils in Bronchoalveolar Lavage (BAL) fluid following exposure of cigarette smoke in guinea pig and mice [126,127]. Cortijo, et al. [128], have also reported that roflumilast prevented bleomycin-induced infiltration of neutrophils and macrophages in mice lungs.

In vitro study has shown that roflumilast and its active metabolite roflumilast N-oxide inhibited neutrophil secretion of IL-8, Leukotriene B4 (LTB4), Matrix Metalloproteinase-9 (MMP-9) and neutrophil elastase [122,129]. PDE4 inhibitors have also been reported to inhibit Interleukin-4 (IL-4) and IL-13 generation by human basophils [130]. Furthermore, roflumilast and roflumilast N-oxide reduced liposaccharide-induced release of chemokines (CCL2, CCL3, CCL4 and CXL10) and TNF- α from human lung macrophages in a dose-dependent fashion [131].

Roflumilast has been reported to suppress secretion of TNF- α from epithelial cells and exerts anti-inflammatory and immunomodulatory effects [132]. PDE4 inhibitors, such as cilomilast and roflumilast have been shown to decrease MUC5AC expression induced by Epidermal Growth Factor (EGF) [133] and roflumilast has been reported to improve ciliary function and mucociliary clearance [134]. *In vitro* study have shown that roflumilast antagonized profibrotic activity of fibroblasts stimulated by TGF- β [135,136]. Hence, PDE4 inhibitors have the potential to prevent progressive subepithelial basement membrane fibrosis and pulmonary fibrosis [135].

Phosphodiesterase 4 inhibitors are appropriate as add-on therapy for patients with neutrophilic asthma, because they suppress immune cell trafficking, activation and degranulation. They also suppress the release of cytokines, chemokines and growth factors which promote subepithelial membrane fibrosis, ASM cell proliferation, ASM hypertrophy and airway remodeling [122]. Long-acting selective PDE4 inhibitors, such as roflumilast have been shown to significantly reduce airway hyper responsiveness [137], which is a key feature of neutrophilic asthma. Oral roflumilast 500 μ g morning or evening has been shown to be beneficial as add-on treatment for fixed airflow limitation in patients with increased ASM mass, AHR and airway remodeling [138]. Similarly, clinical trials have documented that roflumilast improves symptom control, exacerbations, lung function and quality of life [139-141].

Roflumilast helps improve efficacy of other anti-inflammatory agents and bronchodilators, such as corticosteroids, LABA and LTRA. Roflumilast and its active derivative roflumilast N-oxide have been shown to enhance activity of the glucocorticoid receptor activity and glucocorticoid-dependent gene transcription in peripheral blood mononuclear cell of asthmatic patients compared with control [139]. The combination of roflumilast and fluticasone significantly reduced AHR compared with roflumilast dosage alone [138].

Roflumilast can be used as an add-on treatment to ICS and LABA and/or LTRA therapies [141] and is beneficial in reducing gradual decline in lung function associated with increase in ASM hypertrophy and airway remodeling. The GINA guidelines [1], recommends addition of slow-release theophyllines, including the long-acting PDE-4 inhibitor roflumilast for the treatment of asthma at step 3. Roflumilast (Daliresp) is the only approved long-acting selective PDE4-inhibitor for the treatment COPD and asthma and was approved

for these indications by the European Union (EU) in 2010 and in the USA in 2011. It has better selectivity and tolerance.

Macrolide Antibiotics

Patients with neutrophilic asthma are unresponsive to corticosteroids [142], anti-IgE and anti-interleukin Monoclonal Antibody (mAb) therapies targeting eosinophilic asthma. Thus, there is need for alternative anti-inflammatory therapies for patients suffering from neutrophilic asthma [143].

