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Highlights in rhinology

Edited by
Sven F. Seys, Giancarlo Ottaviano and Pongsakorn Tantilipikorn

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Highlights in rhinology

Topic editors
Sven F. Seys — KU Leuven, Belgium
Giancarlo Ottaviano — University of Padua, Italy
Pongsakorn Tantilipikorn — Mahidol University, Thailand

Topic Coordinator
Glenis Kathleen Scadding — University College London, United Kingdom

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REVIEW
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The Nose as a Route for Therapy: Part 1. Pharmacotherapy

*Cemal Cingi[1], Nuray Bayar Muluk[2], Dimitrios I. Mitsias[3], Nikolaos G. Papadopoulos[3,4], Ludger Klimek[5], Anu Laulajainen-Hongisto[6], Maija Hytönen[6], Sanna Katriina Toppila-Salmi[7,8] and Glenis Kathleen Scadding[9,10]**

[1] Department of Otolaryngology, Eskişehir Osmangazi University, Eskişehir, Turkey, [2] Department of Otolaryngology, Kirikkale University, Kirikkale, Turkey, [3] Allergy Department, 2nd Pediatric Clinic, National and Kapodistrian University of Athens, Athens, Greece, [4] Royal Manchester Children's Hospital, Manchester, United Kingdom, [5] Centre for Rhinology and Allergology, Wiesbaden, Germany, [6] Department of Otorhinolaryngology-Head and Neck Surgery, Helsinki University Hospital, Helsinki, Finland, [7] Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland, [8] Faculty of Medicine, The Haartman Institute, University of Helsinki, Helsinki, Finland, [9] University College London Hospitals NHS Foundation Trust, London, United Kingdom, [10] Royal National Throat Nose and Ear Hospital, London, United Kingdom*

This article reviews nasal structure and function in the light of intranasal pharmacotherapy. The nose provides an accessible, fast route for local treatment of nose and sinus diseases, with lower doses than are necessary systemically and few adverse effects. It can also be used for other medications as it has sufficient surface area protected from local damage by mucociliary clearance, absence of digestive enzymes, responsive blood flow, and provides a rapid route to the central nervous system.

Keywords: intranasal route, nasal epithelium, mucociliary clearance, allergic rhinitis, chronic rhinosinusitis, lysine aspirin, saline douche, drug delivery

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Monash Health, Australia

Reviewed by:
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Chulalongkorn University, Thailand
Supinda Chusakul,
Chulalongkorn University, Thailand

***Correspondence:**
Glenis Kathleen Scadding
g.scadding@ucl.ac.uk

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INTRODUCTION

Medicines are usually given orally or systemically by injection: intramuscular or intravenous. Indeed when patients are asked about their drug history the use of inhalers or sprays is often inadvertently omitted, unless specifically requested. However, other routes not only exist, but can prove more effective in placing a drug accurately, often using smaller doses. One such is the intranasal route, now brought to prominence by SARS-CoV2, which uses it to invade the body.

The nose, even though obvious, "it's as plain as the nose on your face" is an English expression, is often disregarded by non-otorhinolaryngologists. However, it has much to recommend it: as an organ for conditioning inspired air, for immune defense, for hosting smell receptors and for application of therapy. The leading role of the epithelium in respiratory diseases such as Allergic Rhinitis (AR) and Chronic Rhinosinusitis with Nasal Polyps (CRSwNPs) has become apparent in recent years and the ability to interact with it by direct application of molecules, rather than allowing them to reach it via the circulation, having been absorbed via the gut or injected into the system, seems sensible.

Part 1 of this review article involves nasal pharmacotherapy. It begins with a consideration of nasal structure and function, including the nature of the pseudostratified columnar ciliated respiratory epithelium. It is important to understand nasal anatomy, histology, innervation, and blood supply in order to assess the nasal cavity as a route for a particular drug. Necessary factors are a large surface area for absorption and high blood flow for transport. Factors which might interfere with drug absorption are vasoconstriction secondary to stimulation of the adrenergic nerves or irritation stimulating the 5th nerve and causing the 7th to respond by increased glandular mucus secretion, washing away the therapeutic product into the nasopharynx, where it is swallowed. Nasal pH and the lipophilicity of a drug are also relevant.

The article continues with various intranasal therapies, varying from those used locally to treat respiratory diseases, to those countering entirely other problems, such as diabetes insipidus. Cheap and simple measures such as nasal saline or lysine aspirin can prove prophylactic, therapeutic or both. Unfortunately the use of nasal adrenalin for rescue in anaphylaxis was considered too commercially sensitive for inclusion in this paper.

Prevention of COVID-19 infection by copper—containing face masks has just emerged as an idea, doubtless other intranasal approaches will follow. The nose, overlooked for so long, is finally becoming prominent.

Part 2 will follow with a consideration of nasal immunology and immunologically—based nasal therapeutics.

NASAL STRUCTURE AND FUNCTION

Nasal Anatomy

The nasal cavity is a midline airway passage of some 15 ml in volume and 14 cm in length in the adult, extending from the nares anteriorly to the post- nasal space. Approximately cylindrical, it is divided by the nasal septum into two nostrils. Above it are sinuses: frontal, ethmoid, and sphenoid, from front to back; maxillary sinuses are present on each side. Its surface area is ∼160 cm^2, but if microvilli are included this rises to nearly 10 $m.^2$

Nasal Histology

The vestibular entrance to the nose is lined internally by a squamous epithelial layer. The lining changes to a pseudostratified columnar epithelium of respiratory type, bearing cilia and with numerous glands of serous and mucinous type, after the first 1–2 cm (1). This lines most of the respiratory tract, including the sinuses, down to the alveoli.

Epithelial Cells (Cilia)

The epithelium functions as a physical block on the entry of pathogens into the deeper tissues. The movement of their cilia occurs in the deeper sol layer of the nasal mucus, with a stiff-armed forward stroke followed by a limp backward one. This pushes the mucus, including the upper gel layer into which particles entering the nose become contained, backwards in the direction of the nasopharynx. These substances are then usually swallowed. This phenomenon is termed mucociliary clearance (2). Intranasal drugs therefore have a short absorption window before being cleared to the throat and swallowed. This clearance mechanism means that corticosteroids applied locally do not cause atrophy, unlike dermal application, provided septal deposition is avoided. Unlike the oral cavity and gut lumen there is no regular secretion of digestive enzymes capable of disrupting peptides into the nasal cavity, though peptidases may be released from epithelial cells upon stimulation by allergen.

The epithelium also plays a role in regulating inflammation by the secretion of cytokines (3).

Figure 1 shows the lateral nasal wall and the direction of mucus movement.

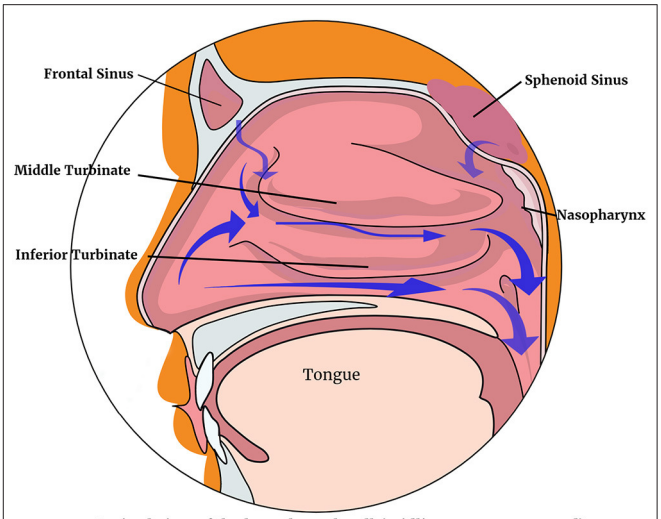

FIGURE 1 | The lateral nasal wall showing the direction of mucociliary clearance.

Endothelium (Sub-epithelial Blood Vessels)

The nasal lining has an abundant vascular supply via capillaries lined by endothelial cells. The endothelial layer is of minimal thickness, so that heat can be rapidly transferred to inhaled air. Encircling the endothelium is a smooth muscle layer, which acts to narrow or widen the vasculature. This regulatory action on vessel diameters is a key feature of inflammation (3).

Mucous Glands

Small serous glands, similar to salivary glands are scattered in the front part of the nose. They secrete watery fluid, sometimes visible as droplets in cold conditions.

Seromucous glands secreting more proteineous secretions are located in the lamina propria elsewhere in the nasal cavity. They deposit mucus onto the external surface of the epithelium. The secreted mucus can immobilize external matter and helps to conserve the integrity of the physical barrier. Parasympathetic nervous impulses result in more mucus being synthesized and excreted. The mucus contains lysozyme and immunoglobulin A, which help to attack potentially invasive microbial organisms (3, 4).

Physiology of the Nose

The nasal cavity has a variety of roles, notably respiratory, olfactory, immunological, and the conditioning of air before entry into the lower respiratory tract. The cavity offers a very extensive, humid surface area which is optimal for adjusting the temperature and humidity of inhaled air prior to its passage toward the oxygen exchanging pulmonary surfaces. Mucus secreted by the nasal lining stops external matter from damaging the epithelial layer, especially in the course of an inflammatory response. The nose is the only human organ where olfaction

occurs and depends on specialized sensory neurones that form part of the olfactory nerve.

Nasal Cycle

There is a continuously operating nasal cycle whereby the two sides of the cavity alternate between congestion and decongestion (3). In adults without health problems, the total resistance to airflow offered by the nose remains fairly constant, although there is an alternating pattern of one side of the nasal interior offering a greater level of resistance to airflow, whilst the other remains fully patent, followed by the inverse (5, 6). This pattern of flow restriction is referred to as the nasal cycle. It is produced by alternating changes in blood flow to the turbinates and the tubercle of the septum. In healthy individuals, this cycle occurs without the person noticing it, since there is no net alteration in how much airflow through the nose can take place. Likewise, the moisture content of inhaled air passing to the lungs does not vary (7). The hypothalamus contains the pacemaker area controlling the nasal cycle (8).

Vascular and Lymphatic Supply

The blood supply to the nasal cavity is extensive, involving six arterial branches, forming a good route for drug administration. There are two main sources of vascular supply to the nose: the internal and external carotid arteries. The former gives rise to the ophthalmic artery and its branches, the anterior ethmoid artery and the posterior ethmoid artery. From the latter arise the sphenopalatine, greater palatine, superior labial, and angular arteries.

The posterior and inferior portions of the interior aspect of the lateral nasal wall receive arterial blood from the sphenopalatine artery, whilst its superior portion is supplied by the ethmoid arteries, both anterior and superior. The septum of the nose receives a vascular supply from these same three arteries. This supply is augmented in the anterior portion by the superior labial artery and in the posterior portion the greater palatine artery makes its contribution. Little's area (also referred to as the Kiesselbach plexus) is an area situated in the most anterior and inferior third of the septum and is where most epistaxes occur. Here the principal arteries providing vascular supply to the nose all anastomose.

The nasal venous network has a similar layout to that of the arteries. The arterial blood flow into the nasal region is overabsorbed into the nasal veins, with the excess draining into the lymphatics, forming a good route for vaccine delivery. The veins do not possess valves and thus communicate directly with the cavernous sinus. In this way, they may render it easy for pathogens and drugs to disseminate within the cranium. Although the nose enjoys a rich vascular supply, smokers suffer from impaired recovery following surgery to the nose.

The lymph vessels originate in the outer layers of the mucosa with drainage from the posterior nasal cavity toward the retropharyngeal nodes and from the anterior cavity to the superior deep cervical or submandibular nodes.

The nasal mucosa contains a network fenestrated veins beneath the mucous membrane. These may provide some humidifying fluid. The epithelium also possesses a network of vascular erectile tissue, which is also cavernous and well-developed over the lower conchae and septum as shown in **Figure 2**, thus providing a good absorption route. Adrenergic vasoconstriction will decrease the rate of absorption, cholinergic vasodilatation may increase it, thus altering drug penetration.

Nervous Supply

The extensive sensory nervous supply to the nose is provided by the initial two branches of the fifth cranial nerve (1): the ophthalmic and maxillary divisions. The latter includes the anterior superior alveolar nerve, which is important in sneezing. The 5th (trigeminal) nerve is responsible for sensing pain and irritation following nasal administration, but it is the 7th (facial) nerve which contains motor fibers and responds to such irritation by stimulating facial movements and glandular secretion.

The 1st cranial (olfactory) nerve is the only site where the central nervous system is directly expressed on the mucosal surface and is hence in contact with the external world. This gives a route for central nervous system (CNS) access for drugs, but also for pathogens.

Parasympathetic Innervation

Parasympathetic innervation occurs via the greater superficial petrosal branch of the facial nerve. This branch combines with the deep petrosal nerve (carrying sympathetic fibers). The deep petrosal nerve emerges from the carotid plexus. Together they make up the vidian nerve within the pterygoid canal. The vidian nerve passes through the pterygopalatine ganglion, but the sympathetic fibers do not make any synaptic connections in the ganglion. Then the vidian nerve joins with fibers from the maxillary division of the fifth cranial nerve to supply the lacrimal gland, the nasal glands and the palate (1).

Osteology

There are twin nasal bones, the superior aspects of which articulate with the frontal bone. The nasal bones articulate with the lacrimal bones on their superolateral aspect, whilst on their inferolateral aspect they articulate with the maxilla on its ascending process. In a posterior and superior direction, the osseous septum of the nose is formed by the ethmoidal perpendicular plate. The septum is thinner centrally and often bent to one side or the other (septal deviation), which may interfere with drug delivery (9). In the posterior and inferior direction there is the vomer, which contributes a portion of the choanae, leading into the nasopharynx. The bony nasal floor is formed by the premaxilla and the palate.

Situated on the lateral walls of the nasal cavity are the three conchae (superior, middle, and inferior), providing the osseous support to the turbinates, projections into the lateral wall which promote turbulent airflow, enabling particle deposition and also act as radiators, warming the inspired air. The medial wall of the maxillary sinus is situated laterally to the turbinates.

Below each turbinate there are apertures, the meatuses, named after the turbinate immediately superior to them. For example, the middle meatus into which most sinuses ventilate and drain is just below the middle turbinate. The conchae and meatus

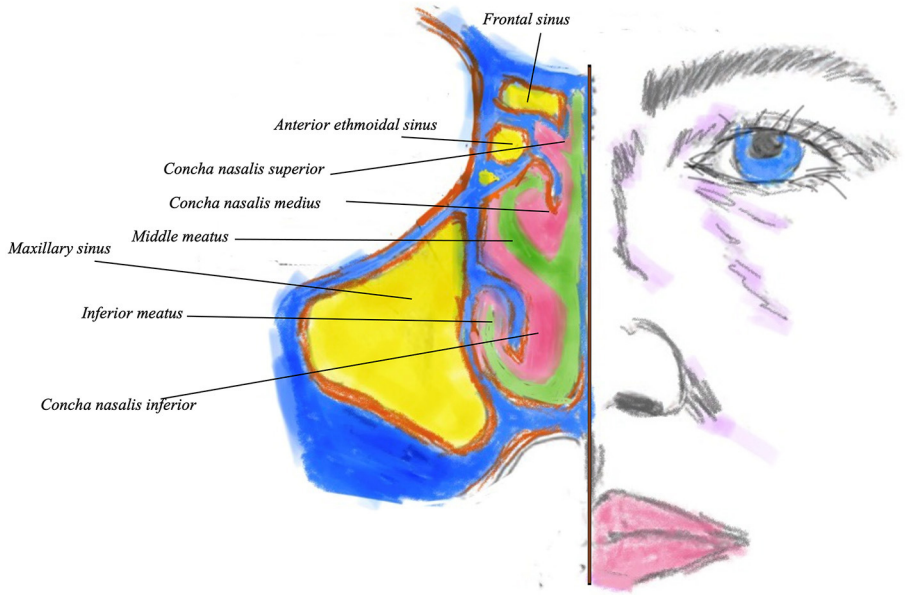

FIGURE 2 | Coronal view of the nostril showing the sinuses (in yellow) turbinates and meati. Bony turbinate structure is in blue, with pink denoting the overlying mucosa. Green indicates the nasal airway.

increase nasal surface area, allowing significant absorption of medications (10) (**Figure 2**).

Viewed from the internal aspect of the nasal cavity, the roof consists of the ethmoidal cribriform plate. Behind and below the roof and angled posteriorly lies the bony face of the sphenoid sinus (1).

Paranasal Sinuses

These develop and enlarge after birth; it is not until some 3–7 years of age that the ethmoid and sphenoid sinuses are of significant size. The frontal sinuses develop last, not reaching full size until adolescence.

The sinuses in human beings exist as four pairs, each of which is lined by epithelial cells of the pseudostratified columnar type. The maxillary sinuses located within the maxilla and inferior to the orbit are the biggest. The frontal sinuses are within the frontal bone and are found above the orbit. The ethmoid sinuses consist of a number of separate pneumatized sacs within the ethmoid bone in between the nasal cavity and the orbit. They are divided into anterior and posterior groups, with differing drainage. The anterior ethmoids drain into the middle meatus via the ethmoid infundibulum; the posterior ethmoids sinuses drain into the superior meatus via the sphenoethmoidal recess. The sphenoid sinuses are inside the sphenoid bone (8).

It is an unresolved issue as to precisely what functions the paranasal sinuses perform, but they appear to accomplish the following (8):

They help to reduce skull weight
They allow the voice to have a more resonant quality
They help to absorb the impact of a blow to the face
They protect against abrupt changes in the temperature of the nasal cavity and thereby prevent injury to some structures that are sensitive to heat or cold
They condition air by adding moisture and warming it before it passes to the lungs
They perform an immune defensive function via the formation of nitric oxide.

NASAL SALINE

Probably the oldest and by far the most frequently used nasal treatment is that of saline. Almost all nasal morbidities [e.g. allergic rhinitis (AR), chronic rhinosinusitis (CRS), infectious rhinitis etc.] are characterized by increased nasal secretions and/or congestion and are empirically countered by patients with nasal douches. Moreover, the concept of nasal irrigation (NI) is supported by many physicians, usually as an add-on to pharmacological treatment. For diseases that follow a chronic course, and therefore need chronic treatment, concerns about drug usage are raised and non-pharmacological approaches are preferred (11, 12). This is of importance in pediatric and elderly populations where parents/caregivers are often skeptical or unwilling regarding protracted pharmacological treatments and adherence is low (13).

Methods

There are many ways to perform NI: sprays, pumps, squeezy bottles, even plain syringes have been used, following various protocols. Moreover, the device may deliver high or low volume of saline, isotonic or hypertonic. NI are widely used and

accepted, being included in therapeutic algorithms for AR and CRS (14, 15).

Mode of Action

This, though not fully delineated, seems to be multiple. First, it humidifies and moisturizes the nasal mucosa and hypertonic saline may reduce mucosal edema. Second, it removes particles, allergens, air pollutants leading to less interaction with the mucosa and, probably, less inflammation. Third, saline seems to make the mucus thinner and more easily expelled and, in turn, mucociliary clearance is improved. Of importance, the release of inflammatory mediators such as histamine, prostaglandins and leukotrienes is reduced and/or receptors, such as ICAM-1 that are used for viral entry to the epithelium (16) are down-regulated. It seems, therefore, that apart from the "mechanical" mode of action, NI may exert immunological effects. Finally, in a post-operative setting, the removal of thick crusts, clotted blood and debris may result in faster wound healing.

Tonicity

The first key issue that needs to be addressed is whether hypertonic solutions (i.e., > 0.9% in sodium chloride) are better than normal (iso-osmotic) saline. Such studies as are available favor the former. Mucociliary clearance is increased (17–19) and clinical studies in children with both allergic rhinitis and chronic sinusitis showed the superiority of hypertonic solutions (20–22); systematic reviews confirm these results (23, 24). In adults with CRS, the advantage of hypertonic solutions albeit probable, is less evident (25–27). Tonicities above 3% may both decrease mucociliary clearance and open tight junctions thus increasing epithelial permeability (18, 28). Post-operatively, where there is no inflammatory/allergic background, the main goal is the removal of crusts and debris. In this setting, high osmolarity seems to be of minor importance (29) whereas the volume of the NI is more crucial (30); nevertheless there are contradictory results (31).

Saline in Allergic Rhinitis

The efficacy of NI in children and adults with AR has been well-studied. Even though these studies are characterized by large heterogeneity, they all point to increased efficacy, either as add-on to pharmacological treatment (antihistamines and/or nasal steroids) or alone, compared to no intervention at all (21, 32, 33). Indicatively, the study of 220 children (aged 5–9 years old) with AR showed the superiority of hypertonic saline (2.7%) compared to normal saline (and even more compared to no intervention) regarding nasal symptoms and turbinate swelling and/or adenoidal hypertrophy. Moreover, NI resulted in reduced antihistamine use, especially in the hypertonic NI group (21). Similarly, 44 children (5–14 years old) with seasonal AR were prescribed hypertonic NI (or not) as add on to antihistamine treatment. The active group had significantly better rhinoconjunctivitis score and less drug usage (32). Recently, 76 children and adolescents (6–18 years old) with seasonal or perennial AR, used NI with a sea-water solution supplemented with algal extracts as an add on to regular treatment. The active group showed significantly improved AR symptom control as judged by CARAT questionnaires, better combined symptom and medication scores using the MASK Allergy Diary (a mobile application designed by the ARIA group) and reduced drug usage (33). Meta-analyses also suggest that NI have no adverse events, lead to less drug usage and can be used as add-on treatment for AR (34, 35).

Saline in Chronic Rhinosinusitis

In CRS, NI have also proven useful (22, 25, 26, 36, 37). Thirty children (3–16 years old) with rhinosinusitis were treated with either hypertonic (3.5%) or normal saline. The first group improved significantly in cough and nasal secretion/post nasal drip score, as well as radiology score, while the normal saline group showed significant improvement only in the post nasal drip score (22). Nasal patency and mucociliary clearance was studied in 80 adult patients with CRS. Both hypertonic and normal saline improved subjective symptoms (i.e., stuffiness and obstruction) and mucociliary clearance (greater effect with hypertonic saline). Nasal patency was increased with normal saline (25). Finally, a randomized control trial of 76 adult patients with chronic sinonasal symptoms showed significantly improved scores [Rhinosinusitis Disability Index (RSDI) and Single-Item Sinus-Symptom Severity Assessment (SIA)] and reduced use of medication, such as antibiotics, for patients with daily hypertonic saline NI (37). Large volume intervention is more efficacious compared to low volume NI (38).

Pregnancy Rhinitis

Hormonal changes during pregnancy alter AR symptoms and approximately one third of pregnant women observe increased morbidity. There also exists a hormonally—induced rhinitis of pregnancy. However, especially during the first trimester, physicians are reluctant to prescribe drugs, let alone in increased doses. NI is not expected to harm the fetus. Hypertonic saline (3% NaCl) was studied in 45 pregnant women, followed for 6 weeks: the active group had significantly better rhinitis score and less antihistamine use after the first week. Similarly, nasal resistance, albeit similar on week 1, was significantly decreased on weeks 3 and 6. No adverse events were reported and therefore, NI seem to be invaluable for the treatment of rhinitis of pregnant women (39).

The Common Cold

The role of NI for the therapy of acute upper respiratory infections (URTI- common cold) is not clearly established. Early studies showed no difference in the use of NI for URTI (40, 41), while a large pediatric study of 401 children indicated faster resolution of some nasal symptoms for those that used NI (42). A subsequent Cochrane review acknowledges possible benefits of NI; this, however, is based on small studies with bias risk (43). Similarly, the most recent EPOS guidelines suggest that NI possibly has benefits for relieving symptoms, mainly in children, and could be a therapeutic option (4). Moreover, NI could be used as prophylaxis for prevention of frequent URTI in both children and adults (42, 44).

Adverse Events

NI does not pose a risk for major adverse events. Epistaxis, nasal and/or aural burning or irritation and middle ear effusions occasionally occur especially with large volume, high pressure, hypertonic solutions. Sodium loading could be problematical in those with concomitant renal or cardiac problems if the solution is swallowed, so advice should be given to spit it out once it reaches the post nasal space. In general NI benefit far outweighs risk.

Conclusion

NI (especially with hypertonic saline) is a useful add-on to pharmacological treatments and can be used alone in pregnant women, small children and those with mild disease. Further studies are needed to delineate NI use in terms of underlying pathology, volume, tonicity, delivery method, supplementary extracts, or minerals.

ALLERGIC RHINITIS

The therapeutic mainstays of Allergic Rhinitis (AR) are antihistamines and corticosteroids, both can be given intranasally, with minimal adverse events, since lower doses can be used. This is particularly important when the severe adverse effects of oral corticosteroid use are considered (45). **Figure 3** shows the EUFOREA treatment algorithm for AR.

Intranasal Antihistamines

Histamine acts in the early phase of allergic responses through H1 receptors (46). Antihistamines are mostly inverse agonists, stabilizing the receptor in an inactive conformation. The H1 receptor is widely distributed throughout the body (besides upper and lower airways in smooth muscle, heart, adrenal medulla, sensory nerves, central nervous system, and others (47) and are G-protein coupled transmembrane receptors that transduce extracellular signals through G proteins to intracellular second messenger systems (48) and may be considered a "cellular switcher," functioning in equilibrium between two active or inactive conformation states.

Azelastine hydrochloride, levocabastine, and olopatadine hydrochloride are the mostly used intranasal antihistamine (INAH) spray formulations in Europe and the US. The pharmacological profile and clinical efficacy of these drugs have been extensively reviewed elsewhere (49–54). INAH are classified as inverse agonists, as they do not antagonize the binding of histamine, but instead bind to different sites on the receptor (55, 56). Binding of antihistamines to the histamine receptor stabilizes the receptor in the inactive state thereby reducing the intrinsic activity of the receptor in response to histamine (46, 49).

They are classed as second-generation antihistamines with high affinities for the H1 receptor and little affinity for the H2 receptor (57, 58) and typically have a fast onset of action (15 to 30 min) (57, 59, 60) with effects lasting up to 12 h (58, 61). In comparison to oral antihistamines, INAH are more effective at

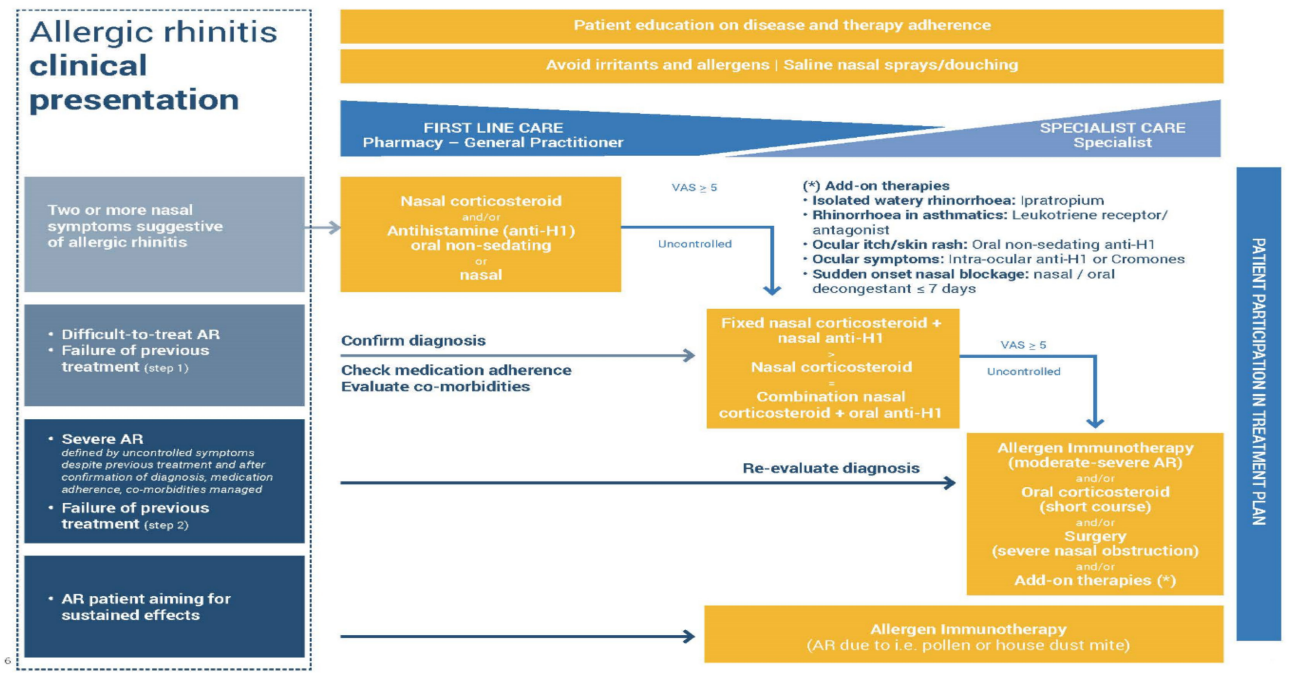

FIGURE 3 | Treatment algorithm for AR as proposed by EUFOREA, taking into account the reality of patient phenotypes and existing international guidelines. EUFOREA treatment algorithm for Allergic Rhinitis (with permission from EUFOREA). The patient should be involved and educated regarding treatment, which starts with allergen and irritant avoidance, plus nasal saline. Further therapies are used as indicated, depending on disease severity and responsiveness to treatment. Failure to control AR should lead to revisiting the diagnosis, the major symptoms, disease extent, and other factors such as patient concordance.

reducing symptoms of itching, rhinorrhoea and sneezing, but less effective at ocular symptoms (62, 63) and have variable effects on nasal congestion (64, 65).

Besides histamine, other mediators released from various immune cells are responsible for amplifying and maintaining inflammation and symptoms. There is some evidence that specific antihistamines including INAH can exert anti-allergic effects beyond inhibiting the action of histamine, including actions on arachidonic acid pathway mediators such as leukotrienes, thromboxanes, inflammatory cells, and mediators (66–70). The mechanisms behind this action have not been fully elucidated but may involve interference with calcium ion channels (50, 54, 71, 72).

The major adverse effect in trials is a bitter taste with azelastine, experienced as severe by a subset (around 10%) of subjects, probably genetic supertasters. It can be mitigated to an extent by correct technique of use as indicated in the manufacturer's advice sheet. The sedating effects of oral azelastine are avoided in the majority of nasal users, since the nasal dose is around one twentieth of the oral one (50).

Intranasal Corticosteroids (INS)

Topical intranasal corticosteroids (INS) are considered the single most effective treatment for AR and suppress most allergic inflammatory reactions (73). INS have been demonstrated to be more effective for relieving nasal symptoms of AR than oral and intranasal antihistamines (74, 75), especially for nasal congestion (76) and are particularly useful for improving ocular symptoms in AR patients (77, 78). INS also reduce bronchial hyperreactivity (79), as with ocular effects suggesting an effect on neurally—mediated distant symptoms via control of local inflammation and mediator release. Not all INS are equally effective (80).

Beclamethasone was the first steroid to be effectively modified for use in a pressurized INS spray in 1972 (81) and 8 compounds for intranasal application have been approved for AR in Europe and USA including triamcinolone acetonide, budesonide, ciclesonide, mometasone furoate, flunisolide, beclomethasone dipropionate, fluticasone propionate, and fluticasone furoate (73, 82).

Glucocorticosteroids diffuse across cell membranes, therefore lipophilicity is an important property, where they bind to the cytoplasmic glucocorticoid receptor (GR) (primary mechanism) (73, 83). On binding of the GR with the corticosteroid ligand, the heat shock proteins dissociate, allowing the GC-GR complex to translocate into the nucleus or interact with transcription factors in the cytoplasm (84). The anti-inflammatory effects are the result of modifications to gene transcription occurring via transactivation or transrepression. In the transactivation pathway, the activated GC-GR complex migrates to the nucleus where it binds as a dimer to the promotor region of palindromic DNA sequences termed Glucocorticoid Response Elements (GRE) (85). Interaction between the activated GR complex and GRE promotes an increase in the transcription of anti-inflammatory genes and of genes encoding proteins that have inhibitory effects on transcription of inflammatory and immune genes (86). The main anti-inflammatory effects of GCs occur via the suppression of multiple genes that encode inflammatory proteins, a process known as trans-repression (87, 88). INS have been shown to inhibit cytokine production in a range of different cell types. Epithelial generated cytokines act as chemoattractants and recruit effector cells such as eosinophils, basophils, and T cells to the nasal mucosa. Fluticasone propionate or fluticasone furoate significantly reduced levels of GM-CSF, IL-6, and IL-8 in stimulated nasal epithelial cells (89–91). Moreover, fluticasone propionate inhibited the release of IL-4, IL-6, IL-8, and TNF-a at an IC50 of <1 nM (92) in stimulated murine mast cells and to significantly reduce IL-4 and IL-5 levels from stimulated peripheral blood CD4C T cells (93). Different classes of steroid drugs (94) induce a different degree of cytokine inhibition with mometasone furoate being the most potent inhibitor of IL-1, IL-6, and TNF-a production among five different ones (mometasone furoate, hydrocortisone, betamethasone, dexamethasone, and beclomethasone).

Corticosteroids may inhibit the maturation of mast cells via regulating the expression of anti- or pro-apoptotic molecules in mast cell progenitors. Glucocorticoid facilitates apoptosis of eosinophils (95, 96) and reduces the numbers of immune cells, production of Th2 cytokines and chemokines and the release of inflammatory mediators in nasal mucosal samples, mostly they seem to actively target Th2 related cytokines (GM-CSF, IL-6, IL-4, IL-5, IL-10, and IL-13) involved in perpetuating the allergic response, in contrast to Th1 cytokines (IFN-g, IL-2) where no effect of steroid treatment was observed (87).

Again the nasal route involves microgram doses, rather than the milligram ones necessary for oral effectiveness. However, INS vary considerably in their systemic bioavailability and the least bioavailable ones: fluticasone propionate, fluticasone furoate and mometasone furoate should be used in children and when long term use is advisable (15). Unlike topical dermal use, there is no local atrophy from properly—applied INS, probably because of the continual movement of any applied drug by mucociliary clearance. Correct application of the spray onto the lateral wall of the nose, with different directions if two squirts are used, should be taught to every person for whom INS are prescribed or to whom they are sold over the counter. Avoidance of the nasal septum, less well-provided with ciliary action, reduces the chance of epistaxis or the extremely rare complication of septal atrophy (15).

Combination Therapy

Recently sprays containing both INS and intranasal antihistamine (fluticasone propionate and azelastine hydrochloride; mometasone furoate with olopatadine) have been formulated and tested in AR patients. Both are more effective on symptom reduction compared to either single molecule alone (97). The low pH of the second combination may cause nasal discomfort. Combining INS with intranasal decongestant is slightly more effective than INS alone and does not appear to cause rhinitis medicamentosa (97).

Intranasal Decongestants

Catecholamines (e.g., phenylephrine) or imidazolines (e.g., oxymetazoline) serve as active agents of intranasal decongestants usually classed as vasoconstrictor sympathomimetic agents (98).

Their decongestion effects exert through direct and indirect activation of postsynaptic a1 and a2 adrenergic receptors on smooth muscles lining nasal capacitance vessels). On activation of these receptors, the smooth muscle contraction constricts blood vessels and thus reduces nasal tissue edema (98–100) followed by rapid reduction of nasal congestion (98, 99) without effect on other symptoms of AR [such as nasal itching, rhinorrhea, and sneezing (63, 100, 101). Prolonged or repeated use of decongestants (>3–5 days) may lead rebound swelling and congestion (101, 102) known as rhinitis medicamentosa. Septal atrophy may result from repeated septal application of such sprays and can also occur with the use of intranasal cocaine (103).

Intranasal Anticholinergics

Intranasal anticholinergic agents (INAA) such as ipratropium bromide can lead to the reduction of rhinorrhoea in AR (104–106) by blocking parasympathetic pathways in the nose that release acetylcholine. Acetylcholine acts on muscarinic receptors on nasal mucus glands to induce hypersecretion (104, 107, 108). Ipratropium bromide is a cholinergic receptor antagonist that blocks the interaction of acetylcholine on muscarinic receptors to inhibit release of watery secretions from mucous glands (104, 107), but has no effect on symptoms of sneezing or nasal congestion or inflammatory responses (104, 109, 110). Side effects include the predictable dry mouth and constipation.

Intranasal Cromones

Cromones are considered mildly effective in relieving symptoms of nasal itching, rhinorrhoea and sneezing, without affecting nasal congestion (83, 101). Their duration of action is short, requiring frequent dosing (up to four times per day) (101, 111).

Both cromoglicic acid, a derivative of chromone-2-carboxylic acid and nedocromil sodium, a pyranoquinolone, are available as intranasal formulations. The exact mechanism of action of chromones is unknown, although several theories have been postulated. Chromones are thought to exert their anti-inflammatory effects by preventing the release of histamine, tryptase and leukotrienes from mast cells following binding of IgE antibodies to the Fc+RI receptor and crosslinking with allergenic peptides (108, 111, 112). Chromones also have reported effects on eosinophils involved in the allergic response (113), but had no significant effect on basophils (114).

NON-ALLERGIC RHINITIS (NAR)

When no allergic or other cause is found for nasal symptoms the diagnosis by exclusion of non-allergic rhinitis is made. This exists in two main forms- with and without eosinophilic inflammation. The former can be treated similarly to AR with saline, antihistamines, intranasal corticosteroids, alone or in combination. The latter is neurogenic and may respond to anti-cholinergics, such as ipratropium, or to capsaicin, an extract from chili peppers which reduces overexpression of a cation channel, TRPV1, in the nasal lining. Capsaicin(8-methyl-N-vanillyl-6-nonenamide) is a natural irritant which initially excites neurones, but then has a long refractory period, during which those neurons are unresponsive, not only to capsaicin, but to a variety of stimuli (115). Capsaicin desensitization performed correctly, is safe and effective for reducing NAR symptoms (number needed to treat = 4; 95% confidence interval [CI], 1 to 22) for several months (116). There is insufficient evidence to compare the effectiveness of capsaicin to other topical or systemic medications.

ACUTE RHINOSINUSITIS (ARS)

This is an inflammatory disease affecting the nose and paranasal sinuses with duration up to 12 weeks. Usually initiated by viral infection (common cold) it can be prolonged (post-viral) and, in a few subjects it is complicated by bacterial infection. Nasal saline, decongestants and ipratropium bromide can be used in the common cold; for post viral symptoms INS may help reduce symptoms in adults, but there is a paucity of evidence for any intranasal treatment when bacterial superinfection occurs (117).

CHRONIC RHINOSINUSITIS

Chronic rhinosinusitis (CRS) is a symptomatic inflammatory disease of the nasal and paranasal mucosa lasting more than 12 weeks (118). CRS has polypoid (CRSwNP) and non-polypoid (CRSsNP) subforms (118). Asthma is a prolonged bronchial inflammatory disease with an increased and variable tendency for bronchial contraction (119). Both CRS and asthma are significant health problems; the prevalence of each is ∼10% (120), and the prevalence of co-morbid asthma and CRS is ∼50% (118). The impact of CRS on the quality of life is significant, analogous to diabetes mellitus (118), and it leads to remarkable costs (121). The main treatment of both CRS and asthma is topically administered corticosteroids and nasal saline douching. Use of corticosteroid locally applied into the maxillary sinus via an indwelling tube was found effective in a group pf HDM sensitive subjects with CRS unresponsive to sinus surgery (122). Corticosteroid- eluting sinus implants reduce polyp size and the need for sinus surgery and are considered an option by EPOS (118). Application using corticosteroids in the nasal douche is now a popular treatment option, but there is no firm evidence for it being more or less effective than when the two are used separately (118).

Topical antifungals and topical antibiotics have been trialed in CRS, without significant benefit, except perhaps in special cases such as tobramycin in cystic fibrosis (118).

However, there is one relatively common CRS subtype in which local nasal therapy, other than saline and corticosteroids, may prove effective.

Nasal Acetylsalicylic Acid Desensitization in Non-steroidal Anti-inflammatory Drug-Exacerbated Respiratory Disease (N-ERD)

Patients with non-steroidal anti-inflammatory drug (NSAID)—exacerbated respiratory disease (N-ERD) have co-morbid asthma, CRS, and NSAID intolerance often with severe disease forms. They are prone to difficult symptoms and recurrent acute exacerbations despite adequate treatment by local or systemic

corticosteroids, nasal saline lavages, antibiotics and sinus surgery. Acetylsalicylic acid (ASA, aspirin) treatment after desensitization (ATAD) may be beneficial. Oral ATAD has been shown to improve the quality of life and sino-nasal symptom scores in patients with N-ERD. However, if ASA is not taken regularly, ATAD is associated with a risk of severe anaphylactoid reactions.

Despite active treatment, some 10–20% of CRS/asthma patients have severe disease, purulent exacerbations and impaired productivity (118, 123, 124). Up to 70% of the uncontrolled cases have Type 2 inflammation, nasal polyps (NP), and/or N-ERD (125–129). The triad of co-morbid CRSwNP, asthma, and N-ERD has previously been called Samter's triad (130). The prevalence of N-ERD is 10–16% in hospital-level CRSwNP patients (131, 132). If endoscopic sinus surgery (ESS) combined with appropriate medical treatment fails, additional therapies including ATAD can be considered to treat N-ERD (118, 133).

Oral ATAD has Level of evidence 1a, although the placebo-controlled studies have had relatively small sample sizes (118). Since ATAD has side- and adverse effects including gastritis, gastrointestinal ulcerations and bleedings, attempts have been made to reduce the risk of side effects. Nasal ATAD (nATAD) tends to have fewer side effects than peroral ATAD both in diagnosis and in therapy (134). The use of nATAD is not suggested (level of evidence 1b-) in the current the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS 2020), since it lacks sufficient evidence for treatment of CRSwNP patients with N-ERD, but further double- blind studies are recommended (118). Here, we will review the nATAD literature, and communicate our own experience regarding its use.

Non-steroidal Anti-inflammatory Drug (NSAID) Exacerbated Respiratory Disease (N-ERD)

N-ERD is an inflammatory airway disease usually consisting of a triad of hypersensitivity to NSAIDs, asthma and CRSwNP (130–133). Patients with N-ERD have severe eosinophilic hyperplastic inflammation and fibrotic tissue remodeling in both their paranasal sinuses and lower airways (130, 135, 136). The age of onset for N-ERD is usually around 30 years, it is slightly more common in females (137, 138).

About 9% of asthmatics have N-ERD, the asthma of N-ERD patients tends to be moderate to severe (133). Compared to other asthmatics, N-ERD patients are more likely to need high dose inhaled corticosteroid treatment or steroid bursts (133, 135), and their asthma is more likely to be uncontrolled and to lead to asthma related healthcare visits, hospitalizations, and intubations (135, 137).

CRSwNP treatment of N-ERD patients consists of saline irrigations, nasal steroids, antileukotrienes, oral steroids, oral antimicrobials (139), and endoscopic sinus surgery (ESS) if conservative treatment is not sufficient (118, 135). The need for recurrent sinus surgeries is common in N-ERD patients (140, 141). ATAD (133), and/or biological agents are also considered if other treatments are insufficient (118).

In N-ERD patients, NSAIDs cause exacerbation of respiratory tract symptoms, provoking nasal congestion, rhinitis and obstruction of the lower airways, usually within 45–60 min of administration, urticaria, dyspepsia, and angioedema can also occur (142). The pathomechanisms behind this are not fully understood; it has been suggested that the hypersensitivity to NSAIDs is not caused by an allergic, immunoglobin E (IgE)—based mechanism, but rather by abnormal metabolism of the lipoxygenase (LO) and cyclooxygenase (COX) pathways (136, 143). Three forms of COX enzyme exist, one of these is COX-1. ASA and its other cross reacting NSAIDs inhibit COX-1, leading to decrease in COX-1 products, including prostaglandins. In N-ERD patients, ingestion of NSAIDs leads to an imbalance in the products of these pathways (143) (**Figure 4**).

Ideally, N-ERD is diagnosed with a NSAID-challenge test. However, if a patient with confirmed asthma and CRSwNP has had multiple reactions with respiratory symptoms within 2 h after two different NSAID ingestions, this history is sufficient for N-ERD diagnosis (133). In unclear cases, for research purposes, or to evaluate for the provocation dose of ASA in oral desensitization, challenge tests are needed (133). The following contraindications for ASA challenge, ASA desensitization (AD), and ASA treatment after desensitization (ATAD) must be appreciated: prior anaphylactic/anaphylactoid reaction(s) due to NSAIDs, gastrointestinal bleeding, renal failure, uncontrolled asthma (Forced expiratory volume in one second [FEV1] <70% of the predicted value), ongoing respiratory tract infection or asthma exacerbation, current treatment with β-blocker, or pregnancy (133).

The initial use of nasal lysine aspirin (in the USA where this is unavailable, ketorolac is used) for challenge, coupled with sensitive upper airway measurements, means that highly sensitive subjects can be identified at a low dose without causing an asthma exacerbation. A negative nasal challenge necessitates oral challenge with larger doses until 300 mg has been tolerated (144).

Acetylsalicylic Acid (ASA)

ASA, also known as aspirin, is a very commonly used drug worldwide (145). It has been used to reduce pain, fever, inflammation, and lately mostly as prevention for cardiovascular diseases, but it may also have other preventive effects (146). The history of acetylsalicylic acid began over 3,500 years ago, when salicylate- containing willow bark was used to treat pain by ancient Sumerians and Egyptians (145). Aspirin was synthesized by Bayer company's chemist Felix Hoffmann in 1897 (145). The molecular formula of ASA is $C_9H_8O_4$ (146) (**Figure 5A**).

Lysine Acetylsalicylate (LAS)

The molecular formula of Lysine acetylsalicylate (Aspirin Lysine salt, Aspirin DL-Lysine, DL-Lysine-acetylsalicylate) is $C_{15}H_{22}N_2O_6$, its component compounds are DL-Lysine and ASA (147) (**Figure 5B**). Lysine acetylsalicylate (LAS) is soluble, it is the only genuinely soluble aspirin preparation (148) and was developed for intravenous administration to treat pain (148, 149).

ASA Treatment After Desensitization (ATAD) in N-ERD Patients

Due to the severity of symptoms in N-ERD, there has been an interest to improve its treatment, one of these developments

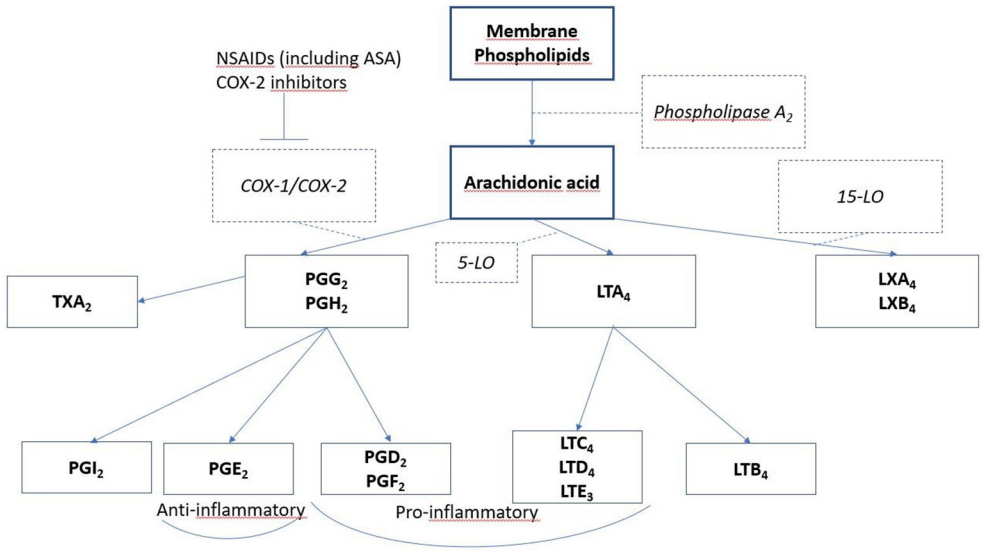

FIGURE 4 | Lipid mediators involved in N-ERD. Arachidonic acid is released from degranulating cells (mast cells and eosinophils) and is metabolized by several routes to form prostaglandins, leukotrienes, and lipoxins. Inhibitors of cyclooxygenase 1, such as aspirin and NSAIDS, block this pathway, reducing bronchoprotective PGE2 and allowing increased pro- inflammatory leukotriene and lipoxin formation.

is ATAD. Since the ASA intolerance of N-ERD patients is not due to IgE-mediated allergy, ATAD is not comparable to allergen desensitization in IgE confirmed allergic diseases. The aim of ATAD is to reduce polyp growth and decrease (upper) airway symptoms. ATAD is considered in N-ERD patients with insufficient response to pharmacological treatment, high recurrence of NPs leading to recurrent surgeries, insufficient control of asthma symptoms with standard medications, need to reduce corticosteroid dose, or in patients who need ASA or NSAID treatment (133, 150).

Our real-world follow-up study showed high discontinuation rates of peroral ATAD with a lack of effect on revision sinus surgery rates, prescribed antibiotics and oral corticosteroid courses (151). The latest EPOS 2020, however, concludes that peroral ATAD improves the quality of life and total nasal symptom scores in patients with N-ERD (118, 147, 152–155).

AD can be performed in an outpatient setting as an extension of ASA challenge, with ATAD continuing straight after the challenge by gradually increasing ASA doses (133, 144, 150). ATAD is usually performed with peroral ASA, with the effective dose varying between 300 and 1,300 mg daily (133, 147, 155–161). Intranasal ASA has been used in both ASA challenge and ATAD (133, 134). There is some evidence, that the surgical removal of NPs, or ESS may be beneficial prior to ATAD (133, 136, 144, 158–162).

Nasal Lysine Aspirin Challenge in N-ERD Patients

Although thus far not a routine part of clinical diagnostics, nasal challenge test with Lysine aspirin (LAS) was introduced for N-ERD assessment in the 1990s (163). The LAS doses, duration of observation period, and criteria for positivity have varied in different studies. One study group performed nasal ASA challenge tests (ASA-NCT) for 51 patients with N-ERD, confirmed by oral ASA challenge (163). The study did not report systemic reactions, including bronchospasm. They concluded that ASA-NCT is highly specific (95.7%) and sensitive (86.7%), that the nasal test is simple, safe, and quick for N-ERD diagnostics, but that negative results do not exclude possible ASA intolerance (163). Another study group performed ASA-NCT with relatively little side effects and showed positive result in 100 of 131 patients with severe CRSwNP and asthma (164). This study concluded that provided patients are carefully chosen and monitored, ASA-NCT is suitable for day-case practice (164).

Nasal Lysine Aspirin Treatment After Desensitization in N-ERD Patients

Nasal Lysine Aspirin treatment after desensitization (nATAD) has been used to treat N-ERD patients, but it has not been in wide clinical use. In the one existing double blind placebo controlled trial by Parikh and Scadding, 22 subjects with ASA sensitive nasal polyposis were enrolled, they were randomized to receive either 16 mg of topical LAS or placebo every 48 h for 6 months before cross-over. Only 11 study subjects completed the study, and no clinical benefit could be demonstrated (165) (**Table 1**). However, a reduction in the characteristically elevated levels of cysLT1 receptors was seen (169) and confirmed in a further study which also showed that this phenomenon did not occur in aspirin tolerant subjects (170).

Prospective, non-randomized studies have shown clinical benefits in LAS treated NP patients (166–168, 171) (**Table 1**). In an n of 1 study 13 N-ERD subjects who were uncontrolled on standard therapy were studied for 3 months and then for a further 3 months with the addition of nasal lysine aspirin, gradually increased to 54 mg daily (168). Significant improvement was seen in nasal inspiratory peak flow rate, $p = 0.014$) and nasal

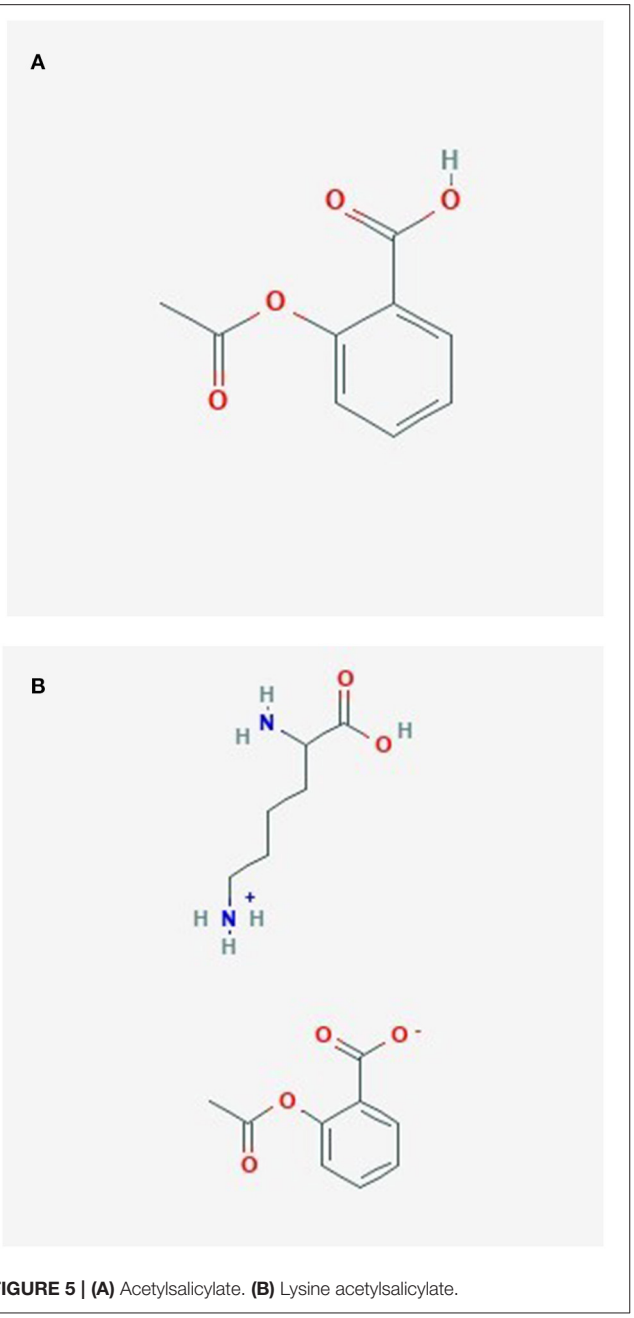

FIGURE 5 | (A) Acetylsalicylate. **(B)** Lysine acetylsalicylate.

nitric oxide levels rose significantly (in both sides, $p = 0.028$), suggesting opening up of the nasal airway and sinus orifices. Exhaled nitric oxide and peak expiratory flow did not change. Compared with the preceding 3 months, adding intranasal lysine-aspirin had an effect on decreasing nasal polyp volume (right side, $p = 0.031$; left side, $p = 0.016$) (168).

Howe et al. (134) performed a non-controlled audit study including 105 N-ERD patients with intranasal LAS in gradually increasing doses following positive LAS challenge. Symptoms improved/stabilized in over 70% subjects at 3 and 12 months, and nasal inspiratory peak flow, olfaction, exhaled and nasal nitric oxide levels were also improved significantly. Asthma outcomes, including use of oral corticosteroids, exacerbations and emergency visits were all reduced in 22 subjects taking lysine aspirin over a year, compared to 20 challenge- positive subjects who ceased using it. Gastrointestinal side effects occurred in 3.8%, which is lower than those reported for oral ASA therapy (145). LAS has also been studied in treatment of patients with CRSwNP but no ASA intolerance. A prospective study involving 20 patients with CRSwNP but no ASA intolerance, receiving 2,000 μg LAS in one nostril and saline in one nostril, showed that polyp recurrence tended to be milder on the LAS treated side. However, a double blind, placebo- controlled trial was negative in aspirin- tolerant nasal polyp patients (172).

Finnish Experience With Nasal Lysine Aspirin Desensitization in N-ERD Patients

Seven nasal aspirin challenge- positive subjects [at 10 mg ($n = 2$) and 20 mg, $n = 5$] were given nasal ASA-desensitization (nAD) according to an earlier published method (134, 173), at the Department of Otorhinolaryngology—Head and Neck Surgery of Helsinki University Hospital.

Six of the seven patients discontinued the nAD (mean duration of the desensitization 19.2 days, range 7–39 days). The known reasons for the discontinuation were severe abdominal pain in three in one accompanied by an asthma exacerbation, and exacerbation of nasal blockage in two. Fortunately, the side effects were transient when nAD was discontinued.

Only one patient continued nAD. Although the dose was low (50 mg) the patient felt symptom relief. Uveitis developed 2 months after onset of nAD, however, according to the ophthalmologist it was not caused by the nAD.

It is possible that the Finnish population does not tolerate ATAD as well as other populations (151), certainly the high reports of gastrointestinal symptoms are concerning. However, it is necessary to warn patients that the desensitization process takes time and that usually they will be worse before their condition improves. Gradual updosing is necessary, with reduction of the dose when adverse events become problematical, as in allergen immunotherapy, although the mechanism of effect of ATAD appears more likely to relate to exhaustion of mediator- bearing cells and to receptor downregulation.

Conclusions

Nasal acetylsalicylic acid treatment after desensitization is a treatment option for N-ERD. The evidence of its benefits for N-ERD patients is not yet convincing, further randomized double-blind placebo-controlled studies are needed. According to the literature, nasal acetylsalicylic acid treatment after desensitization causes fewer side effects than oral ATAD, our own limited experience, however, contradicts this.

INTRANASAL DRUGS FOR DISEASES OUTSIDE THE NOSE

While the intranasal administration of drugs for the treatment of nasal diseases is well-established, intranasal drug delivery is increasingly recognized as being a useful and reliable alternative

TABLE 1 | Trials of intranasal lysine aspirin in nasal polyposis.

References	Study method	Study participants	Dose	Outcome measures	Study results
Patriarca et al. (166)	Prospective, non-randomized controls	20 patients with N-ERD and CRSwNP/43 patients with CRSwNP 191 control patients	2 mg (ASA equivalent) per week	NP relapse	NP relapse rate decreased in LAS group
Nucera et al. (167)	Prospective, non-randomized controls	(1) 28 (N-ERD+CRSwNP)/ out of 76 patients. (2) 14 (N-ER+CRSwNP)/out of 49 patients Control group 191 CRSwNP patients	4 mg (ASA equivalent) 6 times per week	Recurrence of NP (in CT and clinical control)	Recurrence of NPs reduced in LAS group
Parikh and Scadding (165)	Double blind placebo controlled cross-over trial	22 ASA intolerant patients (of these 19 had CRSwNP), 11 completed the study	16 mg (ASA equivalent) every 48 h for 6 months before cross-over	Nasal and pulmonary symptom scores ARM PEF rate PNIF	No significant differences between the groups But cysLT1 receptors reduced
Ogata et al. (168)	Prospective, open n of 1 study	13	54 mg LAS [ASA equivalent 37.8 mg (53)] per day	NP volume NIPF, nNO, eNO, PEFR	NP volume reduced, NIPF, and nNO improved
Howe et al. (134)	Audit	105 AERD + LAS treatment/out of 121 patients with AERD	75–100 mg ASA equivalent per day	Subjective symptom evaluation + VAS PNIF Exhaled + nasal NO Olfaction Spirometry Asthma questionnaire	Symptom improvement Reduced airway inflammation Improvement of olfaction Improvement of asthma outcomes

Nasal Lysine aspirin (LAS) treatment, N-ERD (Non-steroidal anti-inflammatory drug exacerbated respiratory disease) patients with CRSwNP (chronic rhinosinusitis with nasal polyposis). N-ERD, Non-steroidal anti-inflammatory drug exacerbated respiratory disease; AERD, aspirin exacerbated respiratory disease; LAS, Lysine aspirin; CRSwNP, chronic rhinosinusitis with nasal polyps; NP, nasal polyps; ASA, aspirin; CT, computed tomography; ARM, acoustic rhinometry; PEF, peak expiratory flow; PNIF, Nasal inspiratory peak flow; VAS, visual analog scale; NO, nitric oxide.

to oral and parenteral application of drugs for systemic diseases and the nasal mucosa has seriously emerged as a therapeutically viable route for systemic drug delivery. In particular nasal delivery seems to be able to circumvent the blood-brain barrier allowing direct drug delivery in the biophase of central nervous system-active compounds. Also, pharmacologically active compounds with poor stability in gastrointestinal fluids, poor intestinal absorption or unfavorable gastrointestinal and hepatic pre-systemic metabolism are of interest.

Peptide drugs (hormone replacement) treatments in different diseases appear to provide good indications under these circumstances. Different peptide hormones are available as nasal sprays, e.g., authorized products exist for estradiol steroid substitution of estradiol (Aerodiol®) (174, 175) and Gonadorelin hormone for undescended testicle (Kryptocur®) (176).

For the treatment of diabetes insipidus, the peptide analog desmopressin is available for both, nasal and oral administration with a given bioavailability of the commercial tablet of 0.1% and of 3–5% for the nasal spray. It can also be used for nocturnal enuresis in children and in multiple sclerosis. Recently the desmopressin spray was withdrawn from use for mild hemophilia and von Willebrand's disease because of higher than specified dosage[1]. Too much desmopressin can cause sodium levels in the blood to drop sufficiently to result in seizures, coma, and death.

Syntocinon nasal spray containing oxytocin is used to increase duration and strength of contractions during labor and has been investigated for some psychiatric conditions such as anorexia nervosa, autism, anxiety disorders, schizophrenia and alcohol deprivation (177).

Gonadotropin-Releasing-Hormone (GnRH) analogs such as Nafarelin (Synarel®) and busurelin are used for the treatment of endometriosis, precocious puberty, anovulatory infertility, hypogonadotropic, and cryptorchidism (178).

Further authorized products exist for nicotine withdrawal for smoking cessation (Nicotrol NS®) (179).

FUTURE USES OF THE INTRANASAL ROUTE

Anti- viral molecules are under investigation for the reduction of COVID-19 transmission[2].

Since there is some evidence for an intranasal, virally—mediated etiology for some neurodegenerative conditions (Parkinson's and Alzheimer's diseases) it may eventually be possible to prevent these by prophylactic use of a non-toxic intranasal antiviral.

Intranasal adrenalin is under trial for urgent anaphylaxis therapy. It will be necessary to block the nasal mucosa from giving a vasoconstrictive response to the applied adrenalin.

The united airways concept, wherein the nose and lower airways react as one unit to stimuli (180, 181) has led to some attempts to treat both areas via the nose, rather than using both nasal spray and inhaler. As yet this sensible concept has not proved successful.

[1]Available online at: https://hemophilianewstoday.com/2020/10/05/recall-on-ferrings-intranasal-desmopressin-therapies-to-affect-availability-into-2021/

[2]Available online at: https://www.itv.com/news/central/2020-11-16/new-face-mask-that-kills-coronavirus-could-be-available-by-december-says-nottingham-scientist-nottingham-trent-university-dr-gareth-cave?utm_source=upday&utm_medium=referral

Immunologically active intranasal preparations will be considered in Part 2.

CONCLUSION

The nose provides a useful route for therapy of airways diseases and also for other conditions such as CNS and endocrine disorders. Its accessibility, simplicity of use, good blood flow and protective epithelium should allow it to be investigated further as an alternative to systemic administration.

AUTHOR CONTRIBUTIONS

CC and NB contributed the section on nasal structure and function and **Figures 1**, **2**. DM and NP contributed the saline section. LK contributed intranasal drugs. AL-H, MH, and ST-S contributed the section on lysine aspirin. GS conceived the idea of the paper, edited the contributions, and wrote the Abstract, Introduction, and Conclusion. All authors contributed to the article and approved the submitted version.