Macrolides have a macrocyclic lactone ring, whose size and features have been modified from the 14 carbon structure of erythromycin in order to develop newer agents such as clarithromycin and roxithromycin, or the 15 carbon structure to produce azithromycin [144]. They are derived from the product of the microbial order Actinomycetales (*Saccharopolyspora erythrae*, formerly *Streptomyces erythrae*).

Macrolide antibiotics, such as Erythromycin (ERM), Azithromycin (AZM), Clarithromycin (CAM) and Roxithromycin (RXM) and new ketolide antibiotic telithromycin have antibacterial, antiviral and anti-inflammatory effects. They are mostly used to treat infections caused by *Chlamydomphila pneumoniae* and *Mycoplasma pneumoniae*, especially respiratory and genitourinary infections. They now constitute part of guideline-recommended therapy in community acquired pneumonia. Azithromycin and clarithromycin are also used to treat legionellosis.

Chlamydomphila pneumoniae and *Mycoplasma pneumoniae* may play an important role in the pathogenesis of neutrophilic asthma [145]. Moreover, atopic subjects have increase frequencies of detection of *C pneumoniae* in nasal aspirate sampling, independent of symptoms compared with healthy volunteers [146]. Similarly, asthmatic patients are more likely to harbor *M pneumoniae* in their airways [147]. There is cumulative evidence suggesting infections with *Chlamydomphila pneumoniae* and or *Mycoplasma pneumoniae* might play a role in the pathogenesis of different phenotypes of asthma, including neutrophilic asthma. Treatment of these atypical bacteria may be gratifying in preventing severe exacerbations associated with these microbes.

Macrolides and ketolides have been shown to have both *in vitro* and *in vivo* anti-inflammatory activity, including suppression of neutrophil inflammation, which makes them relevant to respiratory conditions associated with airway neutrophilia, such as neutrophilic asthma and atypical bacterial infection.

Treatment with macrolides, such as erythromycin and azithromycin has been shown to reduce neutrophil counts and Bronchoalveolar Lavage (BAL) fluid Interleukin-8 (IL-8) levels in patients with panbronchiolitis [146], bronchiolitis obliterans syndrome (BOS) [148,149] and asthma [150]. The clinical improvement in these conditions occur in the absence of active bacterial colonization, thus confirming the immunomodulatory effects of macrolides.

Several studies have reported that treatment with AZM, CAM and RXM decrease eosinophil and neutrophil numbers, inhibit neutrophil migration and oxidative burst activity and mediator release. Consequently, there is a decrease in the concentrations of neutrophil elastase, metalloproteinase-9, IL-8, IL-6, IL-1 β , TNF- α and Eosinophilic Cationic Protein (ECP) [143,150-155].

Simpson et al. [143], have shown that clarithromycin in patients severe refractory asthma reduced neutrophil count and sputum IL-8 levels, although they did not observe any change in lung function or asthma control [143]. The Azithromycin for prevention of exacerbations in severe asthma (AZISAST) randomized, placebo-controlled trial in patients with severe asthma with history of severe exacerbations, despite receiving high-dose ICS and LABA, studied the effect of AZM on asthma exacerbations [156]. Azithromycin (250 mg daily three times per week) as add-on treatment in patients with non-eosinophilic asthma, defined by normal blood eosinophil counts and normal FeNO, resulted in significantly fewer severe exacerbations during 26-week period compared with controls [156]. Azithromycin significantly reduced severe exacerbations and lower respiratory tract infection in non-eosinophilic asthma phenotype by approximately 67% compared to 38% in placebo group. AZM was ineffective in reducing exacerbations in patients with eosinophilic asthma, who tended to have more exacerbations on AZM treatment [156].

The second randomized double-blinded, placebo-controlled trial (AMAZES) compared add-on azithromycin (500 mg three times per week) with placebo for 48 weeks in patients with symptomatic asthma despite medium-to-high dose ICS and LABA [157]. Treatment with add-on azithromycin significantly reduced the incidence of medium and severe exacerbation by 1.07 versus 1.86 per person-year, for AZM and placebo, respectively. AZM treatment was also associated with an improvement in Asthma Quality of Life Questionnaire (AQLQ) scores in both groups of patients with eosinophilic and noneosinophilic asthma phenotypes [157].

The Telithromycin, Chlamydomydia and Asthma (TELICAST) multicenter, randomized, double-blind, placebo-controlled study in 278 patients with moderate-to-severe asthma reported significantly greater improvement in symptoms and lung function in patients receiving telithromycin, 800 mg once daily, for 10 days compared with placebo [158]. Patients receiving telithromycin had improvement in symptoms from exacerbation at the end of treatment by 51% versus 29% in the placebo treated patients. The FEV1 improved by 0.63 L in telithromycin-treated patients versus 0.29 L in placebo-treated [158].

The Azithromycin Against Placebo in Exacerbations of Asthma (AZALEA) study investigated the effectiveness of azithromycin treatment as add-on to standard therapy for adult patients with with exacerbation [159]. In the AZALEA clinical trial, addition of azithromycin 500 mg daily for 3 days to the standard treatment resulted in no statistically significant clinical improvement, including symptoms and quality of life scores and FEV1 [159].

This large trial in the UK had challenges in the recruitment of subjects, because there was widespread use of antibiotics in the 31 centers enrolled for the study. The study was therefore underpowered because a large number of patients (2044) were excluded because they were already taking antibiotics for their exacerbation [159].

It is possible that the population randomized was not representative of the larger population, because more than 2000 other patients were excluded from the study for other reasons [159].

From the above studies, different macrolides including the dosages of the specific drugs may influence the immunomodulatory

and immunosuppressive response to macrolide antibiotics. Selection and exclusion of asthmatic patients, including phenotypes may also influence outcome of the effects of different macrocyclic lactone ring macrolides, including the 16-membered (spiramycin, jasamycin and midecamycin) [144].

Macrolides and ketolides have proven anti-inflammatory and immunomodulatory effects via activation or inhibition of immunopathological pathways. Macrolides have the ability to alter intracellular signaling, particularly through inhibition of TNF- κ B activation and expression of activator protein-1 [160,161].

Azithromycin has been reported to significantly reduced Nuclear Factor- κ B (NF- κ B) expression, Tumor Necrosis Factor Alpha (TNF- α) RNA levels and TNF- α secretion in a CF-derived airway epithelial cell line [162]. Treatment of Bronchopulmonary Dysplasia (BPD) with AZM has been shown to suppress TNF- α -stimulated NF- κ B activation in tracheal aspirate cells from premature infants with the reduction in Interleukin-6 (IL-6) and IL-8 secretion compared to control levels [163].

Macrolides have several immunomodulatory effects in numerous cells via modulation of intracellular signaling of multiple pathways, including intracellular Ca²⁺ regulation, Mitogen-Activated Protein Kinase (MAPK) signaling pathways and modulation of transcription factor function [164]. The anti-inflammatory effects of macrolides include reduction of cytokine expression, reduction of adhesion molecule expression on inflammatory cells, reduction of chemical mediator release and Reactive Oxygen Species (ROS) and increased apoptosis and efferocytosis [164]. Shinkai, et al. [165] have suggested that macrolides might suppress IL-8 and extracellular signal-regulated kinase related to neutrophil chemotaxis and migration.

Neutrophilic asthma is nonresponsive to corticosteroids. Corticosteroid-resistance is associated with airway hyperresponsiveness and decreased Histone Deacetylase 2 (HDAC2) activity and expression [166]. HDAC2 has been shown to inhibit inflammatory protein coding genes such as granulocyte macrophage colony stimulating factor or cyclooxygenase 2, promoted by IL-1 β , TNF- α and NF κ B kinase [166]. Macrolides reverse corticosteroid insensitivity by restoring the HDAC activity, via inhibiting Phosphoinositol 3 Kinase (PI3K) pathway [167] and by attenuating TNF- α and IL-17 immune responses [168].