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REVIEW
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Review: The Nose as a Route for Therapy. Part 2 Immunotherapy

*Yorissa Padayachee[1], Sabine Flicker[2], Sophia Linton[3,4], John Cafferkey[1], Onn Min Kon[5], Sebastian L. Johnston[5], Anne K. Ellis[3], Martin Desrosiers[6], Paul Turner[5], Rudolf Valenta[7] and Glenis Kathleen Scadding[8,9]**

[1] Department of Respiratory Medicine, Faculty of Medicine, Imperial College Healthcare NHS Trust, Imperial College London, London, United Kingdom, [2] Center for Pathophysiology, Infectiology and Immunology, Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria, [3] Division of Allergy and Immunology, Department of Medicine, Queen's University, Kingston, ON, Canada, [4] Allergy Research Unit, Kingston Health Sciences Centre (KHSC), Kingston, ON, Canada, [5] Faculty of Medicine, National Heart and Lung Institute, Imperial College London, London, United Kingdom, [6] Department of Otorhinolaryngologie, The University of Montreal Hospital Research Centre (CRCHUM), Montreal, QC, Canada, [7] Division of Immunopathology, Medical University of Vienna, Vienna, Austria, [8] Royal National Ear Nose and Throat Hospital, University College London Hospitals NHS Foundation Trust, London, United Kingdom, [9] Division of Infection and Immunity, Faculty of Medical Sciences, University College London, London, United Kingdom

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Pongsakorn Tantilipikorn, Mahidol University, Thailand

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***Correspondence:**
Glenis Kathleen Scadding
g.scadding@ucl.ac.uk

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The nose provides a route of access to the body for inhalants and fluids. Unsurprisingly it has a strong immune defense system, with involvement of innate (e.g., epithelial barrier, muco- ciliary clearance, nasal secretions with interferons, lysozyme, nitric oxide) and acquired (e.g., secreted immunoglobulins, lymphocytes) arms. The lattice network of dendritic cells surrounding the nostrils allows rapid uptake and sampling of molecules able to negotiate the epithelial barrier. Despite this many respiratory infections, including SARS-CoV2, are initiated through nasal mucosal contact, and the nasal mucosa is a significant "reservoir" for microbes including *Streptococcus pneumoniae*, *Neisseria meningitidis and SARS -CoV-2.* This review includes consideration of the augmentation of immune defense by the nasal application of interferons, then the reduction of unnecessary inflammation and infection by alteration of the nasal microbiome. The nasal mucosa and associated lymphoid tissue (nasopharynx-associated lymphoid tissue, NALT) provides an important site for vaccine delivery, with cold-adapted live influenza strains (LAIV), which replicate intranasally, resulting in an immune response without significant clinical symptoms, being the most successful thus far. Finally, the clever intranasal application of antibodies bispecific for allergens and Intercellular Adhesion Molecule 1 (ICAM-1) as a topical treatment for allergic and RV-induced rhinitis is explained.

Keywords: interferon, rhinovirus, microbiome, vaccination, allergen immunotherapy, epithelial barrier, ICAM-1, allergen-specific antibody

INTRANASAL INTERFERONS IN PREVENTION AND TREATMENT OF VIRAL RESPIRATORY ILLNESSES

Interferons (IFNs) are a family of cytokine mediators with unique immune-modulatory antiviral and anti-proliferative properties which has led to their investigation as treatment and prevention against common colds. Early studies using prophylactic systemic high-dose IFN-α have mostly demonstrated success against rhinoviral colds but had varying efficacy as prophylaxis for other respiratory viruses. Subsequently, use of IFN-α for common colds was halted due to adverse effects. Nasal IFNs may provide similar efficacy with reduced side effects. Some studies using intranasal IFN-γ have demonstrated inefficacy as prophylaxis against colds. Intranasal IFN-λ has not been studied in man against common cold viruses but has shown promising *in-vitro* and mouse-model results. Recent studies investigating IFNs as treatment for virally-induced asthma exacerbations demonstrated improvement in some clinical outcomes. Currently IFNs are being investigated for their use in asthma, COPD and the SARS-CoV-2 pandemic.

Interferons (IFNs) consist of a family of cytokine mediators secreted by immune and other cells, in response to infectious and certain malignant stimuli (1–3). IFNs have immune-modulating, antiviral and anti-proliferative properties which make them effective therapeutic agents for various medical conditions (4–7). Consequently, since their discovery, they have been studied extensively for their use in viral respiratory illnesses (8).

IFNs comprise three subfamily types; I, II, and III, classified depending on their sequence relatedness and surface receptor binding (1, 2, 4, 9). The largest subfamily type I IFNs includes IFN-α (leucocyte IFN) and IFN-β (immune IFN), which are secreted by virally-infected cells (9, 10). IFN-γ(immune IFN) is mainly secreted by natural killer and natural killer T-cells after antigen exposure. The type III subfamily includes IFN-λ which has three subtypes (9, 11–13).

IFNs have shown potent anti-viral activity against respiratory viruses *in vitro*, however this has not yet consistently translated into vivo anti-viral effects (2, 5–8, 10–13). The main pitfall of interferons are the associated systemic adverse effects experienced with intramuscular or subcutaneous administration. These include flu-like symptoms (fatigue, fever, myalgia and headaches), pulmonary symptoms, gastrointestinal symptoms, neurotoxicity, and depression (1, 14–16).

Route and Dosing of IFN Administration

Interferons are poorly absorbed orally due to their large amino-acid sequence which is susceptible to digestive enzymes (17, 18). Effective absorption has been noted via intravenous, subcutaneous, intramuscular, and intranasal routes (17). Intravenous, subcutaneous, and intramuscular routes of administration can be associated with serious systemic side effects (5, 17, 18). Intranasal administration is associated with a more targeted effect on the upper airway which limits systemic adverse effects, however it can still result in local side effects such as mucosal irritation, drying, erosion and blood-stained mucus (5, 17, 18). These are dependent on treatment duration and dosing (5).

The Link Between Respiratory Viruses and IFNs

Respiratory viruses such as influenza, respiratory syncytial virus (RSV), human metapneumovirus, parainfluenza, human rhinoviruses (HRV), human seasonal coronaviruses, and SARS-CoV-2 can lead to serious respiratory disease and mortality (19–21). Respiratory viral illness severity ranges from asymptomatic carriage, mild upper respiratory tract symptoms (common cold) to severe pneumonia, bronchiolitis and acute exacerbations of asthma or COPD which can be life-threatening (22). *In vitro* and animal studies have shown successful suppression of respiratory viruses on administration of exogenous IFNs (23–29). Consequently, IFNs have been investigated *in vivo*.

Intranasal IFN-α for Prevention and Treatment of Colds Due to Rhinovirus

Generally, trials investigating either leukocyte-derived human interferon (HuIFN) or recombinant HuIFN-α2 (rHuIFN-α2) as prophylaxis against either experimental or natural rhinoviruses have been positive. Daily dosing of 10 million international units (MU) of rHuIFN-α2 or lyophilized HuIFN-α2 prevent rhinovirus colds and viral replication for both natural or experimentally induced infections (30–36).

Better prophylactic efficacy was noted with higher and or prolonged IFN-α dosing. Furthermore, administration a few hours before experimental rhinovirus infection inoculation conferred better symptom reduction (30, 33–36). High dose intranasal IFN-α2 was associated with increased local adverse effects such as nasal dryness, blood stained mucus and rhinoscopic findings of mucosal damage in most trials (30, 37–39). A trial using high-dose (10 vs. 20-MU vs. placebo) rHuIFN-α2 daily for 5 days was ineffective in treating naturally occurring RV colds. The 20-MU arm experienced prolonged duration of pronounced cold symptoms, more clinically significant adverse effects and secondary complications requiring antibiotic administration in comparison with the placebo and 10-MU groups (39). A study comparing two intranasal methods using a high-dose (9MU) HuIFN-α2 administered three-times daily, for 5 days, did not prevent the development of experimental RV colds. This study did however indicate that nasal drop administration was more effective than nasal spray in improving the clinical course and reducing the duration and quantity of viral shedding (40).

Two placebo-controlled double-blind family studies (performed in America and Australia) assessing the efficacy of a week of daily 5-MU IFN-α2 as post-exposure-prophylaxis against the common cold in exposed household contacts, showed a reduction in incidence of RV infections, but were generally

Abbreviations: IFN, interferon; AIT, Allergen-specific Immunotherapy; EBV, Epstein-Barr virus; Fab, antigen binding fragment; HumAb mice, transgenic mice that produce fully human antibodies; ICAM-1, Intercellular Adhesion Molecule 1; RV, rhinovirus; ScFv, single chain Fragment variable; LDLR, Low density lipoprotein receptor; CDHR3, Cadherin-related family member 3; SARS-CoV-2, the virus causing COVID-19.

ineffective for other viruses (37, 41). In contrast, a family study conducted in Seattle, using the same protocol, showed no reduction in the overall number of colds or secondary colds in family contacts who received IFN-α2. The authors suggested that the lack of preventive efficacy of IFN-α2 could be due to a higher prevalence of influenza B in the study location, Seattle, in comparison with the family studies performed in Virginia and Adelaide where RVs were more prevalent (42). Similarly, another family study, performed in Switzerland, using low-dose rHuIFN-αA (0.3 or 1.5-MU daily for 5 days) resulted in a statistically insignificant reduction in cold transmission, but appeared to almost halve the mean duration of illness ($p = 0.07$) in family contacts (43). Post-exposure prophylaxis for asymptomatic family contacts in a Michigan study using IFN-α2b (5-MU on day 1 and 2.5-MU daily for 4 days thereafter) was ineffective in preventing RV colds. The authors thus concluded that a minimum dose of 5-MU IFN-α2 in family contacts is necessary to achieve acceptable post-exposure RV protection (44). These findings suggest that it is possible for high-dose IFN-α2 to reduce the spread of common colds in family settings, particularly in locations where RV infections are prevalent (37, 41–43).

Intranasal IFN-α as Protection Against Other Respiratory Viruses

IFN-α administration as prophylaxis against other respiratory viruses has shown varying results. Intranasal prophylactic IFN-α administration in experimental coronavirus, RSV and influenza A has shown reduction in virus yield, infection frequency and symptom scores (45–47).

A study using HuIFN-α prophylactically, a day before influenza B inoculation, slightly delayed the onset of infection but did not prevent illness or reduce its severity (31). Assessment of IFN-α2b as seasonal prophylaxis for respiratory infections, demonstrated a significant reduction in the number of rhinovirus, but not parainfluenza, infections (38).

Studies Using IFN-β

Type I-β IFNs have been trialed in the hope that they might have better tolerability and efficacy. There is conflicting evidence regarding the efficacy of recombinant IFN-β-serine (rIFN-βser) as prophylaxis against experimentally induced rhinovirus colds. RIFN-βser has demonstrated *in vitro* anti-viral activity against both rhinoviruses and coronaviruses (38, 48). Most trials using intranasal rIFN-βser have shown efficacy in preventing common colds and are associated with fewer local adverse effects than IFN-α (48, 49).

In contrast, two RCTs in 1986 and 1987 demonstrated rIFN-βser nasal drops to be ineffective as natural cold prophylaxis, even at a higher doses, which were associated with limited local adverse effects (50).

Studies Using Type II IFN-γ

Two studies assessing the effectiveness of rHuIFN-γ as prophylaxis against experimental RV showed no benefit and were associated with symptom worsening and a high rate of local side effects (51).

Type III IFN-λ

IFN-λ was discovered later than the other two types and it plays a key role in respiratory viral infections. It is induced earlier than type I IFNs, mounting an immune response which can overcome viral infection when viral load is low (52). *In-vitro* work has confirmed that IFN-λ is the primary IFN produced by bronchial epithelial cells during the innate response to viral infections (53).

Not only does the specificity of the response to viral respiratory infections with IFN-λ highlight its therapeutic potential, but it may also bring a more favorable side effect profile. In mice, nasal IFN-λ demonstrated a superior anti-influenza therapeutic effect, reducing viral load and crucially not inducing the same pro-inflammatory effect as its comparator, IFN-α (54). Allowing for favorable translation between *in-vitro* and *in-vivo*, murine and human, and subcutaneous and nasal, IFN-λ presents the most promising therapeutic profile of all IFNs. To date, no nasal IFN-λ studies have been identified.

IFNs and Chronic Respiratory Conditions

Asthma

Asthma sufferers of all ages frequently have impaired antiviral immunity. Several studies demonstrate deficient IFN-β and IFN-λ induction after viral infection of primary bronchial epithelial cells (pBECs) as well as deficient induction of IFN-α, IFN-β, and IFN-λ by viral infection of macrophages/dendritic cells (55–60). IFN deficiency may explain virally induced acute asthma exacerbations (55–60) since IFNs suppress viral replication in pBECs (61).

A trial of inhaled IFN-β on asthma symptoms due to viral infections did not show significant improvement of asthma symptoms in the whole study population (62). There were improved morning peak flows, reduction in additional treatment required and enhanced innate immunity as evidenced by serum and sputum biomarkers. A subgroup analysis of moderate/severe asthma did show significant improvement in symptoms and indicated a need for larger trials (62). According to an abstract, a trial investigating SNG001 (inhaled IFN-β) for the prevention or reduction of asthma symptoms after the onset of a respiratory viral infection, have shown that SNG001 maintained antiviral response during the treatment period (63).

Another abstract of a larger trial assessing the use of on-demand SNG001, for treating asthmatic patients with upper-respiratory-tract infection symptoms, did not meet its primary end-point of a reduction of severe exacerbations due a lower than expected number of virally induced severe exacerbations. It demonstrated improved morning peak expiratory flow readings during days 1 to 7 of the treatment period (64).

Chronic Obstructive Pulmonary Disease

Similarly to the IFN deficiency in pBECs of asthmatics, COPD studies have shown impaired virus-induced IFN production in pBECs and bronchoalveolar lavage (BAL) cells (65, 66). This supports a causal relationship between rhinovirus infections and acute exacerbations of COPD (66). A press release for a phase 2 clinical trial investigating inhaled SNG001 in COPD patients with a confirmed respiratory viral infection, has confirmed SNG001 was well-tolerated with enhanced lung anti-viral responses

in comparison with placebo. Furthermore, patients already requiring oral corticosteroids and or antibiotics at the time of randomization into placebo or treatment (SNG001) groups demonstrated significantly better lung function during the treatment period (67).

Recent Trials Using IFNs for Treating COVID-19

Nasal IFNs

SARS-CoV-2 is a zoonotic, enveloped positive- stranded RNA coronavirus first identified in December 2019, now causing a global pandemic. The well-established antiviral properties of IFN have attracted interest in this context. Nasal administration might inhibit SARS-CoV-2 in the nasal epithelium, as well as bolstering the nasal immune barrier (68, 69).

Conversely, *in-vitro* work has implicated viral-driven IFN inflammation in upregulation of ACE2 receptors, the SARS-CoV-2 binding receptor, in nasal epithelial cells (70). Inter-individual IFN responsiveness has been postulated as one factor among a host of others which might explain inter-individual variability in COVID-19 severity. This has led to the hypothesis that blockade of the IFN effect might reduce disease severity (71).

One of the earliest nasal IFN trials was an open-label trial of 2,941 Chinese healthcare workers in one hospital who were split into two groups: those working in areas conferring a high risk of exposure to SARS-CoV-2 and those working in low- risk areas (69). The low- risk group took 2–3 drops of 3,000 μg/mL rhIFN-α in each nostril four times a day for 28 days, and the high-risk group took the nasal drops with an additional weekly subcutaneous injection of 1.6 mg thymosin-α-1, a polypeptide hormone which mediates T-cell response, for 28 days. No new COVID-19 infections were identified by 28 days after therapy ended, which the authors contrast to a "control group" of healthcare workers infected regionally and nationally in other studies spanning the same time frame. The 0% infection rate is striking, and the adverse event profile is promising, limited to "a few… participants experienc[ing] transient irritation of the nasal mucosa," but comes with serious caveats. This trial was not blinded, randomized or conducted with a comparable control arm. The majority (around 80%) of participants were in the low-risk group, and all were reportedly strictly adherent to personal protective equipment guidance, including enhanced PPE measures in the high-risk group. Efficacy of nasal IFN administration against SARS-CoV-2 infection is not substantiated by this preprint alone (69).

Reports from Cuba have described the prophylactic use of intranasal recombinant human IFN-α2b (marketed as Nasalferon) in both asymptomatic travelers arriving at airports and healthcare staff. Very limited information is available, save for a short letter outlining the adverse event profile of the twice daily preparation in 420 participants: 17.4% reported headaches and 3.1% reported weakness. All participants had a negative PCR result at enrolment and no participants were infected at 15 days (defined by examination and PCR result) (72).

Subcutaneous IFNs

There are several studies assessing the efficacy of subcutaneous (SC) IFNs in COVID-19 disease and one of nebulised IFN. A randomized controlled trial used subcutaneous IFN-β1b in conjunction with lopinavir-ritonavir and ribavirin within 7 days of infection (against lopinavir-ritonavir alone), finding that viral shedding, hospital stay and severity of patient observations were markedly reduced, although there was no mortality across groups and insufficient numbers to assess other clinical endpoints (73).

A double-blind RCT of a single dose (180 mg) of SC pegylated IFN-λ (peginterferon-λ) on outpatients with laboratory-confirmed mild to moderate COVID-19 showed an accelerated decline in the SARS-CoV-2 virus, with a high proportion of patients clearing the virus by day 7, in the IFN group in comparison with the placebo (74). In contrast, another trial, on outpatients with mild to moderate COVID-19 disease, using the same dose but SC peginterferon-λ-1a, did not show reduction in the duration of SARS-CoV-2 viral shedding or an improvement in symptoms in comparison with placebo. This result could have been due to the later administration of the peginterferon at a median symptom duration of 5 days (at randomization) with 40% of participants already having positive SARS-CoV-2 IgG results at enrolment; whereas the former trial by Feld et al. had a median time from symptom onset of 4.5 days (SD 1.7) alluding to slightly earlier administration of peginterferon (75). An RCT, in Iran, assessing SC IFN-β1a (12M IU) in the treatment of severe COVID-19 in addition to SOC (standard of care treatment: hydroxychloroquine plus lopinavir-ritonavir or atazanavir-ritonavir) in comparison with SOC alone, demonstrated a reduction in 28-day mortality (PMID: 32661006), with early IFN administration significantly and markedly reducing mortality (76). A second RCT, this time open-label, performed in Iran, assessing the efficacy and safety of longer-term IFN-β1b (250 mg SC every other day for 2 weeks) in the treatment of severe COVID-19 reported a shorter time to clinical improvement ($p = 0.002$), more discharged patients at day 14 ($p = 0.03$) and reduced ICU admission rates ($p = 0.04$) (77).

Another small three-armed study of SC IFN-β1a (12 000 IU on days 1,3,6) and IFN-β1b (8M IU on days 1, 3, 6), comparing them against each other and a control group, reported shorter time to clinical improvement with IFNβ1a against the control group (HR; 2.36, 95% CI 1.10–5.17, $P = 0.031$) while IFNβ1b had no significant difference compared with control; HR; 1.42, (95% CI 0.63–3.16, $P = 0.395$). The median time to clinical improvement for both of the intervention groups was 5 vs. 7 days for the control group. The mortality was numerically lower in both of the intervention groups (20% in the IFNβ1a group and 30% in the IFNβ1b group vs. 45% in the control group) (78).

Addition of SC pegylated IFN α-2b (PEG IFN-α2b) in moderate COVID-19 to SOC did better than SOC alone. Results showed that 19 (95%) subjects in PEG IFN-α2b plus SOC group had achieved clinical improvement on day 15 compared to 13 (68.42%) subjects in the SOC group ($p < 0.05$). Overall, 80 and 95% of subjects in the PEG IFN-α2b plus SOC group had a negative RT-PCR result on day 7 and day 14, respectively, compared to 63 and 68% in the SOC group (79).

The World Health Organisation's large Solidarity trial randomly assigned patients equally to one of four antiviral drugs (remdesivir, hydroxychloroquine, lopinavir, and IFN-β1a) in comparison with control drugs, in hospitalized patients with COVID-19. The IFN-β1a group either received local standard of care (SOC) or lopinavir with ritonavir plus IFN-β1a [three doses 44 micrograms of SC (and in some cases 10 microgram IV doses daily for 6 days when patients were on high-flow oxygen, ventilation or extra-corporeal membrane oxygenation)] IFN-β1a (on the day of randomization, day 3 as well as 6,). Results showed that none of the drugs reduced mortality, initiation of ventilation or duration of hospitalization (80).

Nebulized IFNs

A genetically engineered super IFN-α (rSIFN-co) administered via nebuliser showed a better outcome than regular IFN-α, in a randomized (1:1) trial, in patients hospitalized with moderate-to-severe COVID-19 who received either nebulised rSIFN-co or IFN-α nebulization added to baseline antiviral agents for no more than 28 days. Time to clinical improvement was 11.5 vs. 14.0 days (95% CI 1.10–2.81, p = 0.019); the overall rate of clinical improvement on day 28 was 93.5 vs. 77.1% (difference, 16.4%; 95% CI 3–30%); the time to radiological improvement was 8.0 vs. 10.0 days ($p = 0.002$), the time to virus nucleic acid negative conversion was 7.0 vs. 10.0 days ($p = 0.018$) (81).

Nebulised IFN-α2b was assessed in an uncontrolled exploratory study alone or in combination with arbidol hydrochloride (an antiviral with immune enhancing activity), performed on 53 SARS-CoV-2 PCR positive patients. This showed an apparent shorter duration of viral shedding and a reduction in acute inflammatory markers such as CRP and IL-6 (82).

Another study using IFN-α2b via spray inhalation to 68 patients matched with 36 case controls, with both groups with PCR confirmed COVID-19, did not demonstrate reduced viral shedding but signaled shorter hospitalization times (83).

A randomized controlled trial using inhaled IFN-β-1a showed that in patients with COVID-19 the odds ratio of developing severe disease was 0.28 in the treatment arm compared with placebo ($p = 0.043$). Improvement in the chance of recovery during the treatment period and in symptoms were also demonstrated (84).

Finally, a randomized, open-label parallel group trial of inhaled aerolised Novaferon (a novel interferon manufactured from recombinant antiviral protein) and Novaferon plus Lopinavir/Ritonavir groups demonstrated significantly higher viral clearance rates on day 6 than the Lopinavir/Ritonavir group (50.0 vs. 24.1%, $p = 0.0400$, and 60.0 vs. 24.1%, $p = 0.0053$). The median time to viral clearance was 6, 6, and 9 days (85).

Overall, in COVID-19 disease, there is reasonable biological plausibility for the use of nasal IFN therapy, although as yet very little evidence to support its use. RCTs would be needed to demonstrate both efficacy and acceptable side effect profiles, the latter a factor suggesting that this therapy may be best suited to individuals who are facing the prospect of a high risk of infection (e.g., international air travel, working with symptomatic patients with COVID-19, attending crowded public events). There is however substantial evidence supporting the use of SC IFN therapy in mild to moderate COVID-19 disease, with more benefits evident when administered early. Studies should investigate the use of nasal IFN therapy as both prophylactic therapy and an early intervention to prevent progression. In addition use of nasal IFN might prevent the recently described and nasal colonization by SARS-CoV-2 in patients with prolonged anosmia (86).

The Future of IFNs

IFN therapy is an extremely promising, and in some areas proven treatment for respiratory viral infections and their sequelae. Nasal IFNs bring many of the same benefits without the systemic side effects which are occasionally poorly tolerated by patients. Localized side effects of nasal IFN persist and require strategies for minimization. The therapeutic potential of IFN is pertinent amidst the current global COVID-19 pandemic. Since inhaled IFN-β is well-tolerated and effective in the lung, it is likely that intranasal treatment would also be well-tolerated and effective. IFN-λ is likely to be even better tolerated and trials of intranasal IFN-λ are eagerly anticipated.

INTRANASAL PROBIOTIC THERAPIES FOR RHINITIS AND RHINOSINUSITIS

Over the past decade, there has been an increase in understanding of the importance of bacterial communities present on all body surfaces and cavities (87). These bacterial communities, consisting of trillions of individual bacteria from different species and their genomes, are collectively termed the "microbiome." The term "microbiota," referring only to the microbial taxa associated with humans, should not be used interchangeably with "microbiome." Every surface and cavity of the body has a specific microbiome which can vary dramatically between individuals, for instance, the hand or gut microbiome can be 80–90% different between individuals (88, 89).

The human microbiota consists of 10–100 trillion microbes, primary harbored in the gut (90). In fact, much of what we know about microbe-host interactions and associations between dysbiosis and disease states stems from the gut microbiome. Diversity of the gut microbiome is emerging as a critical determinant of host health, and a loss of diversity has been associated with a variety of gastrointestinal and systemic diseases (91–94) including allergy (95). Dysbiosis is a loosely defined concept referring to any change in the microbiome that adversely affects the health of the host organism. Dysbiosis can be characterized by broad shifts in community microbial compositional structures, reduced species diversity, and changes in the relative proportion of organisms, whereby there is relative lack of "health-associated" bacteria. "Healthy" bacteria are associated with regulation of immune responses, defense against pathogenetic bacteria, and epithelial regeneration or repair of epithelial surfaces (96).

The nasal microbiome has been linked to several immune system disorders and infectious diseases such as allergic rhinitis (AR), chronic rhinosinusitis (CRS), acute respiratory tract

infections (ARTI), otitis media (OM), and asthma. Previously, the persistence of pathogenic bacteria in the nasal cavity was believed to cause disease such as the overabundance of *Staphylococcus aureus* producing superantigens and toxins, impairing immune detection and activation, and ultimately damaging the fragile respiratory epithelium (97, 98). From the perspective of the microbiome, disease can be associated with an imbalance between the commensal microbiome and bacterial pathogens, resulting in a reduction in commensal bacterial diversity, combined with an increase in the growth of microbiomes eliciting an inflammatory response resulting in symptoms of rhinitis. The goal of this review is to contextualize the use of probiotics for the sinus, specifically for AR and CRS, with a focus on pre-clinical studies, due to limited data on the intranasal probiotic formulations in humans.

Dysbiosis and Allergic Disease

AR is an inflammatory disease of the nasal mucosa, triggered by allergen exposure. AR is common and previously estimated to affect 10–30% of the population worldwide (99). A potential role for microbial exposure in allergy risk was identified in the late 1980s with the observation that children from larger households tended to have lower rates of AR and eczema (100). This contributed to the hygiene hypothesis relationship which postulates that a reduction in the frequency of infections, due to reduced exposure to microorganisms, is associated with an increase in the frequency of allergic diseases (100). This hypothesis is supported by robust epidemiological data (100–103). A notable example highlighting the importance of the interaction between the environment, host microbiome, and allergy comes from a comparison of genetically similar populations of Eastern and Western Europe (104, 105). The gut microbiota of infants from Eastern Europe, where the prevalence of atopy is low, and Western Europe, where it is high, have been reported to be distinct (104, 105). Consistent with the sequence of the atopic march, the gut microbiome composition of children with food sensitization from both Western and Eastern Europe has also been found to be distinct from those without atopic diseases from these geographical regions (104, 105). There is even some evidence suggesting a relationship exists with the nasal microbiome, specifically. Ruokolainen et al. examined the prevalence of allergic diseases and both skin and nasal microbiota in 180 children, ages 7 to 11, from Finnish and Russian Karelia. These regions have relatively identical climatic and geographic features, except Russian Karelia is mainly a rural environment and Finnish Karelia is a modernized area. AR, atopic eczema, atopic sensitization, asthma, and self-reported rhinitis were 3- to 10-fold more common in children from Finnish Karelia. Moreover, the nasal microbiome was significantly more diverse among Russian participants than Finnish subjects (106).

A few studies characterize the nasal microbiome in AR, with conflicting results. A 2014 study reported increased bacterial diversity in the middle meatus of seasonal AR participants compared to healthy controls (107). However, these results could not be replicated in a study by Lal et al. (108). More recently, Hyun et al. (109) demonstrated that dysbiosis of the inferior turbinate was associated with high levels of total IgE but not AR occurrence. High levels of total IgE in AR patients were linked to an increased *Staphylococcus aureus* population and decreased *Propionibacterium acnes* in the nose. Dysbiosis of the nasal microbiota was not associated with the number of sensitized allergens or individual allergen specific-IgE levels (109). More studies are desperately needed in this area, especially within the context of a validated disease model such as a controlled allergen challenge facility.

Dysbiosis and Chronic Rhinosinusitis

CRS is considered an inflammatory disease of the nasal and sinus cavities with sinonasal symptoms lasting for 12 weeks or more (110). CRS affects ∼3 to 5% of the Canadian and 12% US populations respectively (111, 112). Several risk factors have been associated with the development of CRS including smoking, lower income, and a history of allergy, asthma, or chronic obstructive pulmonary disease (COPD) (111). Reputed pathological factors include changes in the microbiota, imbalance of the local or systemic immune system, allergens, toxins and genetic pre-disposition (113–116). A 2016 meta-analysis (117) of studies comparing the composition of the bacterial nasal microbiome in CRS patients compared to healthy controls found reduced diversity and less stable bacterial networks in CRS patients (118). These findings have been supported in more recent studies (119, 120). No consistent patterns of one specific microbiome has been observed in all CRS patients, although, previous descriptive studies have shown that the nasal microbiome most frequently includes coagulase-negative *Staphylococcus*, *Pseudomonas aeruginosa*, and *S. aureus*. Importantly, the nasal microbiomes of CRS patients with and without nasal polyps are different in comparison to healthy individuals (108, 121) suggesting the nasal microbiota profile may modulate CRS phenotype (117).

Therapeutic Manipulation of the Microbiome

Taken together, these findings suggest the possibility of improving health by modifying the microbiome to a desirable composition or functional state rather than elimination of the pathogenic bacteria. Perhaps the first example of microbiome supplementation therapy is fecal microbiota transplantation (FMT). FMT involves transferring communities of microbes from a donor to a recipient. Thus far, FMT has been most notably used for treating *Clostridium difficile* colitis, where fecal material from healthy donors is transplanted to patients with the disease (122, 123). Despite the promising results of FMT to treat this condition, several barriers remain with directly transferring live bacteria between humans (124). Excitingly, a small Phase I open-label trial to evaluate the safety and tolerability of oral encapsulated FMT administered open-label over 2 days for the treatment of peanut allergy in 10 adult subjects is currently underway (Clinicaltrial.gov identifier: NCT02960074).

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (125). The most common microorganisms used as probiotics are from the *Bifidobacterium* and *Lactobacillus* genera which are the predominant and subdominant groups

of the gut microbiome, respectively (126). Probiotics exert their beneficial effects by modulating inflammation, secreting small molecules which may act at a distance, and restricting pathogenic bacterial growth via direct inhibition and competition for scarce nutrients. Certain strains have beneficial effects on epithelial regeneration and repair (127). Several probiotic strains, such as *Lactobacillus rhamnosus GG*, *Streptococcus thermophiles*, *Lactobacillus plantarum MB452*, and the gram-negative probiotic strain *Escherichia coli Nissle* 1917 has been shown to increase the epithelial barrier integrity of tight junction- related genes or adherent junction- related genes (128–131). Probiotics interact via their microorganism-associated molecular patterns, with pattern recognition receptors on epithelial cells. This interaction can regulate tight junctions and adherence junctions, which can result in the restoration of epithelial barrier integrity (132). It is important to stress that the biological effects of probiotics are strain specific and therefore, it is vital to use isolates with documented probiotic properties.

Probiotic treatments may be clinically beneficial for individuals suffering from AR, particularly in combination with perennial AR treatment. The literature is well-summarized in a systematic review of probiotics in AR's treatment by Güvenç et al. (133) who concluded that significant evidence suggests beneficial clinical and immunologic effects of probiotics. A caveat is that the probiotic clinical trials evaluated by this review relied on oral dosing, and research into direct nasal probiotics is scarce. Likewise, probiotics treatments have been suggested as an intervention option for CRS; however, the current literature has not supported this concept. One reason for this lack of beneficial effects in CRS could be diverse endotypes and phenotypes in CRS. The pre-clinical and clinical trials described herein support the use of nasal probiotic formulations in AR and CRS.

Probiotics for Sinonasal Disease

Oral administration of *Lactobacillus rhamnosus* GG was previously shown to offer benefits in the context of allergic disease prevention and treatment, both in animal models (134, 135) and in human clinical trials (136–138). Intranasal application of the live probiotic *Lactobacillus rhamnosus* GG bacteria can decrease allergic airway inflammation and lung Th2 cytokine production, and is even capable of preventing airway hyperactivityinduced by repeated intranasal application of birch pollen extract in mice (139). Repa et al. tested the capacity of two lactic acid bacteria (LAB) strains, *Lactococcus lactis* MG1363 (*L. lactis*, a dairy strain) and *Lactobacillus plantarum* NCIMB8826 (*L. plantarum*, a human isolate), to prevent or modulate allergic immune responses. The authors demonstrated that mucosal administration of the two LAB strains—*L. lactis* or *L. plantarum*—applied together with a birch allergen prior or after sensitization, induced a shift toward Th1 immune responses along with a reduction of Th2 dependent basophil degranulation (140). In a follow-up study, Daniel et al. used Bet v 1-producing LAB strains for mucosal prophylaxis in a mouse model of birch pollen allergy (141). They saw reduced allergen-specific IgE concomitant with increased allergen-specific IgA at the mucosae in mice. This suggests mucosal delivery of innocuous recombinant LAB may induce protective immune responses at the site of direct allergen exposure and may represent effective strategy in primary prevention of type I allergy (141). Positive results have also been seen in the context of food allergy. Intranasal administration of recombinant *Lactococcus lactis* strains expressing bovine β-lactoglobulin (BLG), a major cow's milk allergen, has been shown to partially prevent mice from sensitization (142) and when combined with interleukin-12 producing *L. lactis* to inhibit the allergic reaction to BLG (143).

In CRS patients and a subsequent mouse model of sinusitis, Abreu et al. found an increase in the relative abundance of a single species, *Corynebacterium tuberculostearicum* compared to healthy controls. Further, this group found that intranasal inoculation with *Lactobacillus sakei* protected the sinus epithelium, putatively through competitive inhibition of *C. tuberculostearicum*, and may represent a novel therapeutic option for amelioration or prevention of sinus pathology, even in patients with severe sinus microbiome depletion (144).

Besides allergy and CRS, alternative uses for intranasal probiotic therapies have been investigated in animal models and should be noted here. In a neonatal model of influenza virus infection, intranasal application of *Lactobacillus rhamnosus GG* prior to influenza infection dramatically improves survival and provides and early increase in transcription of type I IFNs. The probiotic-related protection is MyD88-dependent and specifically involves TLR4 recognition of LGG (145). As mentioned previously, a major mechanism of action of probiotics is competitive exclusion of pathogens. Following these principles, the intranasal application of *S. epidermidis* has been shown to prevent colonization by methicillin-resistant *Staphylococcus aureus* in mice (146).

Oral probiotic treatments have shown some promise in humans, though this is not the case for CRS. Nasal probiotic formulations may be a more effective drug delivery approach for rhinitis particularly the *Streptococcus spp.*, and *Lactic acid bacteria (LAB),* highlighted in **Table 1**.

S. salivarius and *S. oralis* are alpha-hemolytic streptococci (AHS) isolated from the human pharynx. Together they represent the predominant species in the upper respiratory healthy flora and have been shown to selectively influence the microbiota. Studies in otitis media patients have vetted intranasal administration of *S. salivarius* and *S. oralis* proving it is safe, well-tolerated and able to reduce the risk of acute otitis media in otitis-prone children (147, 148). Whether intranasal administration of AHS is effective as a treatment for otitis media remains controversial (147, 149).

Recently, the ability of *S. salivarius* and *S. oralis* to colonize and modulate the nasal microbiome has been investigated. De Grandi et al. investigated the effects of a 7-day treatment regimen of *S. salivarius* 24SMBc and *S. oralis* 89a in 22 healthy volunteers. After treatment, they a saw significant temporary decrease in *Corynebacterium diphtheriae*, *Haemophilus parainfluenzae*, *Moraxella catarrhalis*, *Prevotella denticola*, *Prevotella melaninogenica*, *Rothia dentocariosa*, *Staphylococcus aureus*, and *Streptococcus pseudopneumoniae*. These findings suggest a potential ability of *S. salivarius* 24SMBc and *S. oralis* 89a to regulate and reorganize the nasal microbiota composition,

TABLE 1 | Human clinical trials investigating intranasal probiotic formulations.

Probiotic type	Probiotic strains	Disease	Treatment regimen	Population	Results	Author	Notes on formulation
Alpha-haemolytic streptococci (AHS)	*S. sanguis, S. mitis,* and *S. oralis*	Acute otitis media (AOM)	One 50 μl puff each nostril OD for 4 months	43 children ≤4 y.o with AOM	No sig. dif. in episodes of AOM than placebo No sig. dif. in nasopharyngeal flora than placebo ↓ *H. influenzae* in the active group	Tano et al. (147)	$\geq 10^7$ CFU/ml in a suspension of 10% skim milk and 0.9% NaCl
	S. salivarius 24SMBc	Healthy adults	Two puffs per QID day at intervals of 4 h One puff: 8 x 10^9 CFU/ml	20 adults ≥ 18 y.o	*S. salivarius 24SMBc* colonized the rhinopharynx tissues in 95% of subjects *S. salivarius 24SMBc* persisted in 55% of colonizers 6+ days from last dose (at 10^5 CFU/ml)	Santagati et al. (136)	5×10^9 CFU/mL in a water solution with dimethicone, without gas
	S. sanguinis 89a, or *L. rhamnosus* (LB21, NCIMB 40564)	Secretory otitis media (SOM)	Two 50 μl puffs per nostril BID for 10 days before trympanostomy tube surgery	60 children 1–8 y.o with SOM and 19 healthy controls	More patients treated with *S. sanguinis* (37%) were cured or much better after clinical recovery than *L. rhamnosus* (6%) or placebo (17%)	Skovbjerg et al. (137)	5×10^9 CFU/ml in skim milk 0.9% NaCl
	S. salivarius 24SMBc and *S. oralis 89a*	Healthy adults	Two puffs per nostril 1 week	20 adults ≥ 18 y.o	↓ in *S. aureus* and other potentially harmful bacteria	De Grandi et al. (138)	*S. salivarius 24SMBc* and *S. oralis 89a* in a 98:2 ratio suspended in a PEG/PPG copolymer and pH 7.00-buffered isotonic solution
Lactic acid bacteria (LAB)	9 *Lactobacillus* spp. and 4 *Bifidobacterium* spp.	Healthy adults	One 100 μL puff to each nostril Single administration	22 adults ≥ 18 y.o	No adverse events (AE) or symptoms No sig. dif. in microflora No inflammatory response	Mårtensson et al. (139)	Spp. obtained from the honeybee Apis mellifera 1×10^{11} CFU/ml in water
	9 *Lactobacillus* spp. and 4 *Bifidobacterium* spp.	CRS	One 100μl puff per nostril BID for 2 weeks (1-week treatment, 1-week sham)	21 adults ≥18 y.o with CRSsNP	No AE or symptoms No sig. dif. in microflora No inflammatory response	Mårtensson et al. (140)	Spp. obtained from the honeybee Apis mellifera 1×10^{11} CFU/ml in water
	Lactobacillus casei AMBR2	Healthy adults	One puff BID for 2 weeks	20 adults ≥18 y.o	*L. casei AMBR2* colonized the nasopharynx in 60–95% of subjects for ≥10–16H after last dose	De Boeck et al. (141)	Spray-dried powder resolved in water One puff: 10^8 CFU/ml
	Lactococcus lactis W136	CRS	One sinus irrigation BID for 2 weeks	24 adults ≥ 18 y.o with CRS refractory to previous medical and surgical therapy	Improvements in symptoms, measures of quality of life, and the mucosal aspect as assessed by endoscopy ↑ *D. pigrum and* ↓ *S. aureus* and ↓ P. aeruginosa	Endam et al. (142)	1.2×10^9 CFU/ml in buffered 0.9% NaCl One sachet: 1.2×10^9 CFU/ml

possibly favoring those microorganisms that may be able to limit the overgrowth of potential pathogens (150).

As described above, several studies investigating intranasal formulations of LAB have produced positive results in murine models. Furthermore, LAB are enriched in the healthy human nose and nasopharynx. In 2016, Mårtensson et al. examined the safety profile of delivering honeybee lactic acid bacteria (HLAB) directly into the nasal passage, using a spray. The administration did not produce any symptoms, or change inflammatory biomarkers of the nasal cavity, and did not alter commensal bacteria (151). The same group repeated such administrations in patients with CRS with nasal polyps for 2 weeks. Treatment was well-tolerated but did not reduce nasal symptom severity or inflammatory markers (152).

Another promising LAB includes *Lactobacillus casei* AMBR2, whose safety for intranasal application in healthy humans was recently confirmed (153). Currently, a clinical trial is ongoing to deliver proof-of-concept that *L. casei* AMBR2 can colonize the upper respiratory tract of health volunteers and CRS patients after daily nasal application via a nasal spray for 2 weeks (ClinicalTrials.gov Identifier: NCT03587545).

Recently, Ednam et al. completed a prospective open-label pilot trial of safety and feasibility for *Lactococcus lactis* W136 and observed positive results. Twenty-four patients received 1.2 billion CFU of *L lactis* W136 self-applied directly to the nasal and sinus passages twice-daily for 14 days via nasal and sinus irrigation. Therapy was well-tolerated and led to improvements in symptoms, measures of quality of life, and improvement in the mucosa as assessed by endoscopy. Gene expression profiling to identify implicated mechanisms suggested enhanced epithelial repair and regeneration and modulation of inflammation. Microbiome profiling using 16s technology showed an increase in the beneficial bacteria *Dolosigranulum pigrum* and reduced in the pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa* (154).

Nasal probiotic formulations may be a more effective drug delivery approach for allergic disease (**Table 1**); however, more studies are needed in this area. Future studies should investigate using a combination of nasal probiotics and immunotherapy to improve pre-existing treatments.

Potential uses for intranasal probiotic therapy may extend to Coronavirus disease 2019 (COVID-19). Intranasal administration of probiotic *Lactococcus lactis* W136 is being investigated as a potential therapy for ambulatory SARS-CoV-2 infection (Clinicaltrial.gov identifier: NCT04458519). It is suspected that innate immune signaling via the TLR1/2/6 motifs present on the bacterial surface and the TLR3 motifs in the cytoplasm could induce interferon gamma production, leading to clearance of COVID-19 infection during its early phases and helping regulate subsequent inflammatory events.

BOX 1 | Challenges to nasal immunization [adapted from Yusuf and Kett (155)].

Exposure:

- Dilution of nasal antigens by mucosal secretions
- Reduced bioavailability due to mucociliary clearance, encapsulation of nasal antigens in nasal mucosal gel and inefficient uptake of antigen across the nasal epithelial barrier
- Degradation of vaccine by local proteases and nucleases

Immunostimulation:

- Need for a relatively large dose to ensure adequate immune response, yet limited delivery volume (typically 100–200 μL)
- Requirement for adjuvants to enhance immunogenicity, which may cause toxicity
- Higher molecular weight compounds (typically above 1 kDa) cannot be delivered via the intranasal route (157)

NASAL IMMUNIZATION

Infection

The nasal route has great potential for vaccination because of its simplicity, painlessness, and ease of administration. The follicle-associated lymphoid tissues in the nasal epithelium induce mucosal immune responses, such as local IgA, in addition to serum IgG. Mucosal IgA neutralizes measles virus and Streptococcus pneumoniae, preventing further infection. In addition intranasal immunization can result in cross-reactive antibodies, possibly capable of cross- protection, thus increasing vaccine efficiency (155).

While there are clearly significant practical advantages to a needle-free vaccine delivered by the intranasal route, there are also some important disadvantages (155). Vaccines need to induce a long-lasting innate and adaptive immune response; however, there are some significant challenges to nasal immunization, as summarized in **Box 1**. A number of delivery systems including those based on liposomes, nanoparticles, virus-like particles and emulsions have been developed to overcome some of these barriers, with varying degrees of success (156).

Probably the greatest success story for intranasal vaccination is the live attenuated (cold-adapted) influenza vaccine (LAIV). In the USA and Europe, this is marketed as Fluenz/Flumist®, however a nasal LAIV has been in use for over 50 years in Russia/USSR (158). Epidemiological data and mathematical modeling indicate children are the main spreaders of influenza infection (159). As a result, the vaccination of children has proven to be a very effective means of interrupting transmission and achieving disease control. Indeed, in two countries (UK and Finland), annual vaccination of children now forms part of the national immunization programmes. The intranasal route facilitates in-school vaccine administration.

LAIV consists of cold-adapted live influenza strains, which replicate locally (mimicking natural immune exposure) in the upper respiratory tract resulting in a mild, subclinical self-limiting immune response. The cold-adaptation prevents viral replication in the lower respiratory tract. The route of administration for LAIV is particularly well-suited to use in children. Furthermore, data suggests that intranasal LAIV results in a higher level of protection in children than the injected alternative (158, 159). However, more recent data from USA has indicated a reduction in efficacy against seasonal H1N1 strains in children (160). Recent changes in the strains included in the vaccine appear to have restored a replicative fitness and a reasonable level of efficacy (161). Perhaps the most noteworthy research finding from these changes has been the realization that serum antibody titres and seroconversion rates are poor correlates of protection in LAIV-vaccinated children (162); this highlights the key differences between assessing mucosal immunity induced by local (intranasal) vaccines and systemic immunity induced by parenteral vaccination.

The intranasal route has also been explored in developing vaccines against SARS-CoV-2 which has caused the COVID-19 pandemic. A number of Phase 1 studies are underway (163). In addition, at least one dual intranasal vaccine for both influenza and COVID-19 has been developed and is being evaluated (164). If successful, these would offer a significant advantage facilitating global mass immunization.

Allergy

In contrast, surprisingly little research has been undertaken assessing the potential for intranasal immunotherapy

against allergic disease, particularly allergic rhinitis. Nasal immunotherapy was first investigated 40 years ago, using both native allergen extracts and soluble allergoids (165). Early data indicated both the potential for efficacy as well as low rate of systemic adverse events (166–169). However, research into this route of administration appears to have been largely superseded by immunotherapy via the subcutaneous (SCIT) or sublingual (SLIT) route. A 2006 review summarized 21 double-blind placebo-controlled studies of local nasal immunotherapy, and reported that in 19, the clinical efficacy was at least equivalent to that reported for SCIT (170). Only one head-to-head comparison seems to have been published: Giannarini and Maggi randomized 45 grass-sensitized patients to either no treatment or open-label immunotherapy using SCIT or via the local intranasal route. There was a high drop-out rate: 37 completed the study, and only 25 (11 for nasal immunotherapy, 7 for the other arms) were evaluated after 2 years. Both local nasal immunotherapy and SCIT resulted in a similar improvement in symptom scores (171).

One concern with nasal immunotherapy is the potential to induce hyperresponsiveness (as is the case following allergen challenge (172) rather than allergen hyporesponsiveness. Reassuringly, the published data has demonstrated that hyporesponsiveness of the nasal mucosa can be achieved following exposure to low levels of allergen (173). At least in animal models, local nasal administration of antigens can induce interleukin-10 release in a manner akin to that seen with conventional subcutaneous immunotherapy (174, 175). Interestingly, in the above-mentioned study comparing nasal immunotherapy to SCIT, a significant reduction in allergen-induced T-cell responses was seen in both active treatment arms, but only SCIT induced an increase in IgG antibody production (175) which more recent studies have suggested is of critical importance in the clinical response to immunotherapy for allergic rhinitis (176).

It is unclear as to why further studies into local nasal immunotherapy have not been undertaken. It has been suggested that there may have been compliance issues due to frequent local nasal reactions, and/or difficulties in controlling the actual dose of allergen administered (165). However, in the study by Giannarini and Maggi, drop-out rates were lowest in the nasal immunotherapy arm. Similarly, dry-powder devices were developed to facilitate dose administration (169) One can only speculate that the nasal route was superseded by SLIT, where local reactions may be less frequent and probably less bothersome.

Finally, there is one report in a murine model of egg allergy where nasal immunotherapy using a liposomal-based delivery system resulted in desensitization to allergen challenge via the oral route (176) While intranasal challenge with food allergens causes a local allergic response (177) it is possible that the intranasal route could also be used to induce a degree of desensitization; this has not to date been formally assessed, and one has to consider whether the intranasal route would be an alternative to oral immunotherapy.

TOPICAL TREATMENT FOR ALLERGEN- AND RHINOVIRUS-INDUCED RHINITIS WITH ANTIBODIES BISPECIFIC FOR ALLERGENS AND ICAM-1

Allergic sensitization and rhinovirus (RV) infections are major causes of rhinitis. We propose intranasal application of antibodies bispecific for allergens and Intercellular Adhesion Molecule 1 (ICAM-1) as topical treatment for both allergic and RV-induced rhinitis. The immobilization of allergen-specific antibodies with ICAM-1-specific antibodies on the nasal epithelium should prevent washing out of the therapeutic antibodies and thus provide sustained inhibition of allergen transmigration through the epithelial barrier and of consequent allergic inflammation in the nasal mucosa. Since the majority of RV strains use ICAM-1 as receptor it should be possible at the same time to block RV infections.

IgE-mediated allergy represents a common health problem affecting around one third of the world population (166). Allergic rhinitis is the most frequent manifestation of allergy. Rhinitis can be classified according to severity and appearance of symptoms as mild or moderate-severe and intermittent or persistent, respectively (178). Intermittent forms of allergic rhinitis are mainly caused by outdoor airborne allergens derived from pollen of grasses, trees and weeds (179–181). Allergic rhinitis is a major burden because it reduces the quality of life of affected individuals heavily (182). Among the non-allergic forms of rhinitis, virus-induced rhinitis, in particular rhinitis due to rhinovirus (RV) infections predominates (183). RV infections and allergen exposure in allergic patients trigger different pathways of inflammation. The majority of RVs infect the respiratory epithelium via binding to their receptor ICAM-1, minor group RVs bind to the low-density lipoprotein receptor (LDLR) and RV-C to cadherin-related family member 3 (CDHR3). Models for the first two virus-receptor interactions are available but RV-C is still difficult to isolate and propagate (184). RV-infections cause damage of the respiratory epithelium and local inflammation of the Th1 phenotype with production of inflammatory cytokines, leukocyte infiltrations and activation of the innate immune system (185). Allergens reaching IgE-sensitized mast cells after penetration of the respiratory epithelium of allergic subjects, cause immediate allergic inflammation by mast cell degranulation leading to release of biological mediators, cytokines and proteases and, upon chronic exposure also induce T cell- and eosinophil-mediate allergic inflammation (**Figure 1A**) (176).

Allergic sensitization, allergen exposure and RV infections can have synergistic effects in inducing rhinitis. For example, it is known that Th2 immunity impairs immune responses against RV infections which may render allergic subjects more sensitive to RV infections (186, 187). On the other hand, it has been shown that RV infections impair the barrier function of the respiratory epithelial cell layer and facilitate trans-epithelial penetration of allergens, thereby increasing submucosal allergen concentrations which potentially may aggravate allergic inflammation (188).

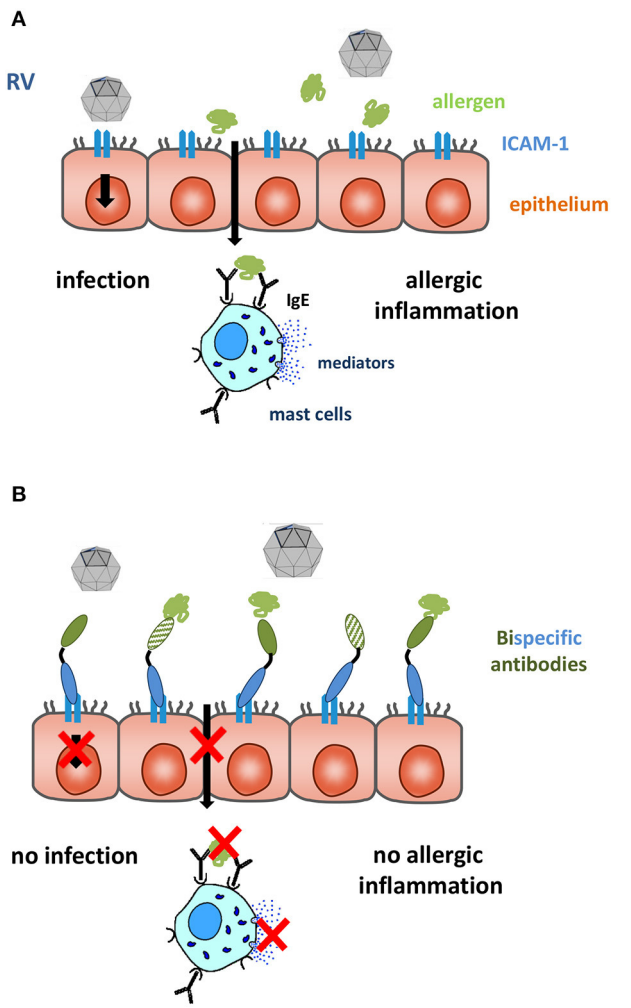

FIGURE 1 | The respiratory epithelium is an important site of contact with allergens and RV. **(A)** Major group RVs infect the epithelium via their receptor ICAM-1 inducing tissue damage facilitating trans-epithelial penetration of allergens. **(B)** Bispecific antibodies binding simultaneously allergens (green) and ICAM-1 (blue) block RV binding to ICAM-1 and capture allergen. This may prevent RV- and allergen-induced inflammation. The immobilization of allergens on the apical side of the mucosa can be achieved by IgE blocking (*full green* domain) and IgE-non-blocking (*cross-hatched green* domain) allergen-specific antibodies, the epitope-specificity of the ICAM-1-specific antibody (full blue domain) decides if RV infections can also be blocked.

The use of antibodies specific for the binding site of RV on ICAM-1 has actually been considered as a possible approach for the treatment of RV infections (189). Regarding allergy, it is established that allergen-specific immunotherapy (AIT) induces allergen-specific IgG antibodies which compete with IgE for allergen binding and thus prevent allergic inflammation (190). The development of allergen-specific blocking IgG in serum is considered as a robust biomarker for success of AIT (191) and it has been shown that after AIT allergen-specific IgG increase also in nasal secretions where they can capture allergens (192). It is therefore reasonable to assume that it may be possible to combine local treatment approaches for rhinitis caused by RV infections and allergic inflammation by creating bispecific antibodies which bind to ICAM-1 and block RV infections and simultaneously capture allergens and prevent them from intruding through the respiratory mucosa as indicated in **Figure 1B**.

The feasibility of such an approach has actually been demonstrated by a series of *in vitro* experiments (193). In the coming paragraphs we will review the concept of allergen-specific blocking IgG in AIT in the context of technological advances made during the last decades regarding the production of human and in particular of human allergen-specific antibodies, which may now create the basis for a combined antibody-based topical treatment for allergen- and RV-induced rhinitis.

Allergen-Specific IgG Antibodies Confer Protection Against Allergy: Historic Aspects

Figure 2 provides a timeline of the studies highlighting the role of allergen-specific blocking IgG for treatment of allergy in the context of technological advances made toward the production of recombinant specific monoclonal human antibodies in general. The first evidence that immune-sera raised in animals against grass pollen allergen extract protect against allergic inflammation originates from a paper by Dunbar (194) (**Figure 2**).

Then R.A. Cooke and co-workers published their experiments demonstrating that immune-sera from AIT-treated patients suppressed allergen-specific skin reactivity in human subjects (**Figure 2**) (195). Loveless showed that blocking antibodies prevent allergen-IgE recognition (**Figure 2**) (196). She further demonstrated an association between the amount of protective antibodies and clinical improvement of AIT and identified IgG antibodies as major isotype involved in blocking (196, 197). The importance of allergen-specific IgG for treatment of allergy was corroborated by the demonstration that passive immunization of allergic patients with IgG derived from non-atopic volunteers who had been immunized with large doses of ragweed-extract protected against allergen-induced inflammation (**Figure 2**) (198).

Monoclonal Antibodies in Allergy Treatment

A milestone toward the development of monoclonal antibodies was the invention of hybridoma technology by G. Köhler and C. Milstein which allowed production of mouse monoclonal antibodies on a large scale (**Figure 2**) (199). Their method was further utilized to generate human IgG antibodies (**Figure 2**) (200). In parallel, Steinitz and associates established human lymphoid lines immortalized by Epstein-Barr virus (EBV) transformation for the production of antibodies with defined antigenic specificity (**Figure 2**) (201). The introduction of these technologies enabled the generation of human monoclonal IgA and IgG antibodies specific for the major ragweed allergen, Amb a 1(202) and shortly thereafter, of human IgG antibodies specific for the major birch pollen allergen, Bet v 1 (**Figure 2**) (203) One of the Bet v 1-specific monoclonal IgG antibodies strongly inhibited IgE binding to Bet v 1 and Bet v 1-induced basophil

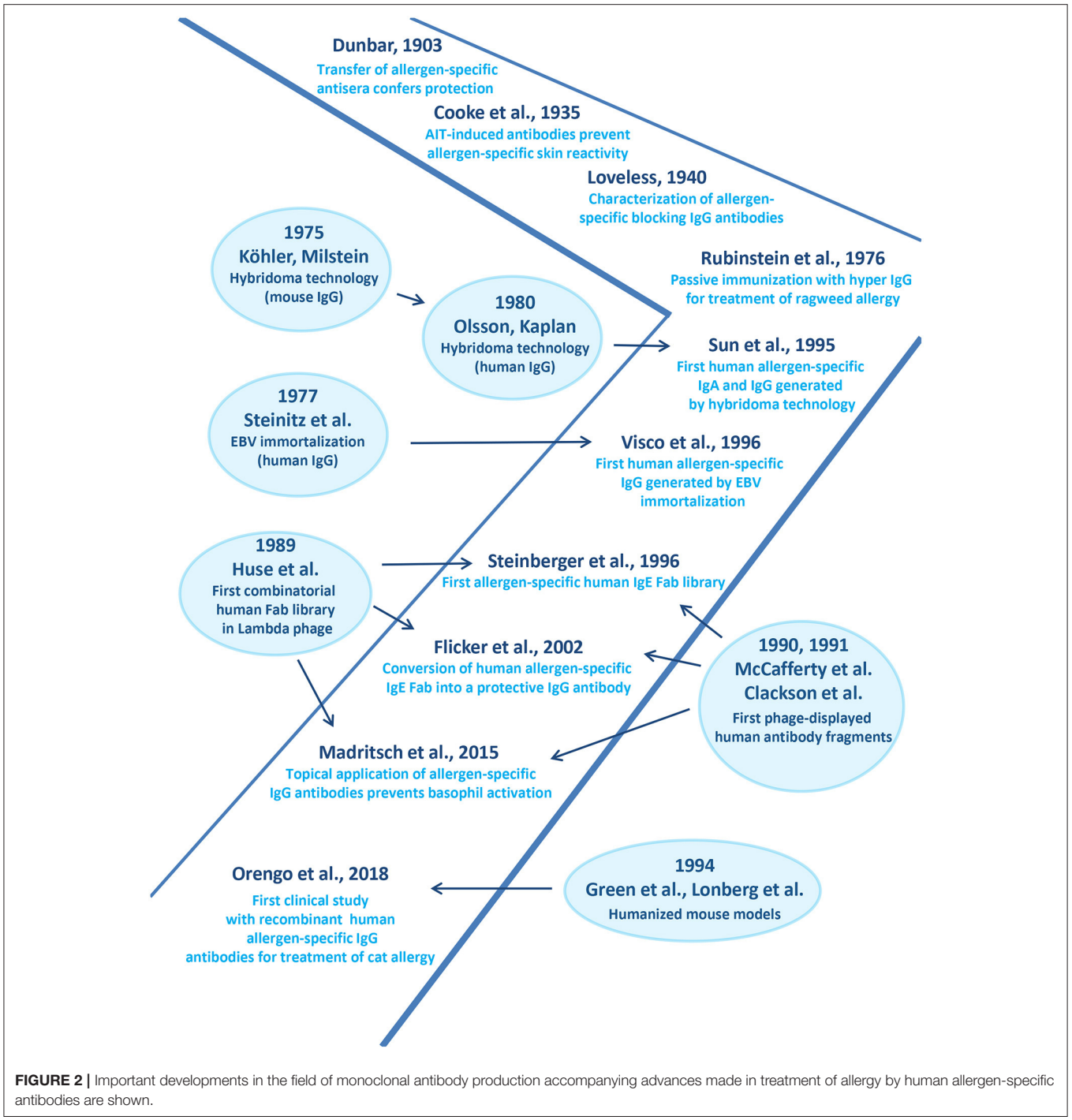

FIGURE 2 | Important developments in the field of monoclonal antibody production accompanying advances made in treatment of allergy by human allergen-specific antibodies are shown.

degranulation and thus was considered a candidate for treatment of birch pollen allergy (203).

In order to be able to generate libraries of antibodies resembling the specificities of a complete organism the combinatorial library technology was developed. This technology was based on the isolation of cDNAs coding for the heavy and light chains from the antibody producing host, their random combination to obtain all possible pairs of heavy and light chain combinations and the isolation of specific antibody fragments (Fabs) or single chain fragments (ScFvs) (204–206). The combinatorial library technology actually allowed for the first time isolation of human allergen-specific IgE and provided access to their variable region sequences (**Figure 2**) (207) conversion of a grass pollen allergen-specific IgE Fab into a complete human IgG antibody it could be shown that this technology can be used to obtain human monoclonal IgG antibodies which block allergic

inflammation *in vitro* highlighting their therapeutic potential antibodies (**Figure 2**) (208).

This human grass pollen allergen (Phl p 2)-specific blocking antibody was then further developed for topical application as described in **Figure 1B** (193). A bispecific conjugate consisting of the Phl p 2-specific IgG and a monoclonal ICAM-1 specific antibody was shown to anchor the conjugate on the surface of a respiratory epithelial cell layer and to prevent the transmigration of the allergen and subsequent allergen-induced inflammation underneath the epithelium (193).

In order to obtain large numbers of allergen-specific human IgG antibodies for treatment, the company Regeneron has used a technology which allows generation of panels of human IgG antibodies by immunization of mice. This technology is based on transgenic mice containing complete human antibody repertoires (**Figure 2**) (209, 210). Based on the first generation of such transgenic mice, further HumAbmouse approaches were established, e.g., VelocImmune mice for the efficient production of fully human antibodies (211, 212). Using this refined technology two fully human IgG4 antibodies specific for Fel d 1, the major cat allergen were generated recently and shown to be effective for the treatment of cat allergy in a clinical trial (**Figure 2**) (213). This proof of principle study showed that a single subcutaneous injection of a mixture of these two human monoclonal IgG4 antibodies significantly reduced allergic symptoms in cat allergic patients and the effect of treatment lasted for ∼3 months (213). This study thus suggested that treatment by passive immunization with allergen-specific IgG which blocks allergic patients IgE binding to the culprit allergen can be an effective treatment for allergy but there are limitations of this approach.

The Basis for Allergy Treatment by Passive Immunization With Monoclonal Allergen-Specific IgG Antibodies and Its Limitations

The mechanisms of action of the monoclonal antibodies used for treatment by passive immunization are similar to those in AIT (191, 213). AIT induces by active vaccination a polyclonal allergen-specific IgG response which competes with the patients' IgE for allergen binding by occupying the epitopes recognized by IgE. As a result of this competition, intruding allergens are captured by IgG and thus cannot trigger IgE-mediated mast cell or basophil activation, they fail to induce IgE-facilitated allergen presentation to T cells and do not boost systemic IgE production (214–217). This leads to a reduction of immediate allergic symptoms, T cell-mediated allergic inflammation and eosinophil recruitment as well as of allergen-specific IgE production (218). It is obvious that polyclonal allergen-specific IgG induced by AIT is more effective in blocking the binding of the polyclonal IgE to the allergen than single monoclonal allergen-specific IgG antibodies. For certain allergens such as the major birch pollen allergen, Bet v 1 it was possible to identify single monoclonal IgG antibodies which potently blocked the polyclonal Bet v 1-specific IgE in the majority of birch pollen allergic patients (203). For the major cat allergen Fel d 1 a profound blocking of cat allergic patients polyclonal IgE to Fel d 1 was achieved with a cocktail of two monoclonal antibodies (213). However, there are several highly potent allergens such as the major grass pollen allergen, Phl p 5 which consists of two flexible IgE-reactive domains (219). For Phl p 5 it was not possible to inhibit patient's polyclonal IgE binding even by a cocktail of several monoclonal IgG antibodies (220). Phl p 5 is only one of the four clinically relevant allergens recognized by grass pollen allergic patients, which comprise in addition Phl p 1, Phl p 2, and Phl p 6 and account for a high percentage of grass pollen-specific IgE (221, 222). Accordingly, it will be very difficult, if not impossible, to define a small-enough panel of grass pollen allergen-specific monoclonal IgG antibodies which are capable of blocking the majority of grass pollen allergen-specific IgE. The same is true for other important allergen sources such as house dust mites in which six important allergens (i.e., Der p 1, Der p 2, Der p 5, Der p 7, Der p 21, and Der p 23) have been identified (223). Regarding cat allergy it is clear that Fel d 1 is the most important allergen, but several other cat allergens have been identified (e.g., Fel d 2, Fel d 3, Fel d 4, Fel d 5, Fel d 6, Fel d 7, Fel d 8) (224). Their clinical relevance has not yet been defined but it is quite likely that blocking Fel d 1-specific IgE alone will be insufficient to treat all cat allergic patients.