Mucus hypersecretion and plugging is one of the characteristic features of COPD and severe refractory asthma, including the neutrophilic phenotype. Macrolides have also been shown to reduce mucus secretion and ion transport in the airways and increase mucociliary function of epithelial cells [169].

Macrolides such as AZM and CAM and telithromycin are suitable as add-on treatment to ICS and LABA in patients with neutrophilic asthma. Clarithromycin has been shown as an effective add-on therapy in prednisone-dependent patients with asthma [170]. However, the use of long-term macrolides particularly in patients on high-dose ICS carries its own risks, such as adverse reactions, including diarrhoea, cholestasis, tinnitus and torsades de pointes ventricular tachycardia. It may also promote antibiotic resistance against macrolides, ketolides, tetracyclines and other related antibiotics [157,171-173].

Bronchial Thermoplasty

Corticosteroids are the mainstay of asthma treatment; however, they do not suppress ASM hyperplasia and hypertrophy which is one of the histopathological features of severe asthma responsible for airway hyperresponsiveness [174]. One of the strategies for the treatment of severe asthma is to target airway smooth muscle hypertrophy, using bronchial thermoplasty, a non-pharmacological procedure aimed at reducing airway smooth muscle mass [175,176].

Bronchial Thermoplasty (BT) is a bronchoscopic treatment for subjects aged 18 and above with severe persistent asthma not responding to high-dose ICS and LABA. Selection and preparation of patients for BT is very important and the procedure should be performed by experienced pulmonologists or bronchoscopists [176-179].

Patients for BT should be in an optimal stable condition, without any asthma exacerbation or respiratory infection for at least 2 weeks. In addition to their standard medical treatment for severe asthma they should be pre-treated with prednisolone 50 mg/day for 3 days before BT, on the day of BT and the day after bronchial thermoplasty [179]. Patients with low FEV1 <80% should have the procedure postponed until their lung function improves. Before the procedure, all patients should be pre-treated with nebulized salbutamol and/or ipratropium bromide [179].

Bronchial thermoplasty is performed under moderate-to-deep sedation or general anesthesia [176-179]. At bronchoscopy a special catheter with a basket design is inserted through the instrument channel which delivers radiofrequency energy generated by a Radiofrequency (RF) generator (Alair™ Bronchial Thermoplasty System) to the airway wall [180,181].

The Alair™ catheter electrode delivers targeted radiofrequency energy to bronchial airway wall and results in reduction of the hypertrophied airway smooth muscle mass which is responsible for bronchoconstriction [178-186]. The procedure also decreases subepithelial fibrosis, extracellular matrix, submucous glands, airway nerve endings, epithelial cells and neuroendocrine cells [176,178,179,185,187]. Bronchial thermoplasty may lead to functional denervation, thus, reducing sensory neural axonal reflex bronchoconstriction [187].

Bronchial thermoplasty is given over three bronchoscopy sessions at approximately 3-week intervals, one for each lower lobe and one for both upper lobes [178,179,182]. Radiofrequency electrical energy delivered by a Radiofrequency (RF) generator (Alair™ Bronchial Thermoplasty system) is applied to the airways distal to the mainstem bronchi between 3 and 10 mm in diameter throughout the tracheobronchial tree, except the right middle lobe [179,182,183,186]. In practice, 40-70 RF activations are delivered in the lower lobes and between 50 and 100 activations in the upper lobes combined depending on the patient's size and airway caliber [179]. Traditionally, the Right Middle Lobe (RML) is typically avoided due to concerns about the right middle lobe syndrome [179]. The right is usually not treated because it has a narrow, more horizontal orifice and RF energy could lead to post-procedure inflammation, obstruction and atelectasis of the right middle lobe (syndrome). Otherwise, the lingula is treated.