Considering that many allergic patients are sensitized to several independent and antigenically unrelated allergen sources it will be difficult to create cocktails of therapeutic monoclonal antibodies which cover the necessary range. This problem exists for AIT which can be used mainly for treatment of patients who have a limited number of clinically-relevant driving allergens.

One possibility to obtain a large panel of monoclonal allergen-specific IgG antibodies resembling a polyclonal IgG cocktail for difficult allergens and complex allergen sources is to make use of humanized mouse models. This will be technically challenging. Alternatively, one can consider immunizing healthy subjects with defined allergen molecules to generate therapeutic immunoglobulin G preparations which are enriched for polyclonal blocking allergen-specific IgG antibodies. In this context it should be mentioned that it was recently demonstrated that non-allergic subjects could be safely immunized with recombinant allergen derivatives to induce polyclonal allergen-specific IgG which strongly blocked allergic patients IgE binding to the corresponding allergen (225). In fact, immunization of non-allergic subjects with hypoallergenic recombinant Bet v 1 was safe and did not induce allergic sensitizations in the vaccinated subjects and the induced IgG antibodies blocked polyclonal IgE binding to Bet v 1.

Another possibility to render treatment with allergen-specific IgG antibodies more feasible would be topical application of the antibodies with the goal to prevent them from passing through the epithelial barrier. Accordingly, allergens would be captured "outside" of the epithelial barrier and would not reach underlying mast cells and T cells (**Figure 1B**). Therefore, for capturing and keeping allergens "outside" one could eventually use monoclonal antibodies which do not compete with allergic patients' IgE binding to the allergen. Experimental evidence for such an approach is outlined below.

TABLE 2 | Passive immunization with or topical application of monoclonal allergen-specific IgG antibody for treatment of IgE-mediated allergy.

Passive immunization	Topical application
Requires IgE blocking antibodies	May be performed with single non-IgE blocking antibodies
Requires full antibodies with long serum half-life	Can be done with antibody fragments or small scaffolds
Works only for certain less complex allergens and allergen sources	Can be used for complex allergens and allergen sources
One systemic administration sufficient for up to 3 months	Daily topical administration
Only for allergy treatment	Suitable also for treatment of RV infections with a blocking ICAM-1 antibody

Topical Application of Monoclonal Allergen-Specific IgG Antibodies for Treatment of Allergic Rhinitis

Topical application of drugs for the treatment of allergy represents a first line treatment for rhinitis, conjunctivitis, asthma and dermatitis. For each of the target organs sophisticated devices for drug delivery have been developed and are available. It is therefore tempting to speculate that topical administration of therapeutic allergen-specific blocking antibodies to the nose for treatment of allergic rhinitis could be an alternative to systemic passive immunization. However, there are a few hurdles which need to be overcome. First of all, topically applied antibodies will be quickly washed out by nasal secretions and the mere formation of allergen-antibody immune complexes will not completely prevent allergens from passing the epithelial barrier. Accordingly, it will be important to build up a shield of protective antibodies on the outer surface of the respiratory epithelium which stays there long enough so that only one or two applications per day are necessary to keep the antibody shield intact. To prevent washing out of topically applied antibodies we therefore considered immobilizing them to ICAM-1 which is a molecule that is highly expressed on the surface of airway and conjunctival epithelial cells in allergic patients and which has a low surface turn-over (226, 227).

In a proof of principle study we generated antibody conjugates bispecific for ICAM-1 and the major grass pollen allergen Phl p 2 by biotin-streptavidin coupling of a monoclonal anti-ICAM-1 antibody and a Phl p 2-specific human monoclonal IgG antibody) (193). We found that the conjugate remained immobilized on the surface of a layer of cultivated respiratory epithelial cells and prevented the allergen from transmigration through the cell layer. The allergen transmission was reduced substantially so that basophil activation with allergen-containing culture medium collected from the basolateral side of the epithelial layer was strongly reduced as compared to that from the apical side (193).

These proof of principle experiments thus demonstrated that immobilization of allergen-specific IgG on epithelial cell layers via ICAM-1 has the potential to prevent trans-epithelial allergen migration and to reduce allergic inflammation in the underlying tissue. These experiments were carried out with chemical conjugates of the two monoclonal antibodies but there are a variety of possibilities to generate bi-specific antibodies or alternative scaffolds of different formats in different expression systems in a quality suitable for clinical application, in sufficient quantities and at reasonable costs to make the topical treatment affordable (228). Moreover, we conducted further experiments in which we used a monoclonal ICAM-1-specific antibody which blocks the binding of major group RVs to ICAM-1 and a monoclonal allergen-specific IgG antibody (229) which did not block IgE binding but has high affinity for the allergen. We found that this conjugate strongly prevented RV infection and in addition could trap the allergen on the apical side of the epithelial cells and prevent allergic inflammation at the basolateral side (230). This result indicates that it may be possible to perform topical treatment with one high affinity monoclonal antibody per allergen without need for a cocktail of IgE blocking antibodies. Keeping the allergen outside the epithelium may be sufficient to prevent allergic reactions. Moreover, the use of ICAM-1 antibodies capable of blocking major group RV binding to respiratory epithelial cells may at the same time prevent RV infections.

We therefore propose topical treatment with ICAM-1 anchored allergen-specific IgG antibodies or scaffolds as alternative to passive immunization with allergen-specific IgG antibodies. **Table 2** summarizes features of the two forms of treatment. Passive immunization will be only effective when IgG antibodies are used which compete with IgE antibodies for allergen binding because they cannot prevent the allergen from crossing the epithelial barrier. By contrast, topical administration of ICAM-1 anchored antibodies can be performed with non-IgE-blocking antibodies because the bispecific conjugates prevent allergen from trans-epithelial migration and thus keeps the allergen outside. Accordingly, one allergen-specific IgG per allergen may be sufficient for topical treatment whereas cocktails of IgE-blocking antibodies will be necessary to cope with complicated allergens and complex allergen sources. Passive immunization requires full length antibodies expressed in mammalian cells with long half-life whereas topical treatment can be performed with small molecules which can be obtained by relatively inexpensive expression in Escherichia coli. However, passive immunization confers long-term protection for months whereas topical treatment will need to be carried out at least once per day. Passive immunization can be used only for treatment of allergy whereas topical application of conjugates bispecific for allergens and ICAM-1 may protect against allergy and certain RV infections.

The technologies for realizing both forms of antibody-based treatment are available and it will hence be possible to bring them into clinical trials. However, it remains to be seen whether any of

the antibody-based forms of treatment is clinically more effective than currently available pharmacotherapy.

CONCLUSIONS

The work described in this review is exciting- and some of it, such as LAIV to prevent influenza, has already provided improvements in human health. Interferons, beneficial bacteria, and bispecific antibodies are promising, but require further trials before being translated into clinical care. Despite the potential importance of the digestive tract in regulating immune responses, results remain controversial for orally-administered probiotics. While several murine studies have demonstrated that probiotics may have beneficial effects in CRS, there are no consistent results in human CRS trials. Responder groups may be hidden within the large diversity in endotypes and phenotypes of CRS. Fortunately the ready accessibility of the nose enables application of materials, in contrast to those which necessitate injection. A nasal vaccine for COVID- 19 would probably speed the delivery of relief from the pandemic. Intranasal therapy is likely to be a growth area.

AUTHOR CONTRIBUTIONS

GS conceived the idea of reviews on the nose as a route for therapy and commissioned the sections of this part 2 paper on intranasal immunotherapy. GS edited the paper, with help from SF, YP, and SL, and wrote the abstract and conclusion. PT wrote on intranasal vaccination. SF and RV contributed the work on bispecific nasal antibodies. YP, JC, OK, and SJ have written the Interferon part. SL, AE, and MD have written the Probiotics section. All authors contributed to the article and approved the submitted version.

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REVIEW
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Allergic Rhinitis in Childhood and the New EUFOREA Algorithm

Glenis Kathleen Scadding[1,2], Peter Kenneth Smith[3], Michael Blaiss[4], Graham Roberts[5,6,7], Peter William Hellings[8,9], Philippe Gevaert[10], Marinda Mc Donald[11], Tania Sih[12], Suzanne Halken[13], Petra Ursula Zieglmayer[14,15], Peter Schmid-Grendelmeier[16], Erkka Valovirta[17], Ruby Pawankar[18] and Ulrich Wahn[19]*

[1] Ear, Nose and Throat Department, University College London Hospitals National Health Service Foundation Trust, London, United Kingdom, [2] Faculty of Medical Sciences, University College London, London, United Kingdom, [3] Allergy Medical Group, Brisbane, QLD, Australia, [4] Department of Paediatrics, Medical College of Georgia, Augusta University, Augusta, GA, United States, [5] National Institute of Health Research Biomedical Research Centre, Southampton General Hospital, Southampton, United Kingdom, [6] The David Hide Asthma and Allergy Research Centre, Newport, United Kingdom, [7] Faculty of Medicine, University of Southampton, Southampton, United Kingdom, [8] Department of Microbiology and Immunology, Department of Otorhinolaryngology, Katholieke Universiteit Leuven, Leuven, Belgium, [9] Department of Otorhinolaryngology, Academic Medical Center, Amsterdam, Netherlands, [10] Department of Otorhinolaryngology, Ghent University, Ghent, Belgium, [11] The Allergy Clinic, Johannesburg, South Africa, [12] Medical School, University of São Paulo, São Paulo, Brazil, [13] Paediatric Allergy, University of Southern Denmark, Odense, Denmark, [14] Karl Landsteiner University of Health Sciences, Krems an der Donau, Austria, [15] Vienna Challenge Chamber, Vienna, Austria, [16] Allergy Unit, Dermatology Department, University Hospital of Zurich, Eidgenössische Technische Hochschule Zürich, Zurich, Switzerland, [17] Department of Lung Diseases and Clinical Immunology, University of Turku and Terveystalo Allergy Clinic, Turku, Finland, [18] Division of Allergy, Department of Pediatrics, Nippon Medical School, Tokyo, Japan, [19] Klinik für Pädiatrie m.S. Pneumologie und Immunologie, Charite-Berlin, Berlin, Germany*

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Edited by:
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***Correspondence:**
Glenis Kathleen Scadding
g.scadding@ucl.ac.uk

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Allergic rhinitis in childhood has been often missed, mistreated and misunderstood. It has significant comorbidities, adverse effects upon quality of life and educational performance and can progress to asthma or worsen control of existing asthma. Accurate diagnosis and effective treatment are important. The new EUFOREA algorithm provides a succinct but wide- ranging guide to management at all levels, based on previous guidelines with updated evidence and has been adjusted and approved by experts worldwide.

Keywords: pediatric allergic rhinitis, antihistamines, intranasal corticosteroids, fixed dose combinations, allergen specific immunotherapy, asthma, sleep

INTRODUCTION

The term rhinitis indicates a symptom complex including two or more of: nasal itching, sneezing, rhinorrhea and nasal blockage. Allergic rhinitis (AR) is IgE-mediated, usually caused by sensitization to inhaled allergens. Other forms of rhinitis are infectious, and non-allergic, non-infectious (1).

AR is the commonest immunological disorder in man, with a prevalence of up to 50% in some countries. Often trivialized, in fact it represents a global health problem causing worldwide morbidity. In children AR can not only reduce quality of life via its symptoms, but can affect contiguous organs such as the sinuses, ears and chest and cause sleep problems, leading to reduced school/ work performance (equivalent to that seen in adults), family difficulties and decreased involvement in outdoor activities (2–4). The burden of pediatric AR is shown in **Figure 1**.

Nasal symptoms and nasal obstruction were more likely to be associated with poor QOL in adolescents than in adults or younger children, respectively (5). In addition AR predisposes to asthma (6), and reduces the control of concurrent childhood asthma, increasing likelihood of

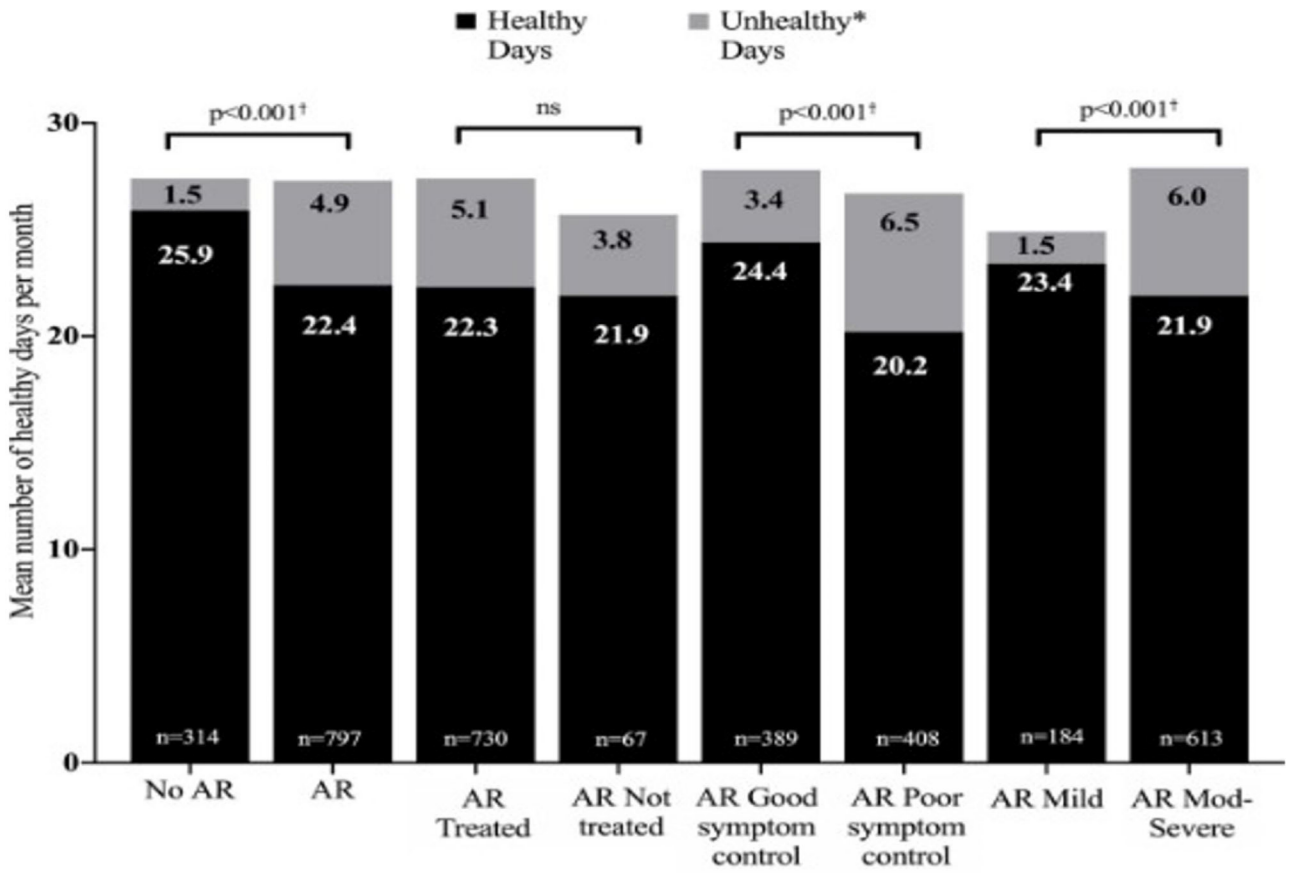

FIGURE 1 | The burden of allergic rhinitis in children aged 6–15 years. Moderate to severe rhinitis and poor symptom control cause over 6 unhealthy days per month. Reproduced from Bosnic-Anticevich S et al. Impact of allergic rhinitis on the day-to-day lives of children: insights from an Australian cross-sectional study. BMJ Open. (2020) 10:e038870. doi: 10.1136/bmjopen-2020-038870, with permission. *Unhealthy days = number of days of poor emotional health and number of days of poor physical health combined. † Statistically significant difference between groups for each metric (healthy days and unhealthy days).

hospitalization [OR = 2.34, 95% CI (1.41–3.91)], physician visits (4.4 vs. 3.4, $p < 0.0001$), asthma drug costs [mean GBP 6.7, 95% CI (6.5–7.0)], use of short-acting beta agonists and use of oral corticosteroids (0.091 vs. 0.146, $p < 0.0001$) (7, 8).

The European Forum for Research and Education in Allergy and Airways diseases (EUFOREA) has the mission to implement optimal care for patients suffering from allergies and chronic respiratory conditions (9, 10). Recently, a pocket guide for adult AR was developed by an extended global panel of EUFOREA experts including a novel treatment algorithm (11). The latter has been developed based on existing guidelines and with the aim to allow all care providers to adequately treat adult AR. The need has arisen to develop a pediatric version because the frequency of the common cold and the protean manifestations of AR mean that the diagnosis is often missed, treatment is inadequate and opportunities to alter the course of allergic disease by allergen-specific immunotherapy (AIT) are being wasted.

This article documents the evidence concerning AR prevalence and natural history, then provides management advice with an algorithm based on an update of existing guidelines (12–15). Initially devised by GS and UW, using searches involving the terms "pediatric allergic rhinitis" and "allergic rhinitis in children" and "childhood rhinitis" each meshed with all possible therapies and with "education," "prevention," "development," "outcomes," "side effects," and "safety," this was then altered and adapted by the other authors until a final version was agreed.

EPIDEMIOLOGY

Most of our current knowledge of pediatric AR epidemiology comes from a widely accepted standardized tool, the International Study of Asthma and Allergies in Childhood (ISAAC) survey, first iterated in 1997 and repeated twice since (16).

In Phase One 156 centers in 56 countries completed the research (17). Prevalence of allergic disease varied more than more than 20-fold between centers (18). Symptoms of rhinitis (and of asthma and eczema) were commoner in some affluent western countries e.g., UK, New Zealand, Australia, but not all,

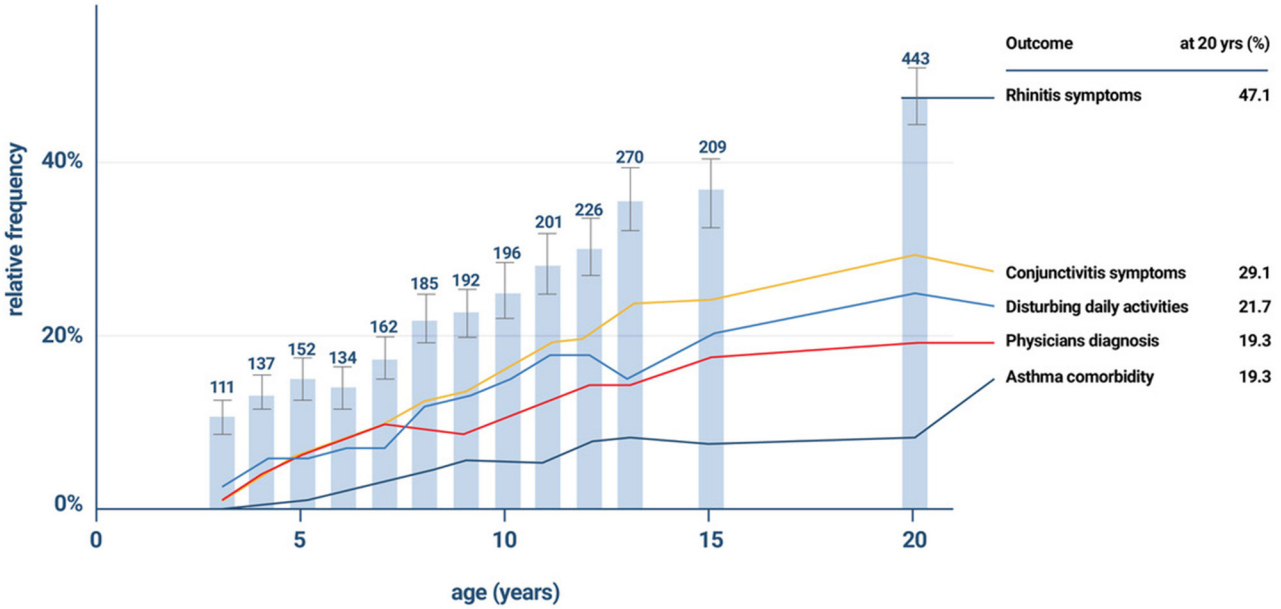

FIGURE 2 | The gradual increase in the incidence of rhinitis symptoms and co- morbidities in the the German Multicentre Allergy Study (MAS), which began in 1990 in five German cities and included 1,314 newborns for the study of the natural course of atopic diseases (26, 27).

e.g., Spain (16–18). Severe symptoms occurred more frequently in lower and middle income countries, particularly in Africa and Latin America (19, 20), illustrating the important and significant morbidity of rhinitis.

In ISAAC Phase Three two thirds of the centers repeated the study and showed that asthma, rhinitis and eczema symptoms had increased substantially over the previous 15 years, especially in younger children. AR often begins in the under 5 s, but its prevalence increased from 8.5% in individuals aged 6–7 years to 14.6% in those aged 13–14 years (21).

ISAAC Phase Two also provided new information about factors potentially affecting symptom prevalence of asthma, rhinitis, and eczema. Environmental, rather than genetic factors appeared the likely cause of the large variations. Fruit, vegetables, fish and a Mediterranean diet appeared protective; children who ate fast food were more likely to have symptoms (22). A very weak relationship was found between allergy (atopy) and rhinoconjunctivitis, especially in less affluent centers (23). However, such underlying factors may be misunderstood if the phenotype of rhinitis is not diagnosed. The core ISAAC question for diagnosis of AR was: "Has your child ever had a problem with sneezing or a runny or a blocked nose when he/she did not have a cold or the flu?" Subsequent enquiries included itchy eyes and whether an AR or hay fever diagnosis has ever been made, as well as the timing of nasal symptoms. The omission of a detailed history and IgE testing gives a fairly low accuracy for AR diagnosis, estimated as about 60% in a recent Korean study which considered that the ISAAC survey overestimates the true prevalence of AR (24). The roles of the innate and acquired immune systems in rhinitis may differ in individuals, with different allergens and in different parts of the globe; atopy may be more relevant in affluent areas (25).

THE NATURAL HISTORY OF ALLERGIC RHINITIS

The best way to understand the natural history of chronic diseases including its major determinants is to observe and prospectively follow cohorts of children for many years, if possible, from birth onwards. Over the last decades a number of birth cohorts have been initiated in Europe and the US. Their main messages regarding allergic rhinitis are as follows:

The incidence of allergic sensitization and allergic (mostly seasonal) rhinitis is very low in the first 2 years. Anecdotal information suggests that very few infants and toddlers develop allergic- type symptoms during any pollen season before the third year of life. In general 2 years (seasons) of environmental allergen exposure seem to be needed before allergic sensitization can be observed by specific serum IgE measurement. The percentage of new cases with seasonal AR increases between the ages of 3 and 12 years at a constant rate of ∼2% per year (26, 27). A positive family history (father or mother with allergic rhinitis) is the best predictor of allergic rhinitis (28). Early in life IgE responses to indoor or outdoor allergen sources may only be directed to a minority of allergens, but the 12 month prevalence of sensitization rises from year to year in the first decade of life (**Figure 2**). A systematic evaluation of the process of sensitization was performed in grass and birch – pollen allergies: The analysis of sequential blood samples for IgE antibodies against

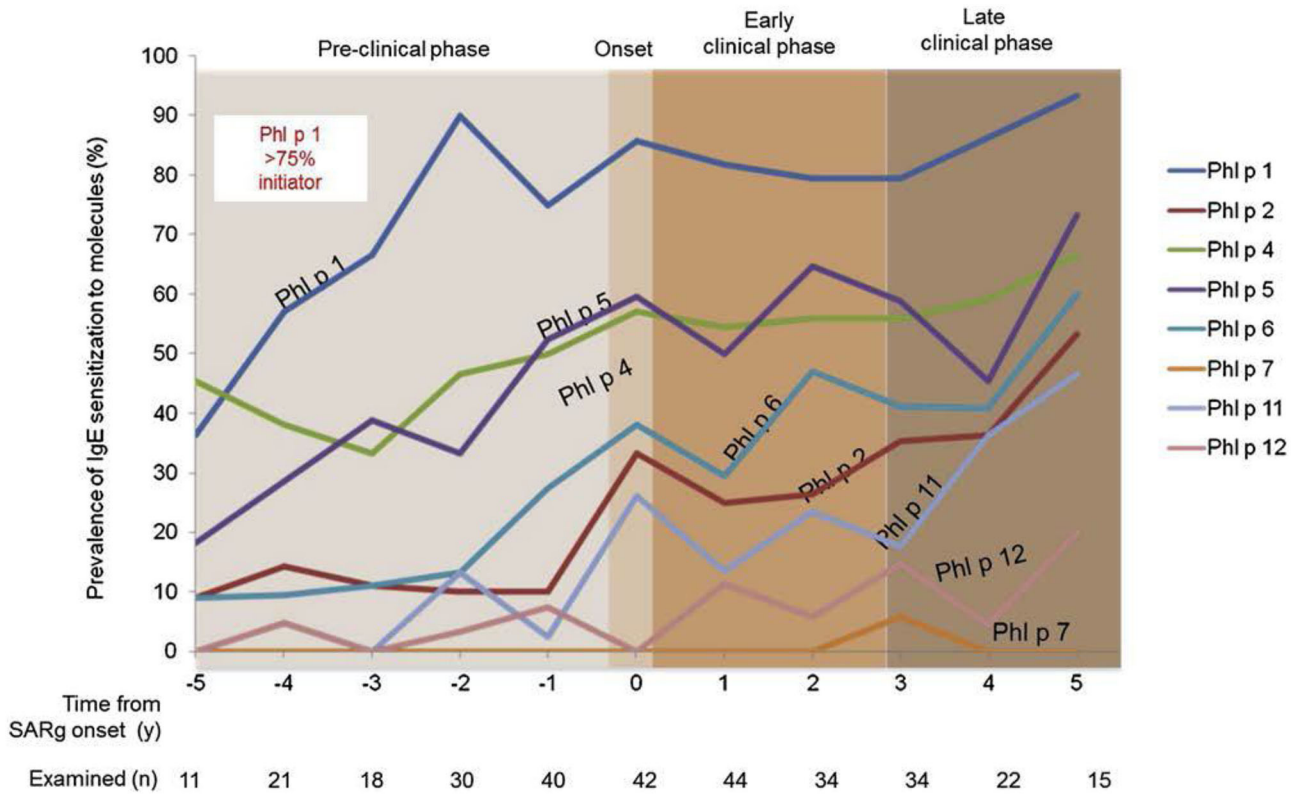

FIGURE 3 | Development of molecular IgE responses of children who developed grass pollen allergy in the first decade of life. Sequential blood samples were obtained in the preclinical phase as well as during the first 5 years with seasonal symptoms. Children were part of the prospective birth cohort study MAS (29).

grass and birch pollen including individual allergen molecules demonstrated the process of sensitization, which precedes the initiation of symptoms by several years. IgE responses to individual pollen allergens increase with time (molecular allergen spreading), and IgE serum concentrations increase during pre-symptomatic years (**Figure 3**). Once sensitization to pollen is established, the probability for symptoms within the next 3 years strongly increases (odds ratio 13.6). Simple detection of preclinical allergic sensitization may therefore allow prediction of the onset of hay fever in an allergen-specific manner (29).

Over 60% of children with AR report accompanying eye symptoms, often poorly recognized as allergic in nature (30), by the age of 20 years. Between one and two thirds of them have severe persistent symptoms (according to the ARIA-definition), affecting their daily life. Boys develop rhinitis symptoms earlier, but during adolescence girls catch up and show higher incidences during and after puberty, reaching comparable frequencies by age 20 years (31). This sex shift is most strongly seen in multimorbid patients with both asthma and rhinitis (31).

In atopic children comorbidity is a characteristic feature already in the first 5 years of life. Many children with allergic rhinitis had eczema in infancy. About half of the children with severe persistent allergic rhinitis report wheezing episodes. These findings are in line with the concept of united airways, which suggests that in young children, as in adults, a progression from rhinitis to wheezing can be frequently found and underlies the importance of treating both sites of allergic inflammation to achieve disease control (32).

Rhinitis in childhood is a strong predictor for adolescent- and adult-onset asthma. In the German MAS birth cohort, rhinitis in preschool children was a risk factor for subsequent wheeze when associated with allergic sensitization. This is also true for perennial chronic rhinitis symptoms, which are associated with sensitization to house dust mites. In these cases a causal relationship between allergen exposure and reported symptoms is more difficult to demonstrate than in exclusive seasonal symptoms (33).

MANAGEMENT OF AR IN CHILDREN

Diagnosis

History

The frequency of common colds in childhood means that AR may be misdiagnosed or ignored. AR is diagnosed by a detailed history, supported by examination of the patient as a whole as well as the nose, plus, if necessary, testing for allergen- specific IgE. The clinical history (see **Boxes 1**, **2**) should note where and when nasal symptoms occur, plus exacerbating and relieving factors. In addition other symptoms, particularly those of asthma, eczema, ENT problems and food allergy should be sought, plus any effects of all these upon sleep and quality of life. A history or a family history of allergic disease and/or immune problems,

BOX 1 | Rhinitis symptoms are nasal running, blocking, itching, sneezing, all of which are common in children due to viral colds. This Box gives the clues to an AR diagnosis.

Rhinitis may be allergic if

- The eyes are involved
- Itching is noticeable- child gives allergic salute, has allergic crease
- Exposure to a known allergen reliably causes symptoms
- Personal or family history of other allergic diseases
- Some children present with a comorbidity (asthma, atopic eczema, rhinosinusitis, hearing difficulties, sleep disturbance, behavior problems, pollen food syndrome). Always ask about nasal symptoms in such patients
- Always ask about asthma in children with rhinitis and vice-versa.

BOX 2 | Red Flags- for specialist attention.

- Children with unilateral symptoms, severe nasal obstruction +- sleep apnoea
- Children under 2 years and those with a history of rhinitis symptoms present continuously since birth (34, 35)
- Children with nasal polyps
- Those refractory to medical management.

together with social history, including a review of treatments tried, those currently being taken and their efficacy, should be taken.

Examination

This should include measurement of height, which needs monitoring, especially in children receiving corticosteroids at several sites (36).

The presence of conjunctivitis, nasal allergic crease, allergic salute or double creases beneath the eyes (Dennie–Morgan lines) all suggest that the patient has an allergic diathesis (**Figure 4**). The ability to breathe through the nose should be tested. In children with moderate to severe AR or uncontrolled symptoms nasal examination is needed, both external and internal. An otoscope will suffice if nasendoscopy is unavailable. Plentiful clear secretions and swollen pale turbinates suggest AR, but the mucosa may be normal or be reddened by INS use. Nasal polyps should prompt testing for cystic fibrosis (37).

ENT referral is advised for patients with bleeding, unilateral disease, high crusting, marked septal deviations and septal perforations as well as those patients who are refractory to medical management (12).

Ear inspection is sensible as otitis media with effusion is a co-morbidity in children with rhinitis, as is auscultation of the chest and an objective measurement of lower airways function, where possible, checking for concomitant asthma and observing the skin for eczema (12).

Investigation

Where there is a clear history of symptoms in relation to known allergen exposure a trial of effective treatment, such as intranasal corticosteroids (INS) may be used as a diagnostic tool, with further investigation if unhelpful.

Allergic sensitization can be demonstrated by skin test or specific serum IgE antibody analysis. Both can in principle be applied at any age. If allergen immunotherapy (AIT) is being considered then testing is mandatory. IgE test results need interpretation in the light of the history, as both false- positive and false- negative results can occur.

Skin prick test sensitivity ranges from 68 to 100% and specificity from 70 to 91% (38).

Component- resolved diagnosis, looking at reactivity to specific molecules within an allergen, such as Phl p 1, Phl p 5, Bet v 1 or Pru p 3 is not routinely used, but can predict persistence of AR and the likelihood of future development of asthma or pollen food syndrome. It may also be useful in deciphering cross-sensitization and enabling accurate vaccine content (39–41).

Other tests such as evaluation of nasal nitric oxide and ciliary beat frequency, nasal allergen challenge, CT scans, nasal smears, nasal cultures and analysis of nasal fluid for β-transferrin may be required to include or exclude different forms of rhinitis (37).

Treatment

Treatments for AR include education, allergen avoidance, pharmacotherapy and AIT (12, 13, 42).

The EUFOREA algorithm (**Figure 5**) includes these and is based upon an update of previous evidence- based guidelines. It covers management of pediatric AR at all levels and of all severities (12–15).

Education

Parent/carer education, as well as that of the child, to improve understanding and concordance is vital and also saves time and costs in allergic diseases (12, 43). It includes nature of the disease, finding and eliminating triggers such as allergens and pollutants, explanation of medication suggested and demonstration of the way to use nasal sprays (**Figure 6**), if prescribed (12). Continuation of patient contact via mobile apps and telehealth may improve outcomes as well as providing data for analysis. If possible children should score their own symptoms, as caregivers usually are less able to adequately capture disease burden (44).

An emoji visual analog scale is currently under investigation for validity (**Figure 7**).

Allergen/Pollutant Avoidance

In the current COVID pandemic the wearing of face masks is advised for older children. These may also reduce AR symptoms and the possibility of viral spread by sneezing (45).

The UK Royal College of Paediatrics and Child Health (RCPCH) systematic review of 221 studies recently provided evidence linking indoor air pollution to a range of childhood health problems including asthma, wheezing, conjunctivitis, dermatitis, and eczema. Sources of indoor air pollution include smoking, damp, the burning of fossil fuels and wood, dust, chemicals from building materials and furnishings, aerosol sprays and cleaning products. Indoor air quality tends to be poorer in low quality housing where ventilation may be inadequate

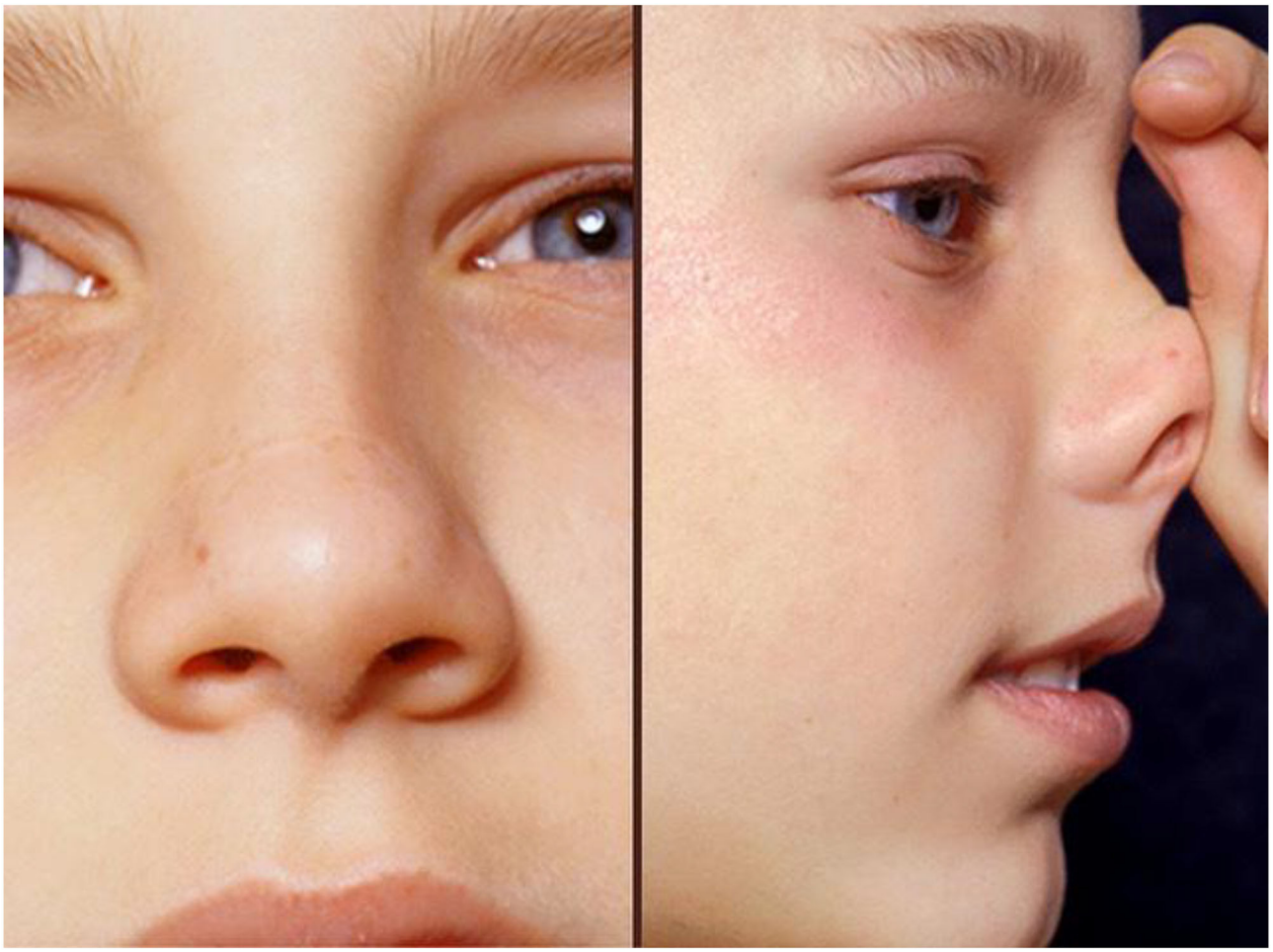

FIGURE 4 | This child shows typical facial changes associated with allergy: he is pale, mouth breathing, with dark circles beneath eyes, a transverse nasal crease, double eye creases and loss of the lateral eyebrow. He is seen giving an allergic salute in the right-hand photo.

or insufficient. Improved ventilation and non-allergenic green plants help to mitigate pollution effects[1].

There is a need for avoidance of allergens and pollutants both inside and outside the home. Persuading parents not to smoke in the home can ease children's symptoms, as can avoidance of gas cookers. Avoidance of major exposures to known allergens, such as pets, house dust mites and mold is sensible, multiple measures do show benefit in AR and asthma, so allergen proof bedding covers and HEPA filters on vacuum cleaners are advised for asthma and AR (https://www.asthma.org.uk/advice/triggers/dust-mites/ and https://www.asthma.org.uk/advice/triggers/indoor-environment/).

The next step is the use of nasal saline which can be universally recommended for all ages. It reduces symptoms and the need for pharmacotherapy and can be used both regularly and/or post-allergen or pollutant exposure. Evidence suggests that seawater or mildly hypertonic saline are most effective (46, 47).

[1]Available online at: https://www.rcpch.ac.uk/resources/inside-story-health-effects-indoor-air-quality-children-young-people.

Pharmacotherapy

Antihistamines

Although widely given as first-line treatment there are problems with pediatric antihistamine use. Firstly there is a paucity of well-controlled studies in AR treatment for some widely available molecules, especially in young children. In particular the first-generation sedating antihistamines lack good evidence of efficacy and are known to have adverse effects such as psychomotor retardation and behavior disturbance and so are not recommended (42).

There is evidence for equivalent efficacy and safety of several second generation antihistamines in pediatric AR. A meta-analysis involving more than 2,500 patients has consolidated the clinical evidence for rupatadine in allergic rhinoconjunctivitis in adults and children (level of evidence Ia, recommendation A). Other recent advances include observational studies of rupatadine in everyday clinical practice situations and approval of a new formulation (1 mg/ml oral solution) for use in children (48). In children aged 6–11, cetirizine, but not loratadine, outperformed placebo (49). However, in a Taiwanese study

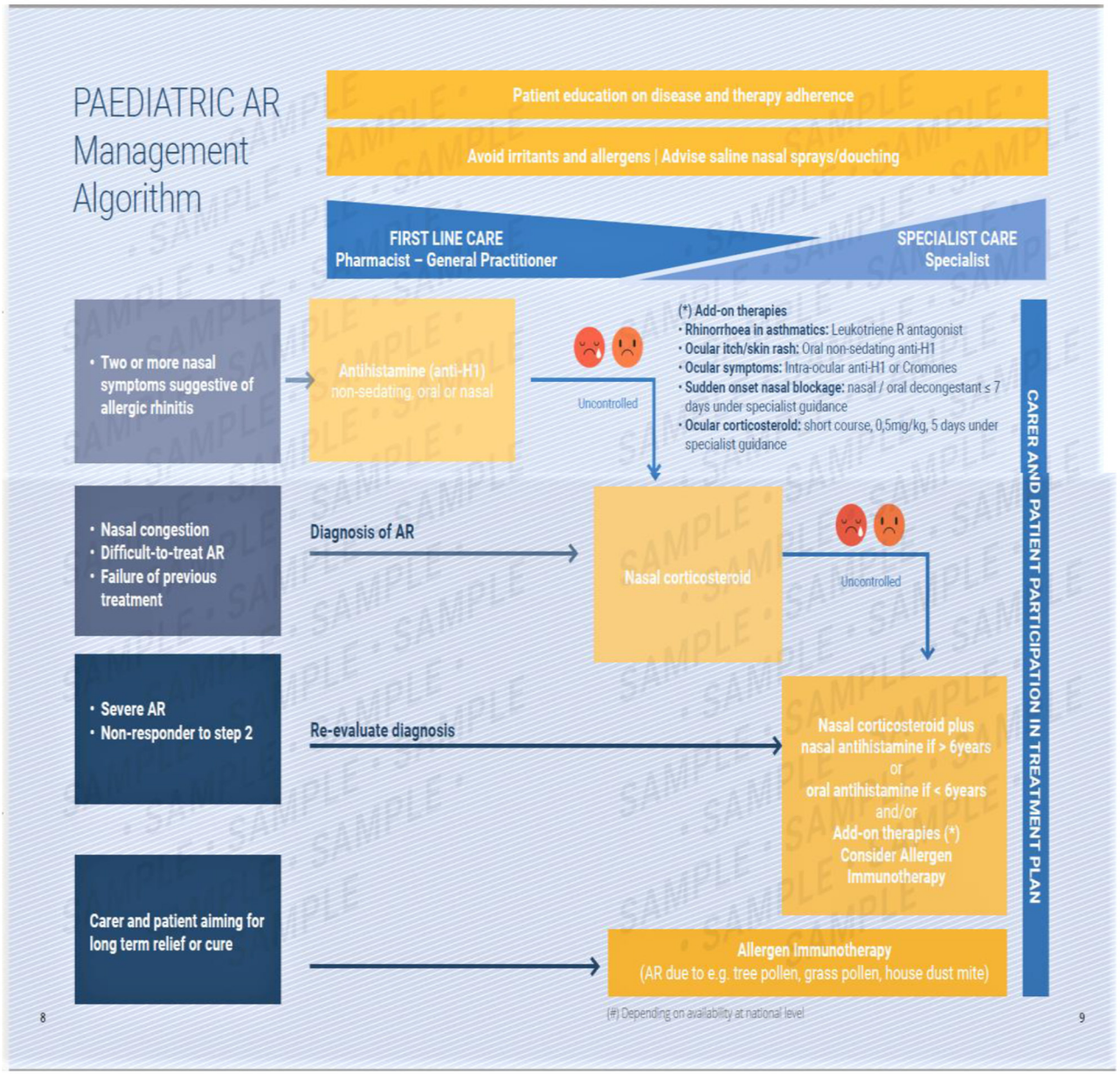

FIGURE 5 | The EUFOREA management algorithm for pediatric allergic rhinitis. This includes measures common to all sufferers and provides a graduated guide to therapy based upon symptoms and their response to therapy. A pictorial visual analog scale is suggested, with poor control being indicated by the two sad faces shown. This requires verification.

loratadine outperformed cyproheptadine (50). There is also good evidence for the use of fexofenadine (51, 52), which, together with bilastine (available in Europe for children over 6 years) shows least brain penetration (53).

A further problem is the fact that in drug trials it is often the parent or carer who is scoring the child's symptoms. A scoring system for children to use is needed. We have proposed one using emojis, this is currently being assessed for validity (**Figure 7**).

Finally antihistamines given orally are only weakly effective in controlling nasal symptoms, so are most suitable for mild AR and where other histamine-mediated symptoms are occurring in the same patient. If one oral antihistamine fails to control symptoms there is no point in trying a different one, the patient should be switched to an intranasal antihistamine or corticosteroid.

Topical intranasal antihistamines act rapidly (15 min) and are more effective than oral ones. Azelastine has shown efficacy and safety in children with AR in 2 European double-blind, placebo-controlled, parallel-group trials and in an open USA study (54). Olopatadine has also shown efficacy in pediatric allergic rhinitis (55). The major adverse effect of intranasal azelastine is a bitter taste, perceived by around 10% of subjects. The taste aversion was less with olopatadine (56).

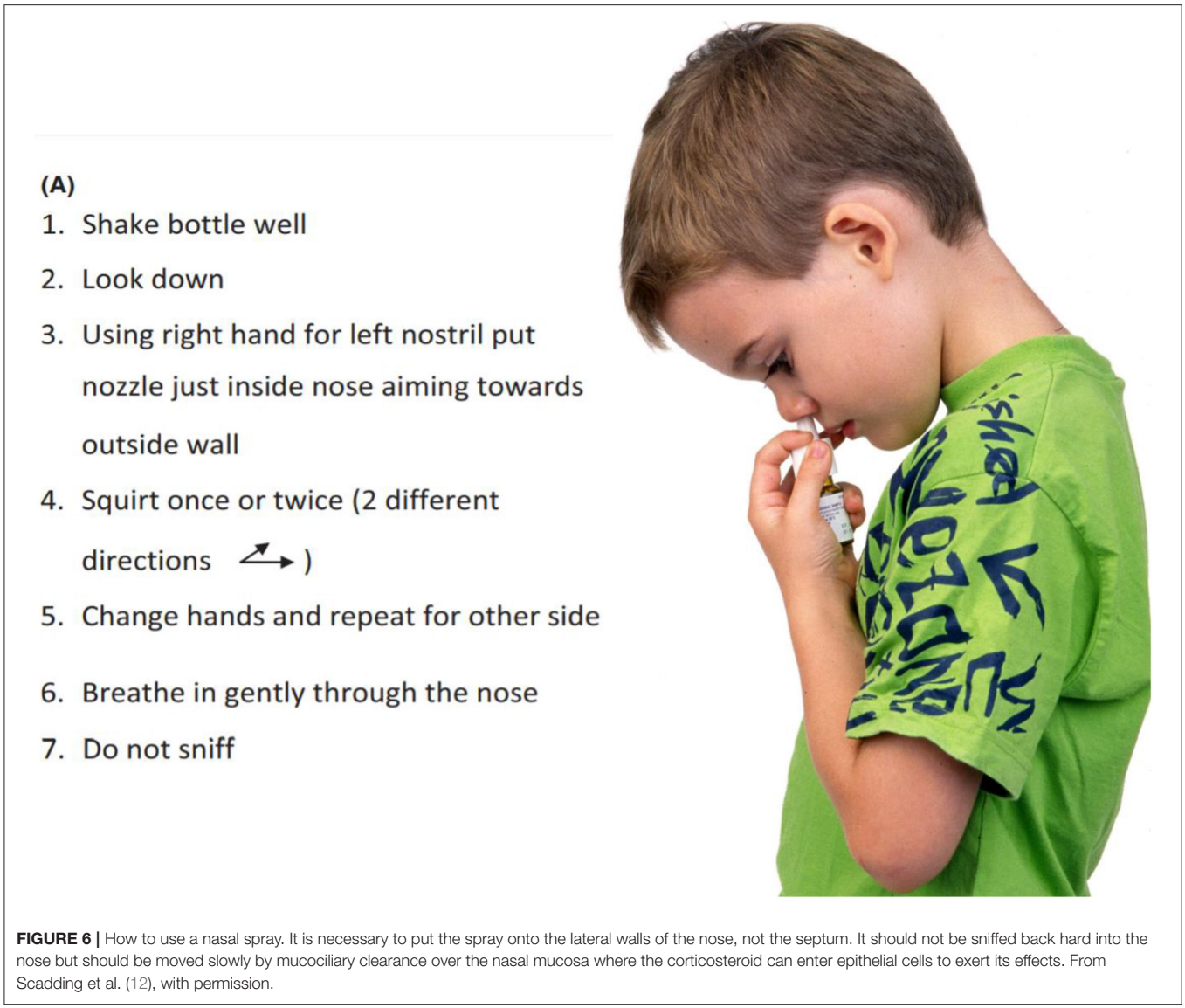

FIGURE 6 | How to use a nasal spray. It is necessary to put the spray onto the lateral walls of the nose, not the septum. It should not be sniffed back hard into the nose but should be moved slowly by mucociliary clearance over the nasal mucosa where the corticosteroid can enter epithelial cells to exert its effects. From Scadding et al. (12), with permission.

FIGURE 7 | A suggested visual analog scale, using emojis, for younger children to express their feelings about their symptoms.

Intranasal Corticosteroids (INS)

Good quality evidence for the efficacy of INS in AR in children exists (12). INS are more effective than H1- antihistamines and leukotriene receptor antagonists, particularly for nasal congestion, although their maximum efficacy requires several hours or days (56). INS are useful first line treatment for AR which is moderate to severe.

The molecules with least systemic bioavailability from the nose are ciclesonide, fluticasone propionate, fluticasone furoate, and mometasone furoate (57). These have good safety data and are preferred for long term pediatric use. Growth in children is a sensitive measure of corticosteroid effects so monitoring it is important.

Teaching correct use of these sprays (**Figure 6**) reduces common adverse events such as nasal irritation, stinging and epistaxis. Long- term INS use does not damage the nasal mucosa (58).

Combination Therapy

For those children whose AR remains uncontrolled despite regular use of an INS the addition of an antihistamine is advised. For those over 6 a fixed dose combination (FDC) nasal spray containing azelastine and fluticasone propionate (MP-Aze-Flu) is available. In a trial this improved quality of life, but not total nasal symptom scores, in all children involved - but did do so in those children who rated their own symptoms, showing the importance of self-assessment (44).

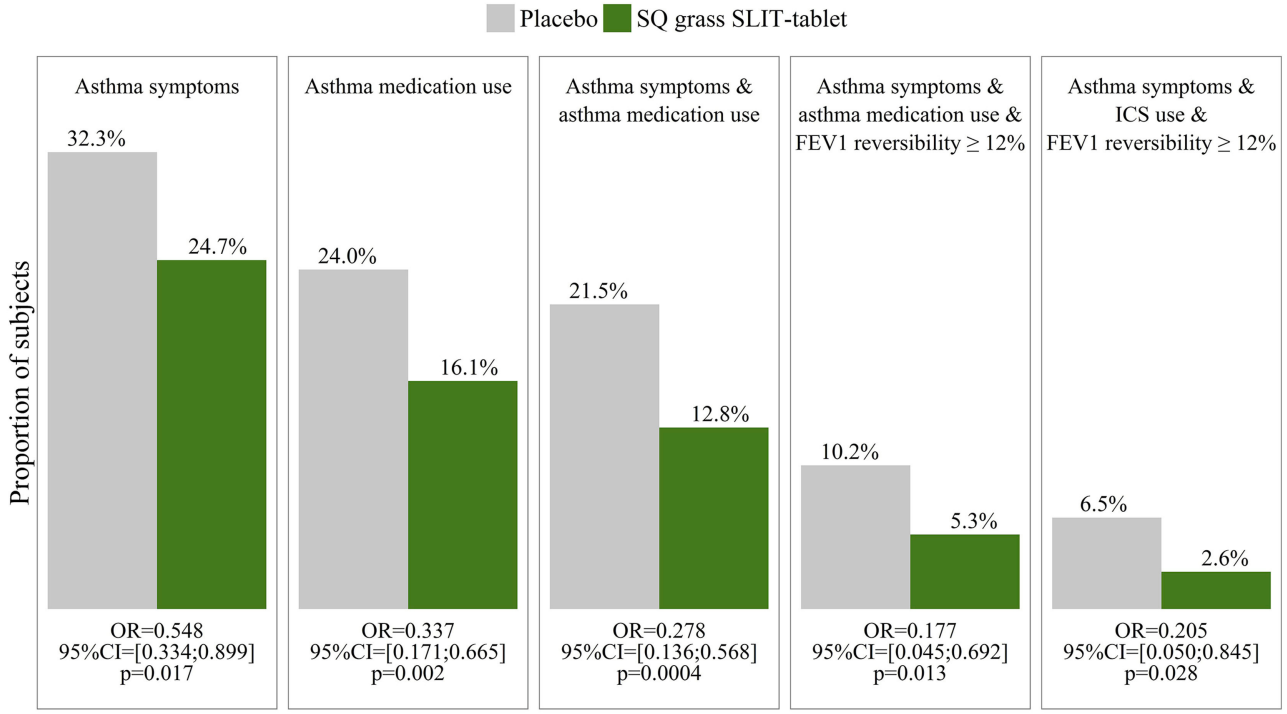

FIGURE 8 | Outcome measures in the GAP study (65) show a reduction in asthma symptoms, medication use and FEV1 reversibility in children with grass pollen allergy who were treated by sublingual immunotherapy (SLIT) to grass pollen, compared to those who received placebo.

In some countries there is also an FDC with mometasone furoate and olopatadine. These FDCs are rapidly active, more effective than the individual compounds administered alone in over 12 s and adults and are largely well-tolerated (apart from a bitter taste in some patients). FDCs may be most useful in patients, such as teenagers, who tend to treat their symptoms intermittently.

Add-On Therapies

INS Plus Oral Anti-histamine

In adults combining oral H1- antihistamines and INS does not increase the efficacy of INS, except occasionally for eye symptoms (59, 60). The combination has not been formally tested in children, however addition of an oral antihistamine to an INS makes sense when there are persisting extra-nasal histamine-induced symptoms.

Anti-leukotrienes

These have evidence of effectiveness similar to that of oral antihistamines in AR, though there is a spectrum of responsiveness, genetically determined (61, 62).

They may provide useful additional help in children with AR plus asthma, but there should be monitoring for possible adverse psychiatric effects (63).

Topical Nasal Decongestants

These cause vasoconstriction and increase the nasal airway but have no effect on other rhinitis symptoms. Regular use can lead to rhinitis medicamentosa. Brief use, under specialist control, is advised when the nose is completely obstructed. This may allow ingress of other therapeutic sprays.

Oral Corticosteroids

Specialist prescription of these may be needed when symptoms are extremely severe. Brief use only is necessary because of possible major side effects (12). Injectable depot corticosteroids have an adverse risk profile and should not be used (12).

Eye Symptoms

INS reduce eye symptoms to some extent. Cromoglycate or antihistamine eye drops are suitable for patients older than 3 years. Olopatadine is a mast cell stabilizer properties licensed for pediatric use in some countries. Severe eye symptoms warrant an ophthalmological opinion, both to check for vernal conjunctivitis and to enable the use of corticosteroid eye drops which can only be used under such supervision because of the danger of herpetic keratitis (12).

Allergen Specific Immunotherapy

While avoidance of environmental allergens, unrealistic in many patients, and antiallergic/anti-inflammatory pharmacotherapy are aiming at symptomatic control, allergen specific immunotherapy (AIT), based on the application of relevant allergens to the allergic patients via different routes, is more ambitious. AIT not only reduces symptoms but there is evidence that it can alter the course of disease.

In children as well as in adults this allergen specific treatment has been demonstrated to lead to symptom reduction and less need for medication, not only for the time of treatment, but also for at least 2 years beyond (64).

Large placebo- controlled trials using subcutaneous and – more recently- sublingual immunotherapy have provided robust evidence for the disease modifying potential of this treatment. In the recent GRAZAX trial which was performed in children with seasonal rhinitis, but no asthma symptoms during the grass pollen season in Europe, it was demonstrated that for a period of 5 years (3 treatment and two follow up years) not only seasonal rhinitis symptoms were reduced, but also the incidence of asthma symptoms as well as the need for asthma medication was reduced for the whole 5 year period (65) (**Figure 8**).

Immunotherapy had been practiced in Europe and the US for decades without solid scientific evidence for efficacy until Frankland and Augustin (66) published the first placebo-controlled study with grass pollen extracts, and it took until the turn of the century until new criteria for safety and efficacy were defined by health authorities prior to market approval. Nowadays the FDA and the EMA request for all immunotherapy products clinical development plans meeting strict criteria for clinical outcomes such as predefined effect sizes etc. Registration today also includes a pediatric investigational plan. For seasonal pollen allergies both pre- and co-seasonal immunotherapy are widely used; for perennial allergies using allergens from domestic dust (e.g., house dust mites) perennial treatment over 3 years is recommended, in order to achieve fewer symptoms, less need for medication and long term tolerance induction, which lasts for years, even after AIT is discontinued.

Long term safety studies in children indicate that, while sublingual and subcutaneous immunotherapy induce local side effects around the allergen application sites, particularly during the first weeks of treatment, there are very rarely any severe systemic adverse reactions (67). Oral reactions may be reduced by application of the tablet to the vestibulum, between inner lip and teeth, where dendritic cells are more plentiful (67).

Therefore, this treatment can be considered safe from the 5th year of life.

Given the impairment of preschool and school children during daytime activities as well as during sleep, given the increased risk of allergen induced asthma in this age period, it should be recommended to consider allergen specific immunotherapy at the latest after 2 years of allergic symptoms. During recent years the robust clinical effects of SLIT and SCIT in patients with seasonal allergic rhinitis could be confirmed by real world data obtained from data banks in Germany and France (68, 69).

What About Biologics?

Thus far, no biologic has been marketed for allergic rhinitis. Several studies in children, which combined allergen specific immunotherapy with anti-IgE indicate, that a strong non-specific therapeutic effect of the monoclonal antibody—in addition to the symptomatic effect of AIT—can be observed (70, 71).

In contrast to AIT biologics are significantly more expensive and do not lead to a long-term modification of chronic disease. It seems, however, promising to consider a future treatment with anti-IgE for very severely affected children who showed insufficient response to SIT.

PREVENTION

While some preventive interventions seem promising in atopic dermatitis or food allergy (72), the options for allergic rhinitis appear limited. In the German prospective birth cohort study, no single modifiable risk factor was linked to AR. The GINI birth cohort did not observe any reduction of allergic rhinitis after dietary modification in infancy. Multiple other approaches (probiotics) failed in demonstrating preventative effect. Therefore, besides allergen-specific immunotherapy interventions aiming at primary prevention of AR are currently not available. However, high levels of butyrate in early life are associated with a certain degree of protection against atopy (73, 74).

DISCUSSION

The advent of new treatments and the underuse of current effective therapies such as INS and AIT has meant that a new management guide for pediatric AR became necessary. We have adapted and updated evidence from previous guidelines and combined this with extensive personal experience. Allowing children more control by monitoring their own symptoms and applying their own nasal sprays should improve concordance and control, but this requires confirmation.

AUTHOR CONTRIBUTIONS

GS and UW researched the evidence and put together the original document. It was then revised and finally approved by all authors.

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Conflict of Interest: PS was employed by the company Allergy Medical Group (Brisbane).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Pathophysiological and Clinical Aspects of Chronic Rhinosinusitis: Current Concepts

Stephan Vlaminck[1], Frederic Acke[2], Glenis K. Scadding[3], Bart N. Lambrecht[4,5,6] and Philippe Gevaert[2]*

[1] Department of Otorhinolaryngology, Centre Hospitalier de Mouscron, Mouscron, Belgium, [2] Department of Otorhinolaryngology, Ghent University/Ghent University Hospital, Ghent, Belgium, [3] The Royal National Throat Nose and Ear Hospital, London, United Kingdom, [4] Laboratory of Immunoregulation, Flemish Institute for Biotechnology, Center for Inflammation Research, Ghent, Belgium, [5] Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium, [6] Department of Pulmonary Medicine, Erasmus University Medical Center, Rotterdam, Netherlands

Adult chronic rhinosinusitis (CRS) is a chronic inflammation of the mucosa of the nose and paranasal sinuses. According to the latest EPOS guidelines CRS should be regarded as primary or secondary with distinction between diffuse and localized disease. Further pathophysiologic research identified different inflammatory patterns leading to the term "endotyping of CRS." The primary focus of endotyping is to define a dominant inflammatory type allowing for better orientation of therapy. The current approach proposes the differentiation between type 2 (eosinophilic) and non-type 2 inflammatory responses. In this review pathophysiological concepts of CRS will be discussed, focusing on the different inflammatory endotypes of T cells with special attention to the eosinophilic type 2 inflammatory response. The contribution of innate and adaptive immune system responses is presented. The possibility of endotyping based on sinonasal secretions sampling is brought to attention because it is indicative of corticosteroid responsiveness and available to most ENT surgeons. Furthermore, the clinical aspects of the three distinct phenotypes are analyzed in view of their characteristics, the related endoscopic findings, typical radiological imaging, histopathology findings, their relation toward allergy and obvious therapeutical implications. This overview will enable clinicians to relate pathophysiological patterns with clinical observations by explaining the different inflammatory mechanisms, hence providing a better understanding of therapy.

Keywords: chronic rhinosinusitis, nasal polyps, eosinophilia, endotyping, allergy

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****Correspondence:***
Stephan Vlaminck
stephan.vlaminck@gmail.com

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INTRODUCTION

Chronic rhinosinusitis (CRS) is a multifactorial inflammatory disease of the nasal and paranasal mucosae presenting with a variety of symptom combinations. Chronic rhinosinusitis may be used to describe conditions ranging from unilateral single sinus disease to widespread sinonasal airway inflammation. The currently recognized definition of primary CRS refers to sinonasal inflammation in which no obvious underlying etiopathogenic event is occurring (i.e., excluding fungal ball, neoplasia, odontogenic or immunodeficiency).

Based on expert recommendations, criteria for CRS were established in the European Position Paper on Rhinosinusitis (EPOS) to sustain uniform epidemiologic studies (1). The EPOS 2012

guidelines describe CRS as an inflammatory disorder defined by the presence of two or more cardinal symptoms [obstruction, drainage (anterior or posterior), smell loss, and facial pain or pressure] for at least 12 weeks duration, confirmed by objective evidence using sinus endoscopy or computed tomography (CT) scan. For study inclusion the guideline requires at least two of four symptoms for at least 3 months duration, one of which must be either nasal obstruction or discharge. According to the new EPOS 2020 classification CRS should now be regarded as primary or secondary, and distinction is made between diffuse and localized disease based on anatomic distribution (2).

Using this definition, epidemiological studies estimated the prevalence of CRS in Europe (10.9%), China (8%), and Brazil (5.5%) (3–5). Studies in the USA, using symptom criteria alone, reported a prevalence of 11.9% resembling the European CRS frequency pattern (6). It is clear however that defining CRS on symptoms alone cannot be sustained as conditions such as odontogenic sinusitis, fungus ball, antrochoanal polyps and others may mimic sustained CRS symptoms and therefore have to be differentiated by adjunctive measures. Recent research showed that epidemiologically defined CRS is not verified by nasendoscopy and CT scan in half the subjects, so the prevalence in Europe is actually 3% if a cut-off score $>$ 4 on the Lund-Mackay scale is used (7). Clinical presentation and CT scanning and/or nasal endoscopy are able to phenotype CRS patients differentiating patients with (CRSwNP) and without nasal polyps (CRSsNP) (8). The European Rhinologic Society and the American Academy of Otolaryngology, Head and Neck Surgery initiated the use of guidelines useful for medical therapy and surgery within a very mechanical understanding of sinus pathology (1, 9). This traditional phenotype-based classification however showed inadequate disease control after medical and surgical treatment probably because it does not mirror the underlying inflammatory disease. Further analysis of this is needed to understand the patient's responsiveness or lack of it to standard treatment.

The recent call for a Precision Medicine Concept now aims for integrated care pathways (ICPs) with treatment protocols adapted to clinical practice (10). Therefore, understanding and identification of different inflammatory types in CRS with proper biomarkers are researched and believed to influence decision making in personalized therapeutic strategies (10, 11). Three phenotypes of primary CRS have been described: allergic, eosinophilic and non-eosinophilic (1, 12). This more pathophysiological view initiated a new term called "endotyping of CRS" and resulted in the search for a more adequate therapy especially for severe and recurrent CRS inflammation (8, 13). An alternative distinction is the inflammatory type dominance, either type 2 (T2) (eosinophilic) or non-T2 (2). T2 can then be subdivided predominantly *via* T helper (Th) 2/allergy/immunoglobulin E (IgE) mechanisms and *via* innate mechanisms (ILCs, innate lymphoid cells) or a mixture of the two (later on in CRSwNP).