The Alair™ Bronchial Thermoplasty System (Boston Scientific, Natick, MA, USA) uses continuous feedback to tightly control the degree of tissue heating to avoid bronchial perforation, scorching and stenosis [179,180,186]. The Alair™ catheter delivers radiofrequency energy in order to warm the airway wall to a targeted temperature of 65°C, which reduces the ASM mass by approximately 50% after 3-6 weeks after the procedure [179, 182,186].

Patients should be screened adequately to confirm a correct diagnosis of asthma (including phenotyping), verify the criteria for severe refractory asthma despite adherence to appropriate pharmacological and non-pharmacological therapies, review and address co-morbidities that could affect asthma control, such as obesity, gastroesophageal reflux disease, post-nasal drip and obstructive sleep apnea [176].

Bronchial thermoplasty requires proper preparation and management of patients pre- and post-thermoplasty. It also requires identification of the right patients, implementation of proper BT technique and intense post-procedural care and follow-up [176]. The procedure should be performed meticulously to avoid bronchoconstriction, airway edema and bleeding. Minor radiological features occur following BT and a chest X-ray should be performed after the procedure.

Bronchial thermoplasty is a safe and effective procedure, however, it is associated with a short-term increase in asthma-related symptoms such as cough and sputum production, exacerbations and hospital admissions for asthma during the treatment phase [176,179,188]. Occasionally, bronchiectasis [189], atelectasis [190] and rarely pneumothorax and lung cysts [191], have been observed as complications following the bronchial thermoplasty. One case report describes hemoptysis associated with bronchial nodule which resolved by the third session of bronchial thermoplasty [192]. Table 6 lists the complications of bronchial thermoplasty.

Worsening of asthma control
Hospital re-admissions
Severe exacerbations
Cough with sputum production
Hemoptysis
Acute CT peribronchial consolidation
Nasopharyngitis
Pulmonary infection
Lung abscess
Central bronchiectasis
Upper lobe atelectasis
Collapse of airway by mucous plugging
Pulmonary cysts and pneumothorax

Table 6: Complications of bronchial thermoplasty.

Several randomized controlled trials and prospective multicenter studies in Australia, Canada, France, Japan, Netherland, Spain, UK and USA in patients with severe persistent asthma have documented improvement in asthma control, fewer exacerbations and hospitalization and better quality of life score which persist up to 5 years following bronchial thermoplasty [178,179,181,191-195].

The first randomized unblinded clinical trial (Asthma Intervention Research [AIR] 1) in 112 patients with moderate-to-severe asthma was done by Cox and colleagues [196]. Patients treated with BT showed a significant reduction in mild exacerbations as compared with baseline, whereas there were no changes in the frequency of exacerbations in the control group. Additionally, there was a significant improvement in asthma control assessed by the Asthma Control Questionnaire and quality of life assessed by the Asthma Quality of Life Questionnaire in the BT-treated patients compared with controls [196]. However, there were no differences in FEV1 or airway hyperresponsiveness (defined as a provocative concentration of methacholine required to lower the FEV1 by 20% (PC20) or less than 8 mg/mL [196].

The second randomized clinical unblinded trial was the Research in Severe Asthma (RISA) trial [197]. This trial which studied 32 patients with severe asthma for the safety and efficacy of BT, reported a significant improvement in the asthma control, quality of life, rescue medication use and pre-bronchodilator FEV1 in BT-treated subjects compared with control. The beneficial effects of BT persisted even after reduction in the dosages of ICS and OCS [197].

The largest randomized double-blind sham-controlled trial was the Asthma Intervention Research 2 (AIR2) trial in 297 patients with severe asthma which compared BT with a sham procedure [198]. The AIR2 trial reported significant improvement in AQLQ scores, reduced frequency of severe exacerbations and decreased emergency department visits and days lost from work or school in the year after bronchial thermoplasty compared with treatment with sham procedure [198].