The general ENT clinician will be confronted with a subpopulation of Severe Chronic Upper Airway disease (SCUAD) resistant to classic therapy. Some of these individuals will have phenotypes such as aspirin sensitivity, allergic fungal disease or vasculitis, each with specific therapeutic possibilities. Blood tests such as total IgE, IgE, and IgG to Aspergillus and anti-neutrophil cytoplasmic antibodies (ANCA) can aid to identify the latter two, whereas the former requires aspirin challenge testing. Nowadays multidisciplinary teams use molecular knowledge and precision medicine, with close follow-up regarding efficiency and quality control upon novel treatment options, such as biologicals (14). The therapeutic challenge especially applies to the even more complex pediatric SCUAD population in which underlying conditions such as cystic fibrosis and primary ciliary dyskinesia are in the differential diagnosis (15). In the adult population, SCUAD with nasal polyposis and T2 signature is the most challenging phenotype in finding a correct therapeutical rationale combining surgery with potential biologicals. Since these molecules are expensive it makes sense to identify characteristics which identify responders to each particular molecule by submitting data centrally to increase patient numbers (16). To date, this has not happened and the decision-making process for the individual CRS patient is based on careful monitoring of any improvement (17). The complexity of CRS pathology is important as correct medical and/or surgical treatment may largely be beneficial on control improvement of bronchial disease (18). Usefully selected biomarkers are not yet available for predicting a type 1 or type 3 inflammation in CRS; however testing for eosinophils in secretions is simple (19).

Pathophysiological concepts will be discussed in the next chapter, focusing on the different inflammatory endotypes of T cells with special attention to the eosinophilic T2 inflammatory response. This chapter is followed by an overview of the three main inflammatory sinonasal phenotypes, focusing on diffuse disease.

PATHOPHYSIOLOGY

To clarify pathophysiological aspects of CRS, some general immunological topics need to be known. Lymphocytes play an important role in the innate and adaptive immune system. Two major types can be distinguished: B lymphocytes originating from the bone marrow and T lymphocytes arising from the thymus. In the adaptive immune response, T cells are generated in the secondary lymphatic tissues to encounter antigens and become antigen-specific cells after proliferation. B cells, after being influenced by T cells, become antibody-secreting cells. Natural killer (NK) cells, activated by interferons (IFNs), are a third type belonging to the innate immune system and recognize changes in the major histocompatibility complex (MHC) class 1 (20). The main focus regarding CRS pathophysiology is on T cells.

T-Cell Physiology

T lymphocytes and their cytokines influence the cell-mediated immune response through activation *via* the T cell receptor (TCR) and the co-stimulatory molecule cluster of differentiation (CD) 28. Activation results in the production of interleukin (IL)-4 and IL-10 facilitating T-cell/B-cell interaction. T lymphocytes can be immunophenotyped in CD3+CD4+ and CD3+CD8+ white blood cells by their cell surface identification molecules.

CD8+ cells, also known as cytotoxic T (Tc) cells, recognize MHC-1 molecules on the surface of infected cells and are bound to eliminate those. CD4+ cells, also known as Th cells, recognize MHC-2 molecules on the outer layer of antigen-presenting cells (B cells, macrophages and dendritic cells). When CD4+ T cells are stimulated by an antigen, further differentiation occurs with different cytokine patterns and distinct cellular function *in vivo*: actual importance is retained in Th1, Th2, Th17, regulatory T (Treg), and T follicular helper (Tfh) cells (21) (**Figure 1**).

Different Types of Immune Responses

Immune polarization is based on T cell cytokine production. The emerging linkage between adaptive and innate immune systems has led to the proposal of type 1, 2, and 3 immune responses (22). Type 1 immune responses are characterized by type 1 innate lymphoid cells (ILC1), and Tc1 and Th1 cells. The crucial role of this type 1 immune response is to deal with intracellular microbes, protozoa and viruses (23). The activation of ILC1, Tc1, and Th1 cells will induce the production of type 1 cytokines IFN-γ and tumor necrosis factor (TNF)-α resulting in the activation of mononuclear phagocytes loaded with potent cytotoxic molecules (22). Type 2 immune responses implicate ILC2s, Tc2, and Th2 cells responsible for the production of IL-4, IL-5, and IL-13 cytokines. Type 2 plays an important role in parasite infection and induces allergic diseases with important contribution of eosinophilic cells, IgE production and goblet cell hyperplasia (22, 24). Type 3 immune responses are currently associated with cytokines IL-17 and IL-22 and controlled by IL-3, Tc17 cells, and Th17 cells. Their role is believed to facilitate immune responses opposing extracellular bacteria and fungi (22). As an overview, CD4+ (Th) and CD8+ (Tc) cells with their cytokines in CRS are described below.

Th1 cells: these cells are mainly activated by intracellular pathogens. Bacterial and viral products are bound to Toll like receptors (TLR) on antigen presenting cells (APC). Hence dendritic cells will secrete IL-12 cytokines leading to the production of typical Th1 cytokines: IL-2, IFN-γ, and TNF-ß expressing a dominant neutrophil pattern.

Th2 cells: during a type 2 immune response Th2 cells produce key cytokines IL-4, IL-5, IL-9, and IL-13, which induce antibody class switching to immunoglobulin (Ig)E and IgG1, and enhance the recruitment of inflammatory cells (predominantly eosinophils, basophils and mast cells). Goblet cell hyperplasia is stimulated and mucus production is induced. Th2 response is essential to fight parasitic infections, but also promotes allergic disease and asthma.

Th17 cells: implication of Th17 cells is considered as an immediate response to extracellular bacteria and fungi. The production of IL-17 and IL-22 cytokines may cause chronic inflammatory disease and autoimmune pathology when dysregulation is present.

Tfh cells: Tfh cells are recognized important in regulating B cells to support antibody response. IL-21 is considered the signature cytokine.

Treg cells: the two cytokines mainly associated with Tregs are IL-10 and tumor growth factor (TGF)-β. Tregs secrete these cytokines and use them to carry out a suppressive function on the immune system. It has shown importance in controlling Th2 responses. Lacking those cells may allow further development of asthma and allergy, as well as autoimmune diseases (25).

Th9 cells: IL-9 has been identified in a subset of T cells distinct from Th2 cells. The production of IL-9 requires the combination of TGF-β (which also promotes Tregs) and IL-4 (known to induce Th2 cells). Interestingly, Th9 cells, which are strongly associated with the immunopathology of asthma, also produce IL-10. IL-9 seems important in promoting mucus production and activation of mast cells as well as eosinophils (25).

Th22 cells: Th22 cells represent a recent separate Th subset and are closely related to Th17 cells. They predominantly produce the cytokine IL-22 and were initially associated with immunopathology of skin diseases. Recent evidence indicates that IL-22 plays an important role

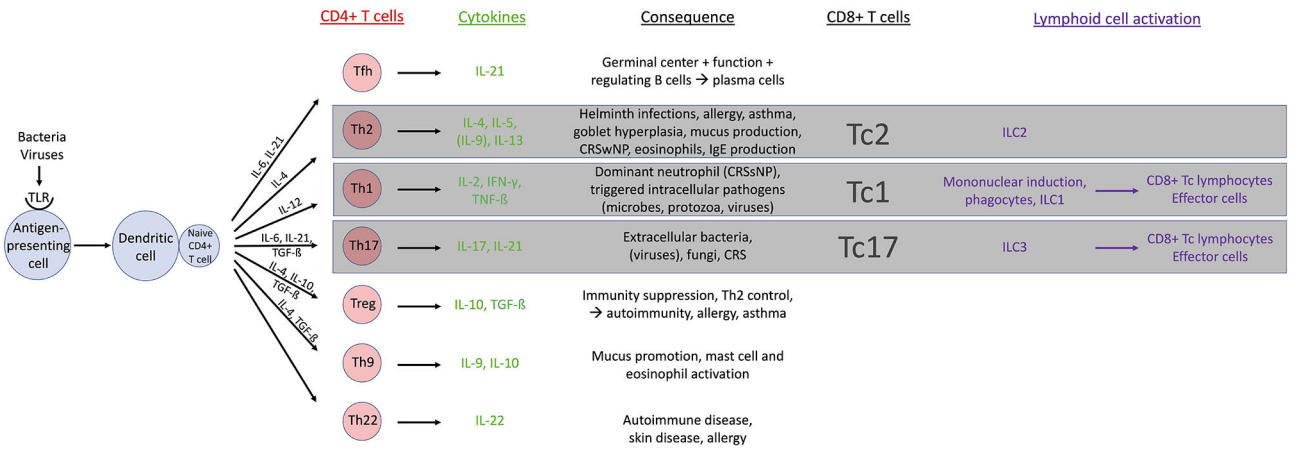

FIGURE 1 | Overview of T cell physiology. CD, cluster of differentiation; CRS, chronic rhinosinusitis; CRSsNP, CRS without nasal polyps; CRSwNP, CRS with nasal polyps; IFN, interferon; IgE, immunoglobulin E; IL, interleukin; ILC, innate lymphoid cell; Tc, cytotoxic T cell; Tfh, T follicular helper cell; TGF, tumor growth factor; Th, T helper cell; TLR, Toll like receptor; TNF, tumor necrosis factor; Treg, regulatory T cell.

in the pathogenesis of autoimmune diseases and allergic diseases (25).

CD8+ cells: The CD8+ Tc lymphocytes seem to mirror the Th cell subset classification based on their transcription factor and cytokine expression patterns forming counterparts toward the CD4+ cell line namely Tc1, Tc2 and Tc17 cells (24, 26).

A large heterogeneity in CRS immune polarization is seen worldwide as the immune responses vary across different geographic areas and populations with distinct racial backgrounds (27–30). Immune responses toward type 1, type 2, and type 3 directions can define certain endotypes and may therefore influence clinical manifestations of CRS pathology (31). CRSwNP and CRSsNP nowadays may be linked to inflammatory patterns associated with Th1 (type 1), Th2 (type 2), or Th17 (type 3). CRSsNP is accepted to exhibit a type 1 immune response (28, 29). In Europe, Caucasian patients with CRSwNP mainly demonstrate a type 2 immune response with high asthma comorbidity (1, 32, 33), whereas in China and other East Asian countries, patients with CRSwNP show ∼50% less type 2 cytokine expression with less eosinophilic inflammation and lower asthma comorbidity (34). Asian patients with CRSwNP predominantly show neutrophil-biased inflammatory patterns (34). The association of type 2 immune responses with the development of nasal polyps is sustained in a clustering analysis of CRSsNP vs. CRSwNP in Caucasian patients (35).

Type 2 Immune Responses and CRS

Most European Caucasian and some Asian CRSwNP patients show increased numbers of Th2 and Tc2 cells, which could be associated with mucosal eosinophilia (34, 36). This type 2 immune response in CRSwNP is supported by the elevation of ILC2, the increased presence of tissue eosinophilia, a clear upregulation of IL-4, IL-5, IL-13, and local IgE, and profound tissue eosinophilia independent of atopy (37) (**Figure 2**).

After stimulation with innate immune-activating stimuli, cytokines, or injurious environmental agents such as proteases, epithelial cells produce thymic stromal lymphopoietin (TSLP) and sometimes IL-33 or IL-25, which activate ILC2. One example is the induction of IL-13 by IL-33 in reaction to the protease activity of Aspergillus Fumigatus (38). Epithelial cell-derived TSLP upregulates OX40 ligand (OX40L) expression on dendritic cells, and then dendritic cells initiate the differentiation of naive T cells into Th2 cells. Th2 cells, ILC2, and Tc2 cells orchestrate eosinophilic inflammation through production of type 2 cytokines. IL-4+ IL-21+ Tfh cells initiate the differentiation of B cells into plasma cells, followed by mast cells activation due to IgE, which is locally produced by plasma cells. Subsequently, mast cells can produce type 2 cytokines. Th2 inflammation can also induce monocytes and macrophage differentiation into M2 macrophages. M2 macrophages produce coagulation factor XIII-A (FXIII-A) that induces excessive fibrin deposition by cross-linking of fibrin and by antifibrinolytic pathways through binding the α2-plasmin inhibitor (α2-PI, also known as α2 antiplasmin) to fibrin (39). Meanwhile, tissue plasminogen activator (t-PA) levels are lowered in Th2 inflammation, causing impaired plasmin generation, which in turn decreases fibrinolysis (40). These events collectively result in the retention of water and the formation of edema in polyps. Th2 cytokine-mediated pendrin expression can increase mucus production. Cytokines IL-4 and IL-13 can decrease the expression of epithelial cell tight junction proteins. Neutrophil-derived oncostatin M (OSM) and eosinophil-derived DNA traps can also contribute to epithelium disruption.

Typical for the type 2 immune response is the increased production of local IgE in association with mucosal eosinophilia (33, 41), as well as the increased mucosal infiltration of B cells with the presence of markers of class switch recombination to IgE in CRSwNP patients (41–43). Of interest, Tfh cells may be found in germinal centers in secondary lymphoid tissue and are important to generate B cell responses. This is supported by the finding of ectopic lymphoid tissue in nasal polyps and the finding of Tfh cells in loco (44).

Literature reports Staphylococcus Aureus enterotoxins to act as antigen and superantigen inducing local IgE production (33). This could not be confirmed in the analysis of Chinese patients with eosinophilic CRSwNP (41). Polyclonal IgE antibodies have been shown to activate mast cells in nasal polyps (45–48) and IgE-mediated mast cell activation is found to be upregulated in eosinophilic nasal polyps (49). The finding of elevated infiltration of basophils in tissue of eosinophilic CRSwNP remains to be analyzed (50).

Type 1 and 3 Immune Responses in CRS

The type 1 immune response of CRSsNP expresses IFN-γ cytokines in Caucasian patients (28, 29). Asian patients and patients with cystic fibrosis-related nasal polyps present with a neutrophil-related inflammation with high levels of IFN-γ and IL-17A expression (12), the latter pointing toward a type 3 immune response. The IFN-γ upregulation could however not be retained in a CRSsNP Chicago study suggesting a geographical variation (51).

In type 1 and type 3 immune responses environmental triggers will stimulate epithelial secretion of osteopontin (OPN) hence triggering dendritic cells to activate Th1 and Th17 cells (52) (**Figure 3**). Together with Tc1 and Tc17, Th1, and Th17 cells orchestrate non-eosinophilic inflammation through production of IFN-γ, IL-17A, and IL-22 (31). IFN-γ induces apoptosis of epithelial nasal cells, disrupts tight junctions and stimulates neutrophils phagocytosis and chemotaxis (52, 53). IL-17A upregulates the expression of IL-36γ in epithelial cells, whereas the latter acts on neutrophils and further exaggerates neutrophilic inflammation by inducing IL-8 [C-X-C chemokine ligand-8 (CXCL8)] production from neutrophils (54). IL-22 induces epithelial cells to produce IL-8/CXCL8, which also acts on neutrophils. Neutrophils might produce OSM, OPN, and TGF-β2. TGF-β2 is supposed to be involved in fibrosis. IFN-γ and OSM could disturb epithelial barrier function by decreasing the expression of epithelial cell tight junction proteins. IFN-γ can induce activated but insufficient autophagy, leading to apoptosis of nasal epithelial cells. IL-17A+ IL-21+ Tfh cells initiate B cell differentiation into plasma cells that produce immunoglobulins G and A (IgG and IgA).

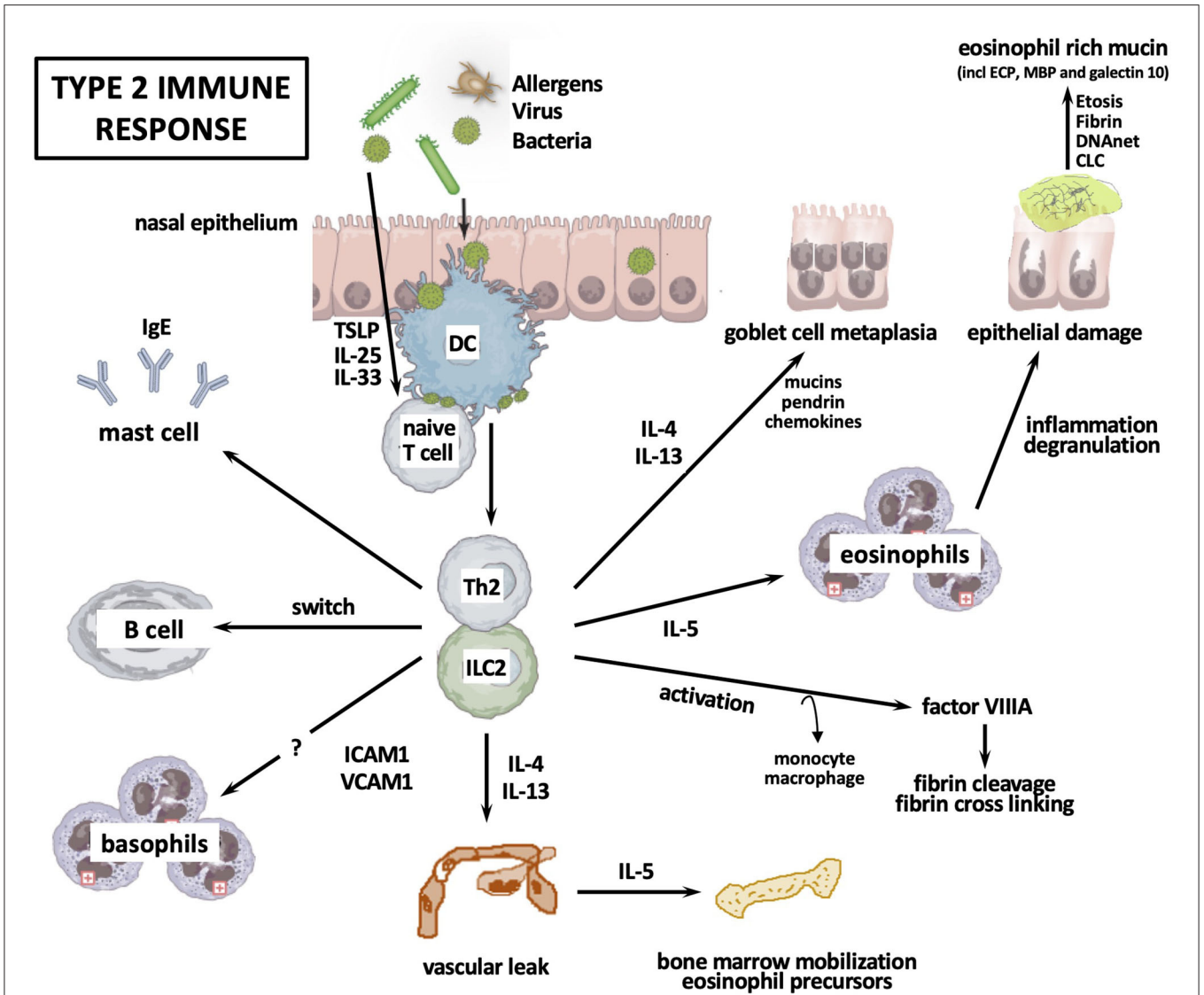

FIGURE 2 | Type 2 immune response. CLC, Charcot-Leyden crystals; DC, dendritic cell; ETosis, eosinophil apoptosis; ICAM, intercellular adhesion molecule; Ig, immunoglobulin; IL, interleukin; ILC, innate lymphoid cell; Th, T helper cell; TSLP, thymic stromal lymphopoietin; VCAM, vascular cell adhesion molecule.

Endotyping CRS – Role for Mucus Sampling

The variety in pathophysiological mechanisms of CRS makes the achievement of a universal analysis regarding clinical characteristics, molecular biomarkers, genetics, histopathology, epidemiology and treatment response difficult. Of interest, although on a small number of patients ($n = 32$), a simplified commercially available 41-plex cytokine-chemokine array on sinonasal tissue allowed the detection of three distinct endotype signatures helpful to decide on individualized therapy (35). To date, most studies are in search of biomarkers based on tissue sampling obtained during invasive procedures (41). A more feasible and possible repetitive approach involves the sampling of mucus (55). In the cluster analysis of the latter study, inflammatory mediators pointing toward types 1, 2, and 3 inflammatory patterns were consistent with results based on tissue analysis (55). Even the presence of the Th2-associated cytokines IL-5 and IL-13 could recently be detected in sinonasal secretions by multiplex cytometric assay, suggesting possible early stratification of CRS subgroups and more personalized therapies (55). Cellular analysis of sinonasal secretions in CRSwNP patients more often shows a Th2 bound inflammation whereas the presence of eosinophil-rich mucin may be considered an easy-to-obtain biomarker for predicting recurrence of CRSwNP with higher need for surgery and for predicting asthma development (56). Recent research highlights the importance of Charcot-Leyden crystals (CLC) as a relevant Th2 marker within CRS secretions

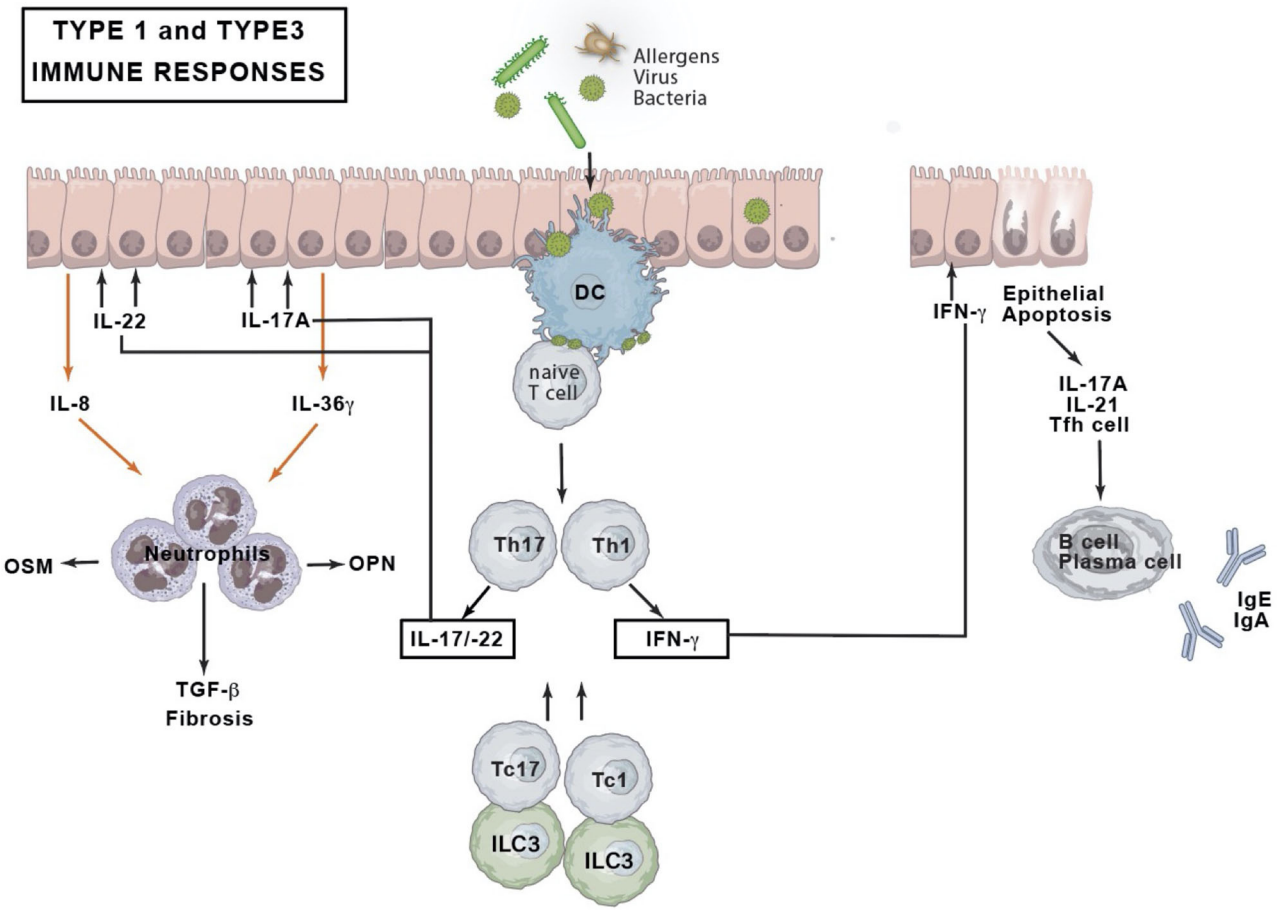

FIGURE 3 | Type 1 and type 3 immune response. DC, dendritic cell; IFN, interferon; Ig, immunoglobulin; IL, interleukin; ILC, innate lymphoid cell; OPN, osteopontin; OSM, oncostatin M; Tc, cytotoxic T cell; Tfh, T follicular helper cell; TGF, tumor growth factor; Th, T helper cell.

and its implications on their finding in sinonasal secretions of CRSwNP patients and in impacted bronchial secretions of eosinophilic asthma patients, opening new targets for therapeuticals (57).

In analogy with sampling of sinonasal secretions Seys et al. clearly could demonstrate in asthmatic sputum a diversity of type 2 cytokines discerning also non-type 2 cytokines concluding a priori the likeliness of type 2 vs. non-type 2 molecular asthmatic phenotypes (58).

CLINICAL PRESENTATION

Inflammatory sinonasal disease may be grossly divided in eosinophilic airway inflammation vs. non- eosinophilic inflammation. Three endotypes show a distinct T2 eosinophilic airway inflammation namely allergy, eosinophilic CRS and CRSwNP vs. a non-eosinophilic T1 inflammation pattern also present in the CRSsNP and some of the CRSwNP population. Those inflammatory patterns will be described below in addition to the concept of united airways. An overview of the characteristics of these endotypes is provided in **Table 1**, whereas endoscopic, CT and histologic (sinonasal secretions) images are shown in **Figure 4**.

Eosinophilic airway inflammation includes allergic disease such as allergic rhinitis in which the sinuses are usually minimally involved. Eosinophilic airway inflammation is also noted in CRSsNP and CRSwNP subdivisions. On the other hand we face non-eosinophilic airway inflammation in CRSsNP and in some CRSwNP subgroups.

The impact of upper airway inflammation on the lower airways is currently investigated for its impact by collecting real-life data and confirms the high disease burden in uncontrolled CRS patients, clearly impacting quality of life. Mobile technology such as Galenus Health opens a new era of real-life monitoring giving valuable clinical information about the relationship between upper and lower airways (59).

Allergic Airway Inflammation

Defining Characteristics

Patients with allergic rhinitis (AR) often show an earlier onset of disease, namely at younger age (<20), and although there is eosinophilic Th2 cell involvement, the disease is mainly

TABLE 1 | Overview of the three main inflammatory sinonasal phenotypes with their characteristics (CRS, chronic rhinosinusitis).

Phenotype	Allergic rhinitis	Eosinophilic CRS	Non-eosinophilic CRS
Type of secretions	Watery secretions	Thick tenacious mucin	Discolored secretions
Appearance of eosinophilic cells	Intact eosinophilic cells	Necrotic eosinophilic cells (ETosis)	Mainly neutrophilic cells (NTosis)
Charcot-Leyden crystals (CLC)	No CLC	CLC present	No CLC
Appearance of granules	No granules, no proteins	Granule proteins	Free eosinophilic granules
Type of cytokines	T2 cytokines	T2 cytokines	T1 cytokines
Typical age of patients	Likely young population	Likely older population	Diverse
IgE involvement	Evidence of IgE-mediated	IgE not necessarily present	IgE not likely
Presence of nasal polyps	Nasal polyposis not likely	Nasal polyposis likely	(Small) nasal polyps possible
State of mucosal lining	No damage of mucosal lining	Possible mucosal damage	Possible mucosal damage
Presence of hyphae	No hyphae	Hyphae possible	Hyphae not likely
Presence of major basic protein (MBP)	No MBP	MBP present	MBP not likely
CT appearance	Typical black halo on CT	Possible CT hyperattenuation	Atypical sinusitis on CT
Presence of asthma	Asthma with early onset	Late-onset asthma, eosinophilic	Atypical asthma
Possibility of oral steroids	Oral steroids rarely	Oral steroids more frequently	Oral steroids rarely
Possibility of vaccination	Possible vaccination	Vaccination rarely	No vaccination considered
Standard oral therapy	Anti-allergic therapy	Steroids, monoclonal antibodies	Antibiotics
Typical evolution	Tendency to disappear with age	Tendency to aggravate with age	Aggravation with age (multifactorial)
Persistence of disease	Restricted lifetime pathology	Lifetime pathology	Multifactorial dependent lifetime

immunoglobulin E (IgE) driven with other signs of atopic disease. Local symptoms are more dominated by itch, sneeze, and watery rhinorrhea. The presence of hyposmia rather suggests chronic rhinosinusitis with or without nasal polyps than rhinitis (60). The symptoms remain corticosteroid responsive and might soften and disappear with age (61). There is only weak evidence supporting a connection between CRS with/without nasal polyps vs. an allergic CRS condition (62). Since sinus involvement is minimal, allergic airway inflammation should probably not be included as part of CRS but be re-designated Persistent Polypoid Allergic Rhinitis (63).

Endoscopy

In patients with AR inhaled allergens are deposited on the head of the middle turbinate with possible inflammation and edema of the mucosa. The middle turbinate edema in more advanced cases can extend to the superior turbinate and posterior nasal septum and narrow or obstruct the more lateral sinus ostia (64). However, the presence of thick eosinophilic mucin as seen in eosinophilic CRS patients is by far less common in this allergic phenotype. Even with extreme polypoid change, there is often near normal ethmoid, sphenoid and maxillary mucosa, and simple trapped mucus is mostly found at surgery (65).

Radiology

The typical "black halo" sign originally described by Lund et al. shows a central thickening of the turbinates and septum with near normal peripheral sinus mucosa and is considered typical for inhalant/IgE driven CRS (66).

Histopathology

On histopathology T2 cytokines dominate and elevated total and serum specific IgE is found. Elevated serum eosinophil count is only rarely observed and tissue sampling is performed with simple hematoxylin and eosin (H&E) coloring (61). Of most importance, eosinophilic mucin and CLC are not found in this condition as being typical to eosinophilic CRS conditions.

Allergy

Patients with AR show higher serum specific IgE compared to other subtypes of asthma (61). A Positive skin prick test or Immunocap/radioallergosorbent test (RAST) sustains the diagnosis. Differentiation has to be made between perennial and seasonal allergic disease when therapy is required (67).

Therapeutic Implications

Current treatment guidelines provided by the Allergic Rhinitis and its Impact on Asthma (ARIA) Task Force propose a stepwise approach with medical treatment and when intractable disease is faced, immunotherapy has to be considered. Only in extreme conditions of tissue remodeling adjunctive surgery might be considered as an ultimate step (64, 67).

Eosinophilic Airway Inflammation

Defining Characteristics

Eosinophilic upper airway pathology is an inflammatory disease based on a T2 response driven by an eosinophilic inflammation. Most of those patients show an adult-onset history mainly from 30 toward 50 years of age (68). This condition is mostly found in CRS patients with nasal polyps (CRSwNP) and is often characterized by eosinophilic inflammation with elevated levels of T2 cytokines (69, 70). The tissue eosinophilia in CRSwNP is frequently associated with extensive sinus disease (71), higher post-operative symptom scores (72), less improvement in both disease-specific and general quality of life (73), and

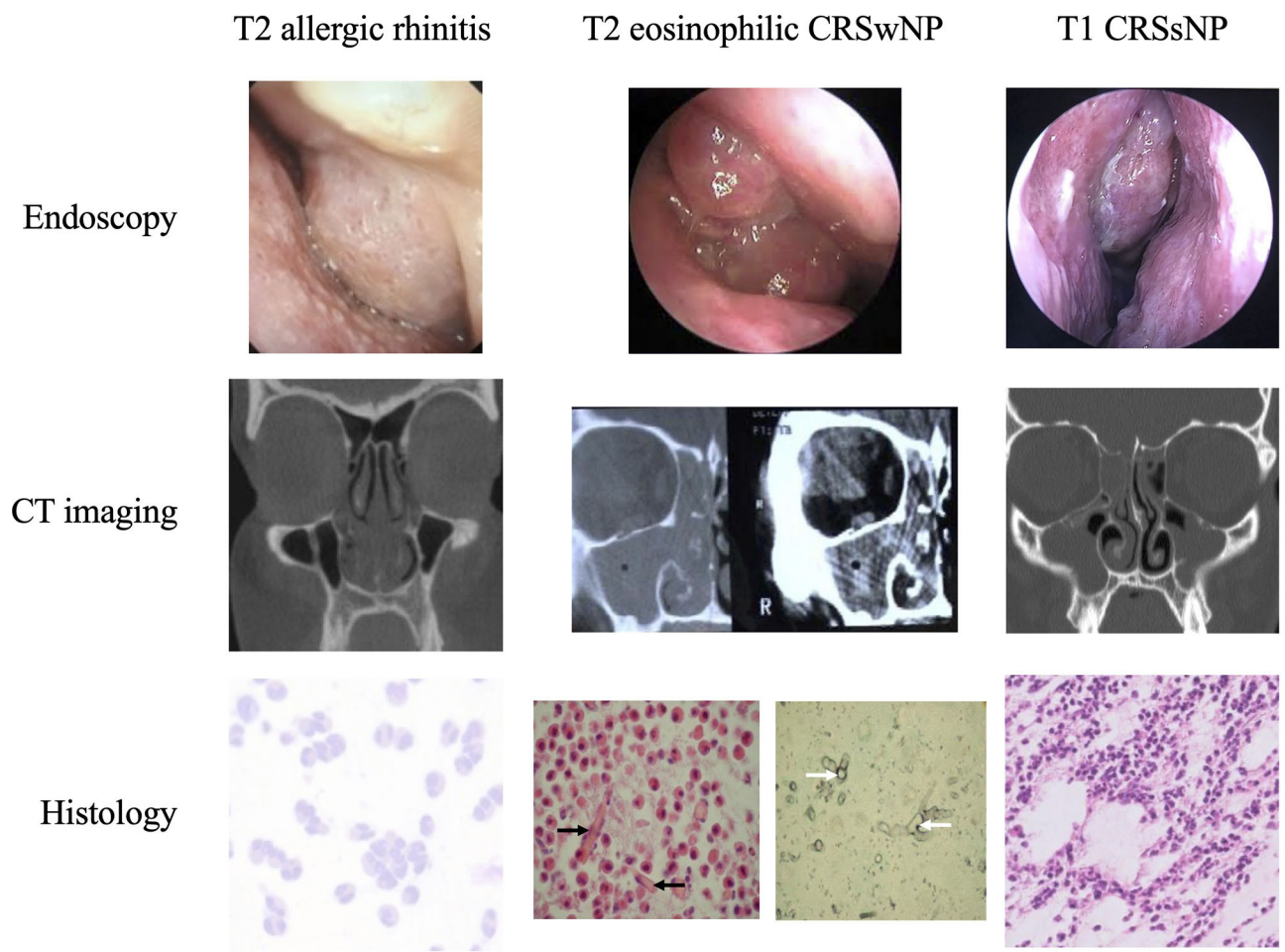

FIGURE 4 | Typical examples of endoscopic, CT and histologic (sinonasal secretions) images of the three main inflammatory sinonasal phenotypes. Images of type 2 (T2) allergic rhinitis show congestion of the nasal turbinates, normal aeration of the paranasal sinuses and mainly eosinophilic cells on a nasal smear. Images of T2 eosinophilic chronic rhinosinusitis with nasal polyps (CRSwNP) show the presence of nasal polyps on endoscopy, opacification of the paranasal sinuses on CT bone window and a hyperattenuation pattern on CT soft tissue setting. Corresponding histology findings are eosinophilic and necrotic patterns with Charcot-Leyden crystals (latter shown by black arrows) on hematoxilin/eosin staining, and fungal hyphae (white arrows) on silver staining. Images of T1 chronic rhinosinusitis without nasal polyps (CRSsNP) show opacification of the paranasal sinuses on CT and a mainly neutrophilic content in the nasal secretions.

a higher polyp recurrence rate (74–76). Moreover, CRSwNP patients with type 2 inflammation show a higher risk of late-onset asthma comorbidities, multimorbidity and recurrence of disease after surgery (75, 76). Noteworthy, a small number of CRS patients without nasal polyps (CRSsNP) may show a T2 signature and yield tissue and sinonasal secretions with eosinophilic infiltration (76). It is not clear if this subgroup holds the initial phase toward the development of nasal polyps. CRSwNP must be differentiated from other nasal polyp conditions like nasal choanal polyps, inverting papilloma and cystic fibrosis.

Endoscopy

At endoscopy patients will frequently present with small or already larger polyps typically protruding from the middle meatus. When attention is given the finding of tenacious eosinophil mucin may be encountered and collected on simple aspiration on consultation or at surgery. This eosinophil mucin helps further identification of a T2 bound inflammation on histopathology.

Radiology

Very often those patients show a pan-sinus opacification with evidence of secondary obstruction on CT imaging. Neo-osteogenesis changes are common even in the non-operated patient. On CT soft window imaging a central density in sinuses may be present compatible with thick mucin. On T2 weighted magnetic resonance imaging (MRI) those densities may appear as a signal void (77).

Histopathology

High eosinophilic blood levels yield a positive likelihood ratio (LR) of 3.28 to predict high tissue eosinophilia but the latter is

not significantly associated with serum allergen specific IgE (78). Few studies have investigated the level of mucosal eosinophil density required to meet the definition of tissue eosinophilia. Various eosinophil numbers per high power field (HPF) were used in different studies with cutoff values ranging from 5 to 350 eosinophils/HPF. A recent systematic review showed a cut off 55 eosinophils/HPF was likely to predict recurrence following surgical intervention (79). The existence of geographic, ethnic, and environmental differences suggest that specific cutoff values may be considered in different populations and regions (80). The Japanese Epidemiological Survey of Refractory eosinophilic CRS presented a new algorithm with diagnostic criteria based on scores comprising bilateral disease sites, nasal polyps, CT findings, and eosinophilia in peripheral blood. Reaching a score of 11 points was considered diagnostic for eosinophilic CRS. Significancy was reached with a cut-off value of more than 10% blood eosinophilia and tissue analysis with 70 eosinophils/HPF. Though the Japanese proportion is almost equal to that observed in Western countries <50% of polyps in Asian patients show tissue eosinophilia (81).

The analysis of sinonasal secretions is of growing interest, as these are easy to obtain in contrast with tissue sampling. Occurrence of eosinophil apoptosis (ETosis) is shown by the presence of eosinophilic free vesicles containing toxic proteins but also by the presence of CLC as a T2 eosinophilic hallmark (82). Recently, the finding of eosinophil rich mucin (ERM) in patients with T2 bound CRSwNP was proven to be a predictor for NP recurrence after surgery, the need for revision surgery and appearance of late onset asthma (56). Very recently the importance of CLC in secretions has been stressed by mouse models and the analysis and research aiming at the dissolution of crystals opens new horizons in human treatment facilities of pulmonary and sinonasal T2 inflammatory disease (57, 83).

Allergy

A large number of these patients show no allergy at all whereas in others IgE sensitization or even a multiallergen sensitization can be found. Mechanisms with local mucosal IgE generation inducing multi-allergen sensitivity have been described (33). Staphylococcus aureus might act as a superantigen in difficult to treat patients with nasal polyps and concomitant asthma (84). CRS patients exhibiting evident allergic reaction to fungi (e.g., a positive skin prick test and/or elevated specific IgE) can still be named allergic fungal rhinosinusitis (AFRS) as this term is commonly used according to EPOS 2020 (2). AFRS is considered a clinical subtype of CRSwNP based on an innate type 2 immune response. It is characterized by the presence of eosinophilic mucin with non-invasive fungal elements; typical imaging signs of CT hyperdensity and signal void on T2 MRI images may be present. The controversy is directed at the presence of at least one positive IgE-mediated allergy to one or more fungi. This IgE-bound inflammation rises the assumption of a possibly different endotype. Treatment is based on oral steroids and surgery with debridement of the sinuses. Good rationale exists for the use of biological agents targeting the eosinophilic inflammation or other type 2 responses (85–87).

Therapeutic Implications

Medical management has been outlined in the EPOS guidelines in a stepwise approach targeting the disease severity (1). The goal is to deliver anti-inflammatory medicine to the site of the disease with the least amount of side effects or systemic exposure. When exacerbations occur with limited burden of disease, intermittent short courses (2–3 weeks) of glucocorticosteroids (GCS) can be offered 2–3 times per year. Although medical management with intranasal and oral glucocorticosteroids has been shown to be effective in mild cases, the side effects of long-term use of GCS urges the need for surgical intervention (88). Some medical treatments of NP patients based on glucocorticoids and doxycyclin therapy show temporary success (89, 90).

Sinus surgery is performed in an attempt to control disease and improve the patients' symptoms and overall quality of life. Proposed surgical techniques vary from the least extensive polyp extraction to the most extensive nasalization procedures (91, 92). Due to the high recurrence rate in CRSwNP patients a tendency toward more extended approaches have been proposed for better access to the sinuses for more adequate local treatment and reducing the inflammatory load (91, 93). After the surgical creation of large open cavities the delivery of high-volume glucocorticoid nasal irrigations showed to be more effective vs. nasal spray in preventing endoscopic evidence of recurrence (94). Over decades, performing endoscopic surgery, it was generally accepted that stripping of the mucosa was to be avoided fearing scarring, chronic osteitis and non-functional mucosa (88). Nowadays a new concept is arising called reboot surgery based on the removal of all inflamed sinus mucosa for type 2 inflammatory CRSwNP (95).

Other therapeutic considerations are necessary as a significant number of patients continue to have upper and lower airway symptoms despite classic medical and surgical treatment. Humanized and fully human monoclonal antibodies (mAbs) such as anti-IgE, IL-5, and anti-IL-4 receptor α are increasingly used (96–98). Not reaching a 100% success rate and the high cost for long-term treatment urge the need for alternative products. Evaluation of biological treatments in CRSsNP patients with signs of type 2 inflammation will be crucial in the development, together with the further search for biomarkers to identify responders to those treatments (99).

The sinonasal outcome test (SNOT)-22 score is recommended as a useful tool in symptom severity scoring by also evaluating emotional and social consequences of the condition. The objectification of eventual individual improvement by medical therapy and/or surgery can be interesting for study purposes as well as in evaluating quality of life (QoL) repercussions (100).

Non-eosinophilic Airway Inflammation

Defining Characteristics

Patients with a non-eosinophilic airway inflammation may be considered as non-type 2 and are mainly characterized by neutrophils in their nasal mucosa (31, 70). These conditions are present in infectious rhinitis, CRSsNP and the Th17 pathway currently addressed now as type 3 immune response. In Asia, non-eosinophilic CRSwNP is frequently observed and is associated with relatively less edema and more fibrosis compared

with eosinophilic CRSwNP (50). Of note, the presence of a mixed Th17/Th2 inflammation in CRSwNP is possible as neutrophil-biased inflammation may be demonstrated in eosinophilic nasal polyps. The combination of high levels of type 2 inflammation mediators combined with high levels of type 1 or type 3 and/or neutrophilic markers seems to predict a more severe inflammatory burden (33). Neutrophilic inflammation can be triggered by infections or chronic irritation, environmental toxins, work conditions and air pollution. Very often tissue neutrophilia is not completely controlled by inhaled GCS (101, 102).

Endoscopy

Small polyps and polypoid edema may be seen in these patients. The aspect will rather show inspissated secretions but not like eosinophil mucin. Those thick discolored secretions can obstruct the sinus outflow tracts and cause retro-obstructive retention in the paranasal sinuses with purulent postnasal drip patterns.

Radiology

This type of neutrophilic inflammation cannot be distinguished from imaging of the eosinophilic type as sinuses can be diffusely involved.

Histopathology

Tissue neutrophilia is significantly higher although some eosinophils may be present. The presence of the non-T2 cytokines in the mucus is correlated with higher culture positivity and age (103). The analysis of sinonasal secretions may contribute to depicting the presence of an active neutrophil extracellular trap (NET) mechanism with the release of chromatin (NTosis) and granule proteins that bind and kill microorganisms (104–106). In contrast ETosis depicts typical free eosinophil granules (FEG) with regulated release of toxic proteins such as major basic protein (MBP) (107).

Allergy

Overall patients will have a negative skin prick and immunocap/RAST testing, and poor clinical evidence of allergen driven symptoms.

Therapeutic Implications

When medical therapy fails patients will benefit from having sinus surgery allowing for saline washings and local application of medical therapy. This type of patients may benefit from long-term low dose macrolide immunomodulation especially CRS patients with limited response to corticosteroids (108, 109).

United Airways

The united airway hypothesis links the entire upper and lower airways as an interconnected system sharing the same inflammatory responses. Although Brown demonstrated the importance of sputum eosinophils in relation to corticosteroid responsiveness in asthma in 1958 (110), the concept of asthma being heterogeneous has only recently gained traction with the advent of biologicals. The term "asthma" is currently considered an umbrella diagnosis for different disorders (endotypes) and phenotypes (e.g., allergic, obesity associated, aspirin-sensitive, fungal allergic, and elderly). It is characterized by reversible airflow obstruction and its main symptoms include wheezing, shortness of breath, cough and chest tightness (111). Asthma endotypes may be broadly regarded as type 2 high or T2-low (111), similarly to CRS. In this way, it follows the heterogeneity of chronic rhinosinusitis which has been known for a long time, probably because of ease of access of the upper airways for examination and investigation.

Most Western asthma patients (78%) have some form of upper airway disease, with similar levels of severity (112). In return, in a recent UK analysis the prevalence of asthma increased from control participants (10%) over CRSsNP (21%) to CRSwNP (47%) and AFRS (73%) (113). Typical associations are pollen asthma together with seasonal allergic rhinitis, and persistent rhinitis and chronic asthma. In one study 84% of severe asthma patients had sinonasal CT abnormalities. The latter correlated with eosinophils in peripheral blood and induced sputum, and with the level of exhaled NO. Sinonasal CT scores also related to lung function measurements: positively to functional residual capacity and inversely to diffusion capacity (114). Western eosinophilic CRS patients, with and without nasal polyps, frequently have asthma with shared histological and immunological features, characterized by an environment high in T2 cytokines (IL-4, IL-5, and IL-13) and in ILC2s (27, 115), suggesting a common immune process involving the upper and lower airways (116, 117). In contrast, T2-low asthma is characterized by neutrophilic (sputum neutrophils >40–60%) or paucigranulocytic (i.e., normal sputum levels of both eosinophils and neutrophils) inflammation and a lack of response to corticosteroid therapy. It has been linked to Th1 and/or Th17 cell activation and their imbalance may play a role in steroid-resistant, severe and neutrophilic asthma. The upper airway component of such conditions is not yet fully identified, and may differ geographically, since CRSwNP can be neutrophilic in the East (30). Absence of asthma may indicate a different pathophysiology, such as the co-existence of CRSsNPs and bronchiectasis, in which alpha 1 anti-trypsin should be measured.

Asthma may precede CRSwNP or parallel the sinonasal disease, however, it may also develop after CRS onset (56). The presence of asthma emphasizes the systemic nature of the underlying pathophysiology and suggests the need for consideration of the disease as a whole. CRS therapy, both medical and surgical, can improve asthma outcomes (118, 119). Further analysis of this relationship is required, with potentially positive effects on both upper and lower airway symptoms the use of specific monoclonal antibodies such as anti-IL5, anti-IL4/13 receptor, and anti-TSLP. The pulmonologist should be encouraged to take an interest in the upper airway, and the ENT surgeon in the lower, including history-taking, examination and specific testing. Good collaboration might help in further resolving the CRS and asthma endotypes, both in an individual patient and in general.

A specific phenotype in which asthma and CRSwNP co-occur in a triad together with hypersensitivity to acetylsalicylic acid, is non-steroidal anti-inflammatory drug (NSAID) exacerbated respiratory disease (N-ERD). It may be considered a type 2 dominated inflammatory airway disorder. N-ERD is slightly

more common in females and has an estimated prevalence of 9% in patients with asthma. The pathophysiology is considered an alteration of the cyclo-oxygenase (COX) pathway (2). The CRSwNP in N-ERD patients shows a higher rate of recurrence, and multiple sinus surgeries at younger age (120). Also asthma is more severe with more frequent exacerbations (118). Aspirin desensitization can be considered if an N-ERD patient has insufficient response to CRS treatment and/or insufficient control of asthma symptoms (121). However, discontinuation of this therapy hampers correct long-term evaluation and still questions its actual value (122). A recent retrospective study concluded that nasal polyp eosinophilia, the frequent need of oral corticosteroid courses and a history of recurrent CRSwNP surgery were consistent factors predicting uncontrolled N-ERD (123).

Outcomes

Real life data and studies may help in understanding the long-term expectations and need for close medical follow-up as we now understand in the CRSwNP phenotype. In a meta-analysis Loftus et al. found a long-term revision rate of ∼14 up to 24% (based on different follow-up periods) and retained important risk factors including AFS, AERD, asthma and prior surgery (124). Over a minimal 10-year follow-up Vlaminck et al. retained a revision rate of 26% (34 of 133) stressing the importance of eosinophilic mucin presence on asthma development or aggravation and nasal polyp recurrence (56). The Utah Population Database was queried for Current Procedural Terminology codes for ESS from 1996 to 2016 by Smith et al. reporting an overall revision rate of 30% (n = 9,177) over those 20 years (125). Also, the presence of comorbid asthma and allergy were significant predictors of revision surgery (125). In spite of the low number of patients Calus et al. reported a revision rate of 36.8% (14 of 38) over a 12-year period, finding comorbid allergic sensitization and tissue IL-5 levels to be significant predictors (126). The help of digital health technology might be considered as it becomes more apparent some clinical features might be associated with specific inflammatory endotype patterns (127).

SUMMARY AND OUTLOOK

CRS and its treatment are considerably better understood by improved understanding of the immune pathways behind various types of inflammation, in addition to clinical signs and symptoms of the disease. The ENT surgeon can investigate nasal secretions for eosinophilia as a guide to likely T2 inflammation and corticosteroid responsiveness. This is probably most useful in CRSsNP where it is an unexpected finding. Switching from an organ-based to a molecular-based classification in immune-mediated inflammatory diseases helps us to explain the involvement of different organs and the differences among diseases affecting the same organ. Therapeutic consequences are largely based on the responses to anti-cytokine monoclonal antibodies and might better address pathophysiological commonalities across these diseases. An approach based on signature cytokine hubs is likely to yield further insights into etiopathogenesis (128). The prediction of treatment still needs further research, and one of the challenges is co-operation to provide big data on CRS immunopathology related to various treatment outcomes. Patients themselves can participate using mobile data and a VAS score to simply evaluate their upper and lower airway symptoms and quality of life along with the therapy being used.

AUTHOR CONTRIBUTIONS

SV drafted the first version. FA and PG edited the manuscript significantly. GS and BL revised the work critically for important intellectual content. All authors made substantial contributions to the design of the work, provided approval for publication of the content, and agreed to be accountable for all aspects of the work.

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REVIEW
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Allergic Rhinitis: What Do We Know About Allergen-Specific Immunotherapy?

Tadech Boonpiyathad[1]*, *Mongkol Lao-Araya*[2], *Chirawat Chiewchalermsri*[3], *Sasipa Sangkanjanavanich*[4] and *Hideaki Morita*[5,6]

[1] Department of Medicine, Phramongkutklao Hospital, Bangkok, Thailand, [2] Faculty of Medicine, Department of Pediatrics, Chiang Mai University, Chiang Mai, Thailand, [3] Department of Medicine, Panyananthaphikkhu Chonprathan Medical Center, Srinakharinwirot University, Nonthaburi, Thailand, [4] Faculty of Medicine Ramathibodi Hospital, Department of Medicine, Mahidol University, Bangkok, Thailand, [5] Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Tokyo, Japan, [6] Allergy Center, National Center for Child Health and Development, Tokyo, Japan

Allergic rhinitis (AR) is an IgE-mediated disease that is characterized by Th2 joint inflammation. Allergen-specific immunotherapy (AIT) is indicated for AR when symptoms remain uncontrolled despite medication and allergen avoidance. AIT is considered to have been effective if it alleviated allergic symptoms, decreased medication use, improved the quality of life even after treatment cessation, and prevented the progression of AR to asthma and the onset of new sensitization. AIT can be administered subcutaneously or sublingually, and novel routes are still being developed, such as intra-lymphatically and epicutaneously. AIT aims at inducing allergen tolerance through modification of innate and adaptive immunologic responses. The main mechanism of AIT is control of type 2 inflammatory cells through induction of various functional regulatory cells such as regulatory T cells (Tregs), follicular T cells (Tfr), B cells (Bregs), dendritic cells (DCregs), innate lymphoid cells (IL-10$^+$ ILCs), and natural killer cells (NKregs). However, AIT has a number of disadvantages: the long treatment period required to achieve greater efficacy, high cost, systemic allergic reactions, and the absence of a biomarker for predicting treatment responders. Currently, adjunctive therapies, vaccine adjuvants, and novel vaccine technologies are being studied to overcome the problems associated with AIT. This review presents an updated overview of AIT, with a special focus on AR.

Keywords: allergic, rhinitis, immunotherapy, allergen-specific, immune tolerance

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Dichapong Kanjanawasee, Mahidol University, Thailand

***Correspondence:**
Tadech Boonpiyathad
tadechb@pmk.ac.th

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INTRODUCTION

Allergic rhinitis is a common upper airway disease. Its prevalence varies around the world. A good epidemiologic study reported that 20 to 30% of adults and up to 40% of children are affected (1). We recognize that allergic rhinitis (AR) has significant effects on the quality of life, sleep, and performance at work and school of patients. AR is not only a disease of the upper airway. It may also lead to inflammatory processes in the lower airways, which is supported by the fact that rhinitis and asthma frequently coexist (2). Allergies are characterized by dysregulated type 2 immunity and epithelial barriers that have increased concentrations of allergen-specific immunoglobulin (Ig) E (3, 4). Type 2 immune responses involve T helper (Th) 2 cells, IgE-producing B cells, group 2

innate lymphoid cells (ILC2s), and small fractions of interleukin (IL)-4-producing natural killer (NK) cells and NK-T cells, basophils, eosinophils, mast cells, and their cytokines (5). Emerging evidence suggests that follicular helper T (Tfh) cells, rather than Th2 cells, play a crucial role in controlling IgE production (6). Upregulation of Tfh cell activities, including a skewing toward type 2 Tfh cells and IL-13-producing Tfh phenotypes, and defects in follicular regulatory T cells (Tfr) have been recognized in patients with allergic diseases (6). Moreover, there is a complex network among type 2 cytokines (IL-4, IL-5, IL-9, and IL-13) which are secreted mainly from type 2 immune cells, and alarmins [IL-25, IL-33, and thymic stromal lymphopoietin (TSLP)] which are released from tissue cells, particularly epithelial cells (**Figure 1**).

Basic AR treatment consists of allergen avoidance, use of medications that provide symptomatic relief, anti-inflammatory therapies, and allergen-specific immunotherapy (AIT). At present, AIT is only disease-modifying, and it is aimed at improving allergen tolerance. AIT also changes the allergic immune response to one of immune tolerance, as in healthy individuals (7). AIT uses general mechanisms of immune tolerance to allergens to normalize allergen-specific T and B cells, regulation of IgE and IgG production, and modification of mast cells, basophil activation thresholds, and the phenotype of dendritic cells (DCs) (8). The main goals are maintaining regulatory T cells (Tregs), regulatory B cells (Bregs), and various other regulatory cells in order to suppress type 2 immune responses and allergic inflammation (**Figure 1**) (9). AIT showed efficacy in selected AR patients with HDM and birch or grass-pollen sensitization (10, 11). Substantial evidence supports the effectiveness of AIT for AR in reducing the symptoms and medication requirements, and its safety and cost-effectiveness (12). AIT applied in the early stage of allergic disease had an excellent preventive effect on disease progression to asthma, especially in young children (13). However, significant limiting factors for AIT were the long duration of treatment, cost, poor patient compliance, and severe life-threatening adverse reactions to the treatment (14). It is hoped that these disadvantages can be mitigated by developing non-allergenic, highly immunogenic allergen extracts, combined usage with novel adjuvant molecules, and new administration routes. Here, we review our current knowledge regarding AIT for AR. In addition, we update relevant topics on the use of AIT in AR that can help physicians in daily practice.

Abbreviations: AAMs, alternatively–activated macrophages; AIT, Allergen-specific immunotherapy; APCs, Antigen-presenting cells; AR, Allergic rhinitis; Bregs, Regulatory B cells; CCL, C-C Motif Chemokine Ligand; DCregs, Regulatory dendritic cells; DCs, Dendritic cells; EPIT, Epicutaneous immunotherapy; FAB, Facilitated allergen binding; HDM, House dust mite; IFN-γ, Interferon-γ; Ig, Immunoglobulin; IL, Interleukin; ILCs, Innate lymphoid cells; ILIT, Intra-lymphatic immunotherapy; NK, Natural killer; PBMCs, Peripheral blood mononuclear cells; PGD2, Prostaglandin D2; SCIT, Subcutaneous immunotherapy; SLIT, Sublingual immunotherapy; TGF-β, Transforming growth factor-β; Tfh, Follicular helper T; Th, T helper; Tregs, Regulatory T cells; TSLP, Thymic stromal lymphopoietin.

THE CELLULAR IMMUNE RESPONSE FOLLOWING AIT

Since AIT acts in an antigen-specific manner, modulation of antigen-specific immune cells, including T and B cells, was thought to be its primary mode of action. However, recent findings suggest that AIT also modulates non-antigen-specific immune cells, including ILCs, monocytes/macrophages, NKs, and DCs. These effects may also contribute to the improvement of symptoms after AIT.

T Cells

AIT induces $FOXP3^+$ and $IL\text{-}10^+$ Treg cells (Tregs), which prevent and inhibit allergic inflammation by expressing their immunosuppressive functions at different levels (15). There are 4 types of the suppressive mechanism used by Tregs: (1) via suppressive cytokines, IL-10, IL-35, and transforming growth factor-β (TGF-β) secretion, (2) disruption of metabolic pathways via CD25, cAMP, adenosine receptor 2, histamine receptor 2 (HR2), CD39, and CD73, (3) suppression of DC activation by membrane-bound molecules, programmed death 1 (PD-1), and cytotoxic T lymphocyte antigen 4 (CTLA-4), and (4) cytolysis (granzymes A, B, and K) (16, 17). IL-10–producing Tregs suppress Th2 type immune responses (IL-4, IL-5, IL-9, and IL-13) and IL-17–producing Th cells (18, 19). Moreover, AIT can inhibit $CD45RB^{low}CD27^-CRTH2^+CD161^+CD49d^+$ T cells (Th2A) and $IL\text{-}21^+$ Tfh cells (20, 21). On the other hand, AIT promotes IL-22– and interferon-γ (IFN-γ)–producing Th cells (22, 23). AIT also involves upregulation of activated Tregs ($FOXP3^+Helios^+CD25^+CD127^-$) and downregulation of dysfunctional Tregs ($ILT3^+CD25^+$ and $FOXP3^+SATB1^+$) (23, 24). Recent studies revealed that AIT improved dysfunctional Tfr ($CD45RA^{low}CXCR5^{high}FOXP3^+$) and reduced type 2 Tfh cells that contributed to aberrant IgE production (25–27).

B Cells

Patients responding to AIT are characterized by the increase of IgA, IgD, IgG2 and IgG4-positive allergen-specific B cells, plasmablasts, and IL-10 or IL-1RA-positive Bregs (28–30). IL-10 suppresses IgE production and augments IgG4-producing class-switched B cells (31). IgG4 blocks IgE antibodies by mopping up free allergen, and IgE fails to trigger Fc receptors. Moreover, IgG4 prevents mast–cell activation through FcγIII. AIT also enhanced local allergen-specific IgA1 and IgA2 in patients with grass–pollen allergy (32, 33). Thus, secretory IgA provides protection by blocking allergens absorbed into the mucosa. AIT induces allergen-specific IgD, and a recent study demonstrated that IgD constrains IgE-mediated basophil degranulation (34). Interestingly, a study in patients with grass-pollen subcutaneous immunotherapy (SCIT) found that AIT could induce nasal IgG4 levels, and blocking activity correlated with the clinical response (35).

Innate Lymphoid Cells

Innate lymphoid cells were recently identified as innate-type immune cells with no antigen receptors, meaning that they are not directly activated by antigens (36). ILCs were activated by

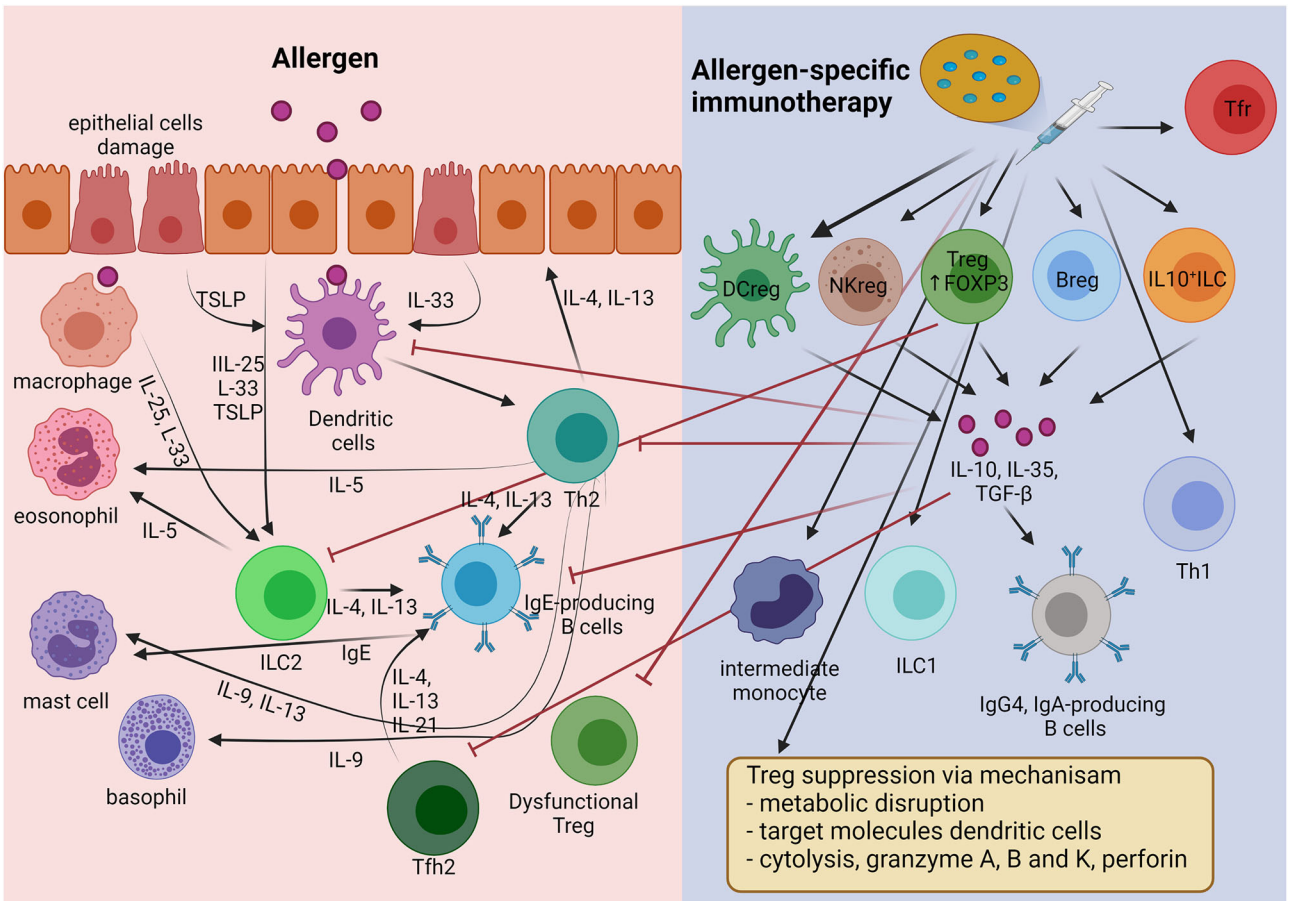

FIGURE 1 | The mechanism of immune tolerance to allergen induces by allergen-specific immunotherapy (AIT). AIT principally induces regulatory cells including Treg, Breg, Tfr, DCreg, NKreg, and IL-10^{+} ILC cells. Treg cells apply four main mechanisms for suppressing inflammatory cells (inhibitory cytokines, cytolysis, metabolic disruption, and targeting DCs). In addition, the regulatory cells produce IL-10 to suppress the type 2 inflammatory cells involved in allergic inflammation, such as Th2, Tfh2, IgE-producing B cells, and ILC2s. Moreover, AIT induces allergen-specific immunoglobulin class-switch, promoting IgG4 and IgA.

various cytokines, neuropeptides, and lipid mediators produced by surrounding cells (37). ILCs were initially divided into three different subsets that resemble Th cell subsets based on the transcription factors and cytokines they produced. Among them, ILC2s resembling Th2 cells were involved in the pathophysiology of various allergic diseases, including asthma and AR, through the production of type 2 cytokines (38). Indeed, the frequency of ILC2s in peripheral blood of seasonal AR patients was increased during the season compared to healthy individuals (39, 40). Local allergen provocation in patients with AR induced accumulation of ILC2s in the nasal tissue, accompanied by increased levels of prostaglandin D2 (PGD2), and IL-5 in the nasal lining fluid (41). These findings suggested that allergen exposure indirectly induces migration and activation of ILC2s through PGD2 synthesis by activated mast cells. AIT reduced the seasonal increase in ILC2s in peripheral blood of patients with seasonal AR (39). Likewise, AIT reduced the frequency of ILC2s in peripheral blood of patients with house dust mite (HDM) AR (42, 43).

Recently, ILCs that produce IL-10 were identified in tissues of both humans (44–46) and mice (44, 47–50). Such cells were rarely detected in the tissues of both humans and mice at a steady state (44, 50). However, they were increased in tissues with type 2 inflammation, such as the nasal tissues of patients with chronic rhinosinusitis with nasal polyps (44), and in the lungs of a murine asthma model (44, 47–50). IL-10-producing ILCs were shown to be converted from ILC2s upon IL-33 and retinoic acid stimulation *in vitro* (44, 46, 47), and they are now considered to be inducible cell types rather than residential cell types. Intriguingly, AIT induced IL-10-producing ILCs in the peripheral blood of patients with HDM (45) and grass-pollen AR (46), and the frequency of those cells correlated with the improvement in the symptom score. These findings suggest that induction of IL-10-producing ILCs is also involved in the mechanisms of AIT. Furthermore, IL-10-producing ILCs were shown to suppress proliferation of ILC2s and T cells through IL-10 and to protect against disruption of epithelial barrier integrity by allergen exposure (44, 46). In murine asthma models,

IL-10-producing ILCs reportedly exhibited an exhausted-like phenotype with reduced capacity for type 2 cytokine production (48, 51). However, the mechanisms underlying the induction of AIT of IL-10-producing ILCs remain unclear.

Dendritic Cells

Dendritic cells are crucial antigen-presenting cells that direct immune responses toward either inducing inflammation or tolerance and are considered to be heterogeneous, both phenotypically and functionally (52). Among them, tolerogenic DCs (tDCs) induce tolerance through various mechanisms, including induction of Tregs (53). Since tDCs are also heterogeneous and may exhibit different phenotypes depending on the organ, the characteristics of tDCs that may be induced by AIT remain unclear. However, some markers related to tDCs, including *complement component 1* and *stabilin*, were upregulated in peripheral blood mononuclear cells (PBMCs) from grass-pollen allergy patients after 4 months of AIT (54), suggesting that induction of tDCs may play a role in AIT. Regulation of DC activation is a key mediated immune response to allergens. Therefore, patients with allergic disease display a tendency to produce fewer tolerogenic IL-10-producing DCs (55). Furthermore, AIT enhanced regulatory dendritic cells (DCregs) and type 1 DCs (DC1s), while decreasing DC2s and DC17s in responder AIT patients (56). Plasmacytoid DCs (pDCs), which play a crucial role in immunity against viral infections, were suggested to be involved in the mechanisms of AIT. Eljaszewicz et al. reported an increase in pDCs and $CD141^+$ myeloid DCs in individuals with allergies (43). In contrast, the number of $CD1c^+$ myeloid DCs in patients with AR decreased during the first year of AIT (43). Also, pDCs in peripheral blood were found to be decreased in number after AIT (57, 58).

Macrophages

Macrophages are heterogenous phagocytic cells that play a vital role in innate immunity and are significant contributors to the adaptive immune system. Macrophages activated by Th1 cells are identified as M1 macrophages, while those activated by IL-4 and IL-13 are named alternatively activated macrophages (AAMs) or M2 cells (59). M2 macrophages can produce IL-4 and IL-13, and IL-10 and TGF-β in response to specific stimulators. M2a cells activate Th2 cells via IL-4 and IL-13 production mediated by C-C Motif chemokine ligand (CCL) 17 and mannose receptor C-Type 1 (MRC1), leading to the development of allergic asthma (60). M2b cells activate Tregs via IL-10 and TGF-β production mediated by CCL24 and MRC1, leading to allergic tolerance and deceased inflammation (60). However, the roles of M2 macrophages in AIT need to be further investigated.

Monocytes

Circulating monocytes are also known to be heterogeneous and include 3 distinct subsets: classical monocytes ($CD14^{++}CD16^-$), intermediate monocytes ($CD14^{++}CD16^+$), and non-classical monocytes ($CD14^+CD16^{++}$). Non-classical monocytes are considered to be proinflammatory cells that produce large amounts of TNF-α. The frequency of non-classical monocytes in peripheral blood was decreased after 3 months of AIT and was more pronounced after 6 months. On the other hand, intermediate monocytes, thought to have anti-inflammatory properties, were increased after 1 year of AIT (61). Sousa et al. also found that AIT enhanced circulating $CD16^+$ monocytes (57).

NK Cells

Natural killer cells can differentiate into 2 distinct functional subsets: NK1 or NK2 cells, which are analogous to the T-cell subsets Th1 or Th2 (62). Moreover, TGF-β and IL-10-secreting NKreg cells might have a role in the immune regulation of allergic inflammation. For example, IL-10-producing NKreg cells significantly suppressed both allergen or antigen-induced T-cell proliferation, IL-13 and IFN-γ-secreting T cells, and reduced IgE production (62, 63). However, a recent study observed no changes in the frequency of NK cells in patients undergoing AIT (43).

ADMINISTRATION ROUTES

Subcutaneous

Until recently, subcutaneous delivery (SCIT) was the standard administration route for AIT (64). The conventional schedule for SCIT using allergen extracts consists of dose build-up by once-weekly injection, followed by maintenance dose injections at 4–8-week intervals, continued for at least 3–5 years (65). The build-up phase can be shortened by following cluster or rush protocols to help the patients reach maintenance (66). In the cluster protocol, multiple injections are given on non-consecutive days. In contrast, in the rush protocol, multiple injections are given on consecutive days, reaching the maintenance phase in few days, but this increases the risk of anaphylaxis (67). Therefore, the accelerated protocols should be applied only in specialized centers.

Sublingual

Sublingual immunotherapy involves administering allergens under the tongue, generally daily. Sublingual immunotherapy (SLIT) is administered *via* liquid drops or as freeze-dried, lyophilized, or film-coated tablets. SLIT tablets contain a single allergen, whereas SLIT drops often contain multiple allergens for the treatment of poly-sensitization (68). At present, SLIT is widely used to treat HDM, and grass and tree-pollen allergies. Also, SLIT can be safely and effectively performed at home, and it does not require a build-up phase (69). The oral mucosa and regional lymph nodes form an elaborate immunological network, which is an essential prerequisite for SLIT. The network includes local antigen-presenting cells (APCs), such as Langerhans cells in the epithelium, and oral dendritic cells (DCs) with the $CD103C^-CD11b^+$ phenotype and macrophages in the lamina propia (**Figure 2**) (70). Oral DCs transport sublingual antigens to the submandibular lymph nodes and induce antigen-specific Tregs. In addition, SLIT induces mucosal and serum-specific-IgA responses, which may contribute significantly to tolerance induction (32, 71). A clear difference between SCIT and SLIT is the effective dosing range of allergen management. SCIT uses a narrow effective dosing range of 5–25 μg of allergen per injection

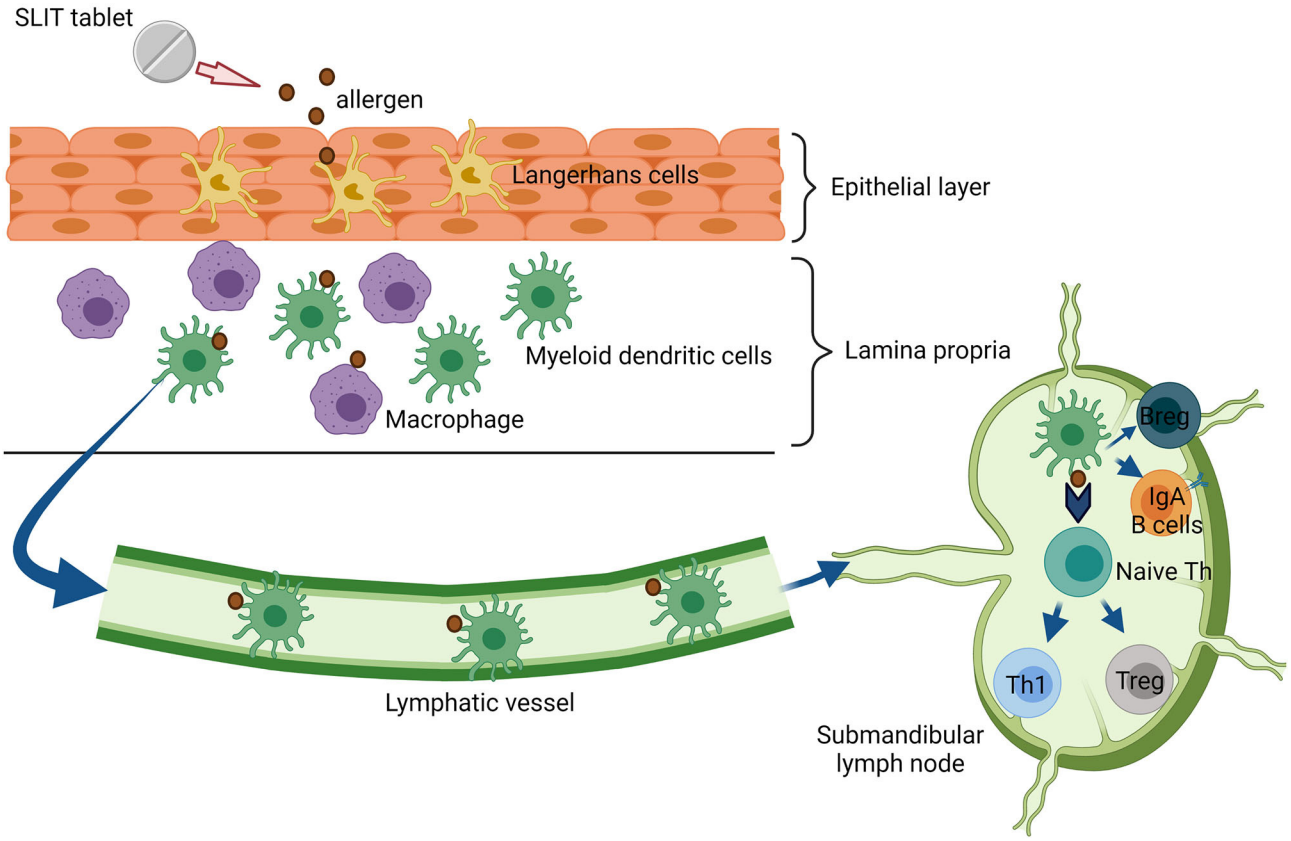

FIGURE 2 | Immunologic mechanism of sublingual immunotherapy (SLIT). Substantial amounts of allergens from SLIT-tablet are capture by Langerhans cells in the epithelial layer. Next, the allergen is processed and migrated transmucosal by myeloid dendritic cells (mDCs) to draining lymph nodes. In the lymph node, these cells interact with T and B cell priming to regulate immune tolerance.

for many allergens, whereas SLIT requires at least 50–100 times more allergen than SCIT to achieve a similar level of efficacy (72). Therefore, long-term compliance with SLIT might be a concern. However, a recent study in Denmark reported similar 1-year compliance of ∼50% with both SCIT and SLIT (73).

Intra-Lymphatic

Intra-lymphatic immunotherapy (ILIT) is the direct intra-lymphatic injection of allergens. ILIT improves the efficiency of AIT by reducing the number of treatment applications and the treatment duration, achieving good compliance and fast symptom alleviation, and showing exemplary safety (74, 75). ILIT requires only three ultrasound-guided injections of a low allergen dose into the inguinal lymph nodes at 1-month intervals (76). The cumulative allergen dose can be reduced 1,000-fold compared to SCIT (77). The disadvantage of ILIT is the requirement for experienced staff for injection under ultrasound guidance.

Epicutaneous

Epicutaneous immunotherapy is a novel therapy that is currently being investigated. Epicutaneous immunotherapy (EPIT) delivers allergens via repeated applications to the skin and APCs in the superficial skin layers (78). Innovative epidermal allergen powder delivery technologies include electronic spreading, ablative fractional laser, and microneedle arrays (79). By targeting epidermal Langerhans cells, but not mast cells or the vasculature, EPIT can reduce both local and systemic adverse effects (80). The following advantages have been noted for EPIT: (1) a high safety profile due to allergen application into the non-vascularized epidermis and subsequent allergen delivery to the less–vascularized dermis, (2) increased convenience for patients due to the non-invasive (needle-free) and self-administrable application method, likely leading to improved compliance, (3) absence of additional potential irritant constituents (e.g., alum, preservatives), and (4) less cost–intensive than conventional AIT (81). Several clinical trials in AR patients used EPIT to deliver allergens of grass and birch pollen (82–85). The patch application time ranges from 8 to 48 h (86–89). EPIT might induce desensitization in patients with pollen sensitization, although at increased risk of local adverse events. However, more data are needed regarding patients with AR and indoor allergen sensitization.

Local Nasal

Local nasal immunotherapy has been extensively investigated in the past 40 years and seems to be effective only on rhinitis symptoms. However, local nasal immunotherapy (LNIT) is not

popular with patients due to local side effects that require topical nasal premedication for their prevention and difficulty of application (90). Currently, LNIT is not recommended for clinical use.

BIOMARKERS

Allergen-specific immunotherapy is considered a precision medicine model for treating allergic diseases because of its individualized approach to treatment based on clinical and immunological profiles of each patient (91). Biomarkers are measurable indicators linking an underlying pathway to the phenotype or endotype of a disease. Identification of specific biomarkers that can identify responders, monitor treatment, predict the durability of therapeutic effects, and determine adverse event risk would aid clinical decisions and the delivery of targeted and effective treatments (91, 92). New potential biomarkers have been discovered with the emergence of advanced immunological, data-driven "-omic," and molecular technologies (93, 94). Here, we briefly review a number of promising candidates that are being evaluated for AIT immune monitoring in the context of clinical trials as well as in real-world clinical settings (**Table 1**).

Specific Immunoglobulins and Their Inhibitory Activity

Measurement of IgE is the first step in the diagnosis of atopic diseases. At present, detection of sIgE, either through measurement in serum or by an *in-vivo* skin prick test, and the manifestation of symptoms on exposure to the sensitizing allergen is the only criteria for allergy diagnosis and starting AIT (95). Several studies showed that the allergen-specific IgE (sIgE) and total IgE (tIgE) levels increase transiently during the initial stages of AIT but then return to their pre-treatment levels during the maintenance phase (96, 97). These trends seem to vary primarily with the duration of AIT and the time of sampling. A slow decrease in those levels may not be accompanied by a favorable clinical outcome (91). Several studies reported that the sIgE/tIgE ratio before AIT might predict the ultimate efficacy of AIT (91, 98, 99). A retrospective study in patients who underwent grass-pollen or HDM SCIT or SLIT found that the clinical response to AIT correlated significantly with the initial sIgE/tIgE ratio ($r = 0.723$, $p < 0.0001$). The sensitivity and specificity of the decision point for a serum sIgE/tIgE ratio of >16.2% were 97.2 and 88.1%, respectively (98). Others also found a similar correlation between the ratio and AIT outcome, but a small randomized controlled open-label study could not replicate those results (99).

Numerous studies have found that the IgG1 and IgG4 levels increase during AIT. Allergen-specific IgG4 (sIgG4) can compete with sIgE for allergen binding, thereby blocking allergen-IgE complex formation and preventing mast cell and basophil degranulation, IgE-dependent cytokine secretion from mast cells, binding of allergen to B-cell receptors on IgE^+ memory B cells, and allergen presentation to T cells (100). A correlation between allergen sIgG4 and clinical outcomes has been reported in some but not all studies (91). Furthermore, sIgG4 levels do not always differentiate between responders and non-responders (101). Thus, an IgG4 increase during AIT may reflect compliance, not clinical efficacy. The absence of sIgG4 induction may also be indicative of poor compliance (91). The sIgG4/IgE ratio may monitor AIT progress and outcome, but it has shown inconsistent utility (102, 103). Intriguingly, sIgG4 fell back to its pre-treatment level within 1 year after discontinuation of AIT, but its inhibitory capacity for serum IgE persisted for several years, together with clinical benefits (104). That suggests that sIgG4 might have either higher avidity or higher affinity (105). Besides sIgG4, allergen-specific IgA (sIgA) is also induced during grass-pollen SLIT and HDM SLIT. sIgA and other subclasses of IgG may have a similar blocking function (97). There are only limited data regarding the roles of other IgG subsets, i.e., IgD and IgA, in serum.

IgE-facilitated allergen binding (IgE-FAB) is a highly reproducible flow cytometry-based bioassay that was developed to detect binding of allergen-IgE complexes to B cells that express surface low-affinity IgE receptor FcεRII (CD23). This bioassay is used to determine the antigen-presenting capacity of B cells to T cells (106). It has been developed as a surrogate for determining IgE-inhibitory activity during AIT (91). In addition to sIgG4, which is responsible for serum inhibition of IgE, there may be other factors that support serum inhibition of IgE because IgG4-depleted serum retained its blocking activity (107). These factors need further study. It was found that serum inhibitory activity determined by IgE-FAB showed potential to predict the clinical response (107). They were reported that changes from the baseline of IgE-FAB at the initiation of the maintenance phase and persist at least 1 year after AIT discontinuation associated with clinical manifestation (104). Inverse correlations were found between the symptom score, the rescue medication score, and the IgE-FAB result (35). These findings suggest that the serum inhibitory activity for IgE could predict the final efficacy of AIT as early as at the start of the maintenance phase of (105). To date, no data are available on the association between the initial level of serum inhibitory activity for IgE-FAB and responsiveness to AIT (91). An alternative test is the enzyme-linked immunosorbent-facilitated antigen binding (ELIFAB) assay, which follows the basic principles of a standard ELISA protocol and is able to detect the inhibitory activity for IgE after AIT (108). Although the IgE-FAB and ELIFAB techniques show good clinical efficacy correlation for AIT, they are both complicated, and their use is limited to specialized laboratories (91, 92).

In addition, the sIgG subclass and sIgA levels can be detected in the nasal lavage of allergic patients (109). An increase in the IgG4 level was significantly associated with reduced nasal sensitivity. A study of grass-pollen AIT patients demonstrated that the nasal sIgG4 level increased during the pollen season. The inhibitory activity for IgE-FAB of the nasal fluid and serum were significantly increased in the SCIT group and correlated with the total symptom improvement, indicating that sIgG4 produced locally in the nasal mucosa can be a potential biomarker for AIT efficacy (35, 110). Moreover, a recent study compared the nasal and systemic grass-pollen sIgG4, sIgA1, and sIgA2 responses

TABLE 1 | The implementation of biomarkers in allergen-specific immunotherapy (AIT).

Biomarkers	Assay	Advantages	Disadvantages
IgE	Total IgE Specific IgE (serum/body fluid)	Serum sIgE is a gold standard of patient selection for AIT. Baseline sIgE/tIgE ratio may be a potential positive predictive marker for AIT.	The relationship sIgE/tIgE ratio and clinical outcome has been inconsistent.
IgG4	Specific IgG4 (serum/body fluid)	Elevation in serum sIgG4 is an indicator for compliance.	sIgG4 may not be related with clinical outcomes.
Inhibitory activity	IgE-FAB (serum/body fluid) ELIFAB	An association between serum inhibition activity and combined symptom-medication scores has been demonstrated.	Serum inhibition activity has restricted the availability requirement of specialized techniques.
Basophil activation	Basophil activation test via flow cytometry	*Ex vivo* test reflects the *in vivo* allergen-sensitized response.	The results are variable with inhibition being shown in some but not all studies. Standardized and optimized assays are needed.
Cytokines and chemokines	Serum/body fluid/*in vitro* cell culture-based by ELISA or Luminex	Serum and local cytokines and chemokines may be useful for exploring mechanisms of AIT and proof of concept at drug development.	Serum and local cytokines are at a low level in concentration.
Cellular markers	Immunophenotyping in *ex vivo* or *in vitro* activating cells, or tissue biopsy	Change in multiple cell subsets may be useful for exploring mechanisms of AIT.	There is not sufficient information to link the presence or function of cell subsets with clinical efficacy. The standardization for the identification of most cell types is deficient.
Clinical biomarkers	Allergen provocation test	Provocation tests have been used as surrogate markers to diagnose local allergic rhinitis and evaluate clinical response to AIT.	Allergen provocation cannot replace natural exposure in phase III clinical trials.

during 2 years of SCIT and SLIT and 1 year after treatment discontinuation. Production of sIgA was shown to be a major biological difference between SLIT and SCIT. Although SCIT induced higher specific sIgG4 levels than SLIT, SLIT led to higher sIgA levels both in serum and nasal fluid. The level of sIgA1 in nasal fluid correlated with the suppression of nasal symptoms of SLIT during nasal allergen challenge. sIgA production may therefore represent a distinct mechanism by which SLIT achieves its therapeutic effects (32).

As stated in the European Academy of Allergy and Clinical Immunology (EAACI) Position Paper, serum-based biomarkers are beneficial for selecting patients for AIT. An elevated sIgE/tIgE ratio is a potential positive predictive marker for AIT. The sIgG4 level is proposed to be an indicator of compliance of patients, but it shows no association with the efficacy of AIT. Serum inhibitory activity for IgE, determined by IgE-FAB rather than the level of sIgG4, might be associated with the symptom and rescue medication scores and predictive of the clinical outcome (91).

Basophil Activation

To determine allergen sensitization, basophils are incubated with a specific allergen, followed by an examination for degranulation. Activation of basophils leads to upregulation of surface markers, which is indicative of sIgE functional activity. Several surface markers indicate basophil responsiveness and histamine release, i.e., CD63, CD203c, CD13, CD107a, and CD164. Intracellular histamine-binding fluorochrome-labeled diamine oxidase can be quantified by flow cytometry. Blocking antibodies, such as sIgG4, are augmented during AIT. They inhibit cross-linking of allergens to sIgE bound to the surface of basophils and hereby suppress basophil activation (111).

The findings regarding basophil activation during AIT in placebo-controlled trials are inconsistent (91). Some studies describe reduced basophil activation after AIT with the decline correlating with clinical score improvement (112, 113), while others failed to show suppression (114). One study found no significant changes in basophil activation after SLIT, despite induction of sIgG4 (114). These contrasting findings may be explained by differences in the immunotherapy route, with SLIT possibly being less effective than SCIT in inhibiting basophils. Also, the methods used to measure the markers of basophil activation may alter the outcome (91).

Cytokines and Chemokines

The mechanism of induction of immunological tolerance by AIT is the redirection of the Th2 phenotype toward a Th1 and Treg phenotype. One would anticipate decreases in Th2 cytokines (e.g., IL-4, IL-9 13, IL-19) and chemokines (e.g., eotaxin), and upregulation of Th1 (e.g., IFN-γ) and regulatory cytokines (e.g., TGFβ, IL-10) (100, 105). However, serum cytokine measurement is difficult due to their low levels, which are often below the limit of detection of current methods. Furthermore, relationships between serum cytokines and the clinical outcome of AIT have not been elucidated (91). Shifts in cytokine production by $CD4^+$ T cells following AIT are quantifiable through *in vitro* stimulation of PBMCs from patients by treating them with allergen extracts at both the protein and the transcript levels (92). High levels of IL-10 transcripts in T cells of patients with HDM allergy predicted the success of AIT (115).

Local rather than serum levels of cytokines may be predictive of the clinical efficacy of AIT. Local cytokine production

following nasal allergen challenge may be an important treatment-related indicator (91). A cross-sectional study found lower concentrations of Th2 cytokines and chemokines in the nasal fluid after nasal allergen challenge following successful AIT compared to untreated controls (110). In a double-blinded randomized controlled trial, both SCIT and SLIT led to a decrease in Th2 cytokines, including IL-4, IL-5, and IL-13 in the nasal fluid after nasal allergen provocation after 2 years of continuous AIT, and those changes were associated with improvement in the clinical symptoms (19). At this stage, local and systemic cytokines, and chemokines are not practical as biomarkers in clinical practice. However, nasal cytokines can serve as markers of the immunological response and be used for proof of concept in drug development (91).

Cellular Markers

Tregs play a key role in immune tolerance to an allergen after AIT (100, 105). There are two main types of Tregs, i.e., natural regulatory T cells (nTregs) that express $FOXP3^+$ $CD4^+$ $CD25^+$, and inducible Treg cells (iTregs) generated in the periphery under different tolerogenic conditions that produce regulatory cytokines such as IL-10 and TGF-β. Different studies have shown the immunomodulating properties of both allergen-specific nTregs and iTregs in blood and tissues after SCIT and SLIT, suggesting that there is a commonality between these subgroups of Tregs (91). In AIT, initiation of peripheral T-cell tolerance presents anti-inflammatory cytokines IL-10 and TGF-β. An increased number of IL-10-expressing T cells during pollen season and a seasonal increase in $TGF\beta^+$ T cells correlated, respectively, with an increase in the serum IgG4 level and an increase in the peripheral circulating IgA concentration (116). Upregulation of activated allergen-specific Tregs (Der p1-specific $FOXP3^+$ $Helios^+IL\text{-}10^+$ Tregs) and downregulation of a dysfunctional allergen-specific Treg cell subset ($ILT3^+$ Tregs), associated with improved clinical response, were recently described in a study of HDM-SCIT treated patients (29). Identification of cell subsets, proteins, transcripts, and epigenetic biomarkers may suggest the prognosis. A recent randomized controlled study investigated epigenetic modification in the FOXP3 promoter region and found that methylated CpG sites within the FOXP3 locus of enriched peripheral memory Treg cells were reduced after SLIT treatment, leading to immune tolerance (117).

A novel effector subgroup of Tregs, i.e., Tfr cells, was recently identified. Tfr cells can suppress Tfh cell-mediated B-cell activation and antibody production (17). Recent evidence shows that AIT modulates the balance between circulating Tfh and Tfr, with Tfr as a potential biomarker for AIT efficacy (25, 27). A study showed increased numbers of circulating Tfr cells, with improved suppressive function, in AR patients after HDM SCIT (23). Therefore, a better understanding of Tfr cells will help in the development of novel strategies for AIT.

The involvement of B cells in allergen tolerance is mainly through regulatory B cells (Bregs). Bregs are a subset of B cells that have immunosuppressive and anti-inflammatory properties, predominantly via the release of IL-10, auxiliary Treg differentiation, IgG4 production, and inhibition of the inflammatory responses facilitated by T cells and DCs (118). IL-10-producing Bregs have been isolated from bee venom-tolerant subjects, and they suppress the proliferation of bee venom-specific T cells. Bee-venom immunotherapy (VIT) increases the number of phospholipase A2 (PLA)-specific IL-10-producing Bregs to a level comparable to in healthy beekeepers. Interestingly, both groups have high levels of PLA-specific IgG4-switched memory B cells, plasmablasts, and PLA-specific CCR5-expressing B cells (7). Recently, a study of grass-pollen SCIT showed an increase in the number of $IL\text{-}10^+$ Bregs that was associated with an increase in the sIgG4 level in the nasal fluid (35). Furthermore, successful HDM SCIT-treated patients were shown to have heightened frequencies of IgA and IgG4-expressing allergen-specific B cells, plasmablasts, and $IL\text{-}10^+$ and/or $IL\text{-}1RA^+$ Bregs (29).

Growing evidence has proven the role of innate immunity in allergic diseases, and there is a heightened focus on how AIT alters ILC2s to induce tolerance. Peripheral ILC2s were suppressed by grass-pollen SCIT. The level of ILC2s correlated with the severity of self-reported symptoms during the pollen season (39). Likewise, ILC2s in the peripheral blood of SCIT-treated, HDM-allergic AR patients were reduced compared with the untreated group (42). More recently, a subset of ILC2s able to produce the regulatory cytokine IL-10 was described (43, 45), and they attenuated Th responses and maintained epithelial cell integrity. $IL\text{-}10^+KLRG1^+$ ILC2s were fewer in patients with grass-pollen allergy compared to healthy subjects. The ability of ILC2s to produce IL-10 was restored in patients who underwent grass-pollen SLIT. Moreover, symptom severity correlated inversely with the number of IL-10-producing ILC2s after immunotherapy (46).

Dendritic cells are specialized antigen-presenting cells with the ability to integrate a variety of incoming signals and subsequently orchestrate adaptive immune responses. Molecular markers associated with polarized monocyte derived DCs that support the differentiation of either effector Th1, Th2, Th17, or regulatory $CD4^+$ T cells (termed DC1s, DC2s, DC17s, and DCregs, respectively) have been identified by comparative transcriptomic and proteomic analyses (91). AIT modulates DCs by up-regulation of DCreg markers and down-regulation of DC2 markers (54). There was also a significant increase in DCs with the DCreg phenotype [assessed by mRNA expression of stabilin-1 and complement component 1Q (C1Q)], with enhanced capacity to generate IL-10 with diminished IL-12 in peripheral blood samples from responders to SLIT (54).

At this stage, no cellular biomarker can serve as a biomarker for monitoring AIT in clinical practice. However, biomarkers may be valuable as indicators of immunological responses in drug development and in AIT mechanistic studies (91).

Clinical Biomarkers

Allergen provocation tests, such as conjunctival provocation tests, nasal provocation tests, and environmental exposure chambers, are used to evaluate target organ responses. APTs are commonly used in clinical practice to assess allergen-specific reactivity of patients and the clinical relevance of IgE-mediated sensitization (91, 119). They are a vital tool for the diagnosis

of local AR (92, 119, 120). These tests can also be used as *in vivo* methods for stratifying patients when investigating the therapeutic effects in AIT trials. Allergen provocation tests (APTs) permit better standardization of procedures, control of environmental factors (temperature, humidity), and avoidance of variability caused by seasonal variations in pollen exposure. They are used as surrogate markers of the clinical response to AIT. APTs are recommended to provide insight into the mechanisms of AIT and biomarkers at both the local and systemic levels (91, 119). The European Medicines Agency (EMA) suggests APTs as primary endpoints in proof of concept and dose-finding trials of AIT (phase II) before proceeding to phase III AIT trials. However, APTs cannot be substituted for assessing symptoms and requirements for rescue medication during natural allergen exposure in phase III trials (91).

THE EFFICACY IN AR

Allergen-specific immunotherapy has been shown to be useful for the long-term reduction of medical expenses because of its sustained, disease-modifying effects. After administration for 3 to 4 years, both SCIT and SLIT effectively improved allergic rhinoconjunctivitis (121), and asthma (122). The rate of new antigen sensitization after 2 years was significantly lower in patients undergoing AIT than in non-AIT patients. Moreover, there was a 2–3-fold reduction in the risk of development of asthma for 2–7 years after stopping AIT (123). Some studies also found that there might be a lower prevalence of allergy in children born to mothers who underwent AIT during pregnancy. A total of 56 homogeneous studies between 2003 and 2013, including SCIT and SLIT, concluded that the recovery rate in AIT groups was 53.67-fold higher than in the placebo groups (124). The rate of reduction of symptoms and the medication score was as high as 80% in SCIT for seasonal AR in many randomized, placebo-controlled trials (RPCTs). Accordingly, the efficacy of AIT depends on the allergen dose and treatment duration. The clinical results have shown a high degree of heterogenicity and responsiveness in individuals. The immunological response was related to the personal dose (125), and long-term improvement after discontinuation was related to the treatment duration (126). There are no definitive diagnostic tools or markers for identifying responder patients, so current practice suggests that physicians discontinue AIT if there is no clinical response after 18–24 months (127). However, a standardized extract dose and clinical data are not available for all extracts. The extracts in each country have different potency, allergen dose, allergen mixtures, and adjuvants. Moreover, data for direct comparisons of AIT and pharmacotherapy are lacking because of a dearth of head-to-head studies.

Comparison of SCIT and SLIT

Subcutaneous immunotherapy and SLIT differ in schedules, route, frequency, amount of allergen, up-dosing, and maintenance dosage. Nevertheless, clinical efficacy is evaluated in the same way by using subjective and objective parameters. SCIT has demonstrated benefits in children and adults with AR. Symptom reduction has persisted for many years after stopping treatment. Meta-analysis comparing SCIT and SLIT revealed both to be effective for seasonal AR. In perennial disease with HDM allergy, SCIT also showed benefit, but SLIT was doubtful (128). Analysis of all randomized studies of SCIT generated a dose-response curve (129). Effective doses were associated with the amount of allergen, but side effects of SCIT also increased.

Meanwhile, SLIT showed a wide range of effective doses (130). Some studies showed improvement in the second year of treatment (131). A meta-analysis that compared the efficacy of SCIT, SLIT tablets, and SLIT drops for grass AR found no difference between SCIT and SLIT tablets, whereas SLIT drops were less effective than the tablets (132). The early study in 20 adults mono-sensitized to grass and treated with either SLIT drops or SCIT showed that the combined symptom and medication scores decreased by at least 50% in both groups compared with the placebo, whereas sIgG4 changed only in the SCIT group (133). However, direct comparisons of 11 randomized studies showed that SCIT was more effective than SLIT compared with the placebo (134). A direct pairwise meta-analysis of 26 double-blind randomized controlled trials to compare the efficacy of HDM AIT using SLIT drops, SLIT tablets, and SCIT in patients with perennial AR to HDM showed that the symptom score was more significantly decreased with SCIT than with SLIT drops or SLIT tablets (135).

However, more head-to-head, large, and well-designed studies are needed to compare the effectiveness of SCIT and SLIT. A head-to-head comparison of SCIT and SLIT in the grass-pollen allergic mice showed that SCIT suppressed Th2 inflammation and induced neutralizing antibodies, whereas SLIT suppressed allergen-induced airway hyper-responsiveness and induced a grass-pollen-specific IgG2a response (136). An evaluation of AR patients who changed from SCIT to SLIT for a variety of reasons found similarity of symptoms in 75%, while SCIT was preferred by 8% and SLIT by 17% (137). Indirect comparisons tend to conclude the superiority of SCIT due to the rapid improvement and immunological change. Adverse reactions to SCIT included a higher risk of local and systemic allergic reactions compared with SLIT. Therefore, the risk with SCIT correlated with a larger injection volume, multiple allergens per shot, and a higher extract concentration. Retrospective data showed that 23% of SCIT patients experienced systemic reactions after injection (138). On the other hand, the safety of SLIT was better. Reactions were recorded in 10–15% of SLIT patients, and most were mild reactions in the early phase of treatment.

ILIT and EPIT

Several clinical trials testing the efficacy of ILIT in the treatment of grass, birch, and cedar-pollen, and cat–dander allergies have shown high therapeutic efficacy (75, 139–141). A systematic review and meta-analysis of 11 randomized controlled trials and 2 cohorts showed short-term benefits of ILIT for seasonal allergic rhinoconjunctivitis (142). ILIT improved the composite score and visual analog scale and increased sIgG4 levels but did not change the quality of life or sIgE levels. A recent study found that 3 injections without an annual booster achieved a substantial reduction in allergic symptoms and use of rescue medication during a 3-year follow-up (143).

Epicutaneous immunotherapy seems effective and safe for rhino-conjunctivitis. In adults with timothy-grass-pollen allergy, applying a Phl p 5 patch for 6 weeks reduced the allergic symptoms and medication use in the treatment group compared to the placebo group (89). EPIT efficacy was dose-dependent, but a high dose was associated with local skin inflammation (144). EPIT for HDM AR was studied in an animal model (145). A number of questions remain, such as the standard dose, time of treatment, type of antigen, and placebo effect, and are in need of further study.

Comparison of AIT for Seasonal and Perennial AR

The meta-analysis in AIT studies found SCIT to be significantly effective in patients with seasonal (93) and perennial AR who were sensitive to HDM (146). SLIT tablets for timothy grass, a 5-grass mix, ragweed, and HDM showed efficacy in relieving symptoms in America and Europe. Asthmatic child patients who underwent SCIT treatment for more than 3 years showed control of the symptoms of seasonal AR for 7 years after discontinuing the AIT (123). In patients unresponsive to regular drug therapy, SCIT reduced symptoms and medication in pre and co-seasonal immunotherapy (147, 148). Subjects started SCIT at least 8 weeks before the season and continued for at least 16 weeks (149). Allergy drops for birch, alder, and hazel also showed benefits in Europe. In pooled analyses, Durham et al. (150) showed that, compared with the placebo, nasal symptoms improved in seasonal AR by 4–27.2% with the 6–timothy–grass SLIT tablet (overall improvement 16.3%) and by 15.2–18.8% with the 2–ragweed SLIT tablet (overall improvement 17.1%).

For perennial AR, nasal symptoms improved by 16.1% with HDM SLIT tablets relative to the placebo. The combined symptom and medication score (CSMS) also decreased significantly in patients using the HDM SLIT tablets for 1 year (99). Thus, HDM SLIT tablets were more beneficial than all pharmacotherapy regimens in perennial AR trials. Medication reduction with SLIT tablets for perennial AR was 1.5–2-fold compared to seasonal AR. SCIT for cat and dog allergies yielded no meaningful data because of low potency and variable standardization of allergens.

Comparison of Mono-Allergen and Poly-Allergen AIT

There is no standardized approach to AIT for poly-sensitized patients. In Europe and Asia, mono-allergens have predominantly been used by choosing allergens that correspond with the symptoms. However, in the United States, all relevant allergens have been given to allergic patients (separate shots or mixed shots). Poly-allergen AIT is performed by administering mixed extracts at a single body site or single extracts at different body sites, simultaneously or at different times. Poly-allergen extracts were effective in SCIT (151), and the therapy is safe if administered in an appropriate setting. However, data for poly-allergen SLIT are scant. Therefore, there has been no head to head clinical outcome comparisons of mono-allergen and poly-allergen SCIT or SLIT. A meta-analysis study of HDM AIT in mono and poly-sensitized patients with AR found no significant differences in the nasal symptom score, medication score, or quality of life between the groups. The study concluded that single-allergen AIT using HDM was clinically effective for both mono and poly-sensitized AR patients (152). Component-resolved diagnosis is essential for avoiding the inclusion of irrelevant allergens in mixed shots. An Expert Committee recommended limiting mixtures to 2 or 3 extracts for patient safety (153). The European AIT guidelines recommend that poly-sensitized patients who are poly-allergic to taxonomically–related homologous allergens be administered either a single allergen or a mixture of homologous allergens (95). Moreover, patients who are poly-allergic to non-homologous allergens should be started on AIT with either the allergen responsible for most of their AR symptoms or separate treatment with the two clinically most important allergens (154).

The Efficacy in AR With Co-morbid Disease

Asthma

Allergen-specific immunotherapy has shown effectiveness against allergic asthma. The common allergens in patients with allergic asthma are similar to AR, including HDM, grass pollen, tree pollen, and animal dander (122). A recent systematic review and meta-analysis of 98 studies showed that SCIT and SLIT significantly reduced short-term symptom scores and medication use in patients with allergic asthma (155). SCIT improved the quality of life (QoL) (156–158), whereas SLIT showed variable results in patients with allergic asthma (155). SCIT did not reduce asthma exacerbation, defined as the number of oral corticosteroids needed to restore asthma control (159), but SLIT also showed inconclusive results. A large randomized controlled trial of HDM SLIT tablets in patients with allergic asthma found the treatment extended the time to exacerbation during inhaled corticosteroid (ICS) reduction in suboptimally–controlled asthma (160). Importantly, there were no reports of severe systemic allergic reactions in SLIT patients (160). Nevertheless, data are limited regarding the ability of SLIT to suppress asthma exacerbation (160, 161).

Allergen-specific immunotherapy significantly improved the forced expiratory flow at 25–75% but not the peak expiratory flow rate (PEFR) or forced expiratory volume in 1 s (FEV1) (155). SCIT improved bronchial hyper-reactivity, but nothing was reported regarding SLIT (155). Conversely, SCIT caused more systemic adverse effects than SLIT (122, 155). Some cohorts showed a benefit of AIT in preventing the onset of asthma in allergic rhinitis patients (162, 163). The EAACI guideline recommends AIT as an add-on to regular asthma therapy in adults with controlled or partially-controlled HDM-driven allergic asthma (164). "Controlled asthma" is defined as daytime symptoms <2 times/week, no night awakenings, relief is needed for symptoms <2 times/week, and no activity limitation due to asthma. "Partially-controlled asthma" is defined as failure to meet the first 2 criteria above. The updated asthma guideline recommends SLIT in adult HDM AR patients with asthma that is suboptimally–controlled despite low to high dose inhaled corticosteroid and FEV1 >70% (160, 165).

Atopic Dermatitis

Many studies have been conducted in AR patients with or without asthma who also have atopic dermatitis (AD). Some patients showed improvement in AD symptoms, and no patients became worse (166, 167). A systematic review and meta-analysis reported a moderate level of evidence for effectiveness in improving the total SCORing Atopic Dermatitis (SCORAD) index over 18 months of SCIT (168). No fatal or near-fatal adverse events were reported in any of the studies assessed. SLIT also improved the total SCORAD (169, 170). However, another systematic review of 12 eligible trials (6 SCIT and 4 SLIT) found no significant differences in the disease severity score or eczema symptoms (171). Therefore, large controlled and randomized clinical trials are needed to study this more. Nevertheless, AIT may be an effective treatment option for selected AD patients (172).

Sinusitis and Nasal Polyps

Allergen-specific immunotherapy also shows good efficacy in AR with sinusitis. A survey study in the United States showed a 72% decrease in days lost from work, a 26% reduction in the use of medications per year, and a mean reduction of 51% in the overall symptom score in sinusitis patients who underwent AIT (173). In addition, AIT for allergic fungal sinusitis resulted in significant improvement in the endoscopic disease score and chronic sinusitis survey symptom score and decreased systemic corticosteroid use (174).

DURATION OF AIT

Many randomized controlled trials show long-term efficacy in improving clinical and immunological change after SCIT and SLIT. Continuous SCIT for 3–4 years resulted in 3 years of persistent improvement in the clinical condition and medication (147, 175, 176). In the SLIT study, 3 years of grass-pollen sublingual drops showed benefit for only 1 year after stopping treatment (177). Three years of grass-pollen SLIT tablets showed a 20–30% reduction in symptoms and rescue medication for 2 years after discontinuation (147, 178–181). When AIT was administered for less than 3 years, allergic symptoms usually relapsed 1 year after discontinuation.

Patients undergoing AIT for more than 3 years showed clinical efficacy beginning after 1 year of treatment (19, 176, 177, 180, 182). A comprehensive 5-year prospective controlled trial that compared 3- and 5-year HDM SCIT found significant decreases in the rhinitis severity score, asthma severity score, and visual analog scale in both groups after 3 years. Moreover, the AIT benefit was maintained in both groups at 5 years (183). All the above evidence suggests that the duration of both SCIT and SLIT should be at least 3 years for long-term clinical benefit.

ADJUNCTIVE THERAPIES IN AIT

Adjunctive therapy in AIT refers to the use of another treatment together with AIT. Its purpose is to improve the efficacy of AIT and decrease its adverse effects (**Table 2**).

Vitamin D

Vitamin D is a major substance that enhances human immunity (200). Vitamin D2 is converted into active vitamin D3, which regulates innate and adaptive immune responses (184). Active vitamin D3 enhances IL-10 production from DCs and induces Tregs (185). The clinical efficacy of AIT was increased when vitamin D was sufficient (201, 202). Skin test reactivity to grass pollen was significantly reduced by grass-pollen AIT with adjunctive vitamin D supplementation compared to the placebo (203). Moreover, children with grass-pollen AR who underwent AIT with vitamin D showed a reduced symptom-medication score and improved lung function compared to the placebo (204). However, the role of vitamin D in AR remains controversial (205).

Monoclonal Antibodies

Omalizumab is a monoclonal antibody that binds to the Fc portion of the IgE molecule. In *in vitro* testing, omalizumab also restored pDCs to Tregs (189). Combined SCIT with omalizumab reduced symptoms and rescue medication during seasonal allergen exposure compared to SCIT alone (190). Moreover, omalizumab reduced the adverse effects of AIT, especially in high-risk asthma patients and with the rush AIT protocol (191–193). Dupilumab is a monoclonal antibody against the IL-4 receptor. A recent study found that AIT combined with dupilumab did not improve the clinical response compared to AIT alone (195). There have been no studies using anti-IL5 receptor or anti-IL5 monoclonal antibodies as adjunctive therapy to AIT in humans.

Probiotics

Probiotics have been proven beneficial for the immunological system. Some species have been shown to increase Tregs, IgA antibody production, and the activity of DCs. Thus, probiotics can help reduce the risk of immunologically-mediated disease, including Th2-mediated allergic responses that play a significant role in allergic diseases. *Lactobacillus* and *Bifidobacterium* are the main genuses used for the preparation of the products tested in several studies. Strain-specific probiotics were used for adjunctive treatment of AIT, specifically either probiotics or recombinant probiotics. However, the data are still limited. Probiotics may be ineffective after enzymatic degradation of allergens by the oral route. Combination recombinant probiotics producing the allergoid may be better to use only an allergoid for AIT treatment of AR patients because of a safer and more effective. Most studies of recombination probiotics in murine models as pre-clinical studies showed reduced sensitization in both newborn and adult mice (196). Intranasal vaccination of adult mice with *Lactococcus lactis* strains resulted in decreased sIgE antibodies and increased sIgA antibodies (197). Also, oral treatment of adult mice with *Lactobacillus acidophilus* strains increased sIgG antibodies (197). It appears that recombinant probiotics can modulate the immune response, shifting it toward a Th1 and Treg-specific immune response, but it remains unclear whether long-lasting immunological tolerance is induced.

Several human studies have shown that probiotics reduce symptoms and improve the quality of life in AR patients.

TABLE 2 | AIT and adjunctive therapy.

Adjunctive therapy	Immunological mechanism	Clinical benefit	References
Vitamin D (VitaminD2 and D3)	1. Decrease DCs function by stimulating IL-10 production. 2. Increase production of Treg cells 3. Regulate innate and adaptive immune responses.	1. Improve symptoms in the patients with AR and allergic asthma patients. 2. Laboratory improvement of regulatory cells and decrease type 2 inflammatory cells	(23, 184–188)
Anti-IgE	Restore pDCs to Treg cells	1. Decrease allergic symptoms, rescue medication during seasonal exposure. 2. Decrease adverse events from immunotherapy, especially in high-risk asthma and rush protocol.	(189–194)
Anti-IL5 and Anti-IL-5 receptor	No current study using Anti-IL5 receptor or anti-IL5 monoclonal antibody as adjuvant therapy to AIT in human		–
Anti-IL4/IL-13 receptor	Monoclonal antibody against IL-4 receptor	Do not improve clinical response compared to AIT alone	(195)
Probiotic	1. Increase Treg cells, IgA antibodies production, and activity of DCs. 2. Conversion Th2 to Th1 response.	Additional AIT treatment with strain-specific probiotics might help clinical improvement in allergic patients.	(196–199)

A systematic review by Zajac et al. (206) found that the duration of probiotic administration varied from 4 weeks to 12 months. However, probiotics did not affect either tIgE or sIgE. In another study, SLIT with adjunctive probiotic treatment showed significantly higher Tregs than in the SLIT only group (198). Overall, the mechanism and efficacy of probiotics in AR management remain unclear. Nevertheless, probiotics have the potential as adjunctive therapy in AR management.

ADJUVANTS IN AIT

Adjuvants are substances that precipitate with an allergen extract in AIT vaccines (**Table 3**). The aim is to skew a robust Th2 immune bias toward the cytosolic inflammatory pathway for enhanced antigen cross-presentation and IgG production or toward the vacuolar pathway with a clear Th1 shift and active tolerance (226). Also, adjuvants can prevent the too rapid systemic distribution of allergens at the injection site.

Toll-Like Receptor Agonists (TLRs)

Toll-like receptor ligands comprise the innate immune system that responds to pathogen-associated molecular patterns (PAMPs). AIT with adjuvant TLR shows benefits and can reverse allergic inflammation (207). TLR4 and TLR9 have been tested for TLR-activating properties in allergic diseases (208, 227). TLR4 ligands are monophosphoryl lipid A (MPL) and lipopolysaccharide (LPS) (208). MPL can promote a shift in the immune response toward a Th1/Treg response (208). MPL is now being investigated in a clinical phase III study by both subcutaneous and sublingual routes (209). Laboratory markers showed a significant decrease in the IgE level and increased production of IgG4. The symptom score also improved more than with AIT without MPL (209). TLR4 has been used as an adjuvant in vaccines for cancer and infection. However, TLR4 as an adjuvant of AIT for AR or asthma is unclear, but LPS has been used to stimulate TLR4 in many animal studies. LPS can promote human DCs to produce IL-12p70 and IP-10 and is a potent Th1-biased stimulus (210). *In vitro* models using human cord blood cells also showed downregulation of Th2 responses due to reduced IL-13 after LPS administration (211). Clinical studies are needed to determine the effectiveness of LPS as an adjuvant of AIT in humans.

CPG-ODNs

Unmethylated deoxycytidyl-deoxyguanosine oligodeoxynucleotides (CpG-ODNs) are PAMPs that mimic bacterial DNA. CpG-ODNs stimulated TLR9 (228). CpG-ODNs were previously considered to be potential vaccine adjuvants (227). CpG-ODNs can shift human allergen-specific Th2 cells to a Th1/Th0 phenotype. In a murine model, CpG-ODNs decreased Th2 inflammation and IgE secretion (212) and increased Tregs (213). The USFDA approved CpG-ODN as an immunoadjuvant in the hepatitis B vaccine (229). CpG-ODNs are also used as immune modulators in many cancer immunotherapies. Recently, AIT cat allergen Fel d 1 with high-dose CpG-ODNs reduced all allergic symptoms in a murine model. Moreover, pDCs were increased and migrated from the injection site to periphery sites (213). CpG-ODNs showed long-term clinical effectiveness in patients with ragweed AR in phase II clinical trials (214), but its efficacy was lacking in phase III controlled clinical trials (215). A randomized controlled trial in humans is needed to generate more information.

Aluminum Hydroxide

Aluminum hydroxide is the most common adjuvant used in vaccines and AIT (230). Aluminum hydroxide in AIT can induce allergen immunogenicity and increase IgG and IgE titers (216), and create a sustained-release antigen depot leading to greater safety (230). Aluminum hydroxide also induced greater inflammation due to the recruitment and activation of APCs at the injection site (217). The adverse effects of aluminum hydroxide constitute a significant problem: acute and chronic inflammation at the injection site was found in more than 15% of AIT patients (218). At present, there is no clear consensus regarding the benefit and serious adverse events of using aluminum hydroxide as an adjuvant in AIT (219).

TABLE 3 | Adjuvant in AIT.

Vaccine adjuvant therapy	Immunological mechanism	Clinical benefit	References
TLR agonist (MPL)	1. Reverse immune toward Th1/Treg response. 2. Significant decrease IgE level and increase production of IgG4 level.	Improve symptom score.	(207–209)
TLR agonist (LPS)	1. Promoted human DCs to produce IL-12p70 and IP-10 and potent Th1-biased stimulus. 2. Downregulate Th2 responses by reducing IL-13.	Clinical benefit in human needs to study more.	(207, 210, 211)
CPG-ODNs	1. Shift human allergen-specific Th2 cells to Th1/Th0 phenotype. 2. Reduce Th2 inflammation and IgE secretion. 3. Increase in regulatory Treg cells.	1. Reduce symptoms of allergic asthma in the mouse model. 2. Show long-term clinical efficiency in patients with ragweed AR in phase II clinical trial but in phase III controlled clinical trial show lack of success in efficiency.	(212–215)
Aluminum hydroxide	1. Increased allergen immunogenicity and IgG and IgE titers 2. Recruitment and activation of APCs at the injection site.	Inconclusive	(216–219)
Calcium phosphate	Adsorb antigens and increases IgG levels.	Induce local adverse reactions.	(220, 221)
Microcrystalline tyrosine	Increased IgG production and limited increases of IgE levels	Safe in using as adjuvant of AIT in humans.	(222)
Fungal compounds	Stimulate the innate immune system and induce cytokine for the adaptive immune system.	Inconclusive	(223)
Heat-labile toxin (LT) from *Escherichia coli*	Stimulate the innate immune system	Inconclusive	(224)
Parasite molecules	Suppression of host antigen-specific immune response	Inconclusive	(225)

Calcium Phosphate

Calcium phosphate is a mineral salt that could be used as an adjuvant in AIT (216) because it can adsorb antigens and increases IgG levels (220). However, it might cause local adverse reactions. Calcium phosphate will be considered as an alternative to aluminum hydroxide, but with lower adjuvant activity (221).

Microcrystalline Tyrosine

Microcrystalline tyrosine has been used as an immunomodulator and adjuvant. The product released from the injection site is L-tyrosine. Microcrystalline tyrosine (MCT) increased IgG production while suppressing the IgE level (222). L-tyrosine is safe when used as an adjuvant of AIT in humans. However, caution is required in regard to possible tyrosine metabolism disorder (222).

Fungal Materials

Compounds of fungal origin, i.e., fungal immunomodulatory proteins (FIP), such as glycophosphopeptical, have been shown to stimulate the innate immune system via non-specific receptor recognition molecules and induce cytokines for the adaptive immune system (223). However, the results for FIP are inconclusive because most studies of FIP add-on to AIT have shown no superior clinical improvement in AR patients compared to AIT alone.

Heat-Labile Toxin

Patch delivery of a combination of birch-pollen allergen and rBet v 1 with the heat-labile toxin from *Escherichia coli* was superior in inducing allergen-specific IgG compared with subcutaneous alum-adsorbed rBet v 1 in an animal model (224).

Parasite Proteins

Helminths can evade host immunity by suppressing the antigen-specific immune response of the host. For example, *Brugia malayi* TGF-β homolog-1 and *Brugia malayi* TGF-β homolog-2 can bind to human TGF-β receptor (225) and mimic human TGF-β. Such parasite molecules might be able to serve as adjuvant carriers for AIT in the future.

DIFFERENT TYPES OF AIT

It is hoped that advanced technologies will be able to be combined with AIT to achieve greater efficacy and safety. The aims are IgE-activity reduction, allergenicity reduction, and induction of allergen-specific blocking IgG antibodies. Methods would include bypassing IgE, targeting T cells, modification of natural extracts, and use of multiple recombinant allergens.

Component-Resolved AIT

The proportion of poly-sensitized AR patients has increased along with cross-reacting allergens (i.e., profilin, polcalcin, lipid-transporting proteins, tropomyosin, etc.). Allergen sensitization varies among different age groups, study populations, and geographical regions. In many countries, allergen extracts for immunotherapy still use whole extracts. Component-resolved diagnostics (CRD) has been brought to identify sensitization to allergenic proteins and to improve AIT efficacy in poly-sensitized patients (231). In a murine model of cockroach allergy, component-resolved immunotherapy using Per a 9 found reduced levels of Per a 9 sIgE, whereas sIgG1 and sIgG2 antibodies did not show significant change (232). In a human

study, 1,263 Spanish patients with seasonal AR to grass and olive pollens underwent AIT based on skin prick tests in 73% or CRD IgE antibodies in 56.8% of the patients. The results showed that AIT prescribed based on CRD was more accurate and reduced the cost of immunotherapy (233).

Recombinant Proteins

Recombinant allergen-based vaccines that use allergen-encoding DNA have been developed for both SCIT and SLIT. The aim is less induction of IgE response and good induction blocking allergen-specific IgG antibodies. Advances in molecular cell biology enable the use of recombinant wildtype allergens (which contain mainly conformational IgE epitopes that eliminate the problem of poor quality of natural allergens), recombinant hypoallergens (which, by DNA technology, convert allergens to abolish IgE activity but leave the T–cell response), and recombinant fusion proteins (carrier proteins and non-allergenic allergen-derived peptides that contain tolerogenic epitopes) (234). Significant benefits accruing from recombinant proteins are more effective immune responses and fewer systemic reactions following AIT. The recombinant hypoallergen Bet v 1 was reported to significantly increase Bet v 1-specific IgG1 and IgG4 antibody levels and decrease the medical symptom score in AR patients compared with non-AIT groups (235). Long-term efficacy was seen in patients with allergic rhinoconjunctivitis more than 3 years after completion of treatment (236). Non-allergenic peptides from the major grass-pollen allergen and the major HDM allergen induced allergen-specific IgG antibodies in allergic patients. A novel recombinant fusion protein might be able to be used with inactivated *Escherichia coli* as the expression system, and rhinovirus-derived coat protein or hepatitis B as a carrier protein. However, results in humans are inconclusive due to scant data, and variations in extract preparation, dosing, the dosing interval, and the reaction products.

Recent technology has already been developed for AIT with allergen hybrids or mosaic antigens by fusion of different protein sources, such as pollen, animal dander, and various foods. The hybrid allergens are modified to be hypoallergenic while still being able to induce T–cell tolerance. A Fagales pollen hybrid (birch, hazel, alder, oak, and hornbeam) molecule for AIT was more efficient in raising a T-cell response and showed lower IgE-binding capacity compared with the crude extracts in a murine model (237). In a study in rabbits, a recombinant hybrid molecule consisting of the major birch allergen (Bet v 1) and grass-pollen allergen (Phl p 5) increased IgG antibodies and reduced allergenicity (238). A clinical trial that administered a vaccine containing major grass-pollen allergens (Phl p 1, Phl p 2, Phl p 5, and Phl p 6) to patients with allergic rhinoconjunctivitis found significantly increased grass-pollen-specific IgG and a decrease in the total nasal symptom score (TNSS) (239). However, a small study in patients with allergic rhinoconjunctivitis did not find differences in the combined medication score or pollen sIgG1 and sIgG4 (240). Recombinant allergen hybrids help to reduce the administered dose, long-term immunogenicity, and treatment duration, but late-phase reactions are still seen due to the preservation of T-cell epitopes. Preclinical evaluation for application in AIT needs further study.

Nanoparticles and Virus-Like Particles

Using nanoparticles and virus-like particles, allergens can be delivered so as to activate the innate and adaptive immune responses. Nanoparticles (<100 nm in size) such as liposomes, polyamides, polysaccharides, and polyesters, and virus-like particles can be used to encapsulate allergens to protect them from IgE-binding, direct covalent conjugation, or adsorption, and they are then delivered to APCs (241). Encapsulation is preferred for the mucosal and oral routes. Nanomedical platforms have the potential for achieving effective permeation in the cases of epicutaneous and intranasal delivery, and for their ability to form a depot, protect against enzymatic degradation and stimulate allergen-specific tolerance (242). *In vitro* data have shown promotion of Th1 stimulation and enhancement of maturation of APCs without any Th2 response. Patients undergoing HDM SCIT in which the allergen was encapsulated in viral particles showed 50% improvement in the medical and symptom scores compared with adjuvant alone (243).

Nucleic Acid-Based Vaccines

Deoxyribonucleic acid and mRNA encoding the desired allergen are inserted into a bacterial plasmid. The plasmid contains non-methylated CpGs so that it can stimulate an acquired immune response. When the shot is injected, the gene contained in the plasmids is delivered to the APCs of the host. Animal models have shown immunomodulatory effects by driving Th1 induction of IFN-γ and IgG2a antibodies and suppressing Th2 sensitization (244). These vaccines are aimed at reducing severe systemic effects of AIT. mRNA vaccines are safer than DNA vaccines because foreign sequences in the DNA may fuse into a genome of a patient. Most studies had been conducted in murine models. In a study in humans, a CryJ2-LAMP plasmid vaccine administered to Japanese red cedar atopic subjects appeared to be a safe and effective treatment (245).

T- and B-Cell Peptides

Synthetic allergen peptides containing T-cell epitopes do not activate IgE antibodies but induce T-cell tolerance. A clinical study of HDM and ragweed and grass-pollen allergies demonstrated some benefits and safety. On the other hand, increased nasal and bronchial symptoms were found in cat-allergic patients (246). B-cell peptides aim to establish protective humoral antibodies that are independent of IgE antibodies. For the development of recombinant hypoallergenic allergen, B-cell peptides that do not react with IgE antibodies are conjugated with a carrier to be used for AIT with the goal to make a safer, resulting in the generation of protective allergen-specific IgG antibodies without stimulating IgE antibody production which can block the interaction between patients IgE and natural allergen (247).

Allergoids

The term "allergoid" refers to an allergen that was chemically modified by substances such as glutaraldehyde or formaldehyde but retains the ability to elicit an immunological response. The modification results in less-reactive B-cell epitopes by reducing IgE-binding but leaves T–cell epitopes unaltered. Allergoids thus show decreased allergenicity while improving immunogenicity.

Allergoids are used primarily in allergic patients undergoing AIT. The dose-escalation phase of conventional AIT lasts up to 6 months, whereas when using allergoids, up-dosing is significantly shortened to only 4–8 weeks (248). Efficacy of allergoids has been shown for HDM, birch pollen, and grass pollen. In a real-life study from Germany, patients who underwent allergoid SCIT had significantly fewer AR and asthma symptoms than the non-AIT control group after 6 years of follow-up (249). Another study showed an increase in sIgG4 antibodies in the allergoid treatment group that was about 1.4–2.8 fold above the baseline (250). Grass-pollen allergoid also showed efficacy for nasal symptoms in the first pollen season, persisting until the third season. There was no difference in basophil activation between the allergoid and standard grass-allergen extract. Of note, immunogenicity was significantly lower in the allergoid group than in the control group (251). Allergoids have been demonstrated to be more cost-effective than and preferable to other AIT options. However, we have a poor understanding of the mechanism of action with different allergoids, and the chemical modification method has not been standardized. SCIT with allergoids appears to be efficacious and more cost-effective and provides benefits that persist for at least 1 year after cessation of AIT.

AIT IN THE COVID-19 PANDEMIC

The COVID-19 pandemic has taken an extreme human toll, and the economic and social impacts of the pandemic are being felt globally. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Co-morbidities such as obesity, hypertension disease, chronic obstructive pulmonary disease, and cardiovascular disease are associated with severe COVID-19 (252), but AR is not a risk factor for severe disease. Currently, no immunologic or clinical evidence is available on how AIT and SARS-CoV-2 interact (253). SCIT and SLIT should be continued as long as there is no contraindication. SCIT can be an option for patients who wish to start AIT and in clinics where social distancing can be practiced (253). Confirmed COVID-19 cases should discontinue AIT, whether SCIT or SLIT, independent of disease severity until the symptoms have completely resolved and/or adequate quarantine has been put in place (254). After patients have recovered from COVID-19 and are asymptomatic, AIT can be started up again as scheduled. SLIT offers the option of self-treatment at home, thus avoiding the need to travel to or stay in an allergy clinic or hospital. Data are needed regarding patients switching from SCIT to SLIT during maintenance–phase AIT.

CONCLUSION

Allergen-specific immunotherapy has been recommended in practice to treat severe AR patients who do not respond to conventional drug treatments. AIT induces allergic immune tolerance by enhancing various regulatory cells to control type 2 inflammation. AIT has been shown to be effective in alleviating allergic symptoms, reducing medication requirements, decreasing allergen reactivity, improving the quality of life, and preventing the development of asthma. However, conventional SCIT has disadvantages of requiring numerous injections and visits to the clinic, high cost, and systemic allergic reactions. Multiple administration routes for AIT provide alternatives and help to improve patient compliance and safety. New biologicals and advanced technologies are being developed to further improve the effectiveness of AIT.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Patients Unmet Needs in Chronic Rhinosinusitis With Nasal Polyps Care: A Patient Advisory Board Statement of EUFOREA

N. Claeys[1], M. T. Teeling[1], P. Legrand[1], M. Poppe[1], P. Verschueren[1], L. De Prins[2], L. Cools[2], L. Cypers[2], W. J. Fokkens[3,4], C. Hopkins[3,5] and P. W. Hellings[3,4,6,7]*

[1] Patient Advisory Board of the European Forum for Research and Education in Allergy and Airway Diseases, Brussels, Belgium, [2] Patient Liaison Officers of the European Forum for Research and Education in Allergy and Airway Diseases, Brussels, Belgium, [3] The European Forum for Research and Education in Allergy and Airway Diseases Scientific Expert Team Members, Brussels, Belgium, [4] Department of Otorhinolaryngology, Head & Neck Surgery, Academic Medical Center (AMC), Amsterdam, Netherlands, [5] Guy's and St Thomas' Hospital, London Bridge Hospital, London, United Kingdom, [6] Department of Otorhinolaryngology, Head and Neck Surgery, Universitair Ziekenhuis Leuven, Leuven, Belgium, [7] Department of Otorhinolaryngology, University of Ghent, Ghent, Belgium

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Giancarlo Ottaviano, University of Padua, Italy

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Jo Rimmer, Monash Health, Australia
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***Correspondence:**
L. Cypers
lindsay.cypers@euforea.eu

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Background: European patients with chronic rhinosinusitis with nasal polyps (CRSwNP) have had only limited occasions to unite to have their voices heard, hence missing the opportunity to contribute to the improvement of CRSwNP care.