Chupp, et al. [199] compared the outcome of BT after a follow-up of 3 years in 190 PAS2 (Post-FDA Approved Clinical Trial Evaluating Bronchial Thermoplasty in Severe Persistent Asthma) subjects with 190 bronchial thermoplasty-treated subjects in the AIR2 trial at 3 years of follow-up. At year 3 after BT, the percentage of PAS2 subjects with severe exacerbations, emergency department visits and hospitalizations significantly decreased by 45%, 55% and 40% respectively [199], resembling the AIR2 results [198]. The PAS2 study showed similar improvements in asthma control after BT compared with the AIR2 trial despite enrolling subjects who had poorer asthma control [199]. After 3-year follow-up, PAS2 subjects were able to significantly reduce their mean ICS dose to 2070 µg/day, whereas, the AIR2 subjects significantly reduced their mean ICS to 1841 µg/day [199]. Previous observational studies on the effectiveness of bronchial thermoplasty for severe asthma have reported reductions in exacerbations and/or a step-down in treatment in 50-75% of patients undergoing the procedure [192-194,197].

A systemic review of the long-term safety of BT in the AIR, RISA and AIR2 trials demonstrated no long-term decline in FEV1, no change in the number of emergency room visits or hospitalization for adverse respiratory events [200]. The reduction in exacerbations seen in the first year after remained stable for up to 5 years [176,201-203] and follow-up CT scans performed on the subgroup of the treated patient cohort demonstrated no evidence of bronchiectasis of bronchial stenosis [203]. Recently, Chaudhuri, et al. [204] have reported that after 10 years post-thermoplasty the AQLQ scores and frequency of severe exacerbation were comparable to those recorded 1 year after bronchial thermoplasty. This suggests that the beneficial effects of bronchial thermoplasty may be sustained for up to 10 years or longer.

Bronchial thermoplasty has a long-term safety profile and may be considered for patients with predominant chronic airflow obstruction and patients who do not respond to anti-IgE, anti-interleukin biologics, or macrolides [176,205,206]. Patients with neutrophilic phenotype of asthma are suitable candidates for bronchial thermoplasty because they have excessive ASM hypertrophy, hyperplasia and hyperresponsiveness. They are also unresponsive to treatment with high-dose ICS, LABA, LTRA and interleukin antagonists targeted against eosinophilic asthma.

The US Food and Drug Administration (FDA) approved BT in 2010 as a safe procedure indicated for the treatment of severe persistent asthma in patients 18 years and older, that is not controlled with high-dose ICS and LABA [180]. It is also approved in several EU countries, Australia, Canada, Japan, UK and USA.

The GINA guideline recommends bronchial thermoplasty for the treatment of severe corticosteroid-resistant asthma at step 5 [1]. The British guideline on the management of asthma states that bronchial thermoplasty can be considered for the treatment of adult patients (aged 18 and over) with severe asthma who have poorly controlled asthma despite optimal therapy [207].

Conclusion

Neutrophilic asthma is a phenotype of asthma that is severe and persistent, with frequent exacerbations and hospitalizations. It is characterized by the presence of high levels of neutrophils in the lungs and airways, fixed airflow obstruction and low FEV1. Histopathologically, it is characterized by ASM hyperplasia and hypertrophy, increase in ECM proteins and subepithelial basement membrane fibrosis, which all lead to fixed airflow limitation. Neutrophilic asthma is unresponsive to high-dose ICS, LABA and LTRA and to interleukin antagonist targeted against eosinophilic asthma. This phenotype of asthma requires specific therapeutic interventions aimed at prevention and reducing airway remodeling, such as novel anti-inflammatory agents, including long-acting PDE 4 inhibitors and macrolide antibiotics; and reduction of ASM mass which is the main cause of severe bronchoconstriction, by bronchial thermoplasty.

Conflicts of Interest

The author reports no conflicts of interest in this manuscript.

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