Aims: To identify unmet needs in CRSwNP from the perspective of CRSwNP patients from the Patient Advisory Board (PAB) of the European Forum for Research and Education in Allergy and Airways diseases (EUFOREA).

Methodology: Semi-structured interviews were conducted individually with 15 European patients with CRSwNP and with a disease history of more than 2 years. Patients shared their burden of the disease and frustrations related to CRSwNP care, experiences with key pillars of current treatment options, shortcomings of the current care pathways and recommendations for improvement of care. A panel of 30 members of the Patient Advisory Board reviewed the interview report and provided further input during 2 virtual meetings.

Results: CRSwNP patients indicated the need for greater awareness from society and physicians of the disease burden with impact on social function and well-being. Along with a loss of ability to smell and the continuous presence of secretions in the nose, most patients reported poor sleep quality and psychological impact as the most bothersome symptoms. Patients' frustrations relate primarily to the underestimation of the disease burden, the lack of coordination of care and the limited treatment options available to them. Treatment options with oral corticosteroids and/or sinus surgery both have positive and negative aspects, including the lack of long-lasting efficacy. Better coordination of care, more patient-centered care, greater public awareness, increases in research on

the disease mechanisms and better therapeutic options would be warmly welcomed by CRSwNP patients.

Conclusions: This statement of the EUFOREA Patient Advisory Board on CRSwNP provides novel insights on the underestimation of the burden of CRSwNP and shortcomings of current care. Multiple recommendations made by the patients can underpin action plans for implementation of better care for CRSwNP among all physicians treating patients with this disabling disease.

Keywords: nasal polyps, oral corticosteroids, quality of life, unmet needs, chronic rhinosinusitis

INTRODUCTION

Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) represents a chronic inflammatory condition of the nose and paranasal sinus cavities with major impact on well-being and social function which is greatest in the young adult to middle aged populations (1). With an estimated prevalence of 3%, CRSwNP represents a common health problem in the Western world (1, 2). Despite international evidence-based guidelines for treatment (EPOS2020), a substantial group of patients remain uncontrolled with recurrent needs of oral corticosteroids (OCS) and/or endoscopic sinus surgery (ESS) (3).

The significant economic and clinical burden of CRSwNP highlights the need for better treatment options and reorganization of the current care pathways. A recent Dutch study revealed that the annual direct and indirect costs per patient were € 1,501 and € 5,659, respectively (4). The high financial impact of the disease resulted from costs related to health care utilization, absenteeism and lost work productivity (4, 5). Patients suffering from CRSwNP experience symptoms of nasal obstruction, smell dysfunction with anosmia in a large proportion, continuous nasal discharge and facial pain (1). Besides the sino-nasal symptoms, CRSwNP is associated with an increased incidence of depression and social dysfunction (6). Existing literature has found the impact of CRSwNP on quality of life (QoL) to be comparable to other chronic diseases such as chronic obstructive pulmonary disease (COPD), congestive heart failure and diabetes (7, 8).

In contrast to asthma, respiratory allergies and atopic dermatitis (9–11), few international patient initiatives have been undertaken to bring the burden of disease and other relevant factors of CRSwNP to the attention of health policy makers, to the general public or to physicians. Although it is widely recognized that upper and lower airway diseases are interrelated with inflammation in part of the airways (12), limited international initiatives have been undertaken to highlight the patient view and impact of diseases in both upper and lower airways. To meet these major unmet needs in the respiratory field, the EUFOREA Patient Advisory Board (PAB) was launched in 2017. The board is composed of 30 European patients from 8 European nationalities who suffer a long disease journey with chronic upper and lower airways diseases for more than 2 years. Patients of the PAB are regularly asked by the EUFOREA board and expert team leaders to share patient views on the burden of disease and care pathways, to advise experts on novel guidelines for respiratory care, and to help define strategies for better care (13–16).

Few qualitative studies on the patients' experiences and perspectives of current management of CRSwNP have been published. These studies identified patients' frustrations with delayed referral, poor communication, inconsistency of advice, incorrect medication use, adherence to intranasal steroids and lack of recognition of the impact of CRS (17, 18). This EUFOREA initiative aims at raising the CRSwNP patients' voice on the disease burden and key pillars of CRSwNP care. This 'Unmet needs in CRSwNP care' is launched as a valuable project to have the patients' voice heard, and to reflect on the current care pathways in all aspects. Fulfilling our mission to ease the burden that CRS patients have to manage, is the ambition of this EUFOREA project.

Abbreviations: CRSwNP, Chronic Rhinosinusitis with Nasal Polyps; ESS, Endoscopic Sinus Surgery; EUFOREA, European Forum for Research and Education in Allergy and Airways diseases; OCS, Oral Corticosteroids; PAB, Patient Advisory Board; QoL, Quality of life; GP, General practitioner.

MATERIALS AND METHODS

Patient Selection and Procedures

Fifteen European patients with CRSwNP of the EUFOREA Patient Advisory Board (PAB) were randomly selected by PH from the 30 PAB members for being interviewed by LD in March 2021. The number of participants was predetermined. Patients were selectively recruited based on a wide range of characteristics such as age, gender, nationality, severity of disease, duration of disease and CRS management, and willingness to be interviewed. The diagnosis of CRSwNP had to be confirmed by a local Ear, Nose and Throat specialist prior to recruitment, with only secondary or tertiary care patients interviewed and participating in this initiative. A list of contact data was provided by the EUFOREA patient liaison officers LCy and LCo. Invitation emails were sent to patients to request their consent and participation in a 20 min. telephone interview on the impact of CRSwNP on their daily life.

In-depth and semi-structured one-on-one interviews were conducted in English, French or Dutch language in order to facilitate the inclusion of diverse demographic characteristics and to avoid the limiting factor of a language barrier. All interviews were carried out by one trained trilingual female clinician (LD, MD in training) who was not involved directly in

the participants' care. The objective of the study was explained to the participants. Patient characteristics including age, gender, nationality, symptom duration, presence of comorbid asthma, severity of CRS symptoms on a visual analog scale ranging from 0 (no symptoms) to 10 (worst thinkable symptoms) and current treatment were questioned.

An open-ended questionnaire was designed by PH and approved by the EUFOREA experts CH and WF. This predefined template was used as a guide to generate discussions and to document all aspects of CRSwNP that affected the participants. Field notes were made during the interview to provide context. Participants were asked to reply to the following predefined questions:

- What is the major burden of CRSwNP?
- What are your major frustrations regarding CRSwNP care?
- What do you consider the benefits and shortcomings of OCS?
- What do you consider the benefits and shortcomings of ESS?
- What are the shortcomings of the current CRSwNP care pathways?
- What suggestions do you have for overall improvement of care for CRSwNP?

Additional comments, corrections, suggestions and approval was provided during two virtual meetings with a review panel of 30 patients of the PAB. This review was performed to validate the findings from the interviews. No repeat interviews were carried out.

Analysis

The interview sessions were audio-recorded and transcribed. Transcribed recordings were managed using oTranscribe software. All transcripts were analyzed qualitatively by one researcher and the qualitative analysis was reviewed by multiple participants. Frequently occurring and important statements were highlighted and categorized for similarities in content. Themes were identified in parallel with the interview questions and determined according to the responses collected. Significant direct quotes were noted separately to illustrate general opinions. Clear summary figures were designed based on the reported strengths, weaknesses, shortcomings, and suggestions for current care pathways with the aim of concisely presenting the unmet needs from the patient perspective.

Ethical Considerations

All patients have provided written consent for participation in this analysis, and those listed as co-author have been interviewed and explicitly approved to be listed as a member of this PAB initiative via written consent.

RESULTS

Interview Specifics

This study recruited 15 patients to participate in a one-on-one interview in April 2021. Phone calls ranged in length from 10 to 50 min with a mean duration of 17 min 18 sec. All interviews were conducted in March 2021.

TABLE 1 | Patient characteristics.

	Total (n = 15)
Age (mean, range, in years)	52 (18–69)
Gender	
Female	7
Male	8
Diagnosis period (mean, range, in years)	22 (2–52)
Comorbid asthma	
Yes	9
No	6
Severity of CRS (mean, range, on scale 0–10)	6 (2–8)
Current treatment	
Nasal treatment	14
Oral treatment	10
Inhaled treatment	9
Previous sinus surgery	
Yes	14
No	1
Biological	
Yes	2
No	13
Nationality	
Belgian	8
Dutch	2
German	1
Swedish	1
Greek	1
Luxembourgish	1
Danish	1

Participant Characteristics

The mean age of participants was 52 years and 53% were men. Estimated history of CRS symptoms ranged between 2 and 52 years with a mean of 22 years. Nearly two out of three study patients suffered from comorbid asthma. Participants rated the severity of their CRS disease by an average of 6/10. The selected patients represented 7 different European nationalities. Baseline characteristics of the patients included are presented in **Table 1**.

Various themes were identified such as major burden and frustration of CRSwNP, experiences with CRPwNP treatments and the role of the EUFOREA Patient advisory board.

CRSwNP in Daily Life—Major Burden and Frustrations

Most patients experienced a major impact of CRSwNP on their daily lives as a result of a wide variety of disease symptoms. Overall, participants were incredibly frustrated about the underestimation of the burden of disease, with the perception of others often being that.

> *"I think there still is not enough knowledge in primary care. CRSwNP is still too often compared to a common cold or a small headache."*

As many as 12 of the 15 interviewees acknowledged living with a lack of smell (and taste) capacity. This olfactory dysfunction limited their pleasure of sharing a dinner with family, colleagues or friends. In addition, it placed patients in awkward and potentially dangerous situations.

"I don't notice the smell of a grandchild's dirty diaper." "Living with no sense of taste and smell is like watching TV in black and white in the 21st century. [. . .] *You miss a lot of impressions of the surroundings. You don't get any input."*
"When I go to friends for dinner, I often don't taste the food. I don't dare to say this then, because the problem is so difficult to explain and no one understands it."

Besides smell reduction, participants reported suffering from other typical symptoms such as nasal obstruction/blockage, rhinorrhea/postnasal drip, sneezing, hearing impairment, teary eyes, bad breath, and facial pain/pressure. Participants, especially those with comorbid asthma, felt embarrassed because these clinical symptoms may be mistaken for symptoms of COVID-19 (19). Some patients also mentioned that they avoided drinking alcohol in order to escape an increase in nasal symptoms. The issues mentioned above were identified as the cause of reduced social contact and social embarrassment.

"I am never able to leave the house without handkerchiefs."
"It seems like I'm continuously out of breath."
"I have to blow my nose all the time, this made customers at work think I had COVID-19. That was very confronting."
"Nobody wants to go to a party when they have a cold."
"I cannot drink half a beer. It makes me blow my nose all the time and I can't talk anymore."
"I feel ashamed of my nasal voice."

Overall, participants agreed with the remarkable psychological impact of CRSwNP. The recurrent upper respiratory tract infections in addition to the continuous presence of physical and mental symptoms caused stress and a depressed mood. One out of five participants had a physician-diagnosed depression.

"Imagine having a cold for 20 years, this breaks you down slowly. [. . .] *You never get a break, it never goes away."*
"I'm afraid my children will have to go through the same thing."
"I can never be just normal. I had to find another way of living: a steady rhythm, spreading the load, learning how to dose, going to bed on time. . . "

The burden of poor sleep quality was mentioned numerous times. While some patients associated nasal obstruction and snoring with their sleep dysfunction, others felt that post-nasal drip was the leading cause. Daytime somnolence and increased fatigue had harmful effects on both individual productivity, social function and work performance.

"I can't focus on anything for 100%. [. . .] *I feel really tired when I wake up, that's the worst time of the day. That's why I always look and feel exhausted."*
"I feel embarrassed to sleep among others because of my snoring."

Experiences With Current CRSwNP Treatment Options—OCS and ESS

Figure 1 shows an overview of the strengths and shortcomings of OCS and ESS. When asked about treatment related factors, several patients described the adverse effects of their treatments as disabling.

"I am often more sick from my medication than from my symptoms."

A majority of patients felt frustrated about the lack of an effective treatment and emphasized the need for a treatment that targeted the cause of their disease.

Some participants reported the underestimation of the importance of alternatives to OCS such as nasal rinses with Saline.

"I haven't discovered anything that works yet, nothing helps."
"Conventional medicine fails, it has no answer. I started to look outside conventional medicine for tools to improve my quality of life. E.g., kinesiologist, yoga, mindfulness osteopathy. . . "
"My current treatment does not target the cause of the disease, it just obscures my symptoms."

OCS

One out of three respondents regarded the use of oral corticosteroids as effective. This group regained their ability to smell and taste, was relieved from facial pressure, headaches, nasal drainage, and experienced a significant improvement of sleep quality. However, others mentioned the low efficacy of this medical treatment, and some patients experienced a reduced effect over time. Multiple specific side effects were described including hyperactivity, insomnia, swelling of the face, mood swings, weight gain, reduced bone mineral density, and anxiety symptoms. Patients regretted the overuse and prescription of long-term OCS. The risk of addiction to OCS given the good mood was mentioned several times.

"The OCS only worked for a couple of weeks."
"It's like choosing between the plague and cholera. When I take OCS, I am relieved of my nasal secretions and I regain my smell. The downside is that I gain weight and I cannot sleep well anymore because it makes me hyperactive. I need to weigh the pros and cons against each other."

ESS

Fourteen patients underwent sinus surgery, of which 8 have had more than one surgery. Only a few among them described the surgery itself as a positive experience. However, almost all patients experienced a significant improvement of quality of life afterwards.

"Symptoms were as good as gone, I regained my smell function."
"I still suffered from nasal blockage, but the periods were shorter."

The occasionally only temporary and unpredictable outcome in combination with the consequent need for lifelong surgeries was

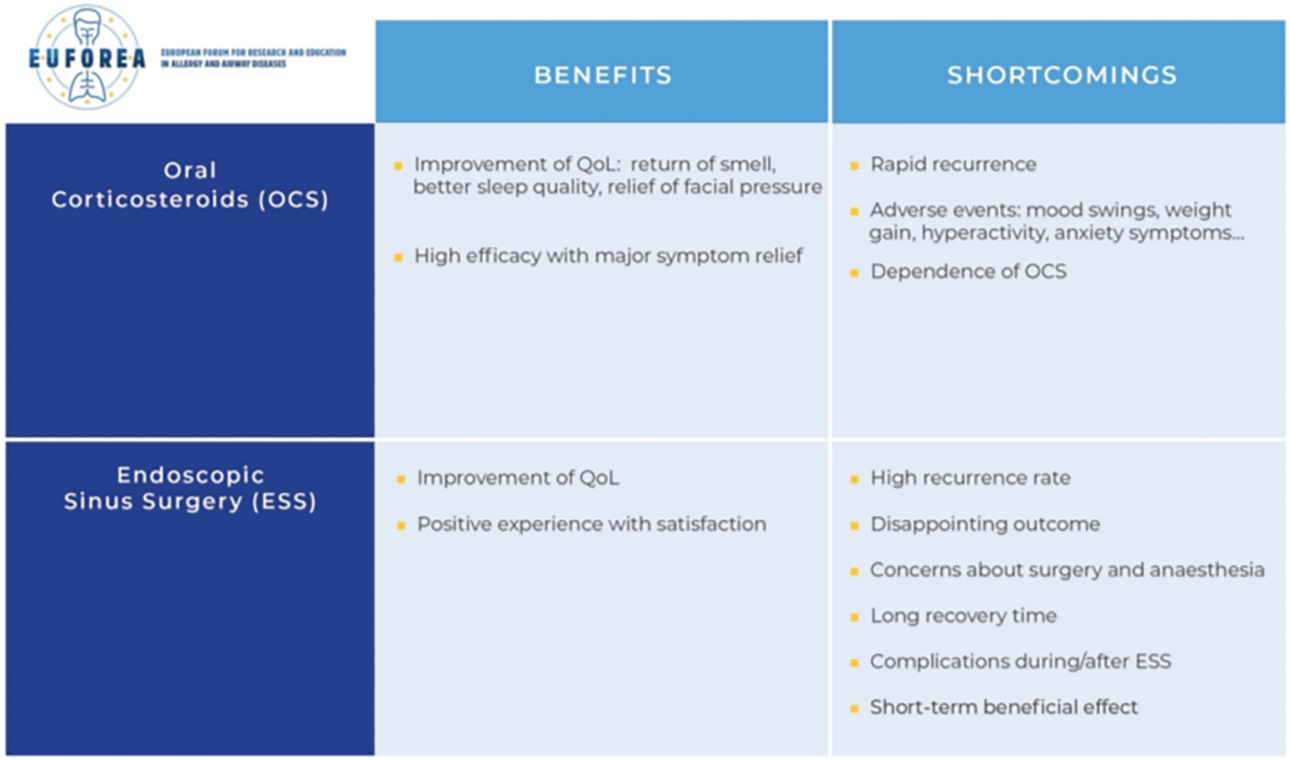

FIGURE 1 | Strengths and weaknesses of the current options with OCS and ESS.

described as most disadvantageous, followed by the often long recovery time/loss of workdays. Patients acknowledged feeling concerned about the possible complications of sinus surgery. Only a minority of patients actually experienced complications of surgery and/or general anesthesia.

"I was very disappointed about the result of the operation."
"I have to take 14 days off each time after surgery. Then I'm not able to work."
Patients' view of the current CRSwNP care pathway–Major shortcomings and suggestions

Major Shortcomings

Patients considered the lack of coordination in care as a major shortcoming in the current health care organization. The inconsistency between specialists in how patients are managed was a source of frustration. Many patients suffered from impactful comorbidities of which asthma was the most common. Among these patients, few felt comfortable about the care they received for their comorbidities; the majority reported a lack of attention to comorbidities.

"I have already seen over six specialists. Every doctor has his own vision about the treatment of the disease, doctors contradict each other. They should be more consistent among each other."

Some patients admitted they felt the search for appropriate help was long and tough, whereas-others felt that their referral from the GP to the specialist proceeded smoothly. Several patients expressed having a feeling that there is a lack of knowledge in care. According to the patients, there is a lack of personalized care and patient participation and they expressed a desire to feel more independent.

"Conventional medicine searches for diagnosis and treats them without looking at the patient itself. Classic medicine does not search enough for the cause."
"It feels like I have more knowledge about myself/my disease, than the doctor has."
"Doctors do not pay attention to things that are not scientifically proven. I believe that a change of lifestyle and nutritional supplements can help to reduce my symptoms."
"I find it tiresome that I have to make an appointment for new prescriptions every time, while I have been taking this medication for 10 years already. This requires a lot of time and money."

Patients also faced difficulties in being taken seriously by healthcare professionals. They perceived an underestimation of the social- and psychological aspects of CRSwNP as well as a general lack of awareness about the disease. Some patients pointed out the financial impact of CRS, due to the ongoing need for multiple medicines and the impact on employment.

"Please don't understand me wrong… cancer is a very serious illness, it's logic that a huge budget is released for this. I know you don't die from CRS, but this disease does have a huge impact on my life… day in day out. It would be nice if we received some attention and financial resources too."

As cited above, patients reported the lack of reimbursed access to new treatments. In particular, the unavailability of new treatment options including biologicals such as Dupilumab was mentioned.

"I experienced the good effects of Dupilumab during a clinical trial. Now I know there is a solution, but I don't have access to it. That is really frustrating."
"I'm afraid my children will also have CRS, I hope that by then there will be a treatment that can cure the disease."

Patients reported the inconsistency within European countries as frustrating. Some of them needed to travel to other countries to purchase the medication they need.

"Flixonase nasules are not available in Belgium. I need to buy them in the Netherlands, which is a long journey every time and they are not refunded."
"I explored the European market to find the best price for Flixonase nasules. I order them in Spain."

Suggestions on Future Care

According to patients, the pooling of expertise and conferences on the latest developments in the field of CRSwNP will be essential to share information and to provide the most appropriate care. Patients proposed training for GP's to ensure faster diagnosis and referral more accurately. Respondents reported the implementation of joint clinics as a good solution to optimize the approach of multimorbidities. In parallel, the mention of CRSwNP in asthma/pulmonology guidelines was proposed.

"My GP, pulmonary specialist and ENT specialist must work together."
"Doctors need to get in line with each other."
"They need to organize extra courses for specialists and GPs to inform them about the latest treatment options."

Participants highlighted the benefit of proper training on correct medication use as well as the implementation of smartphone applications into clinical practice.

"Can't they make a device to link my symptom recording and medication lists to my medical file?"

Patients emphasized an individual approach in the CRS care pathway, and some even begged to have more attention paid to the diversity of patients. Discovering which environmental factors trigger symptoms was a request of multiple patients.

"Specialists should experiment with the different treatment options to see what works the best for each individual."

Another patient-reported idea was the development of specified psychological services to improve the attention for mental health. Patients expressed the demand for research on new treatment options. They hoped to see the availability and reimbursement of novel therapies increase in the future.

"Please develop a therapy against my runny nose. There is no treatment on the market that relieves me from that."
"There's no treatment that can control my symptoms."

Role of EUFOREA Patient Advisory Board

Patients mentioned that EUFOREA can raise public awareness for the CRS burden that many people still underestimate even in 2021. It was reported by participants that more attention is needed for the impact of CRS on school- and work performance.

"I repeated a grade in high school because of multiple infections and a sinus surgery that year. Teachers need to know that you can't control this. You don't do this on purpose."
"I would like to maximize the outreach of this initiative, beyond the local group of EUFOREA."

According to the PAB patients, EUFOREA can play a more important role in the advocacy to health policy makers. Patients believed that EUFOREA can make the patients' voices heard at a policy level. They highlighted the importance of putting pressure on the approval of biologicals as well as on the release of budget for further research. Patients believed that EUFOREA can, as a leading organization, initiate the development of European guidelines on the reimbursement and availability of treatment options. From the patients-view, EUFOREA was the perfect medium to focus on the empowerment of patients through education.

"EUFOREA could inform patients about the possible side effects of their medication."
"Maybe the PAB could draw up a list with tips and tricks from patients to patients e.g., alcohol avoidance, patient experiences with treatments… Inside information about the possible risk factors and triggers would be very valuable."

A summary of the shortcomings and suggestions for improvement of current care pathways is shown in **Figure 2**.

DISCUSSION

CRSwNP is a common chronic disease with a major impact on the daily lives of patients (2). This initiative of the PAB of EUFOREA provides unique insights into the burden of CRSwNP, patients' views on the strengths and shortcomings of the current care pathways and patients' priorities and preferences to overcome these unmet needs in future CRS management. CRS patients reported some very important insights in addition to the classic CRSwNP symptoms presented in previously reported studies (2, 18). They noted impaired sleep quality, along with mental dysfunction and reduced smell capacity, as the most frustrating aspects of the disease. Further typical CRSwNP

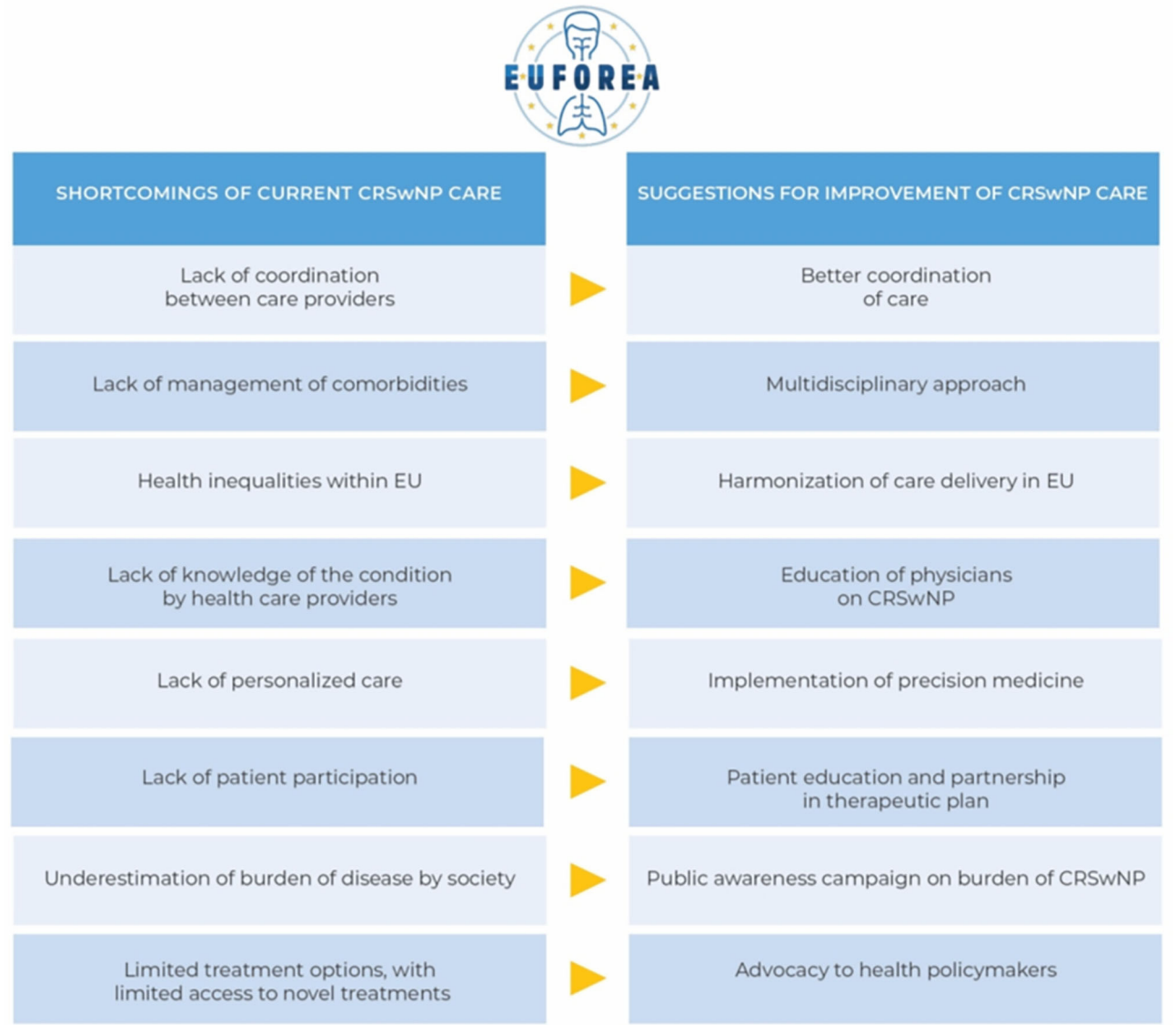

FIGURE 2 | Shortcomings and suggestions for improvement of current care pathways.

symptoms include nasal congestion, nasal drainage and facial pressure (20).

Our findings highlighted coordination in care as one of the key unmet needs. A multidisciplinary approach, in particular the improvement of communication between healthcare professionals, the inclusion of CRSwNP in asthma/pulmonology guidelines and the introduction of joint clinics in hospitals, can help to foster a holistic and multidisciplinary patient approach (21). Management should be applied according to guidelines and thus according to generally accepted consensus on the best treatment for any specific patient (22). Residents of different countries in Europe do not enjoy the same rights and conditions regarding the availability and reimbursement of treatments. European homogenization on availability of medication, treatment options and plans according to guidelines may be a good step forward.

A more patient-centered approach is an additional factor that many patients are asking for. In accordance with previous reports, precision medicine and in particular personalized medicine and shared decision-making will increase satisfaction, therapy compliance and control of disease (23).

Supporting patients in better management of their health demands more insight and advice on environmental factors that aggravate the disease. A broad-minded view of physicians on life-style and preventive medicine would be appreciated, although the limited evidence to support such approaches must be acknowledged (1). In addition, an individual approach can identify and correctly manage the impactful symptoms

and comorbidities from which participants suffer (21). Patients strive for more independence in disease management. This empowerment can be achieved both by implementing mobile health apps, organizing patient training on correct medication use and customizing the legislation regarding prescriptions for chronically ill patients (21, 24).

Another important finding was the underestimation of the burden of disease. The importance of the psycho-social aspect including mental health, sleep quality, and social function is often forgotten. The development of specified psychological services to teach patients how to cope with their disabling disease would be a great advance in health care. Public awareness can help to create a more empathetic environment (13).

Despite the many positive effects of sinus surgery and oral corticosteroids, there are many limitations including disappointing outcomes and adverse events associated with the 2 major pillars of current treatment (2, 3). The dependence on drugs and recurrent surgeries restrict patients in their freedom. In fact, the absence of causal therapies is a clear unmet need. Therefore, as indicated by patients, future research on deeper understanding of pathophysiological mechanisms and potential novel treatment options is needed.

EUFOREA represents a European forum to help overcome these variously experienced shortcomings. The organization is built to develop solutions to bridge the gap between guidelines and daily practice (13). EUFOREA's comprehensive website contains a wide range of educational material and evidence-based information about the symptoms, diagnosis and possible treatments of CRS (1). In the future, this website will be expanded to include information on prevention, environmental triggers, comorbidities of CRS, life-style advice and potential side effects of different medical/surgical treatments. Focus on prevention rather than treatment can reduce the financial burden that patients experience (23, 25). The organization of diverse events for both patients and health-care providers can be further expanded by EUFOREA (13). Themes cited by patients for this include trainings for patients on how to use their medication, testimonials from peers, campaigns to raise awareness on a public level, the education of GPs on how to speed up and optimize the referral process, tools for implementing the concept of precision medicine, and courses for specialists on the latest treatment options. EUFOREA has already developed a multitude of projects to address all these issues, but to increase the awareness of the EUFOREA organization itself remains a future ambition. Patients see EUFOREA as an important tool for raising awareness among all stakeholders and health policy makers. Raising the disease higher on the political agenda may release additional funding for research and may also expedite the process of approval and reimbursement of novel therapies.

A certain limitation of this study includes the participant selection. Participant recruitment based on voluntary involvement and membership of the PAB may imply that these patients have more interest in their disease. Nevertheless, except the fact that almost all patients have undergone surgery, the characteristics of this participant group were balanced and heterogeneous which is one of the strengths of this study. The diverse patients' unmet needs of CRSwNP reflect the major burden on patients' quality of life and care plans that are often inappropriate. Patients hope that joining forces with EUFOREA through this unique initiative can influence political discussions and raise public awareness. EUFOREA plays a leading role in bringing these valuable statements to health policy makers, implementing them into daily practice and thereby improving the quality of future health care.

CONCLUSION

This statement of the EUFOREA PAB on CRSwNP provides novel insights into this underestimated and undertreated disease. Multiple recommendations made by patients can underpin action plans for implementation of better care for CRSwNP amongst all physicians treating patients with this disabling disease.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethische commissie onderzoek Universitair ziekenhuis/Katholieke universiteit Leuven. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LD and PH have designed the questions and proposed these questions for approval and discussion by the PAB members. All authors have revised and approved the content and outcomes of the patient interviews.

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REVIEW
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Pharmacological, Technological, and Digital Innovative Aspects in Rhinology

Rosanna Ruggiero [1,2], Giovanni Motta [3], Giuseppe Massaro [3], Concetta Rafaniello [1,2], Alberto Della Corte [2], Antonella De Angelis [2], Annalisa Capuano [1,2], Gaetano Motta [3†] and Francesco Rossi [1,2†]*

[1] Campania Regional Centre for Pharmacovigilance and Pharmacoepidemiology, Naples, Italy, [2] Department of Experimental Medicine, Section of Pharmacology "L. Donatelli", University of Campania "Luigi Vanvitelli", Naples, Italy, [3] Department of Mental Health and Public Medicine, Section of Otorhinolaryngology—Head and Neck Surgery, University of Campania "Luigi Vanvitelli", Naples, Italy

Innovation refers to the introduction of a product, a process, a service or a solution resulting in something new or significantly improved compared to the already available alternatives. In the clinical context, it is strictly related to the identification of a new added value in terms of quality, therapeutic efficacy and safety. Over the years several innovative approaches have been introduced in the clinical practice, revolutionizing the treatment and the management of important rhinologic conditions. Innovative tools, including new drugs, biomaterials, and mobile applications seem to be able to improve the clinical outcomes and the quality of life of many patients affected by (often relapsing) rhinologic diseases. Among the main modern pharmacological innovations, mention must be made of the biological drugs like monoclonal antibodies (mAbs). Recently, new mAbs have been introduced and investigated as useful arms in the treatment of some inflammatory/infectious or oncological diseases affecting the nasal cavities and paranasal sinuses. The already approved or still investigated mAbs work inhibiting different type 2 inflammation pathways, including those mediated by IgE (omalizumab), IL-4/IL-13 (dupilumab), and IL-5 (mepolizumab). Moreover, considering the higher expression of PD-L1 in nasopharyngeal carcinoma, the use of PD-1 inhibitors, such as nivolumab, or a dual CTLA-4/PD-1 blockade (ipilimumab plus nivolumab) appear to be an effective strategy for the treatment of this cancer form. The implants with bio-absorbable biomaterials represent new interesting available technological innovations. Moreover, advanced technologies such as the artificial intelligence, the machine learning as well as the augmented or virtual reality have also proved useful in rhinologic field with main impacts on precision medicine and surgery. Finally, the development and use of mobile-Health tools represent a winning strategy in monitoring of the therapy success, safety and tolerability as well as the progress of chronic disease including chronic rhinosinusitis with nasal polyps. Supporting the research of innovative tools and strategies (including pharmacological, technologic, or digital ones) is essential to improve the management of chronic diseases that significantly affect the patients' quality of life.

Keywords: pharmacological innovation, digital innovation, technological innovation, monoclonal antibodies, dupilumab, mepolizumab, chronic rhinosinusitis (CRS), rhinology

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***Correspondence:**
Rosanna Ruggiero
rosanna.ruggiero@unicampania.it

† These authors share senior authorship

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INTRODUCTION

Innovation refers to the introduction of a product, a process, a service or a solution resulting in something new or significantly improved compared to the already available alternatives (1). Regarding drugs, innovation is strictly related to the identification of an added value in terms of quality, therapeutic efficacy and safety, established on the basis of the results emerging from randomized clinical trials. The research and development of innovative medicines is fundamental to address persisting unmet therapeutic needs. Both the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) support it through the so-called Early Access Programs (EAPs) (2). In Italy, the marketing authorization of a medicinal product, the possible recognition of its reimbursement or its innovativeness are not automatically consequential. Even if substantially based on the same evidence, they represent three distinct procedures. Using a multidimensional approach, the therapeutic need, the added therapeutic value and the quality of evidence represent the three variables to consider in the innovative evaluation (3).

Current innovations can involve three overlapping domains: *pharmacological*, referred to the discovery of new molecules with innovative mechanisms of action or the recognition of new therapeutic indications for already authorized drugs, *technological* considering new release/administration systems of already available drugs, and *digital* such as new medical software or applications. Today, we are experiencing a renaissance of innovation. Recently, several innovative drugs have been approved and introduced into clinical practice, revolutionizing the treatment of important diseases, such as hepatitis C or several types of cancer. Still other new innovative drugs are going to be authorized (new monoclonal antibodies for the treatment of Alzheimer's, neoplasms, asthma, chronic obstructive pulmonary disease and cardiovascular diseases). Pharmaceutical innovation allowed important therapeutic results. Thanks to innovative drugs, it has been possible to increase the life expectancy of many patients, transforming lethal pathologies into chronic ones. The increase in the 5-year mortality rate for various oncological diseases as well as the reduction in the mortality rate of HIV/AIDS are unequivocal examples. Likewise, innovative drugs are enabling continued advances in the management of the COVID-19 pandemic. Such progress is also made possible by the introduction of innovative research models. Over the years, the Research and Development sectors of biopharmaceutical companies evolved from a *closed innovation* model, where innovation was centralized within the company, to arrive at an *open innovation* enabling collaborations outside the company. Today, the companies are increasingly concentrated in *network innovation* activities, i.e., the acquisition of research and development services (R&D extra muros), machinery and software aimed at innovation and skills from other companies or institutions (4).

Pharmacological innovation has also involved the field of rhinology. A variety of conditions affect the nose and sinuses, including inflammatory diseases, i.e., rhinitis, sinusitis, nasal polyposis, up to tumors of the nasal cavities and paranasal sinuses (**Figure 1**). The traditional treatments for inflammatory rhinological diseases include symptomatic therapies, based on antihistamine drugs and nasal decongestants, and disease modifying treatments, such as topical corticosteroids. New innovative drugs are able to improve clinical outcomes and quality of life of many patients affected by (often relapsing) rhinological diseases. Some of the main advances achieved in terms of pharmacological, technological as well as digital innovation applied to the field of rhinologic diseases are described below.

PHARMACOLOGICAL INNOVATION

Among the main modern pharmacological innovations, biological drugs are revolutionizing the treatment of several pathologies, finding application in many therapeutic fields, including the rhinologic one (5). A biological drug is characterized by an active substance (generally a high molecular weight protein) produced by a living organism (microorganisms or animal cells) or using a biological source through the use of recombinant DNA techniques (biotechnological drugs). Biologics are more complex molecules than chemical drugs. Their major complexity is associated with an increase in their structural dimensions. Among the main classes or categories of biologicals, monoclonal antibodies (mAb) are worthy of note (6). Over the years, the approved mAb therapies have seen incredible growth, evidenced by the fact that in 2018, globally, six out of 10 best-selling drugs were mAbs (7). Today, in the COVID-19 pandemic context, mAbs represent an important part of the therapeutic armamentarium useful against SARS-CoV-2. Since they are able to block the viral attachment of SARS-CoV-2 to host cells, they seem to be promising tools in patients at early stage of COVID-19, preventing its progression and reducing the morbidity and mortality of infection such as the frequency of hospitalizations (8, 9). Overall, excellent efficacy profiles and lower frequency of adverse reactions characterize these drugs. Recently, some mAbs have been introduced and investigated as useful arms in the treatment of some inflammatory/infectious or oncological diseases affecting the nasal cavities and paranasal sinuses.

Innovative Drugs for the Treatment of Inflammatory/Infectious Rhinological Conditions

The rhinological diseases sharing inflammatory features, such as airway eosinophilia, local IgE formation, and a TH_2 cytokine profiles, are evaluated as possible indications for some mAbs (10). Nasal polyps (NP), asthma, rhinitis and sinusitis, individually and in their various possible associations, represent some of these clinical challenges. Moreover, these pathologic conditions are often comorbid, with serious effect on the quality of life of patients. The already approved or still investigated mAbs work inhibiting different type 2 inflammation pathways, including those mediated by IgE, IL-4, IL-5, and IL-13 (**Figure 2**). Such mAbs represent useful tools for a precision medicine approach in the evaluation and management of severe chronic inflammatory conditions of upper respiratory tract (11), such

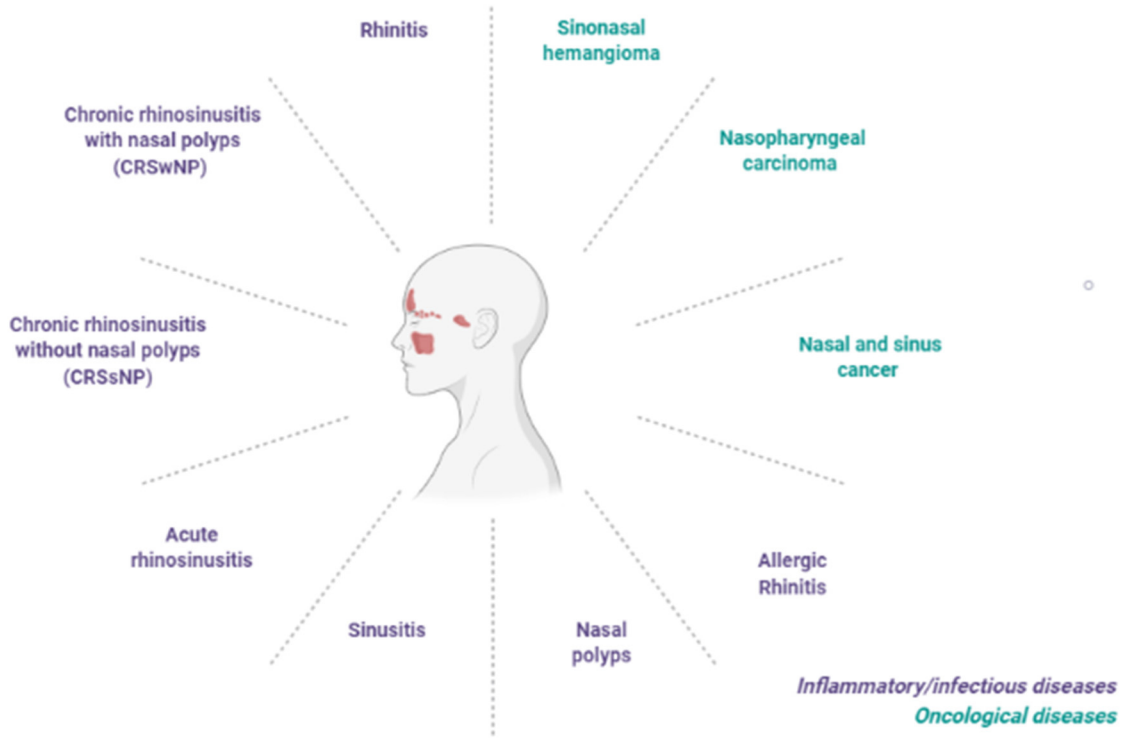

FIGURE 1 | Main inflammatory/infective and oncological conditions affecting the nose and sinus cavities or upper respiratory tract.

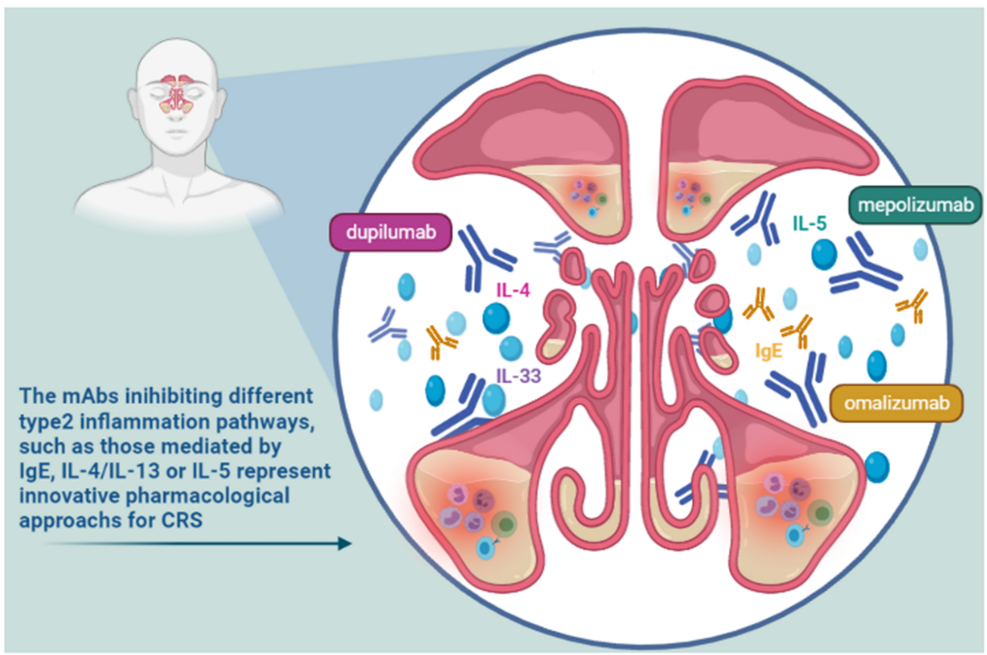

FIGURE 2 | Some target pathways target of mAbs effective in chronic rhinosinusitis (CRS).

as chronic rhinosinusitis (CRS) (11, 12). CRS is characterized by local inflammation of the upper airways and sinuses which persists for at least 12 weeks (13). It affects ∼3% of the population worldwide (14). It is often associated with several co-morbidities including nasal polyps, asthma, acute infection, and obstructive sleep apnea. Based on the associated presence

of nasal polyps, CRS was classified into two phenotypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) (15). The investigated mAbs seem to be particularly effective in the management of CRSwNP. This phenotype is predominantly an adult disease, with an average onset between 40 and 60 years old, frequently associated with severe asthma. It is difficult to treat, as often relapsing, even after surgery. CRSwNP is a debilitating disease accompanied by complete anosmia, headaches, often requiring chronic therapies with douching, topical corticosteroids, systemic corticosteroids and antibiotics, plus repeated surgical polypectomies to control the disease (16). This disease shows a substantial clinical and economic burden, significantly impacting on patients' lives and often causing missed work, and hospitalizations (17). Most patients affected by CRSwNP show a type 2 inflammatory form in the nasal and paranasal sinus mucosa. In particular, the degree of type 2 inflammation is correlated with disease severity of CRSwNP. On the other hand, in about 80% of patients chronic rhinosinusitis is characterized by the absence of nasal polyps. This disease phenotype is primarily associated to type 1 inflammation (13). Neverthless, increased levels of IL-4, IL-5, and IgE have been recently observed also in some patients with CRSsNP. So, some mAbs targeting on these pathways might be effective also in this patients population (18). In the light of this recent evidence, in the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020 the previous phenotype-based classification of CRS has been replaced, highlighting the anatomic distribution (localized or diffuse) and endotype dominance (type 2 or non-type 2) (19). To date, IgE, IL-4, and IL-5 represent the main targets of identified effective mAbs. Moreover, other possible targets for biological treatment of eosinophil and mast cell-related diseases such as CRSwNP seem to be IL-33, IL-17, thymic stromal lymphopoietin (TSLP) (**Table 1**). Overall, biologic therapy with mAbs targeting IgE (omalizumab), IL-4Rα (dupilumab), or IL-5 (reslizumab, mepolizumab) led to the improvement of several clinical outcomes, including reduction size of nasal polyps, favorable impact on quality of life, nasal airflow capacity and smell. Overall, the use of these agents was found to be safe and well-tolerated (20). Recently, a Cochrane Review focusing on the clinical management of patients with NP and CRS evaluated studies referred to three main biologics dupilumab, mepolizumab, and omalizumab. Disease-specific health-related quality of life (HRQL), disease severity and serious adverse events were the primary outcomes. All the patients enrolled in the included studies were using topical nasal steroids. According to the results (summarized in **Table 2**), dupilumab represents the mAb inducing more improvement in all considered primary outcomes (16).

mAbs Targeting the IgE Pathway

Omalizumab is an anti-IgE humanized mAb produced by recombinant DNA technology. It was already approved in 2002 for the treatment of allergic asthma in adults, adolescents and children aged ≥6 years. Since February 2014, it was also approved for the treatment of chronic spontaneous urticaria in patients aged ≥12 years. Finally, in July 2020, omalizumab obtained another extension of indication as add-on therapy for the treatment of adults (age ≥18 years) with severe CRSwNP for whom only intranasal corticosteroid therapy does not provide adequate disease control (21). Omalizumab dosing reflects the personalized approach to which biological therapy is aimed. In fact, as reported in the Summary of Product Characteristics, it is determined considering the baseline serum IgE level (UI/mL) and body weight (Kg) of each patient. Based on these determinations, the dosing (from 75 to 600 mg) and the time intervals for its subcutaneous administration (every 2 or 4 weeks) are identified[1]. Binding selectively to IgE, omalizumab reduces the concentration of free IgE in blood and in tissue, surface IgE on basophils and mast cells and, consequently, blocks the effects of IgE on dendritic cells[1]. IgE is involved in some biological functions and mechanisms relevant for several diseases, including allergic rhinitis and nasal polyposis. Overall, omalizumab treatment is able to induce a reduction of nasal polyp size, an improvement of symptoms and the inhibition of underlying type 2 inflammation[2]. Several randomized, double-blind, placebo-controlled trials such as real-life studies showed omalizumab efficacy in the disturbances of nasal and/or sinusal mucosa. In particular, the results of a randomized, double-blind, placebo-controlled study showed omalizumab efficacy in improving airway symptoms (including nasal congestion, anterior rhinorrhea, loss of sense of smell, wheezing, and dyspnea) and quality-of-life scores in patients with nasal polyps and comorbid asthma. Its clinical efficacy occurred irrespective of the presence of allergy (10). Moreover, according results of a recent real-life study, omalizumab was able to treat both CRSwNP and asthma. The induced improvements in CRSwNP control were rapid and similar to that obtained with upper airway surgery (17). Finally, a recent study aimed to analyse in a real-life setting the therapeutic outcomes of mAb treatments, including omalizumab, was published. Moreover, the authors tried to identify possible predictive biomarkers for successful therapy. Their results confirmed the biologicals as promising treatment option of CRSwNP, especially in severe cases not responding to conventional therapy (22). Ligelizumab and quilizumab are other anti-IgE mAbs, mainly investigated as treatments for chronic spontaneous urticaria. Actually no trials for ligelizumab in rhinologic diseases have been yet initiated, while the evaluation of quilizumab efficacy in patients with allergic rhinitis is still in its early stages (**Table 1**) (23).

mAbs Targeting the IL-5 Pathway

IL-5 is another key driver of local type2-inflammation, produced by Th2 cells and group 2 innate lymphoid cells (ILC2s), stimulating the production, activation and maturation of eosinophils (24, 25). Approximately 85% of nasal polyps (NPs) are characterized by prominent eosinophilia. So, IL-5 inhibition with specific mAbs represent an innovative therapeutic approach in patients with NP or CRSwNP (26). To date, three mAbs targeting IL-5 (*mepolizumab* and *reslizumab*) or α-subunit of its receptor (*benralizumab)* have been developed for clinical

[1]CHMP. *Annex I Summary of Product Characteristics*.
[2]Omalizumab (2021). Available online at: http://www.ncbi.nlm.nih.gov/pubmed/30000860 (accessed April 23, 2021).

TABLE 1 | Main pathways target of recent innovative pharmacological treatments, their approved indications in Europe and United States and study phase for other inflammatory/infective sinonasal conditions.

Pathways	mAbs	Target	Approved indications in EU by ema	Approved indications in us by FDA	studies phase in NP, CRS, or CRSwNP, CRSsNP, AR
IgE	Omalizumab	IgE	- CRSwNP - CIU - Allergic asthma	- Nasal polyps - CIU - Allergic asthma	Phase 3 (AR)
	Ligelizumab	IgE	-	-	No trials yet
	Quilizumab	IgE	-	-	Phase 1 (AR)
IL-5	Mepolizumab	IL-5	- Eosinophilic asthma	- Eosinophilic asthma - HES - EGPA	Phase 3 (NP)
	Reslizumab	IL-5	- Eosinophilic asthma	- Eosinophilic asthma	Phase 3 (CRS)
	Benralizumab	IL-5Rα	- Eosinophilic asthma	- Eosinophilic asthma	Phase 3 (NP) Phase 3 (CRSwNP)
IL-4/IL-13	Dupilumab	IL-4Rα	- Atopic dermatitis - Asthma - CRSwNP	- Atopic dermatitis - Asthma - CRSwNP	Phase 3 (CRSnNP) Phase 4 (CRSwNP)
	Tralokinumab	IL-13	- Atopic dermatitis	- Atopic dermatitis	No trials yet
	Lebrikizumab	IL-13	-	-	No trials yet
IL-17	Brodalumab	IL-17RA	- Psoriasis	- Psoriasis	No trials yet
IL-33	Etokimab	IL-33	-	-	Phase 2 (CRSwNP)
TSLP	Tezepelumab	TSLP	-	-	Phase 3 (CRSwNP)

CIU, chronic idiopathic urticarial; AR, Allergic Rhinitis; NP, nasal polyps; TSLP, thymic stromal lymphopoietin; EGPA, eosinophilic granulomatosis with polyangiitis; HES, hypereosinophilic syndrome; CRS, chronic rhinosinusitis; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps, EU, Europe; US, United States; EMA, European Medicines Agency; FDA, Food and Drugs Administration.

TABLE 2 | Main results of a recent Cochrane Review on the clinical management of patients with NP and CR with biologics.

mAb compared to placebo	Disease-specific HRQL	Disease severity	Serious adverse events
Dupilumab (anti IL-4)	Improve	Results in a reduction	May result in a reduction in number
Mepolizumab (anti IL-5)	May improve	Very uncertain difference	Very uncertain difference
Omalizumab (antiIgE)	Probably improve	No evidence	Very uncertain difference

use. Considering the important role of IL-5 in the development of bronchial hyper-responsiveness, all three mAbs have been evaluated in large-scale clinical trials as treatment for severe asthma. However, major studies evaluating the efficacy in patients with NP and CRSwNP have been conducted for mepolizumab. According the results of a meta-analysis, anti-IL5 therapy with mepolizumab induces a reduction in nasal polyp score in patients with CRSwNP (27).

Mepolizumab is a humanized mAb that binds IL-5, preventing its interaction with the α-chain of the IL-5 receptor (IL-5Rα). It was authorized by the EMA and the FDA in 2015 as an add-on treatment for asthma (28). Mepolizumab is innovative also for its pharmaceutical form of pre-filled syringe or pre-filled pen authorized by the EMA in 2019 representing the first European biologic drug for which self-administration in severe eosinophilic asthma was possible (29). In 2020, the regulatory approval for new additional indications for mepolizumab was submitted to EMA. These included three other eosinophil-driven diseases such as CRSwNP, hypereosinophilic syndrome (HES), and eosinophilic granulomatosis with polyangiitis (EGPA) (29). In the United States, mepolizumab has been already approved as treatment for adult patients with EGPA and represents the first and only biologic treatment for HES approved by the FDA. Moreover, it still waiting for FDA authorization as treatment for CRSwNP. Mepolizumab efficacy in nasal or sinus disturbances has been investigated in several studies. Already in a first randomized, double-blind, placebo-controlled study emerged that mepolizumab reduced the need for surgery at Week 25 and induced a greater improvement in symptoms compared to placebo. For this study patients were enrolled with recurrent eosinophilic nasal polyposis receiving topical corticosteroids and who required surgery. Mepolizumab's efficacy was accompanied with a safety profile comparable with placebo (30). Moreover, the efficacy and safety of mepolizumab as treatment of recurrent, refractory severe bilateral CRSwNP in adult patients was assessed in the SYNAPSE study, a multicentric randomized, double-blind, placebo-controlled, parallel-group, and phase 3 trial (31). According to the results of this study, mepolizumab represents an effective add-on treatment option to standard of care for CRSwNP. In particular, 414 patients enrolled in this study were randomly assigned (1:1) to receive mepolizumab subcutaneously (100 mg) or placebo once every 4 weeks in addition to standard

of care (mometasone furoate intranasal spray, saline nasal irrigations, systemic corticosteroids or antibiotics, or both), as required. At week 52 from baseline, endoscopic nasal polyp score and nasal obstruction VAS score were significantly improved in the mepolizumab group compared to the placebo one.

Reslizumab is another humanized monoclonal antibody approved in Europe and the USA for adult patients as add-on maintenance treatment for severe asthma with an eosinophilic phenotype (32). Reslizumab binds IL-5 with a picomolar affinity, reducing consequently survival and activity of eosinophils (33). Regarding its efficacy in nasal or sinus mucosal diseases, few data are available. In 2016, a first double blind, randomized, placebo-controlled, phase III trial started in California with the purpose of determinating whether reslizumab treatment was effective also for the chronic sinusitis. To date, although the study has passed its completion date, its status on Clinicaltrial.gov database results unknown (34). Another study evaluating the efficacy for the chronic rhinosinusitis symptoms in asthma patients undergoing reslizumab treatment was conducted in United States by the Department of Otolaryngology Head and Neck Surgery, University of Rochester. Its primary objective was to monitor the CRS symptoms in this patient population. This was a prospective observational study started in 2017, but subsequently withdrawn (34). Finally, a third mAb targeting on anti-IL5 pathways, **benralizumab,** is actually authorized in Europe as an add-on treatment in adults with eosinophilic asthma inadequately controlled despite high-dose inhaled corticosteroids plus long-acting β-agonists. This humanized monoclonal antibody targets IL-5Rα with high affinity and specificity. The IL-5 receptor is specifically expressed on the surface of eosinophils and basophils. Benralizumab reduces eosinophilic inflammation by inducing the apoptotic process of eosinophils and basophils, through enhanced antibody-dependent cell-mediated cytotoxicity (ADCC)[3]. Recently, a sub-analysis of the Phase IIIb ANDHI trial has been published, whose results extend benralizumab's efficacy to severe eosinophilic asthma patients with comorbid NP (any severity) (35). In particular, improvements in the annualized Sino-Nasal Outcome Test-22 (SNOT-22), asthma exacerbation rate (AER), FEV 1, Asthma Control Questionnaire 6 (ACQ-6), and St. George's Respiratory Questionnaire (SGRQ) total score were observed with benralizumab treatment compared to placebo. Likewise, benralizumab efficacy and safety profile in patients with severe NP was confirmed by the results of another randomized, double-blind, placebo-controlled trial conducted by Tversky et al. For this study, 24 patients with severe NP (defined by endoscopic grade 5 or more out of 8) and elevated eosinophils, with a history of previous surgical or endoscopic polypectomy, were enrolled. Benralizumab achieved a statistically significant reduction in nasal polyp size, sinus occupancy, symptoms and improved sensation of smell for 83% of patients. Moreover, it was well-tolerated (36). Recently, Humanitas Clinical and Research of Rozzano Hospital (Milan, Italy) conducted a pilot, prospective, double-blind, placebo-controlled, phase III-b trial in order to assess benralizumab clinical efficacy after week 24 of treatment. For this study, benralizumab 30 mg was subcutaneously administered in patients with CRSwNP (allergic and non-allergic), every 4 weeks for the first 3 doses and then every 8 weeks. Moreover, in order to identify any possible predictive biomarker of response, an inflammatory e molecular phenotyping of responders to benralizumab was performed. Its results have been included in the aforementioned Cochrane Reviews, Biologics for chronic rhinosinusitis (16).

[3]Fasenra and European Medicines Agency. (2021). Available online at: https://www.ema.europa.eu/en/medicines/human/EPAR/fasenra#product-information-section (accessed April 30, 2021).

mAbs Targeting the IL-4/IL-13 Pathway

IL-4 and IL-13 are two Th2-associated cytokines with a mutual and important role in the type 2 inflammation. They share a same heterodimeric receptor, consisting in the combination of two subunits, IL-13Rα1 and IL-4Rα chain. This can be activated by both IL-4 and IL-13 (13). In particular, IL-4 and IL-13 pathways induce effects on keratinocytes (impairing their differentiation), eosinophils (inducing their activation), fibroblasts (increasing the production of eotaxin), B cells (IgE production), Th2 cells (increased the differentiation and survival). They play a fundamental role in the pathogenesis of nasal polyposis. Dupilumab, binding to IL-4Rα, blocks signaling of both the IL-4 and IL-13 pathways, resulting in a powerful inhibition of Th2, eosinophil recruitment, and IgE production. **Dupilumab** is a completely human mAb, administered as a subcutaneous injection every 2 weeks. Initially dupilumab has been authorized for the treatment of asthma and atopic dermatitis, as Th2 mediated diseases. Subsequently, it was also approved, first by the FDA (in June 2019) and then by the EMA (November 2019), as the first biological medicine for the treatment of inadequately controlled CRSwNP in adult patients. These authorizations were based on the results of two Phase 3 studies, SINUS-24 and SINUS-52 studies, which evaluated the effects of dupilumab administration (300 mg) every 2 weeks plus intranasal corticosteroids compared to placebo plus intranasal corticosteroids, at 24- and 52-weeks, respectively (37). According to the results of these studies, dupilumab significantly improved the signs and symptoms of severe CRSwNP. In particular, it induced improvements in nasal polyp size, sinus opacification and health-related quality of life (HR-QOL). The major symptoms of CRSwNP, including nasal congestion or obstruction, nasal discharge and loss of smell, were relieved. Moreover, it allowed a reduction in the use of systemic corticosteroids and nasal polyp surgery, being generally well-tolerated. Furthermore, dupilumab has also been shown to improve lung function in asthmatic patients. It is important to highlight this result since many patients with CRSwNP also suffer from asthma. Recently, Laidlaw et al. reported the results of a randomized, double-blind, placebo-controlled trial according to which dupilumab improved both upper and lower airway outcome measures and HRQoL in patients with severe CRSwNP and comorbid asthma. This study also confirmed its positive tolerability profile. The most common adverse events were nasopharyngitis, headache, injection-site erythema, worsening of nasal polyposis, and asthma. These were more frequent with placebo group than dupilumab (38).

Given the high prevalence of chronic respiratory diseases and the high cost asscociated with biological products, patient selection is crucial. During the European Forum for Research and Education in Allergy and Airway Diseases (EUFOREA) in 2019, a multidisciplinary Expert Board proposed indications for biological treatment use in CRSwNP. Some indicative criteria for biological treatment in CRSwNP patients were identified. These included evidence of type 2 inflammation with biological biomarkers, need for systemic corticosteroids (2 or more courses in the past year), significantly impaired quality of life, significant loss of smell, or diagnosis of comorbid asthma. Based on a previous history of surgery, the use of a biological treatment was suggested in patients with presence of bilateral nasal polyps if 3 or 4 aforementioned criteria are found, respectively (39). Moreover, **lebrikizumab** and **tralokinumab** are other two antibody therapeutics that prevent binding of IL-13 to its receptors. In particular, lebrikizumab targets IL-13 with high-affinity, preventing the formation of the IL-13Rα1/IL-4Rα heterodimer receptor signaling complex. Since lebrikizumab does not prevent the binding of IL-13 to the IL-13Rα2 receptor, it does not interfere with the endogenous regulation of IL-13 (40). Actually, they have been investigated only in other pathologic conditions, in particular asthma (including allergic type) and atopic dermatitis. No trials in sinus or nasal cavities diseases have yet been conducted.

Innovative Drugs for the Treatment of Oncologic Rhinological Diseases

The nasal sinus neoplasms consist of a heterogeneous group of benign or malignant tumor histotypes which require different diagnostic-therapeutic management. Among the benign forms, the sinonasal hemangioma represents a rare vascular-type tumor of endothelial cells. Recently, a case report describing the administration of bevacizumab (50 mg) as treatment for a recurrent sinonasal hemangioma has been reported in the literature. The administration was performed by intralesion injection under endoscopic visualization in a 67-year-old patient. After 10 months, a reduction in the tumor size, a complete resolution of epistaxis and nasal obstruction were observed (41). Bevacizumab is a mAb that, by binding the growth factor of vascular endothelial cells (VEGF), blocks its biological activity. It is indicated as treatment for several types of solid tumor. It induces regression of the tumor vascularization, inhibits the formation of new vascularization, with consequent arrest of tumor growth (42).

The major rhinologic field of application of innovative drugs is **nasopharyngeal carcinoma** (NPC). This is a rare type of head-neck cancer. There are ∼129,000 new cases of NPC each year worldwide. Over 70% of such cases are reported in South China and Southeast Asia. This tumor is etiologically associated with the Epstein-Barr virus (EBV). It represents an "inflamed tumor" archetype, showing often a dense lymphocytic infiltrate and increased expression of the programmed death ligand (PD-L1) (43). For this reason, the patients with NPC are potentially suitable for treatment with immune checkpoint inhibitors (ICIs). The ICIs are newly introduced mAbs that have literally revolutionized the treatment of several solid tumors. Cancer cells are able to evade recognition and subsequent elimination by the immune system through a series of adaptive responses, including the overexpression of various immunosuppressive molecules in the tumor microenvironment. Some of these molecules, such as CTLA-4, PD-1, and its PD-L1, are targets of ICIs. By blocking these immunosuppressive molecules, ICIs induce the reactivation of cytotoxic T lymphocytes able to destroy cancer cells. ICIs treatments showed significant clinical benefit for different types of cancer, establishing immunotherapy as an important advance in cancer treatment (44). In order to evaluate the efficacy in nasopharyngeal carcinoma of some anti-PD1 agents, such as pembrolizumab, nivolumab, camrelizumab, several clinical studies were conducted. Other ones are still in progress (43). Among ICIs, **nivolumab** has promising activity in nasopharyngeal carcinoma. Recently, the results of an international phase 2 study evaluating the antitumor activity of nivolumab in the treatment of NPC were published. This study was conducted in 44 patients with pre-treated recurrent or multiple metastatic NPC treated with nivolumab until disease progression. A complete response was observed in one patient, while eight patients showed a ≥30% decline in tumor dimension, defined as partial response. The disease control rate was 54.5%. The 1-year overall survival rate was 59% (95% CI, 44.3–78.5%) and the 1-year progression-free survival (PFS) rate was 19.3% (95% CI, 10.1–37.2%) (45). Recently, the findings emerging from a first phase 2 study of ipilimumab/nivolumab combination in NPC were presented in the context of ESMO Asia Virtual Congress 2020. According to these results, this ICI combination provide durable responses in patients with recurrent or metastatic NPC (46).

TECHNOLOGICAL INNOVATION

Drug-Eluting Implants

Various types of devices are available for nasal drug delivery systems. Biomaterials and sinus implant are some of these. Thanks to the incessant progress of technology, new biomaterials and sinus implants have been investigated, providing postoperative effective local corticosteroids into the sinuses. Over the years, the biomaterials have been used in the CRS post-operative management settings. Polylactide sinus implants, polyurethane foam, and carboxymethylcellulose were commonly used biomaterials (47). The **bio-absorbable implants** represent an example of innovative pharmaceutical technologies. In particular, these implants allowing local release of corticosteroids (CS) could be useful in the post-operative management of endoscopic sinus surgery (ESS). In patients with CRS, even more with CRSwNP, postoperative wound healing following ESS is an important factor for procedural success. After surgical treatment, topical or systemic CS therapy, and revision surgery are the available treatment options. However, these latter have significant risks and limitations. The topical nasal CS therapy ensure more effective and lasting symptomatic benefits, as well

as reducing the size and number of polyps and preventing polyp recurrence. However, the distribution of topical steroids in the nasal cavity and sinuses is highly variable, depending on the delivery device as well as on the anatomy of the sinus drainage pathways. Steroid-releasing bio-absorbable implants have been extensively investigated for their ability to dilate and restore patency of the sinus by local and controlled release of CS. In the literature different CS-releasing bioabsorbable implants are described. A bio-absorbable, fluticasone propionate (FP)-eluting implant (SinuBand FP) resulted well-tolerated and effective in patients with CRS and nasal polyps. In particular, the results of a first-in-human, randomized, partially double-blind, single-tertiary-referral-center, controlled trial showed its local, and ocular safety. Compared to a standard nasal pack, or to a SinuBand without FP, SinuBand FP allowed significantly better polyp score ($p = 0.03$) and a better trend of inflammatory process. Patients receiving the bioabsorbable, fluticasone propionate-eluting implant reported lower pain (48). Moreover, bioabsorbable mometasone-eluting implants were also investigated (49, 50). In a prospective, randomized, double-blinded, placebo-controlled study, the endoscopic appearance in the healing process of CRSwNP after ESS was improved in patients receiving mometasone furoate (MF)-impregnated biodegradable nasal dressings (BNDs) (51). A comprehensive, up-to-date literature review reported a novel, mometasone furoate (MF) sinus implant such as useful treatment for patients with recurrent CRSwNP after ESS, playing an important role in the management (52). MF implants were also evaluated with additional topical nasal spray therapy. According to the results of a pooled analysis of data from 2 randomized controlled trials (RCTs), it emerged that this association has allowed more favorable results, in terms of subject than objective endpoints, compared to topical therapy with nasal spray alone, being useful in the management of patients with NP, especially those who have allergic rhinitis, expanded polyposis, altered odor or ESS <24 months (53). So, according clinical evidences, steroid-eluting bioabsorbable implants result safe and effective in the reduction of polyp size, symptom burden, and the need for revision sinus surgery. Favorable safety profile and efficacy of bioabsorbable steroid-impregnated implants in improving the healing process following ESS emerged from a recent meta-analysis including eight randomized controlled trials (54). About 14% of CRS patients undergoing surgery require ESS revision for a variety of reasons, including recurrence of nasal polyps and inflammation, adhesion formation, middle turbinate lateralization. So, the use of the nasal bioabsorbable implants appears to have a favorable economic impact. In fact, considering the substantial annual revision ESS costs, the use of the implant instead of revision ESS could result in considerable cost savings (55). **Sinus implants** made up of bioabsorbable polymers represent another new method to optimize surgical outcomes and to treat recurrent nasal polyposis after ESS. They allow sustained-release corticosteroids to be delivered locally directly to inflamed sinus tissues. Once implanted, these expand to fit different sizes and shapes, adapting to the space after surgery (47).

Super-Selective Intra-Arterial Infusion of Chemotherapy With Concomitant Radiotherapy

Another innovative technological approach emerged also for the treatment of the maxillary sinus cancer (MSC). It is represented by the super-selective intra-arterial infusion of chemotherapy with concomitant radiotherapy (RADPLAT). It was developed in order to overcome some patients' problems related to advanced MSC surgical procedures, such as impaired facial function and significant facial deformity. Moreover, it is useful therapeutic strategy also for those patients with stage T4b MSC for which there is no indication of surgical resection (56, 57). The super-selective intra-arterial chemotherapy with radiation therapy reflects a precision medicine approach associated with a low risk of side effects. This effective procedure might be useful to avoid highly invasive surgery (58). However, the published studies refer to small patient samples (56–59). The introduction of technological innovations has allowed a significant expansion of outpatient rhinology (60).

Balloon Catheter Dilatation

Innovation refers also to improved old technologies, like balloon-dilatation. Since 2005 the balloon catheter dilatation (BCD) represents a useful intervention for the management of CRS. BCD is a minimally invasive procedure aimed to restore physiological sinus drainage, safely dilating sinuses through microfractures (61). BCD has become a common treatment for chronic sinusitis in the United States (62). It is among the most common office-based rhinological procedures (60). Over the years, it has been renewed in several aspects, including innovations in ergonomics and lighted guidewires in order to make the utilization more effective and safer. Now, the new devices are equipped with suction and irrigation capabilities and allow multisinus applications using just one device. Moreover, the previous tools used fluoroscopy for localization with the consequent risks of exposure to radiation. Today, transillumination and real-time 3-dimensional image guidance have been introduced to overcome these problems. Some studies suggest the use of BCD as a safe tool in the management of pediatric CRS (pCRS). However, they refer to small samples and show methodological limitations (63–65). According to recent meta-analyses and systematic reviews, more evaluations are needed to demonstrate its clinical usefulness in terms of improving the quality of life and the comparative efficacy of BCD compared to standard treatment regimens in specific patients' subgroups such as children (61, 65–67). Moreover, as with all interventions, BCD, although minimally invasive, can be associated with adverse events such as cerebrospinal fluid leaks, mainly reported with frontal sinus procedures (62).

Artificial Intelligence and Machine Learning

In the era of big data, the application of artificial intelligence (AI), the machine learning (ML), and particularly deep learning, represent increasingly relevant topics of the health care research, also in the rhinology field. Recently, in order to efficiently use

all recorded data, AI and ML technology has been used in some studies of chronic rhinitis and allergic rhinitis, providing some exciting new research modalities. Regarding the application of AI and ML technology, few reports describe their use in rhinology. Only recently (since 2015) a slow increase in their descriptions emerged in literature. In the majority of the rhinologic studies in which an AI approach was used, cluster analyses were performed, i.e., to predict surgical vs. medical treatments for CRS in patients who did not have successful outcomes after initial medical treatment (68). Regarding the ML technology, the majority of algorithms are divided into supervised or unsupervised learning. This latter has been reported as a novel tool in the investigation of CRS. It represents a paradigm shift from the traditional approach based upon the clinically recognized phenotypes of CRS "with polyps" and "without polyps." Instead, an unsupervised learning approach using the application of complex mathematical models is able to derive other different subgroups which can then be further examined (69).

Augmented and Virtual Reality

Finally, also the augmented reality (AuR) and the virtual reality (VR) represent other new technological approaches applied to the rhinology field. The main difference between these new technologies consists in an enhancement of a user's natural vision obtained with AuR, that instead, in VR, comes completely replaced. Today, the VR can be used in the surgical simulation, allowing modernization of training and its transition from the practice of simple exercises into a fully-immersive environment experience. In a recent study, the use of a virtual coach was tried guiding a group of surgeons using surgical videos, auditory, and visual cues (70). In AuR, the real-world environments are combined with computer-generated sounds, text, and graphics. So, the AuR represents a tool for the surgeons that improve visualization, location, and orientation allowing improvement of surgical outcomes in terms of operating time, precision, and increased surgeon confidence. AuR can also represent a tool for procedure simulations or anatomy education, allowing the students to learn head and neck anatomy, often difficult to conceptualize. It has grown rapidly and continues to expand (71). Rarely, the AR has been used as a diagnostic and treatment tool through specific AuR-based platforms described in some studies.

DIGITAL INNOVATION

Today, we are in the digital era. Digital tools and devices are ubiquitous. We are learning to exploit the goals achieved in terms of connectivity and connection also for the management of health and, therefore, of diseases. Thanks to the achieved technology progress, therapeutic treatments can take advantage of software and devices. The digital approach is particularly able to obtain a real-time control and support of behavior and health status, improving quality of health care in the long term by greater patient involvement. We are witnessing to the introduction of **digital therapeutics**, as clinically validated treatments designed to complement or potentially replace traditional therapies (72). Moreover, digital advances have allowed innovative, almost futuristic approaches such as that of **digital twins**. These latter represent an engineering concept which can be applied to different complex systems, including that of human physiology (73). Digital twins are built on computer-based models that are fed individual and population data. The translation of the digital twin concept to patients aims to improve diagnostics and treatment in order to deliver data-driven personalized medicine. Beyond these revolutionary paths, digital progress is applied even to innovative approaches that are much more accessible. These take the form in eHealth, mobile-Health (mHealth) such as the telemedicine based on the obtained connectivity of mobile devices with the internet (74–77). Today, given the current COVID-19 pandemic, several professional societies are encouraging the maximization of the use of telemedicine in current practice (78). This approach introduced a new way for generating health and medical data—by the individual, in real time, in a real-world environment. Although these features are interesting, the benefits of digital medicine have to be proven through rigorous research, especially validation through randomized, controlled clinical trials. The **mHealth**, as a branch of eHealth defined as "medical and public health practice supported by mobile devices," can be effective to facilitate communication between primary care providers, able to overcome geographical and temporal barriers as well as to treatment accessibility and availability. It can use different tools, including smartphone applications (app), SMS text messaging with a support service, physical symptom tracking through wearable technologies, and receiving virtual therapy. The mHealth tools are developed to improve patient empowerment *via* education and self-management and will hopefully contribute to better patient adherence, quality feedback to the physician and improved patient health literacy. As reported by mHealth users, it is advantageous compared with face-to-face therapy, allowing them to be more open and honest (79). Moreover, mHealth therapy allows rapid adaptation of the treatment strategy based on the symptoms, concomitant medications and key events that may impact the disease. In particular, mobile applications are achieving a prominent position in the management of chronic diseases. For chronic respiratory diseases, most of the apps have been developed for lower respiratory diseases such as asthma or COPD. Recently, Bodini et al. have identified 5 Digital Therapeutics (DTx) for asthma and COPD which combine sensor devices, mApps for patients, and cloud-based software for healthcare professionals (80). They consent to record if/when/how the patient uses the inhaler, to alert for use the inhaler, to receive information from the sensor, providing a personalized support and remote monitoring. To date, mySinusitisCoach is a mobile app available for patients with sinus disease (81). This tool has been launched during the European Rhinology Research Forum (ERRF) 2017. It was designed, developed and implemented to support CRS patients in monitoring their symptoms and to provide patients with a digital support platform containing reliable medical information about their disease and treatment options. MySinusitisCoach has been developed thanks to a collaboration between CRS medical experts, patients, general practitioners and community pharmacists. This collaboration was sought to obtain a tool that would meet the needs of both

patients and healthcare professionals. Its functionalities include the monitoring of symptoms and consumption of drugs, the visualization of the disease control level, providing unbiased information on chronic sinusitis and asthma. Moreover, the easy sharing of data with the doctor in order to obtain a real-time connection between the patients and health workers, allows optimization of treatment. Recently, a cross-sectional evaluation of data obtained by users of mySinusitisCoach. This real-life assessment confirms the high disease burden in uncontrolled CRS patients, which can be supported by mobile technology in the real-life monitoring (82). The mobile apps allows not only a continuous and remote monitoring of the patient's health status, but also an important collection of real-world data that will help in clinical studies validating patient stratification as well as understanding of the socio-economic impact of CRS, in order to improve treatment strategies. Recently, mySinusitisCoach has been replaced by Galenus Health, a mobile app developed by a team of internationally recognized doctors designed for anyone with asthma, respiratory allergy or chronic sinusitis, often concomitant diseases. Finally, the use of digital approach with a smart language can also be used for the improvement of patient education. In fact, its use can positively impact on patient outcomes such as anxiety, pain and satisfaction in relation to the perioperative patient experience. Online education materials are often too complex, inaccurate or misleading to be useful to the patient. A recent study has been conducted by University of British Columbia in order to evaluate the effect of patient education videos on perioperative anxiety in patients undergoing endoscopic sinus surgery. The enrolled patients received four short YouTube videos explaining chronic rhinosinusitis and endoscopic sinus surgery. Patients of the control group received the standard of care patient education with verbal and written education. The study is completed but results not yet available (83, 84).

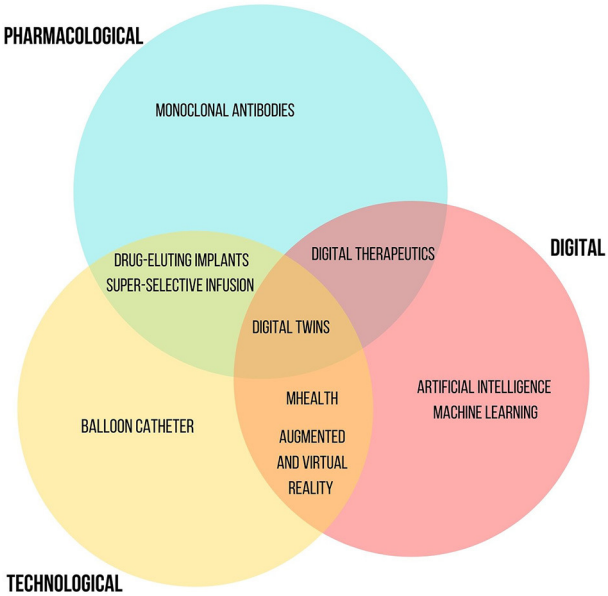

FIGURE 3 | Overlapping nature of each innovative tool in Rhinological conditions.

CONCLUSION

Innovative aspects in rhinology involve new drugs, technologies for their administration as well as digital applications. Each different innovative tool has an important impact and allows an improvement in several clinical and patients outcomes, including quality of life, efficacy and safety. The discussed innovative tools show an overlapping nature among the considered fields (**Figure 3**). The emerging innovative drugs include mAbs targeting on characteristic pathways of type 2 inflammation, such as those of IgE, the IL-5, and IL-4/IL-13 which are involved in several pathologic condictions including CRSwNP or allergic rhinitis. Dupilumab (anti IL-4), mepolizumab (anti IL-5), and omalizumab (antiIgE) represent the main mAbs developed such as innovative treatment options for patients with NP and CRS. They seem to allow improvement in terms of quality of life, disease severity and tolerability of treatment. Other mAbs are in the advanced research stages like etokimab (anti-IL33) or tezepelumab (targeting on TSLP). Moreover, another important application field of mAbs is the oncological immunotherapy. Considering the higher expression of PD-L1 in NPC, the use of PD-1 inhibitors, such as nivolumab, or a dual CTLA-4/PD-1 blockade (85) appear to be an effective strategy for the treatment of this cancer form. However, current studies are not yet at in advanced stages. The careful monitoring of patients with regard to the autoimmune toxicity related to them should not be underestimated. Regarding the technological innovation, the implants with bio-absorbable biomaterials represent new interesting available technologies. In particular, those allowing the topical administration of corticosteroids drugs (fluticasone and mometasone) are useful treatments for patients with recurrent CRSwNP after ESS, playing an important role in its management. Moreover, considering the substantial annual costs of ESS, their favorable economic impact is worthy of note. Advanced technologies such as, AI and ML, as well as AuR and VR have also proved useful in the rhinologic field with their main impacts on precision medicine and surgery. Finally, the development and use of mHealth tools represent a winning strategy in monitoring therapy success, safety and tolerability as well as the progress of chronic disease including CRSwNP. They seem to be efficient and effective mainly in the improvement of patients' outcomes. The mobile apps allow to improve patient empowerment by an active participation in the decision-making process of the therapeutic plan. Likewise, their use allows collection of real-world data that will help to improve treatment strategies in a greater perspective of personalized and precision medicine. So, supporting the research of innovative tools and strategies (including pharmacological, technologic, or digital ones) is essential to improve the management of chronic diseases that significantly affect the quality of life of patients. Further studies are strongly needed in order to support their use

in real life context. In the future, the use of combined innovative approaches is desirable.

AUTHOR CONTRIBUTIONS

FR, AC, and RR: drafting the work and revising it for important intellectual content. RR, GioM, and GiuM: substantial contributions to the acquisition, analysis, or interpretation of data for the work. FR, CR, and ADC: final approval of the version to be published. AC, AD, and GaM: agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. FR and GaM: developed the concept and designed the study. RR and GioM: wrote the paper. All authors contributed to the article and approved the submitted version.

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Biologic Responses to House Dust Mite Exposure in the Environmental Exposure Unit

Lubnaa Hossenbaccus[1,2], *Sophia Linton*[1,2], *Jenny Thiele*[2,3], *Lisa Steacy*[2], *Terry Walker*[2], *Crystal Malone*[2] *and Anne K. Ellis*[1,2,3]*

[1] Department of Medicine, Queen's University, Kingston, ON, Canada, [2] Allergy Research Unit, Kingston Health Sciences Centre–KGH Site, Kingston, ON, Canada, [3] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

Introduction: Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa that can be modeled using Controlled Allergen Exposure Facilities (CACF). Recently, we clinically validated the house dust mite (HDM) Environmental Exposure Unit (EEU) facility. In the current study, we aimed to assess biological responses in the blood following HDM exposure in the HDM-EEU.

Methods: Fifty-five participants passed a screening visit, where they provided consent and completed a skin prick test (SPT), then attended a modest or higher HDM exposure session. Baseline and post-exposure blood samples were collected. Complete blood counts with differentials were measured, and isolated serum was used to determine *Dermatophagoides farinae*- and *Dermatophagoides pteronyssinus*-specific IgE (sIgE) and cytokine concentrations (IL-4, IL-5, IL-6, IL-10, IL-13, TNF-α).

Results: HDM-allergic participants had significantly greater SPT wheal sizes than healthy controls. sIgE concentrations were significantly greater in allergic participants, with a strong correlation between *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. Serum eosinophil counts were significantly decreased post-exposure for allergic participants. White blood cell, neutrophil, and lymphocyte counts were significantly increased for both allergic and non-allergic participants post-exposure. Serum IL-13 concentrations were significantly reduced post-exposure in allergics while TNF-α was significantly reduced in non-allergics.

Conclusion: The HDM-EEU is a useful model for investigating biologic mechanisms of HDM-induced AR. Allergic participants produced measurable biological changes compared to healthy controls following allergen exposure, specifically with serum expression of eosinophils and related markers, namely IL-5, which promotes the proliferation and differentiation of eosinophils, and IL-13, a cytokine released by eosinophils. The exact mechanisms at play require further investigation.

Keywords: allergic rhinitis, Environmental Exposure Unit, house dust mite, cytokines, skin prick test, immunoglobulin E, Der p, Der f

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***Correspondence:**
Anne K. Ellis
ellisa@queensu.ca

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INTRODUCTION

Allergic rhinitis (AR) is a nasal inflammatory disease triggered by exposure to seasonal or perennial allergens, such as animal dander and house dust mite (HDM), which are present year-round. Common species include the American (*Dermatophagoides farinae*) and European (*Dermatophagoides pteronyssinus*) HDMs, and the prevalence of sensitization to these mites is reported to be up to 90% in various countries (1). Over 35 allergens have been isolated from the feces of HDM, with Der p 1, Der p 2, Der f 1, and Der f 2 being the main culprits in the induction of AR symptoms (2). Diagnosis of an HDM allergy involves a review of clinical history and physical examination as well as diagnostic testing including skin prick testing (SPT) and HDM-specific IgE testing.

Allergic sensitization involves the processing of allergens by antigen presenting cells, such as dendritic cells, and presentation on major histocompatibility complex (MHC) class II molecules on the cell surface. Through the T cell receptor (TCR), naïve T cells are primed and differentiate to type 2 helper (Th2) T cells, elucidating a predominantly Th2-mediated immune response (3). Cytokines released by Th2 cells, such as interleukin (IL)-4, IL-5, and IL-13, stimulate IgE production and class-switching and encourage the differentiation of eosinophils to promote allergic inflammation (4–6).

Following re-exposure, allergens crosslink to cell-bound IgE on mucosal mast cells, resulting in degranulation and the release of pre-formed cytoplasmic inflammatory molecules such as (but not limited to) histamine (7). The release of these molecules characterizes the early phase AR response, occurring in a matter of minutes and resulting in the clinical symptoms of nasal itching, sneezing, and rhinorrhea, through increased vascular permeability and mucous secretion.

Mast cells also contribute to the late phase AR response, occurring between 4 and 8 h following allergen exposure (8). Cytokines further promote the recruitment of other inflammatory mediators and cells from the peripheral blood to the nasal mucosa (9, 10). As a result, the nasal mucosa is primed for further allergen exposure and causes persistent symptoms including nasal obstruction or congestion. These late phase inflammatory processes affect tissue remodeling.

Allergic rhinitis can be modeled using Controlled Allergen Challenge Facilities (CACFs). These facilities are custom designed and specifically engineered to control variables including, but not limited to, air quality, temperature, humidity, allergen type, and most importantly allergen concentration with a large group of participants. In addition to highly accurate and complete symptom reporting, CACFs also permit the collection of biologic samples, including blood and nasal specimens. These may provide insights into serum cytokine concentrations throughout or following allergen exposure and genetic or epigenetic changes (11, 12).

The Environmental Exposure Unit (EEU) was the first CACF to be built in North America. Established in the late 1980's, and currently located at the Kingston Health Sciences Centre–KGH site, the EEU has been used extensively for the evaluation of

TABLE 1 | SPT Sensitization.

Allergen	Number of allergic participants
D. pteronyssinus	44
D. farinae	44
Timothy Grass	22
Ragweed	32
Birch	25
Cat	22
Dog	7
Oak	9*
Alder	12**
Alternaria	9

*Out of 25 participants.
**Out of 24 participants.

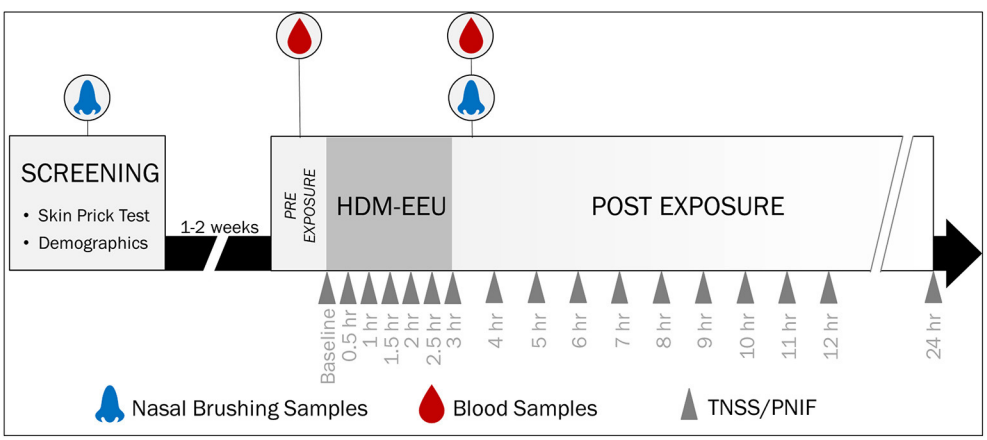

FIGURE 1 | Study Design and Sample Collection. Participants were recruited for a screening visit, where skin prick testing was completed to evaluate allergic sensitization to a panel of allergens. Eligible participants were invited to a modest or higher 3-h HDM exposure session in the HDM-EEU. Blood and nasal samples were collected pre- and post-HDM exposure. Hossenbaccus et al. (17).

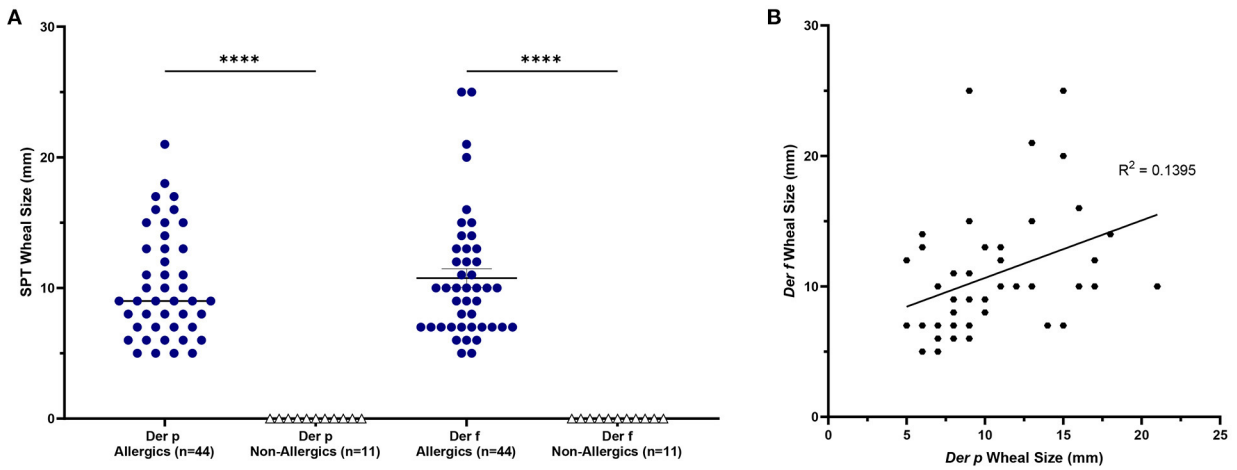

FIGURE 2 | SPT wheal sizes were significantly elevated for allergics. Allergics had significantly elevated SPT wheal sizes for both Der p and Der f compared to non-allergics **(A)**. A weak correlation ($R^2 = 0.1395$) was observed between wheal sizes of the two HDM allergens **(B)**. ****, $p < 0.0001$.

ragweed, grass, and birch allergy (13–16). A specially designed facility now housed within the main EEU was developed to study perennial allergens, the HDM-EEU, and can host 5 to 35 participants per session.

In August 2019, we clinically validated the HDM-EEU, demonstrating that it can generate AR symptoms in HDM-allergic individuals (17). We exposed participants to a modest [(Der f 1) = 2.67 ng/m^3 and (Der p 1) = 2.07 ng/m^3] or higher HDM [(Der f 1) = 3.80 ng/m^3 and (Der p 1) = 6.66 ng/m^3] target for 3 h and measured symptoms for up to 24 h post-exposure. Allergic participants exposed to a higher HDM target experienced a significantly greater peak in mean TNSS at 2.5 ($p < 0.05$) and 3 h ($p < 0.01$) compared to modest target allergics. Compared to healthy controls, allergics experienced significantly elevated TNSS and TRSS from 1 to 5 h following the onset of allergen exposure, irrespective of allergen concentration. Blood samples were collected pre- and post-HDM exposure using the HDM-EEU and here we report the biologic responses of HDM-allergic and non-allergic participants.

MATERIALS AND METHODS

Study Design

Participant recruitment and study inclusion/exclusion criteria for this study were previously published (17). In short, sixty-eight participants 12 to 65 years of age were recruited and attended a screening visit where SPT was completed (**Figure 1**). Fifty-five participants passed screening, with forty-four HDM-allergics and eleven non-allergic controls, who were not sensitized to any allergen evaluated on the SPT panel. Thirty eligible participants attended a modest and twenty-five attended a higher HDM exposure session in the HDM-EEU. Blood and nasal samples were collected before and after HDM exposure. Peripheral blood collected in PAXgene Blood RNA tubes (PreAnalytiX) pre-exposure are reported elsewhere (18) and nasal sample findings are not reported here.

Skin Prick Testing

SPT was performed at screening on the volar surface of the participant's forearm using allergen extracts prepared in a Duotip-Test® II Dipwell tray. The allergen panel included *D. pteronyssinus* [ALK-Abelló; 10,000 allergy units (AU)/mL], *D. farinae* (ALK-Abelló; 10,000 AU/mL), Timothy grass [ALK-Abelló; 100,000 bioequivalent allergy units (BUA)/mL], ragweed [ALK-Abelló; weight per volume (w/v) 1:20], birch (ALK-Abelló; w/v 1:20), cat (Hollister-Stier; 10,000 BAU/mL), dog (ALK-Abelló; w/v 1:20), oak (ALK-Abelló; w/v 1:20), alder (ALK-Abelló; w/v 1:20), and *Alternaria* (ALK-Abelló; w/v 1:20). Histamine and glycerin phenol-saline were the positive and negative controls, respectively. The panel was administered using sterile plastic bifurcated Duotip-Test© II devices. Results for *D. pteronyssinus* and *D. farinae* were determined to be positive if the wheal diameter was 5 mm of greater than the negative control. For all other allergen extracts, a positive result was classified as a wheal diameter of 3 mm or greater than the negative control.

Complete Blood Count With Differential

Hematology samples collected in EDTA tubes (BD) were used for complete blood count (CBC) with differential analysis, processed by the Kingston Health Sciences Centre–KGH site Core Laboratory.

Serum Samples

Blood samples were collected pre- and post-exposure, consisting of a serum separator tube (SST, BD).The SST tubes were allowed to clot at room temperature for 30 min (19). The clot was separated from the serum following the centrifugation of the tubes at 1,500 g for 15 min at room temperature. The serum was aliquoted into 3 microfuge tubes (Sarstedt) using a 1,000 μL pipette, such that each contained ∼500 μL of sample. The tubes were frozen and stored at −80°C.

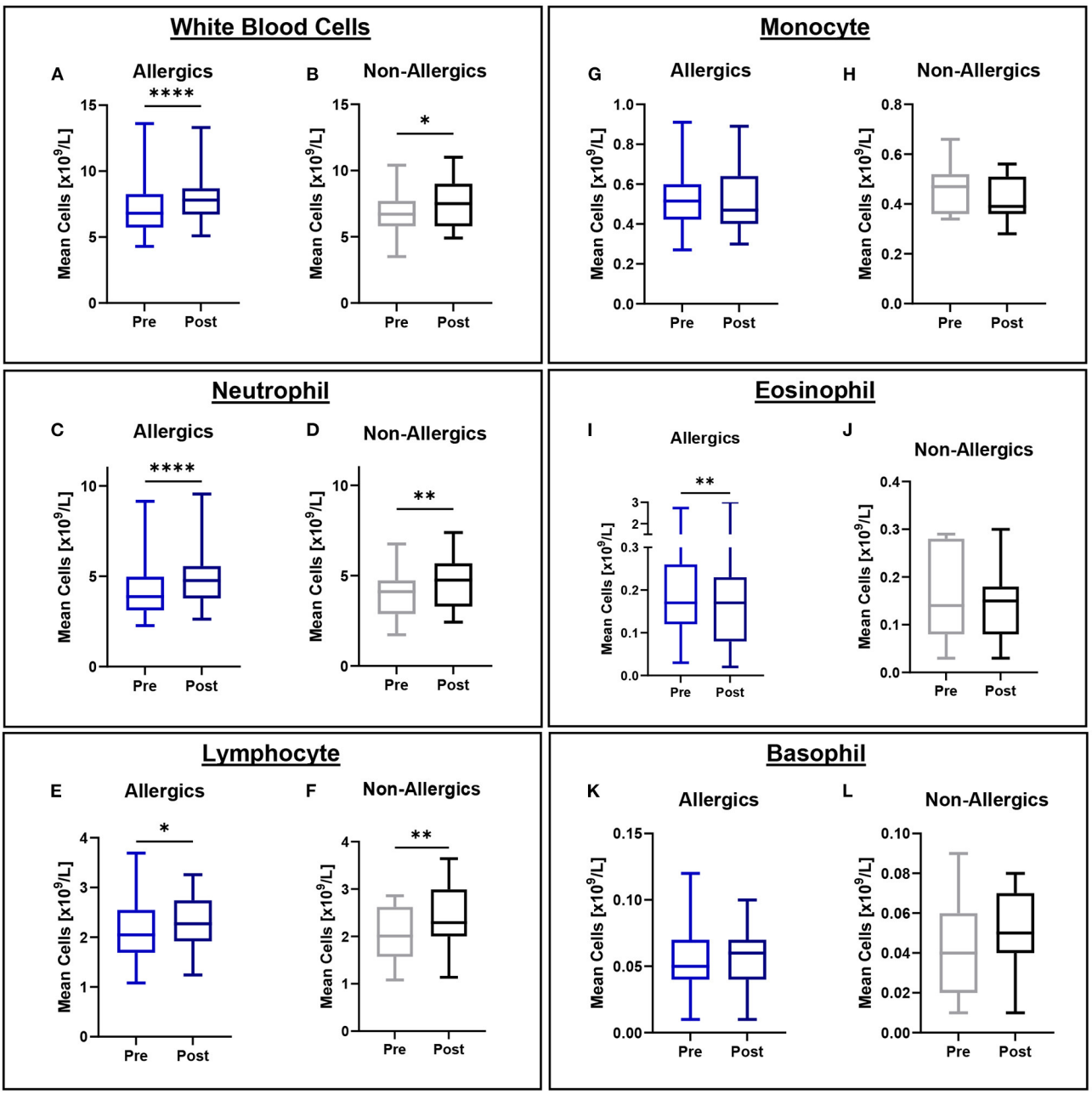

FIGURE 3 | White blood cell counts in peripheral blood collected pre- and post-exposure for HDM-allergic and non-allergic participants. Concentrations of white blood cells **(A,B)**, neutrophils **(C,D)**, lymphocytes **(E,F)**, monocytes **(G,H)**, eosinophils **(I,J)**, and basophils **(K,L)** were evaluated pre- and post-HDM exposure for allergic and non-allergic participants in these paired analyses. Eosinophil concentrations were significantly decreased only for HDM-allergic participants **(I)**. *, $p < 0.05$, **, $p < 0.01$, ****, $p < 0.0001$.

Serum HDM-Specific IgE

Frozen serum samples were thawed and 400 μL was aliquoted into test tubes. The Phadia™ 100 and ImmunoCAP® assay (Somagen™ Diagnostics) were used to measure the concentration of *D. pteronyssinus* and *D. farinae*-specific IgE. Calibrators (0.001, 0.35, 0.70, 3.50, 17.5, and 100 kUA/L), two curve controls, quality controls (low, medium, and high), a negative control, and positive internal controls were used. The fluorescence of the eluate was measured to determine the concentration of sIgE in the samples, relative to a calibration curve established in the first run. Two curve controls were used in subsequent assays against the same calibration curve. The assay procedures were all completed by the instrument.

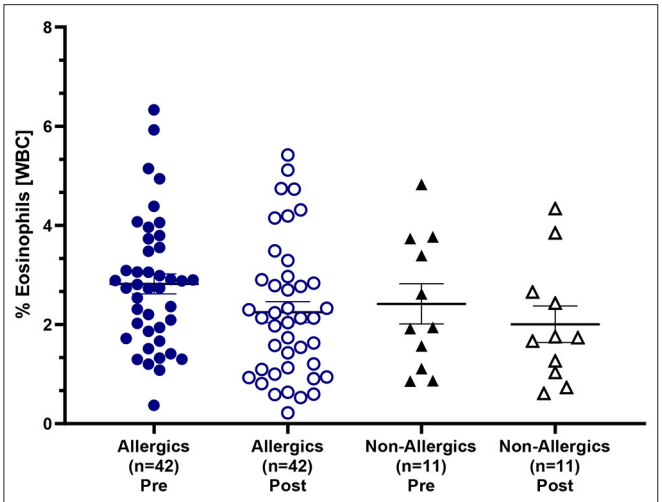

FIGURE 4 | Eosinophil counts as a percentage of white blood cells in the peripheral blood. Blood samples collected pre- and post-HDM exposure were evaluated for complete blood counts with differentials. Eosinophils were evaluated as a percentage of all white blood cells in the peripheral blood. Both allergic and non-allergic participants experienced a decrease in % eosinophils post-exposure compared to pre-exposure.

Serum Cytokine Concentrations

Frozen pre- and post-exposure serum samples were thawed, and the following cytokines were evaluated using a Human High Sensitivity T Cell Magnetic Bead Panel assay: IL-4, IL-5, IL-6, IL-10, IL-13, and TNF-α. The assay was performed as per the manufacturer's protocol. The plate was run on the Bio-Plex® 200™ using the Bio-Plex Manager 6 software.

Statistical Analysis

Pre- and post-exposure mean cell counts ($x10^9$/L) and cytokine concentrations were plotted for allergics and non-allergics, analyzed using Wilcoxon matched-pairs signed rank tests. Change in mean cell counts and cytokine concentrations for allergics and non-allergics, as well as when stratified by HDM exposure level (modest vs. higher) were evaluated using Mann-Whitney tests. Percent eosinophil counts relative to white blood cell concentrations and sIgE concentrations were analyzed using a Kruskal-Wallis test with Dunn's multiple comparisons. Correlations were evaluated using Pearson correlation coefficients. GraphPad Prism 9.2.0 software was used for analysis and graphing.

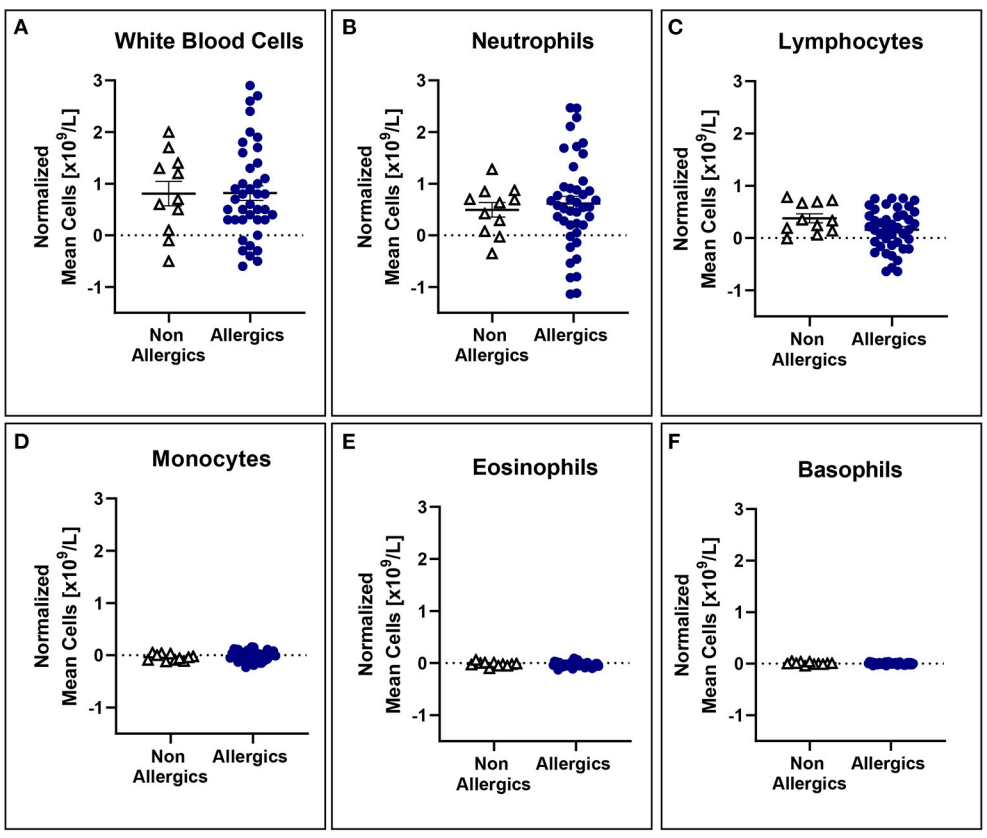

FIGURE 5 | White blood cell counts normalized to baseline are comparable for both allergic and non-allergic participants. No significant differences were observed in white blood cell **(A)**, neutrophil **(B)**, lymphocyte **(C)**, monocyte **(D)**, eosinophil **(E)**, and basophil **(F)** counts normalized to baseline in the peripheral blood.

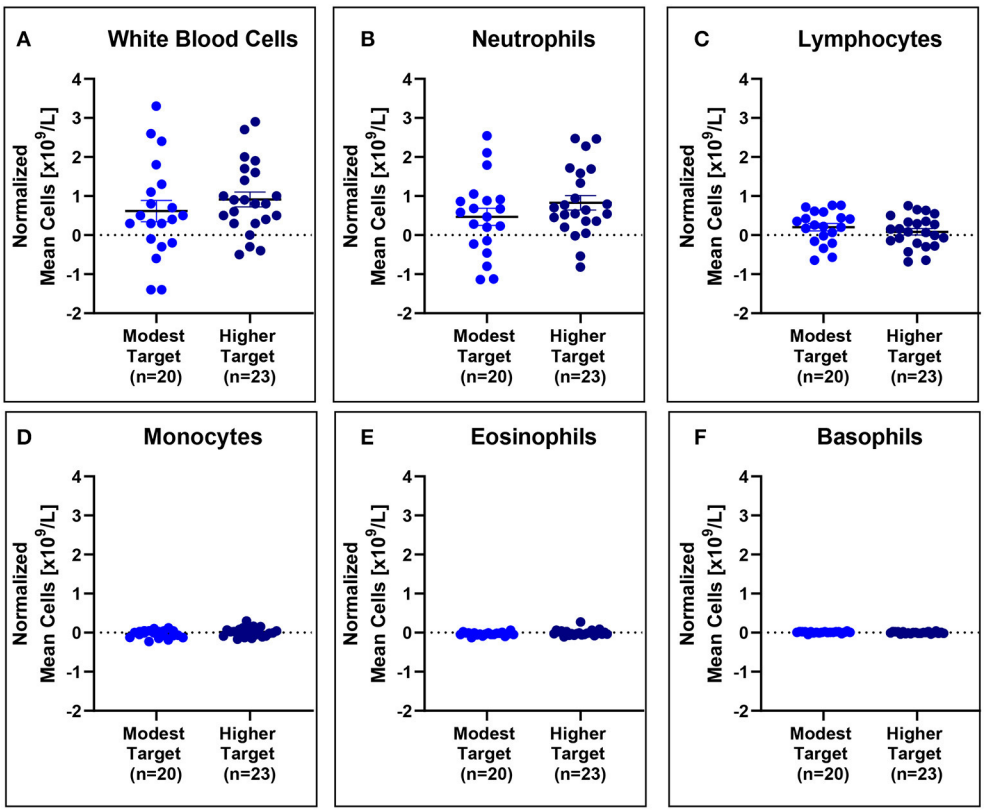

FIGURE 6 | White blood cell counts are comparable for modest and higher target allergics. No significant differences were observed in change in white blood cell **(A)**, neutrophil **(B)**, lymphocyte **(C)**, monocyte **(D)**, eosinophil **(E)**, and basophil **(F)** counts normalized to baseline in the peripheral blood post-exposure for allergics exposed to a higher vs. modest HDM target.

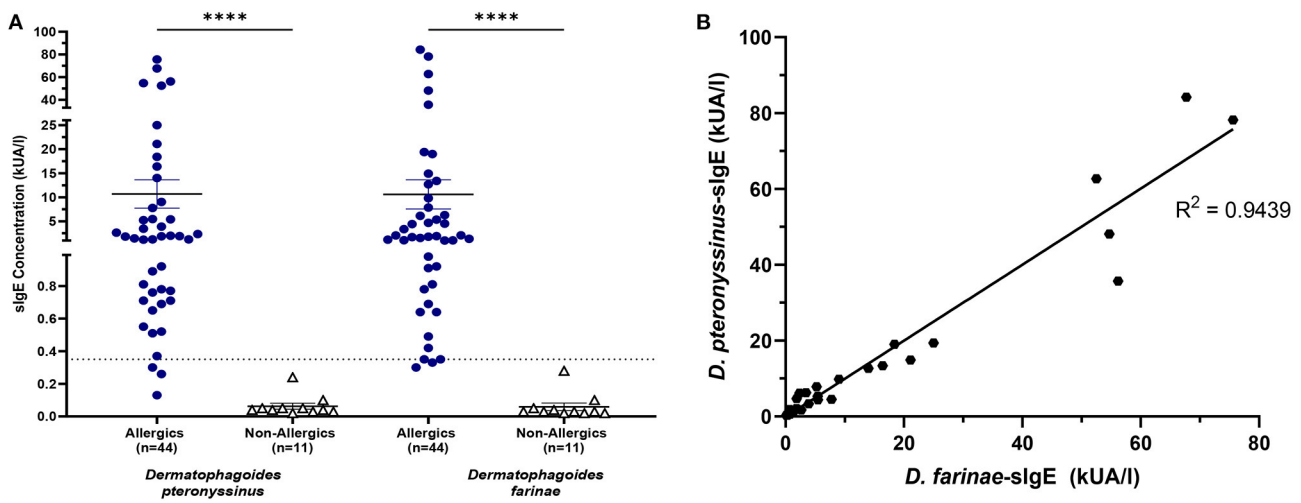

FIGURE 7 | HDM-specific IgE concentrations were significantly increased for allergics. Allergics had significantly elevated *Dermatophagoides pteronyssinus*- and *Dermatophagoides farinae*-sIgE compared to non-allergics **(A)**. Concentrations of *D. pteronyssinus*- and *D. farinae*-sIgE were strongly correlation **(B)**. ****, $p < 0.0001$.

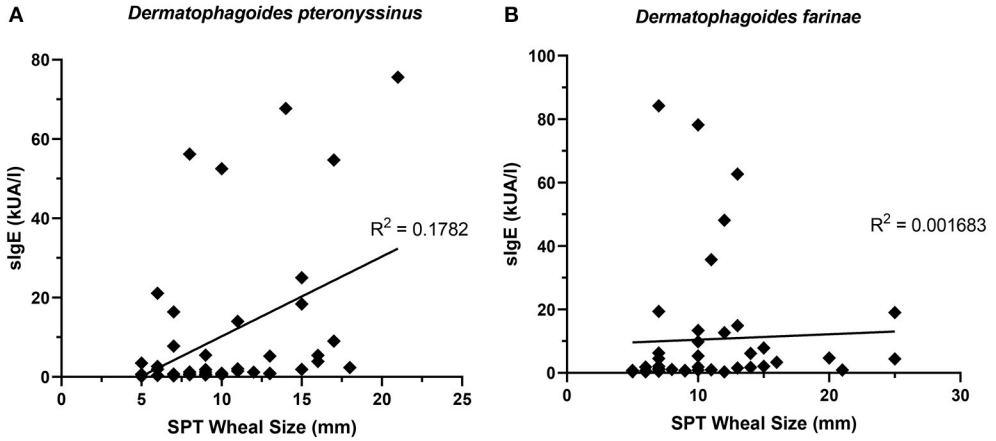

FIGURE 8 | sIgE concentrations and SPT wheal sizes were poorly correlated. SPT wheal sizes and sIgE were poorly correlated for both *Dermatophagoides pteronyssinus* **(A)** $R^2 = 0.1782$ and *Dermatophagoides farinae* **(B)** $R^2 = 0.001683$.

RESULTS

Skin Prick Test Findings

Fifty-five participants successfully completed this study, with twenty-four allergics and six non-allergics attending the modest HDM allergen target concentration session and twenty allergics and five non-allergics attending the higher HDM target concentration session. Most allergic participants were polysensitized to various allergens evaluated using SPT, with only 4 participants who were monosensitized to just the two HDM allergen extracts (**Table 1**).

HDM-allergics had significantly bigger ($p < 0.0001$) SPT wheal diameters for both *D. pteronyssinus* and *D. farinae* extracts than non-allergic controls (**Figure 2A**). Wheal sizes between the two allergen extracts were poorly correlated (**Figure 2B**).

Allergic participants showed greater allergic sensitization to HDM than non-allergic controls.

Complete Blood Counts With Differentials

Mean eosinophil counts were significantly decreased ($p < 0.01$) in the peripheral blood of only allergic participants post-exposure compared to baseline (**Figures 3I, J**). When evaluating eosinophils as a percentage of all white blood cells, no significant differences were observed (**Figure 4**).

Mean white blood cell (WBC), neutrophil, and lymphocyte counts were significantly elevated for both allergics ($p < 0.0001$ for WBC; $p < 0.0001$ for neutrophils; $p < 0.05$ for lymphocytes) and non-allergics ($p < 0.05$ for WBC; $p < 0.01$ for neutrophils; $p < 0.01$ for lymphocytes) post-HDM exposure compared to baseline (**Figures 3A–F**). Mean monocyte and basophil counts were not significantly different for either non-allergics or allergics (**Figures 3G,H,K,L**).

No significant differences in mean white blood cell counts normalized to baseline were observed (**Figure 5**), even when stratified based on HDM exposure concentration (**Figure 6**).

Complete blood counts with differential show the occurrence of non-specific inflammation as well as post-exposure changes in eosinophil concentrations in allergic participants.

Serum HDM-Specific IgE

HDM-allergics had significantly greater ($p < 0.0001$) *D. pteronyssinus* and *D. farinae* compared to non-allergic controls (**Figure 7A**).

The presence of one HDM-specific IgE was strongly correlated ($R^2 = 0.9439$) with the other (**Figure 7B**), though poor correlations were observed between SPT wheal sizes and serum sIgE concentrations for *D. pteronyssinus* (**Figure 8A**) and *D. farinae* (**Figure 8B**).

Serum Cytokine Concentrations

Serum IL-13 concentrations were significantly reduced ($p < 0.05$) in allergics post-exposure (**Figure 9I**) while TNF-α was significantly reduced ($p < 0.05$) in non-allergics post-exposure (**Figure 9L**) in paired analyses. No other significant differences were observed (**Figures 9A–H**; **J, K**).

IL-5 concentrations normalized to baseline were significantly reduced ($p < 0.05$) for allergics compared to healthy controls (**Figure 10B**), though not for the other cytokines (**Figures 10A, C–F**).

Serum cytokine concentrations reveal post-exposure changes in concentrations of eosinophil-associated mediators (IL-5 and IL-13) (**Figure 11**).

DISCUSSION

HDM-allergic participants had significant changes in biological responses compared to non-allergic controls following HDM exposure in the HDM-EEU.

HDM-allergic participants had significantly elevated and more variable concentrations of *D. pteronyssinus*- and *D. farinae-sIgE* when compared to non-allergic participants, and a strong correlation between the two species. This finding is intriguing

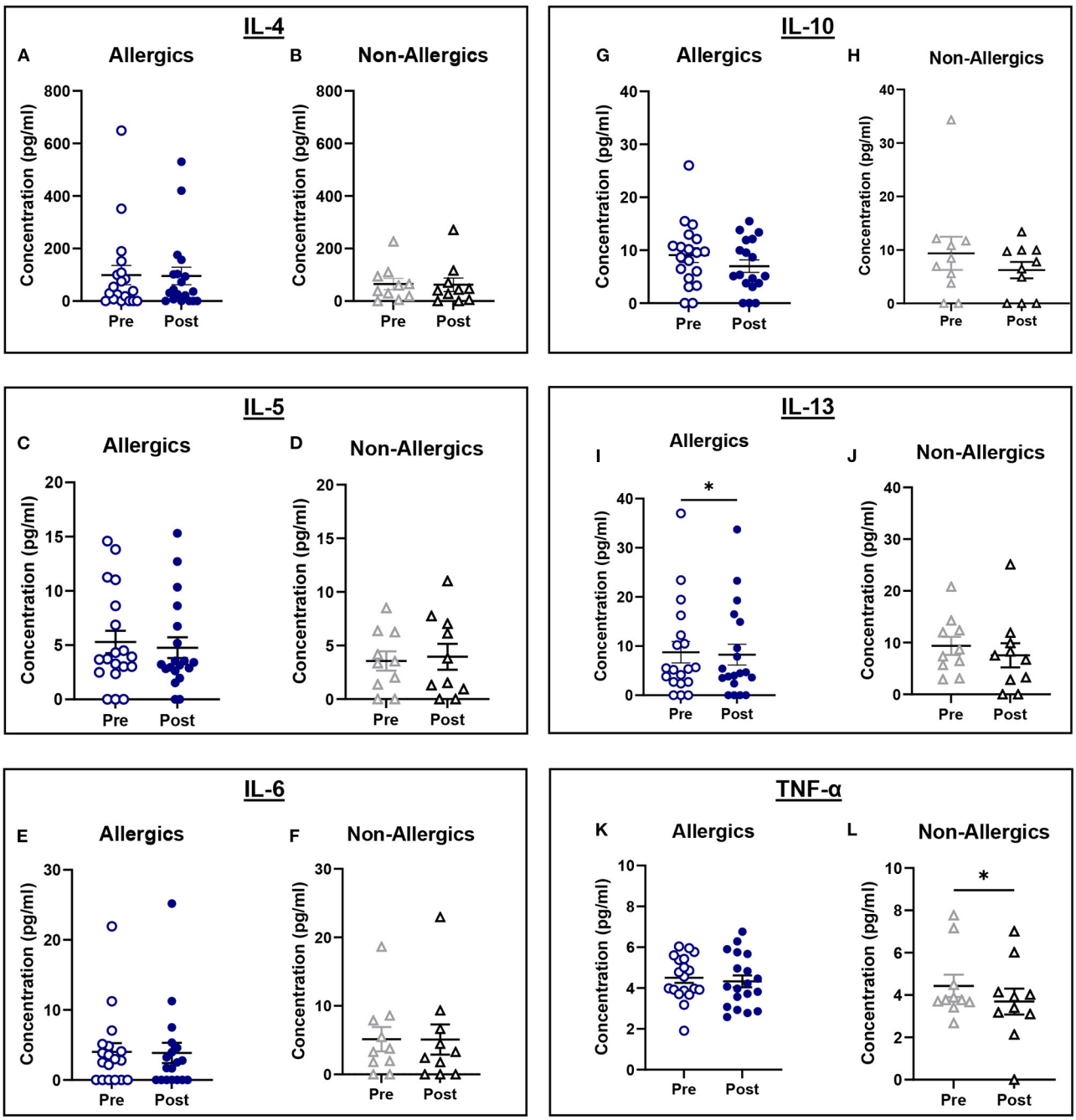

FIGURE 9 | Serum cytokine concentrations collected pre- and post-exposure for HDM-allergic and non-allergic participants. Concentrations of IL-4 **(A,B)**, IL-5 **(C,D)**, IL-6 **(E,F)**, IL-10 **(G,H)**, IL-13 **(I,J)**, and TNF-α **(K,L)** were evaluated pre- and post-HDM exposure for allergic and non-allergic participants in these paired analyses. IL-13 concentrations were significantly decreased only for HDM-allergic participants **(I)**, while TNF-α was significantly decreased in non-allergic participants. *, $p < 0.05$.

as *D. pteronyssinus* is the European HDM, whereas *D. farinae* is the American HDM. Many studies have shown that most North American homes contain measurable levels of both *D. pteronyssinus* and *D. farinae*, and it raises the question as to how the European HDM has become so prevalent in North America (20). In contrast, a European study found a weak correlation between the concentrations of Der p 1 and Der f 1 in homes in two German cities (21). While this is a more epidemiological consideration, it illustrates the effect of travel and globalization on the spread of allergic disease. Additionally, cross-reactivity between the two species may be associated with the strong correlation observed.

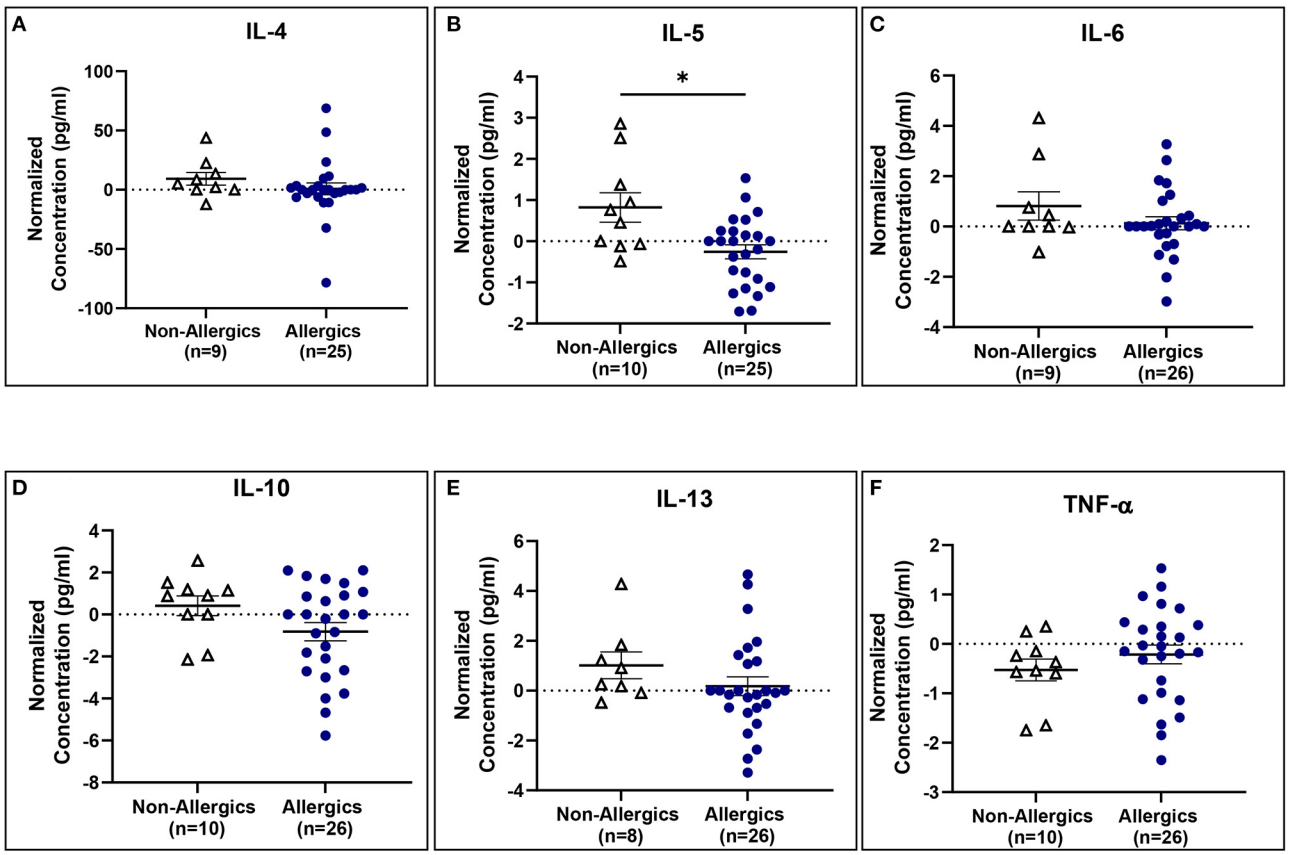

FIGURE 10 | Serum IL-5 concentrations normalized to baseline are significantly reduced for allergics compared to non-allergic participants. Concentrations of IL-4 **(A)**, IL-5 **(B)**, IL-6 **(C)**, IL-10 **(D)**, IL-13 **(E)**, and TNF-α **(F)** normalized to baseline were evaluated pre- and post-HDM exposure for allergic and non-allergic participants. IL-5 concentrations normalized to baseline were significantly decreased for HDM-allergic participants compared to non-allergic controls. *, $p < 0.05$.

sIgE was not well correlated to SPT wheal sizes. While SPT and the sIgE assay are important measures of participant's allergen sensitization and are used diagnostically, they are not interchangeable (22). SPTs reflect a targeted, localized immune response to an allergen *in vivo* and are corrected for skin histamine sensitivity by subtracting the negative control (23). SPT has the clear advantage of having a rapid turn around time, they are relatively inexpensive, and are highly sensitive (22). In our cohort, three symptomatic allergic participants had very low (<0.35 kUA/L) *D. pteronyssinus*-sIgE concentrations, two of whom also had very low *D. farinae*-sIgE concentrations, despite positive SPT results.

Paired analyses of whole blood CBCs for allergic and non-allergic participants revealed significant post-HDM exposure increases in white blood cell, neutrophil, and lymphocyte concentrations. This non-specific inflammation may be associated with small amounts of endotoxin exposure and generalized nasal irritation to the HDM allergens. Allergic participants had significantly decreased eosinophil counts post-exposure, unlike healthy controls, and had elevated percentages, though not significantly so, of eosinophils in the peripheral blood both pre- and post-exposure compared to their non-allergic counterparts. A drop in blood eosinophils may indicate cell migration to the nasal mucosa, as previous findings following Bermuda grass challenge in the NAC revealed significantly increased nasal eosinophil counts from nasal lavage samples (24). Eosinophil cationic protein (ECP), a marker of eosinophil activation, has also been found to be significantly increased by twofold in nasal fluid samples of individuals with perennial AR compared to controls (25).

Paired analyses of pre- and post-exposure serum cytokine concentrations for allergic and non-allergic participants were generally comparable, with all except for IL-5 for non-allergics decreasing post-HDM exposure. The pro-inflammatory cytokines related to Th2 activation (IL-4, IL-5, and IL-13) were expected to increase in allergic participants compared to non-allergics, but there was a significant decrease in post-exposure IL-13 concentrations for allergics. While surprising, the decrease in IL-5 and IL-13 aligns with the observed drop in blood eosinophils, may support the hypothesis that eosinophils from the peripheral blood may have migrated into the nasal mucosa.

The anti-inflammatory marker, IL-10, is responsible for the downregulation of the immune system following activation to prevent tissue damage and restore homeostasis. Given that IL-10 expression has been found to be negatively correlated with the development and severity of AR, HDM-allergic participants

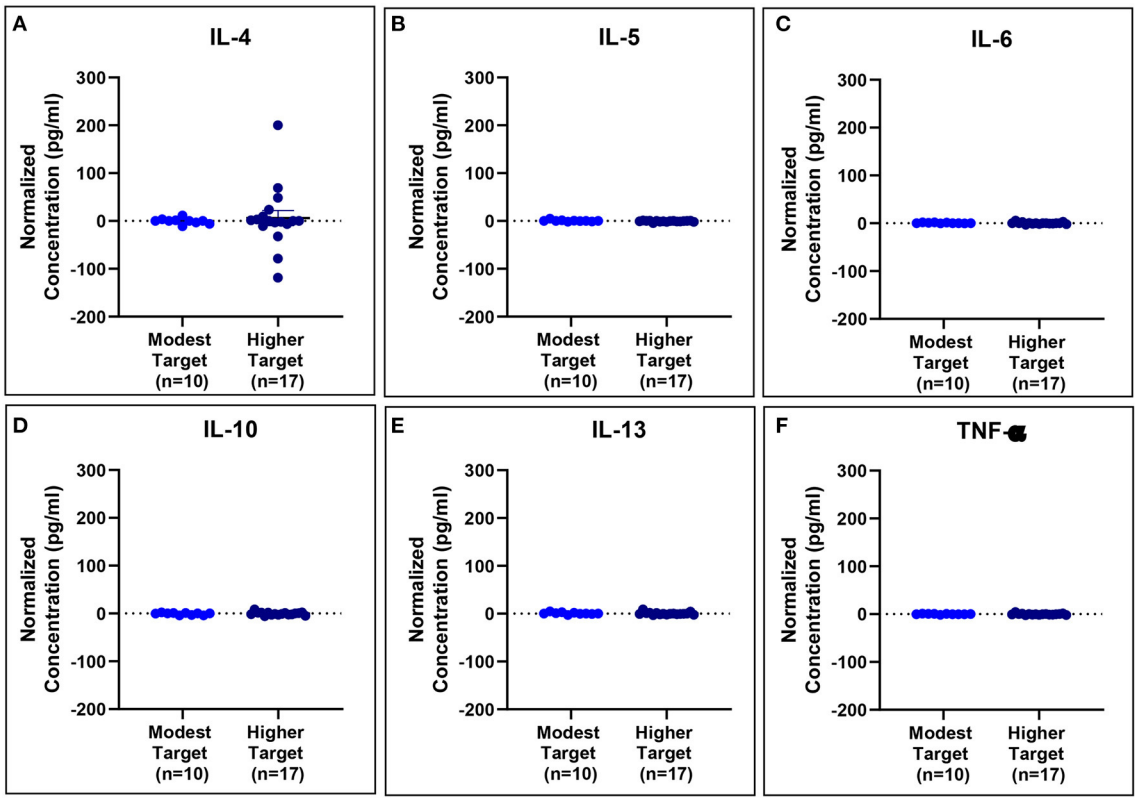

FIGURE 11 | Serum cytokine concentrations normalized to baseline are comparable for modest and higher target allergics. No significant differences were observed in IL-4 **(A)**, IL-5 **(B)**, IL-6 **(C)**, IL-10 **(D)**, IL-13 **(E)**, and TNF-α **(F)** concentrations normalized to baseline in the peripheral blood post-exposure for allergics exposed to a higher vs. modest HDM target.

would be expected to have decreased IL-10 serum concentrations (26). Change in IL-10 concentrations does show a decrease for allergics (mean = −0.8188) though not to a significant degree. IL-6 and TNF-α are pro-inflammatory cytokines that play a role in B cell regulation. These cytokines are also stimulated following endotoxin exposure and although HDM extracts typically contain lipopolysaccharide endotoxin among the allergen proteins, the concentration present in the HDM used in the HDM-EEU was within a reasonable limit (27). No significant differences were observed in the change of IL-6 and TNF-α serum concentrations between allergics and non-allergics, though non-allergics had significantly decreased TNF-α concentrations post-exposure in the paired analysis.

These results are variable, which is not unexpected as there is natural variability in individuals' cytokine expressions. Previous studies involving participants with perennial AR showed a significant decrease in IL-4, an increase in IL-5, an increase in IL-6, a decrease in IL-10, and unchanged IL-13 concentrations compared to controls; however, these were evaluated in nasal fluid samples (28). Time of collection may be another reason why the serum cytokine results observed in this cohort differ from what is reported in the literature. The post-exposure blood samples were collected soon after participants completed the 3-h exposure in the HDM-EEU. A longer timespan between exiting the facility and post-exposure blood sample collection may have allowed the localized immune reaction in the nose to better spread systemically into the peripheral blood, as the late-phase AR response is thought to occur within 4–6 h of allergen exposure (29). However, even in nasal fluid following NAC, cytokine expression levels for perennial AR do not appear to be as distinctly changed as for seasonal AR (28).

As the majority of our allergic participants were polysensitized, it is a possible confounding factor that they may have been exposed to other allergens prior to the HDM challenge. Efforts were made to mitigate this, including running the study at the end of grass season, prior to the beginning of ragweed season. However, as HDM is a perennial allergen, participants may have likely been exposed to it outside of the HDM-EEU, such as in their homes. Chronic exposure to perennial allergens may result in decreased sensitivity to allergen so for some allergic participants and this may have impacted the biologic responses to allergen exposure.

These findings establish the applicability of the HDM-EEU for studying mechanisms of HDM-induced AR, as it can produce measurable biological changes in allergic participants. We've shown that SPT wheal sizes confirmed allergic status of participants, for the purpose of this study, and sIgE levels

were significantly greater and more variable among the allergic participants in comparison to healthy controls. CBCs and serum cytokine concentrations demonstrate decreased eosinophils and eosinophil-related markers in the peripheral blood post-HDM exposure specifically for allergics.

While AR is not a life-threatening condition, it greatly affects quality of life for patients and their families. Translational clinical models, such as the HDM-EEU, serve to reproduce AR symptoms in a controlled manner and allow for pathophysiological changes upon allergen exposure to be further evaluated.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

This study was reviewed and ethics clearance was granted by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (DMED-19149616). All participants reviewed and provided signed consent prior to study enrolment.

AUTHOR CONTRIBUTIONS

AKE developed the protocol, oversaw the study, and ensured critical revision of the manuscript. LH contributed to the conduct the study, conducted the statistical data analyses. SL contributed to the conduct of the study and co-drafted the manuscript with LH. JT contributed to the conduct of the study and edited the manuscript. LS contributed to the development of the study protocol, management of the trial, and revisions to the manuscript. CM was responsible for participant recruitment and revisions to the manuscript. TW was responsible for all operations related to the HDM-EEU and contributed to the manuscript. All authors have read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/falgy.2021.807208/full#supplementary-material

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ORIGINAL RESEARCH
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Xenon-Enhanced Dynamic Dual-Energy CT Is Able to Quantify Sinus Ventilation Using Laminar and Pulsating Air-/Gas Flow Before and After Surgery: A Pilot Study in a Cadaver Model

Sven Becker[1], Tilman Huppertz[2], Winfried Möller[3], Miriam Havel[4], Maria Schuster[4], Anne Merle Becker[4], Martin Sailer[1], Uwe Schuschnig[5] and Thorsten R. Johnson[6]*

[1] Department of Otolaryngology, Head and Neck Surgery, Tübingen University Hospital, Tübingen, Germany, [2] Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Centre, Johannes Gutenberg University Mainz, Mainz, Germany, [3] Institute of Lung Biology and Disease, Helmholtz Center München, Helmholtz Association of German Research Centres (HZ), Munich, Germany, [4] Department of Otorhinolaryngology, Head and Neck Surgery, Ludwig-Maximilians-University, Munich, Germany, [5] Pari Pharma GmbH, Gräfelfing, Germany, [6] Institute for Clinical Radiology, Ludwig-Maximilians-University, Munich, Germany

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Pongsakorn Tantilipikorn, Mahidol University, Thailand

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***Correspondence:**
Sven Becker
sven.becker@med.uni-tuebingen.de

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Background: Chronic rhinosinusitis is a common disease with a significant impact on the quality of life. Topical drug delivery to the paranasal sinuses is not efficient to prevent sinus surgery or expensive biologic treatment in a lot of cases as the affected mucosa is not reached. More efficient approaches for topical drug delivery are, therefore, necessary. In the current study, dual-energy CT (DECT) imaging was used to examine sinus ventilation before and after sinus surgery using a pulsating xenon gas ventilator in a cadaver head.

Methods: Xenon gas was administered to the nasal cavity of a cadaver head with a laminar flow of 7 L/min and with pulsating xenon-flow (45 Hz frequency, 25 mbar amplitude). Nasal cavity and paranasal sinuses were imaged by DECT. This procedure was repeated after functional endoscopic sinus surgery (FESS). Based on the enhancement levels in the different sinuses, regional xenon concentrations were calculated.

Results: Xenon-related enhancement could not be detected in most of the sinuses during laminar gas flow. By superimposing laminar flow with pulsation, DECT imaging revealed a xenon wash-in and wash-out in the sinuses. After FESS, xenon enhancement was immediately seen in all sinuses and reached higher concentrations than before surgery.

Conclusion: Xenon-enhanced DECT can be used to visualize and quantify sinus ventilation. Pulsating air-/gas flow was superior to laminar flow for the administration of xenon to the paranasal sinuses. FESS leads to successful ventilation of all paranasal sinuses.

Keywords: chronic rhinosinusitis, CRS, dual energy CT, xenon, ventilation imaging, sinus ventilation, pulsating airflow

INTRODUCTION

Chronic rhinosinusitis (CRS) is a common disease affecting approximately 15% of the population in western countries (1, 2). Conservative treatment options include topic or systemic corticosteroids, oral long-term antibiotics, and nasal douche. In more recent years, biological treatment for severely affected patients is a highly effective new opportunity (3). However, the number of surgical interventions in the patients with CRS is high. Apart from medical history and nasal endoscopy, CT of the paranasal sinuses is considered as the gold standard to diagnose CRS (4). CT images provide a precise impression of the bony structures and the surrounding tissues, for example swelling of the lining mucosa (5). On the other hand, no functional analysis for instance of sinus ventilation is possible, when standard CT protocols are used. To better estimate treatment success, knowledge about sinus ventilation could be helpful when using topical corticosteroids in form of an aerosol generated by a nebulizer. Paranasal sinuses are non-actively ventilated cavities were local deposition of drugs remains challenging. To improve gas and aerosol transport into the sinuses pressure gradients between the two sides of the ostia are necessary (6, 7). This effect can be attained with a pulsating airflow generated by nebulization devices (7–11).

The introduction of dual-source CT systems [dual-energy CT (DECT)] has improved material differentiation. This is achieved by different tube voltages which are able to generate different X-ray energy spectra (4, 12). Radiopaque stable xenon gas leads to an increased absorption with decreasing photon energies as a result of photoelectric interactions (atomic number of stable xenon gas Z054) (13). Furthermore, the concentration of xenon is linearly associated with its X-ray attenuation (14). Hereby, selective xenon gas visualization can be reached. Furthermore, xenon wash-in and wash-out dynamics using successive CT datasets can be provided. Earlier studies investigated the efficiency of xenon as a CT contrast agent by multiple CT dataset acquisition measurements for the evaluation of xenon wash-in and wash-out characteristics in the paranasal sinuses (15, 16). Our working group could demonstrate sinus ventilation using pulsating gas flow by DECT and dynamic CT imaging in a rudimentary nasal plastic cast. Thus, the aim of the current pilot study was to visualize and quantify sinus ventilation using laminar and pulsating airflows in a cadaver head by DECT before and after surgery.

METHODS

Cadaver Head

For the ventilation examinations, a formalin-fixed female cadaver head from the Anatomical Institute of the Ludwig-Maximilians University, Munich, Germany was used. The mandible with tongue and floor of the mouth was removed to get a better access to the nasopharynx which was occluded by a silicon plug. Endoscopic evaluation revealed no significant septal deviation or any signs of previous surgical procedures on the paranasal sinuses. The study was approved by the local ethics committee.

After the first measurement without manipulation, endoscopic sinus surgery was performed on the cadaver head. Following standard approach of functional endoscopic sinus surgery (FESS), an operation on all sinuses was performed. Thereafter, a second measurement was performed.

Xenon Application System

A pulsating airflow was generated using the PARI SINUS system (Pari GmbH, Starnberg, Germany), which is based on a PARI BOY (PRONEB Ultra in the USA) aerosol drug delivery device. The system includes a compressor with an integrated pressure wave generator. It produces amplitude of 25 mbar with a frequency of 45 Hz. The device was connected to a tank with 100 % xenon (Linde, Munich, Germany, purity 99.996 %). The gas flow rate was 7 L/min in both settings with and without pulsation. The whole setup was coupled to both nostrils of the cadaver head. Xenon gas and pulsation were administered to the left nostril, returning gas from the right nostril was captured in a collecting tank and again insufflated *via* the left nostril (**Figure 1**). This way gas consumption could be decreased.

Dual-Source CT System

A dual-source SOMATOM force CT system (Siemens Healthcare, Forchheim, Germany) was used. Cadaver head and xenon supplying nebulization system were positioned on the patient table of the SOMATOM CT system (**Figure 1**).

DECT Examination

For xenon DECT measurements, 46 subsequent CT series were scanned at a frame rate of 1.5 s. The imaging range covered nostrils and the nasal cavity as well as the frontal, maxillary, and sphenoid sinuses. The examination was started with a laminar flow of room air, and imaging began while continuous alternating table movement. The laminar flow was switched from room air to 100% xenon after 10.5 s of imaging for 42 s. After 24 s of xenon supply, pulsation of xenon flow was started for 18 s. At 52.5 s, xenon influx and pulsation were stopped, and laminar flow with room air was continued. At 63 s after beginning of the examination, pulsation with room air was switched on to washout the xenon gas from the sinuses. The acquisition of images stopped at 69 s.

After performing FESS on the cadaver head, 33 subsequent CT series were captured at the above-mentioned frame rate covering the same anatomical structures as before surgery. Once more, the examination started with a laminar flow of room air in the course of continuous alternating table movement. Approximately, 10.5 s after starting the examination laminar room air flow was switched to laminar 100% xenon flow. Further 24 s later, pulsation was initiated until the end of the measurement. These measurements were abbreviated at 50 s, since complete ventilation with and without pulsation could be seen. The wash out phenomenon was, therefore, not documented.

The CT setup and DECT image reconstruction were comparable to the setup of a previous study in a rudimentary plastic cast model of our working group with following parameters (4): Tube voltage of tube A with a tube current–time product of 100 effective mAs was 100 kV; voltage of tube B (generating a hardened 140 kVp spectrum using a tin filter) and a tube current–time product of 85 effective mAs was 140 kV;

FIGURE 1 | Experimental setting. Fixated cadaver head with connected xenon supplying nebulization system (blue nebulizer + xenon tank and gas recirculation system) on the patient table of the SOMATOM CT system.

slice collimation, 128 mm × 0.6 mm; rotation time, 0.28 s; pitch, 0.55 (4).

Dual Energy CT Image Reconstruction

For the reconstruction of the acquired images, a soft kernel (B30f) at a slice thickness of 1 mm with 0.7-mm increment was used. Post-processing of the reconstructed image datasets from the two different energy tubes was performed after transferring the datasets to a syngo Multi Modality Workplace (Siemens Healthcare, Germany). Xenon enhancement was color-coded by a specific DE post-processing software. Afterward, these enhancement maps were fused with the axial images (4).

Data Analysis

Regions of interest (ROIs) were placed inside, both nasal cavities, and the different adjacent sinuses on both sides (frontal, sphenoid, and maxillary sinuses) (see **Figure 2**). Overall 46 xenon concentrations could be measured in each ROI with a time interval of 1.5 s. Hounsfield units (HU) were recorded from each imaging serie, and time–density curves were generated. Based on the time–density curves, a first-order exponential function was fitted, and the characteristic time constant for the xenon concentration change within the sinuses, τ, was determined for xenon wash-in and wash-out for each sinus separately (4, 16). The enhancement level was calculated by the difference between the HU values and −1,000 HU (value of room air). The enhancement value of 100% xenon was defined as the maximum enhancement within the input nostril. Xenon concentrations were determined for each time point and each ROI by calculating the ratio of the respective xenon enhancement and that of 100% xenon (4).

RESULTS

Dual Energy CT Measurements in the Nasal Cavities

Beginning with laminar airflow with room air at 0 s and with 100% xenon from 10.5 s onward, a rise in xenon concentration could be detected in both nasal cavities reaching a plateau at around 20 s (**Figure 3**, blue and black line). At that time point, nasal cavity is filled with nearly 100% xenon gas corresponding to −750 HU. Room air has around −1,000 HU. The start of pulsation at 34.5 s had no further effect on the xenon concentration in the nasal cavity. After xenon influx was stopped and room air was again delivered at 52.5 s xenon was rapidly cleared out of both nasal cavities. A renewed start of pulsation at 63 s did not change the concentration (**Figure 3**, blue and black line).

Dual-Energy CT Measurements in the Maxillary, Sphenoid, and Frontal Sinuses

Beginning with laminar airflow with room air at 0 s and with 100% xenon from 10.5 s onward, a rise in xenon concentration could be detected in the left sphenoid sinus beginning around 15 s (**Figure 4**). In the other sinuses, no rise in xenon concentration was registered (**Figures 3–5**). With the start of pulsation at 34.5 s,

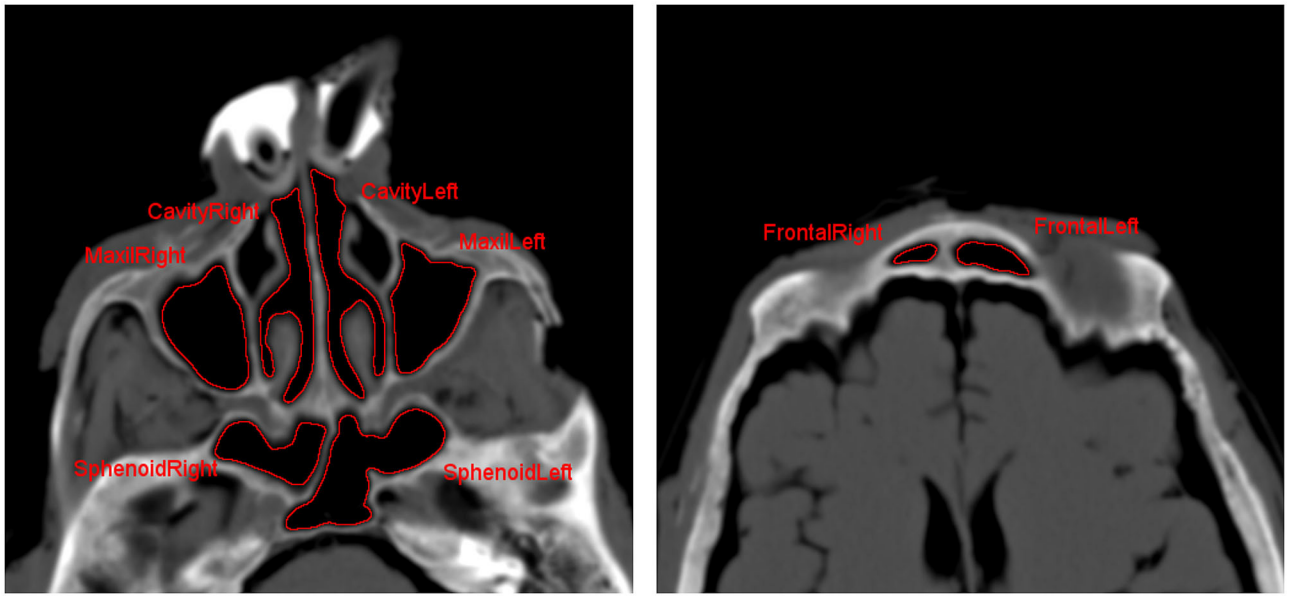

FIGURE 2 | Axial CT scan for placement of regions of interest (ROIs) in nasal cavity as well as in the different paranasal sinuses.

a steep rise could be seen in both maxillary sinuses and the right sphenoid sinus (**Figures 3**, **4**). In the frontal sinuses, the rise was less pronounced but measurable (**Figure 5**). The concentration in the left sphenoid sinus showed a further rise.

After 52.5 s, xenon inflow and pulsation were stopped and laminar airflow with room air continued. The xenon concentration in all sinuses slowly declined. Approximately 63 s after the beginning of the experiment, pulsating airflow with room air was started again and a drop in xenon concentration in all paranasal sinuses could be seen, resembling an active washout. Again this was especially marked in the maxillary sinus with a τ of 6 s (**Figure 3**).

The absolute xenon concentrations reached are between 50 and 90% in the maxillary and sphenoid sinuses, with the sphenoid sinuses reaching slightly higher values. In the frontal sinuses, the maximum xenon concentration reached is around 10%.

Overall, the left-sided paranasal sinuses reached higher xenon concentrations and the rise in xenon concentration was faster, best seen in the maxillary sinus with a τ of 7 s on the left and a τ of 18 s on the right side (**Figure 3**).

Dual-Energy CT Measurements in the Maxillary, Sphenoid, and Frontal Sinuses After FESS

After performing FESS, measurements were repeated. Beginning with laminar airflow with room air at 0 s and with 100% xenon from 10.5 s onward, a steep rise in xenon concentration could be detected in both nasal cavities, in the maxillary sinuses, and in the right sphenoid sinus reaching a plateau between 20 and 30 s (**Figures 6**, **7**). The xenon concentration reached was around 90%. The start of pulsation at 34.5 s had no effect on the xenon concentration. In the left sphenoid sinus and the frontal sinuses, the increase in xenon concentration was delayed and not as marked. Still it was significantly higher than before surgery. This measurement was abbreviated at 50 s, so that washout was not documented.

DISCUSSION

In this study, we could show that visualization and quantification of sinus ventilation using laminar and pulsating air-/gasflow in a cadaver head is possible by xenon-enhanced DECT. Exchange phenomena of the contrast agent between the nasal cavity and the paranasal sinuses under laminar and pulsating xenon gas flow could be seen. Also, we could show that pulsating gas flow leads to xenon influx into the non-operated paranasal sinuses in contrast to laminar flow, similar to our results with a nasal cast and as described in the literature (4, 7, 9–11). By using pulsation, it was possible to transport xenon into the non-operated paranasal sinuses. Ventilation time constants of approximately 10 s showed a nearly two orders of magnitude faster distribution than passive diffusion. Hence, the effects of passive diffusion can be ignored when using a time schedule as applied in the current study.

Xenon-enhanced CT to evaluate the sinus ventilation was first described by Kalender et al. in 1985 (15). Sinuses were filled with Xenon by placing a ballon-tipped catheter in each nostril and positive pressure insufflation during intermittent apnea. Sinuses were then imaged by single energy CT during normal breathing and physiological washout rates of xenon were calculated. Further studies improving the existing protocol followed but were all focused on physiological ventilation while normal breathing of mainly the maxillary sinus (16–18). Paulsson

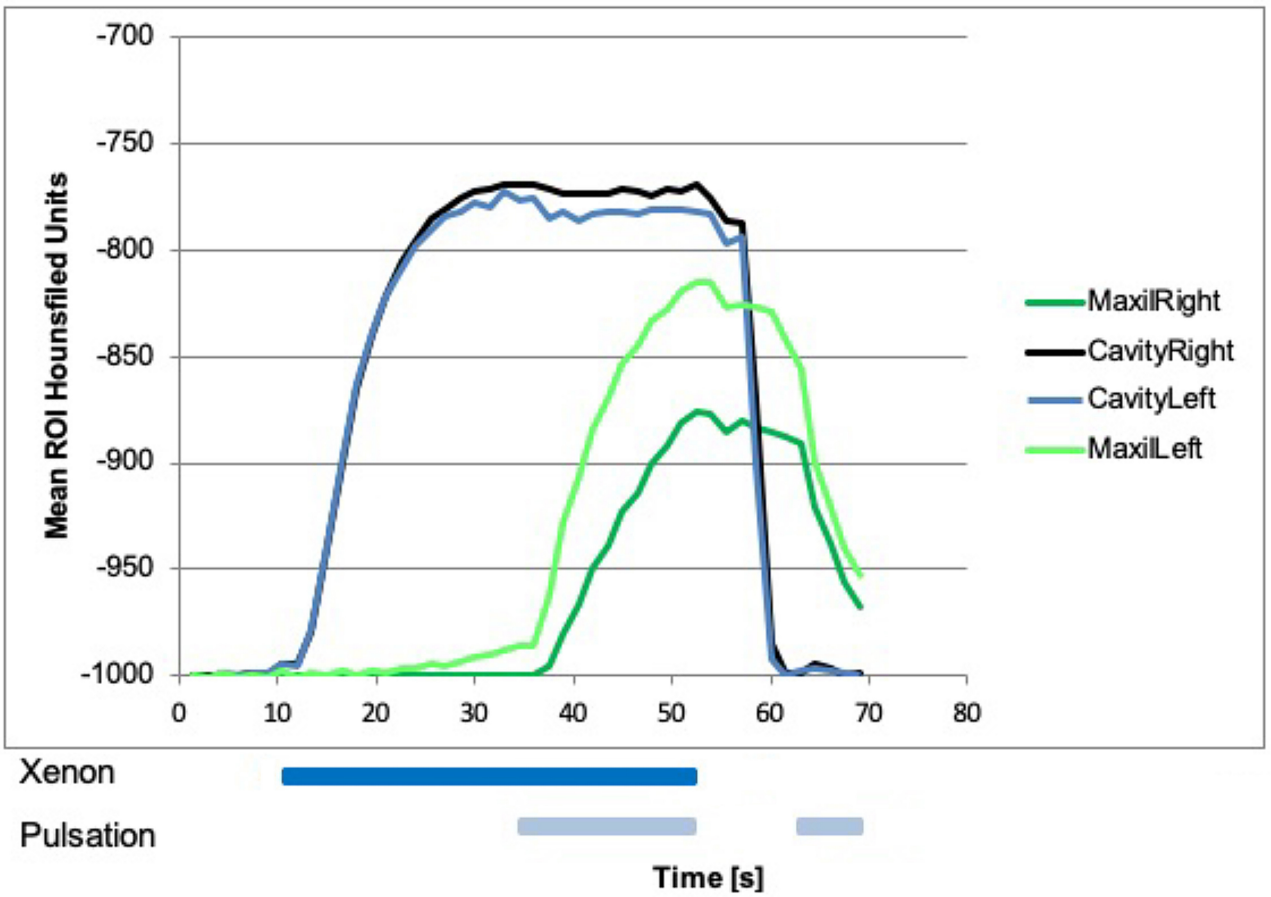

FIGURE 3 | Xenon concentration in the nasal cavities, with a steep rise after influx of 100% xenon at 10.5 s on both sides (black and blue line). Start of pulsation had no effect on the concentration. Xenon influx was stopped at 52.5 s then a steep fall of xenon concentration in both nasal cavities could be seen. Xenon concentration in the maxillary sinuses with a rise after the influx of 100% xenon, and the start of pulsation at 34.5 s (green lines) could be seen. Influx of xenon without pulsation had no effect on the concentration in the maxillary sinuses. After that, xenon influx and pulsation were stopped at 52.5 s, the xenon concentration was slowly declined. With a renewed start of pulsation with room air at around 63 s, a steeper decline in both maxillary sinuses could be seen.

et al. examined the influence of sinus surgery on xenon wash-out and could demonstrate that surgery leads to improved sinus ventilation with faster xenon wash-out from the sinuses (19). Also, Brumund et al. could show that surgical widening of the ostium of the maxillary sinus improves ventilation in a sheep model. Interestingly, a small antrostomy produced a statistically significant increase in maxillary sinus ventilation. No further significant increase was obtained by creating a large antrostomy (20). Beside us, no other working group used xenon-enhanced DECT to examine sinus ventilation during application of laminar and pulsating airflow before and after sinus surgery.

In the current study, the left sphenoid sinus showed uptake of xenon about 5 s after laminar flow with xenon began which is probably due to an anatomical variation with a sufficiently wide natural ostium and the fact that gas influx was given on the left side. This might have resulted in a direct flow of xenon into the left sphenoid sinus. Begin of pulsation did not influence further uptake of xenon into this sinus.

Only in the frontal sinuses xenon uptake under pulsation was very low. This might be explained by the anatomy of the frontal sinus with a canal of firm bone that might not be as easily accessed by gas and might also not meet criteria necessary for an exchange in adjacent compartments as described by Helmholtz due to the thickness of the surrounding bone making a vibration of this compartment difficult (21, 22). The so-called Helmholtz resonator is an acoustical device composed of a sphere cavity attached to an narrow tube also known as the neck (22). When this resonator is exposed to an external acoustic field, the air plug inside the neck oscillates at a frequency equal to that of the external field. The amplitude of the air plug in the neck oscillates according to the different frequencies of the external acoustic field. Maximum gas exchange between the cavity and the surrounding media occurs when the frequency of the external acoustic field equals the so-called "resonance frequency", a specific frequency for each resonator (21–23). Transferred to the anatomy of the human head, each sinus with its ostium and

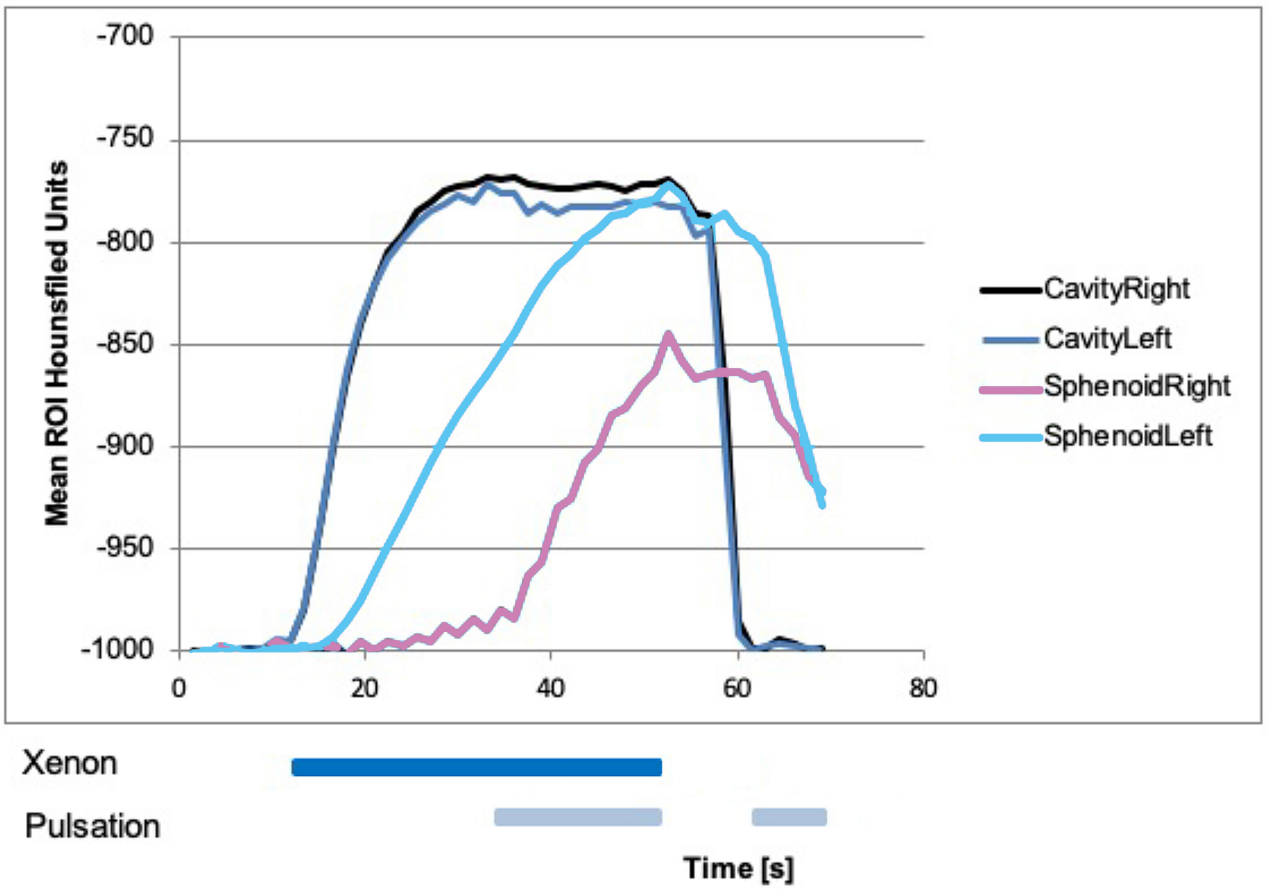

FIGURE 4 | Xenon concentration in the sphenoid sinuses, with a rise in the left side after influx of 100% xenon and no difference on the right side. With the start of pulsation at 34.5 s, there was also a rise in the right side. After that, xenon influx and pulsation were stopped at around 52.5 s, the xenon concentration was slowly declined on both sides. With a renewed start of pulsation with room air at around 63 s, the steeper decline in both sphenoid sinuses could be seen.

the adjacent part of the nose (e.g., frontal sinus plus frontal recess) has its own resonance frequency due to form, thickness of bone, and mucosal properties. To reach all sinuses in an optimal way, a single frequency as applied in the current study is, therefore, not sufficient and can lead to enormous differences in sinus ventilation as seen between the maxillary and the frontal sinuses. To overcome this problem, future nebulization devices could use a frequency sweep from deep frequencies (45 Hz) as used in the current study to higher frequencies up to 300 Hz or more within a 2 min therapeutic inhalation to reach each individual resonance frequency of the different sinuses for a short time. Maniscalco et al. for example could show that the deposition of drugs on the wall of the maxillary sinus can be increased by 3-, 3.5-, and 4.4-fold when laminar nebulized aerosol flow to the nostril was superimposed by pulsation of 45, 120, and 200 Hz, respectively (24). Similarly, positive results could also be achieved by nebulizers using a pulsation of 100 Hz (25, 26). Pourmehran et al. tried to maximize drug deposition in a single-sided maxillary sinus model by optimally suit frequency, amplitude, and flow rate of applied 12 μm aerosol particles by controlled repeated measurements (27). They were able to increase drug delivery by 75-fold when using a frequency of 328 Hz with an amplitude of 126 dB re 20 μPa and a flow rate of 0.267 ml/min showing that further developments in nebulization devices could have the opportunity to substantially improve topical drug delivery. As most of the studies focus on the maxillary sinus, further studies covering the frontal sinus are necessary to get a better understanding of how this also surgically more difficult to address cavity can be sufficiently reached.

Drug delivery to the sinuses is not only influenced by pulsation parameters of the applied aerosol flow, but also influenced by the breathing patterns, size of the aerosol particles, how the nebulizer is connected to the nose (inclination of the nosepiece) and the anatomy of the nasal airway itself (26–28). Last-mentioned could be shown by Hosseini and Golshahi in anatomical 3D printed nasal airway models of 2-, 5- and 50-year old human subjects (29). In their study, a pulsating airflow was applied with 44.5 Hz frequency and 24 mbar amplitude comparable to

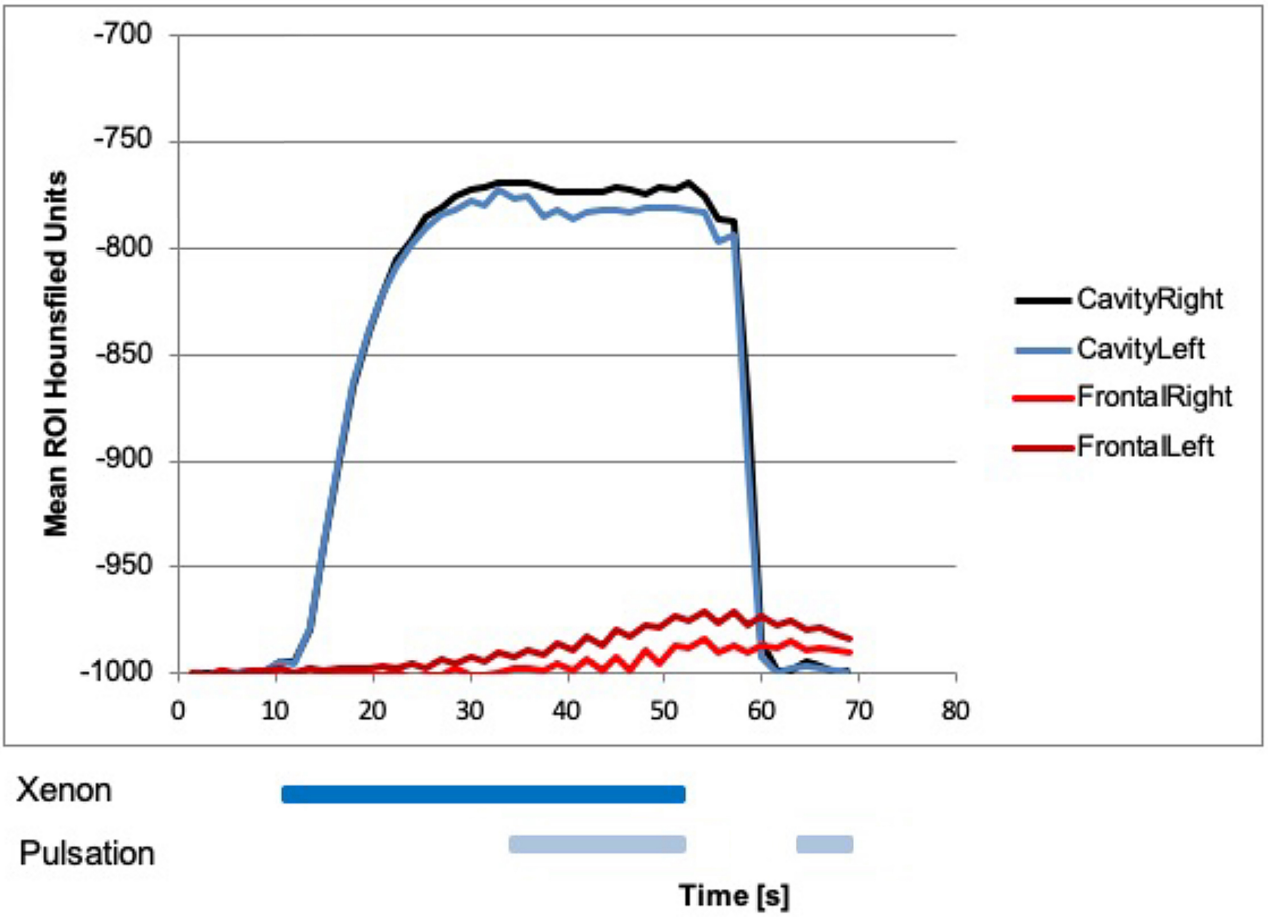

FIGURE 5 | Xenon concentration in the frontal sinuses, with a rise after the influx of 100% xenon and start of pulsation at 34.5 s. Influx of xenon without pulsation had no measurable effect on the concentration in the frontal sinuses. After that, xenon influx and pulsation were stopped at around 52.5 s, the xenon concentration was slowly declined. With a renewed start of pulsation with room air at around 63 s, a further decline in both frontal sinuses could be seen.

our study. Drug delivery to the maxillary sinus in the adult subject increased 4-fold when using pulsation in comparison to a laminar airflow without pulsation. They could show that drug deposition in the anterior part of the nose in the two younger subjects was higher than that in the adult model. This leads to a decrease of 3–11% in drug deposition to the maxillary sinus, and a 25% decrease in lung deposition showing the effect of anatomy/age on sinus drug delivery. They could also show that a bidirectional breathing administration technique can significantly increase the paranasal drug delivery when pulsating airflow is used (29).

Although there are numerous experimental studies on different nebulization devices with pulsation properties, clinical studies on the effectiveness of this kind of drug application in CRS patients are missing. To our knowledge, there are only two registered studies comparing corticosteroid application *via* nasal spray with nebulization plus pulsation in CRS patients with (EudraCT-Nr. 2013-002414-12) and without nasal polyps (EudraCT-Nr. 2013-002421-30). The first results from the latter study were promising and providing estimates for the sample size calculations to conduct a pivotal study in the future (30).

Having the above-mentioned anatomical and functional parameters in mind diagnostic approaches covering these aspects are necessary to evaluate if a patient is suitable for topical sinus drug delivery by nebulization devices or not and to further improve the nebulization parameters itself. In the future, xenon-enhanced DECT could be used for this purpose in patients with CRS to functionally evaluate sinus ventilation properties and sinus anatomy at the same time. Furthermore, it could help to optimize the nebulization parameters in standardized models and pave the way for improved topical corticosteroid delivery. Earlier studies in healthy participants could demonstrate the feasibility of dynamic assessment of paranasal sinus ventilation using xenon-enhanced CT (15, 16, 18). But xenon has anesthetic properties in higher concentrations and is used as an inhalation general anesthetic agent for this reason (31, 32). Therefore, a systematic use of xenon at high concentrations for imaging the paranasal sinuses has to be carefully evaluated. Otherwise, deep

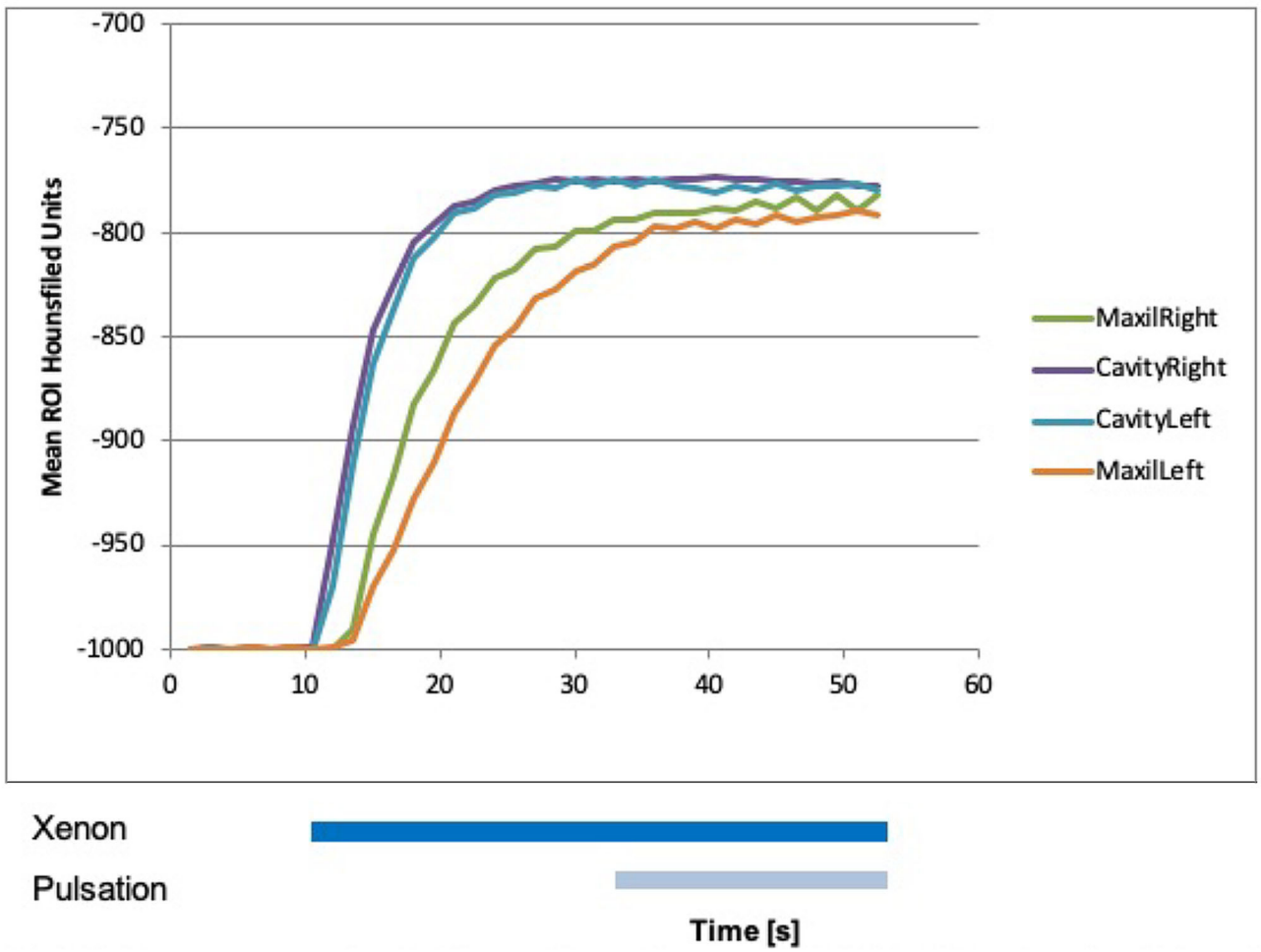

FIGURE 6 | Xenon concentration in the maxillary sinuses after functional endoscopic sinus surgery (FESS), with a rise after the influx of 100% xenon at 10 s. The start of pulsation at 34.5 s did not change influx.

inhalation of xenon is not necessary for imaging sinus ventilation, as patients should close their soft palate during nebulizer use. A former study examined maxillary sinus ventilation in a dynamic protocol over 30 min. Subjects had to breath 30% xenon and experienced side effects like nausea with vomiting or lightheadedness (16). As already proposed, a study design with a short ventilation time but higher xenon gas concentrations while closing the soft palate could reduce the above-mentioned side effects (4).

After FESS, there was a very efficient influx of xenon into all sinuses with the frontal sinuses showing the smallest uptake. By widening the ostia of the sinuses to a maximum extend by FESS, the sinuses became part of the directly ventilated areas like the nasal cavity. Pressure gradients between the two sides of the ostia to ventilate the sinus *via* resonance properties were no longer necessary, so pulsation did not change uptake anymore. Therefore, measurements were abbreviated after 50 s and wash out of xenon was not documented. These findings are in line with existing literature (33, 34) and could be recently confirmed by computational fluid dynamics modeling (35). Our results underline the necessity to adjust nebulization characteristics post-operatively due to anatomical and functional ventilation changes depending on the extend of surgery.

Limitations of our study include using a cadaver head where the nasopharynx is firmly sealed by a silicon plug which is probably more efficient than closure achieved in a person who is asked to obstruct the pharynx with the soft palate. This might influence the measurements and lead to slightly better results than can be expected in real life.

Moreover, xenon gas is of higher viscosity than air. That could result in a systematic underestimation of sinus ventilation due to different ventilation time constants for xenon gas in comparison to normal air. On the other hand for therapeutic purposes, ventilation has to be achieved with an aerosolized drug with a droplet size that exceeds any gas molecule in size to be able to achieve a therapeutic effect. This might lead to a not quite as efficient ventilation of the sinuses as demonstrated with

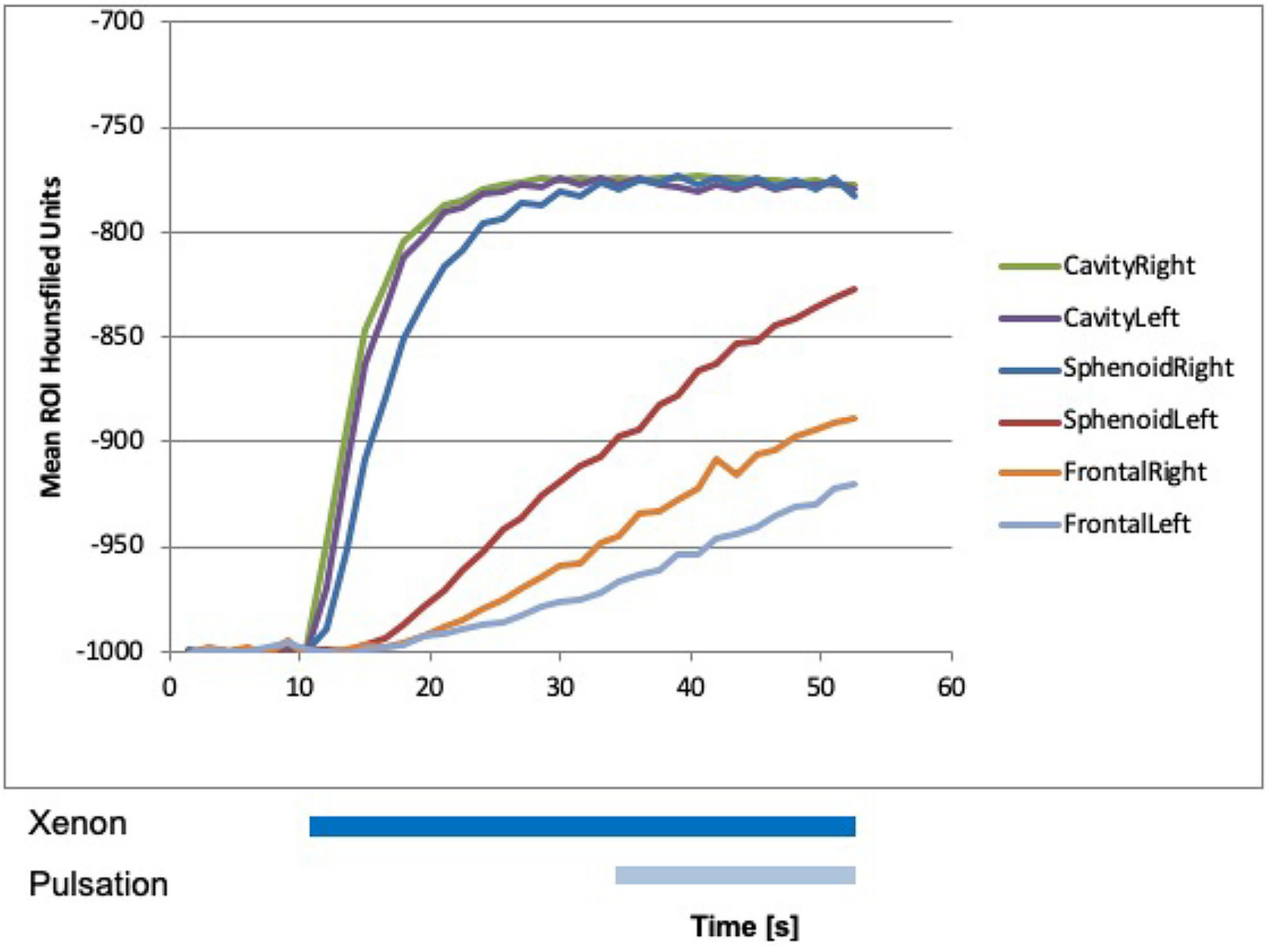

FIGURE 7 | Xenon concentration in the frontal and sphenoid sinuses after FESS, with a rise after the influx of 100% xenon at 10 s. The start of pulsation at 34.5 s did not change influx.

xenon. To overcome these problems, reduction of droplet size (1–3 μm) and improvements in the acoustic properties like changing frequencies, amplitudes, and nebulization flow rates have to be further investigated to increase the sinus drug deposition in the future (27, 36).

A further limitation of the study was that we were able to use only one specimen, what is not sufficient to generalize the results in a wide patient population with very heterogeneous sinus anatomy.

Finally, we were able to visualize and quantify the paranasal sinus ventilation by xenon-enhanced dynamic DECT using laminar and pulsating air-/gas flows in a cadaver model. The superiority of pulsating gas flow over laminar flows to achieve ventilation of the paranasal sinuses in the non-operated setting could be confirmed. FESS is highly effective in improving the ventilation of the sinuses and eliminating the need for pulsation in the postoperative setting. To evaluate the potential advantages of xenon-enhanced DECT for imaging sinus ventilation and to show that pulsating flow is also more efficient in drug delivery to the paranasal sinuses in comparison to the conventional nasal spray application, more patient studies in clinical settings are required.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethikkommission der Medizinischen Fakultät der Universität München, Pettenkoferstrasse 8a, 80336 München. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SB, WM, and US contributed to conception and design of the study. TJ organized and run the CT-scans. SB and MSa performed the statistical analysis. TH wrote the first draft of the manuscript. AB, MH, and MSc wrote sections of the manuscript. All authors

contributed to manuscript revision, read, and approved the submitted version.

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We dedicate this publication to our friend and colleague WM, who passed away far too early. He gave tremendous input to this study as well as to the field of aerosol therapy in general. Winfried, rest in peace!

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Conflict of Interest: The xenon supplying nebulization system used in the study was provided bei Pari Pharma GmbH, Gräfelfing, Germany. US is employee of Pari Pharma GmbH.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Glenis Kathleen Scadding,
University College London, United Kingdom

REVIEWED BY
Maciej Kupczyk,
Medical University of Lodz, Poland
Aleksandar Peric,
Military Medical Academy, Serbia

*CORRESPONDENCE
Patricia K. Kakobo
✉ patkitombole@gmail.com

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Determinants of uncontrolled allergic rhinitis in Kinshasa hospitals

Patricia K. Kakobo[1]*, Hilaire K. Kalala[1], Joseph K. Kelekele[2], Paulin B. Mutombo[3], Dieudonné T. Nyembue[1], Peter W. Hellings[4,5,6] and Jean-Marie N. Kayembe[7]

[1]Department of Otorhinolaryngology, University of Kinshasa, Kinshasa, Democratic Republic of Congo, [2]Ophthalmology Department, University of Kinshasa, Kinshasa, Democratic Republic of Congo, [3]School of Public Health, University of Kinshasa, Kinshasa, Democratic Republic of Congo, [4]Department of Otorhinolaryngology-Head and Nose Surgery, University Hospital of Leuven, Leuven, Belgium, [5]Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, [6]Upper Airways Research Laboratory, University of Ghent, Ghent, Belgium, [7]Department of Pneumology, University of Kinshasa, Kinshasa, Democratic Republic of Congo

Objective: To identify the determinants of uncontrolled allergic rhinitis (UCAR) in a hospital setting in Kinshasa, Democratic Republic of Congo.
Methods: Hospital-based cross-sectional study of 153 patients with allergic rhinitis (AR). The diagnosis of AR was based on clinical grounds according to the Allergic Rhinitis and its Impact on Asthma (ARIA) criteria. Categorization into controlled AR (CAR) and UCAR was based on the visual analog scale (VAS with cut off point of 5). Binary logistic regression was used to identify factors associated with UCAR.
Results: Patients with UCAR (60.1%) proportionally outnumbered those with CAR (39.9%). There were significantly more patients younger than 30 years of age among patients with UCAR. Factors significantly associated with UCAR were age below 30 years (OR = 3.31; 95% CI: 1.49–7.36; $p = 0.003$), low serum vitamin D level (OR = 3.86; 95% CI: 1.72–8.68; $p = 0.001$), persistent form (OR = 3.11; 95% CI: 1.39–6.98; $p = 0.006$) and moderate to severe form of AR (OR = 4.31; 95% CI: 1.77–10.49; $p = 0.001$).
Conclusions: Factors associated with UCAR in this study population were younger age less than 30 years, low vitamin D level, and persistent as well as moderate to severe AR. Further studies are needed to elucidate the underlying mechanisms favoring the occurrence of these factors.

KEYWORDS

uncontrolled allergic rhinitis, vitamin d, persistent form, moderate to severe form, Kinshasa

Introduction

Allergic rhinitis (AR) is a disease of public concern, given its negative impact on patients' quality of life and socioeconomic power (1, 2). Poor control of AR is a leading cause of morbidity and mortality worldwide and accounts for 43% of the global disease burden (1). AR is generally underestimated, poorly controlled and undertreated (3). Although the symptoms of AR can be controlled with adequate treatment in most patients, recurrence is very common (3).

Currently, the most widely used tool to assess the severity and control of AR symptoms is the visual analog scale (VAS). Patients with a score ≥5 on this scale are considered to have uncontrolled AR (UCAR) (4, 5). Several studies have reported an increase in UCAR frequency in some countries such as France (71.7%) (6), Italy (>60%) (7), Tunisia (62%) (8), and the Democratic Republic of Congo (DRC) (75.5%) (9). On average, one-fifth of

patients with AR have bothersome symptoms of AR despite adequate medical treatment abiding to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines (10).

AR Treatment is aimed at controlling symptoms and risk factors for poor progression but also at improving the quality of life of patients. However, despite various recommendations for AR management, most patients remain inadequately controlled for several reasons, including noncompliance, comorbidities, misdiagnosis, and inadequate treatment. Poor control of AR can be caused by several factors such as asthma, rhinosinusitis, atopic dermatitis, and allergic conjunctivitis (11). A quick literature review indicates a clear lack of data on the risk factors for poor control of AR in sub-Saharan Africa (SSA) in general and in DRC in particular. The present multicenter study aims to determine the risk factors for poor control of AR and AR severity level in a hospital setting in Kinshasa.

Patients and methods

The present study included patients with AR. It was conducted from November 2019 to May 2020 in otolaryngology departments of three Kinshasa hospitals, namely Cliniques Universitaires de Kinshasa (CUK), Centre Médical Diamant and Centre Hospitalier Monkole. The study was approved by the Biomedical Ethics Committee of the School of Public Health of the University of Kinshasa, abode to the guidelines of the Declaration of Helsinki, and all patients provided a written informed consent. *Patients were excluded for any of the following reasons: positive skin allergic test without any symptom of allergy; presence of allergic symptoms with a negative skin allergy test with un; pregnancy; current antihistamine treatment; any comorbidity or treatment affecting serum vitamin D level.*

Diagnosis and classification of AR

The clinical diagnosis of AR was based on the ARIA classification and was confirmed by a positive allergen skin prick test (AST) (12). AR was then categorized into controlled allergic rhinitis (CAR) and UCAR based on the VAS in the last two weeks preceding the consultation. This categorization required AR patients to be adequately treated.

Patients scored their own symptoms on the VAS using a ruler graduated from 0 (total absence of symptoms) to 10 cm (maximum presence of symptoms) (12). Any patient with a score ≥5 was considered to have UCAR, whereas one with a score <5 was classified as having CAR (12). UCAR was intermittent if symptoms lasted less than 4 days/week and 4 weeks/year. On the other hand, it was persistent if symptoms lasted more than 4 days/week and 4 weeks/year (13). In addition, it was labelled as mild or moderate to severe depending on whether the symptoms were not very annoying or had an impact on quality (13).

Allergic skin tests and serum vitamin D determination

The AST (Alyostal, Barcelona, Spain) consisted of a battery of nine allergens, namely dermatophagoides farinae, dermatophagoides pteronyssinus, blomia, 5-grasses, cat epithelium, dog epithelium, alternaria, aspergillus, and roach. The test was positive when the diameter of the skin papule induced by at least one allergen was equal to or greater than 3 millimeters, or equal to half the positive control (14).

25-hydroxyvitamin D3 was measured by radioimmunoassay using a Cobas E411 automatic well gamma counter (Roche Diagnostics International AG, Totkreuz, Switzerland) calibrated for iodine 125. For simplicity of analysis, serum vitamin D level was stratified into normal (≥30 ng/ml) and abnormal (<30 ng/ml).

Body mass index (BMI) was used to assess patients' nutritional status. Patients were further classified as underweight (BMI <18.5 Kg/m^2), normal (BMI: 18.5–24.9 Kg/m^2), overweight (BMI: 25–29.9 Kg/m^2), and obese (BMI ≥30 Kg/m^2) (15). The latter two groups were combined and analyzed as a single group.

Statistical analysis

SPSS version 26.0 software was used for statistical analyses. Categorical variables were expressed as frequency and percentage, while quantitative variables were expressed as mean and standard deviation. Student's t-test was used to compare means of quantitative variables. Comparison of parameters of interest between patients with CAR and those with UCAR was performed using Pearson chi-square. Binary logistic regression was used to identify the determinants of UCAR. In the univariate model, gender, age groups, occupation, education level, residence (urban or semi rural), smoking (yes/no), BMI, number of allergens to which the patient is sensitized (mono vs. polysensitized), serum vitamin D level (normal vs. abnormal), allergic conjunctivitis (yes vs. no), asthma (yes vs. no), rhinosinusitis (yes vs. no), dermatitis (yes vs. no), high blood pressure (yes vs. no), number of people sharing the same room with the patient (≤2 vs. >2), use of an air conditioning system (yes/no), existence of pets (yes/no), presence of cockroaches in the house (yes/no), presence of trees and/or flowers in the house yard (yes/no), duration of illness (intermittent vs. persistent), and severity of illness (mild vs. moderate to severe) were used as predictors of AR control. Only variables that showed a significant association in the univariate model were analyzed in the multivariate model. The strength of association was estimated using the odds ratio (OR) at the $p<0.05$ significance level.

Results

Patients' sociodemographic and clinical characteristics

A total of 153 patients with AR were included in this study. The mean age was 32.1 ± 13.4 years for the whole group, 34.6 ± 13.1

years for patients with CAR, and 30.4 ± 13.3 years for those with UCAR. The other sociodemographic and clinical characteristics are shown in Table 1. Half of the patients were either under or at least 30 years old. Significantly more patients were female (62,7%), slightly more than half of the patients (54.9%) lived in urban areas, 56.9% reported a family history of atopy, and 69.3% had a university education. Most patients were sensitized to more than one allergen (60.8%), had a low serum vitamin D level (58.8%), shared the same bedroom with more than one other person (68.6%), and reported the existence of cockroaches in the house (64.7%) and trees in the yard (61.4%). Allergic conjunctivitis and rhinosinusitis were present in 52.9% and

TABLE 1 Sociodemographic and clinical features in patients with controlled and uncontrolled allergic rhinitis.

Variables	Total	CAR	UCAR	Chi-square	*p*-value
	n = 153 (%)	*n* = 61 (%)	*n* = 92 (%)		
Sex					
Male	57 (37,3)	27 (44,3)	30 (32,6)	2,13	0,144
Female	96 (62,7)	34 (55,7)	62 (67,4)		
Age range (years)					
< 30	76 (49,7)	22 (36,1)	54 (58,7)	7,51	0,006
≥ 30	77 (50,3)	39 (63,9)	38 (41,3)		
Occupations					
Unemployed/Housewives	22 (14,4)	13 (21,3)	9 (9,8)	6,97	0,074
Paid occupations	60 (39,2)	25 (41,0)	35 (38,0)		
Tradesmen	20 (13,1)	9 (14,8)	11 (12,0)		
Students/Pupils	51 (33,3)	14 (23,0)	37 (40,2)		
Level of study					
Primary	6 (3,9)	4 (6,6)	2 (2,2)	5,54	0,063
Secondary	41 (26,8)	21 (34,4)	20 (21,7)		
University	106 (69,3)	36 (59,0)	70 (76,1)		
Township of residence					
Urban	84 (54,9)	31 (50,8)	53 (57,6)	0,32	0,574
Urban-rural	69 (45,1)	30 (49,2)	39 (42,4)		
Smoking	6 (3,9)	2 (3,3)	4 (4,3)	0,11	0,739
AR in the family	87 (56,9)	36 (59,0)	51 (55,4)	0,19	0,661
BMI					
Lean	16 (10,5)	4 (6,6)	12 (13,0)	1,67	0,433
Normal	66 (43,1)	27 (44,3)	39 (42,4)		
Overweight/Obesity	71 (46,4)	30 (49,2)	41 (44,6)		
Number of allergens					
Monosensitized	60 (39,2)	27 (44,3)	33 (35,9)	1,08	0,298
Polysensitized	93 (60,8)	34 (55,7)	59 (64,1)		
Vitamin D level					
Normal	63 (41,2)	33 (54,1)	30 (32,6)	6,99	0,008
Reduced	90 (58,8)	28 (45,9)	62 (67,4)		
Allergic conjunctivitis	81 (52,9)	25 (41,0)	56 (60,9)	5,82	0,016
Asthma	32 (20,9)	8 (13,1)	24 (26,1)	3,73	0,053
Rhinosinusitis	106 (69,3)	38 (62,3)	68 (73,9)	2,33	0,127
Dermatitis	52 (34,0)	18 (29,5)	34 (37,0)	0,91	0,341
GERD	55 (35,9)	20 (32,8)	35 (38,0)	0,44	0,507
HBP	25 (16,3)	13 (21,3)	12 (13,0)	1,83	0,176
Number of people in the same bedroom					
≤ 2	105 (68,6)	48 (78,7)	57 (62,0)	4,02	0,029
> 2	48 (31,4)	13 (21,3)	35 (38,0)		
AC use	56 (36,6)	18 (29,5)	38 (41,3)	2,20	0,138
Domestic animals	75 (49,0)	25 (41,0)	50 (54,3)	2,62	0,105
Presence of cockroaches in the house	99 (64,7)	35 (57,4)	64 (69,6)	2,38	0,122
Trees in the parcel	94 (61,4)	33 (54,1)	61 (66,3)	2,31	0,129
ARIA classification					
Intermittent	47 (30,7)	29 (47,5)	18 (19,6)	13,48	< 0,001
Persistent	106 (69,3)	32 (52,5)	74 (80,4)		
Mild	38 (24,8)	24 (39,3)	14 (15,2)	11,43	0,001
Moderate to severe	115 (75,2)	37 (60,7)	78 (84,8)		

AR, allergic rhinitis; BMI, body mass index; GERD, gastroesophageal reflux disease; HBP, high blood pressure; AC use, air conditioning use.

69.3% of the patients, respectively. Patients with UCAR had a persistent form and a moderate to severe form of the disease in 69.3% and 75.2% of cases, respectively.

Comparison of sociodemographic and clinical characteristics of patients with UCAR and CAR

Data in Table 1 also indicate that UCAR and CAR were present in 60.1% and 39.9% of patients, respectively. In patients younger than 30 years, UCAR was significantly more frequent than CAR ($p = 0.006$). There were significantly more patients with abnormal serum vitamin D levels among patients with UCAR than those with CAR ($p = 0.008$). A similar observation was made for patients with concomitant allergic conjunctivitis ($p = 0.016$). The persistent form ($p < 0.001$) and the moderate to severe form ($p = 0.001$) were also significantly more seen in patients with UCAR than in those with good AR control. Similarly, the proportion of patients who shared the same bedroom with more than 2 other people was significantly higher among those with poor than those with good AR control ($p = 0.029$).

Factors associated with UCAR

We also sought to identify factors associated with poor control of AR. In univariate logistic regression (Table 2) including the sociodemographic and clinical variables listed in Table 1 as explanatory variables and the level of AR control (CAR vs. UCAR) as a dependent variable, age <30 years ($p = 0.007$), a low serum vitamin D level ($p = 0$, 009), sharing the same bedroom with more than 2 other people ($p = 0.031$), having concomitant allergic conjunctivitis ($p = 0.017$), permanent nature ($p < 0.001$), and moderate to severe severity of AR ($p = 0.001$) were significantly associated with UCAR. In the final multiple logistic regression model, only age <30 years, a low serum vitamin D level, permanent form, and moderate to severe form remained associated with UCAR. Specifically, patients younger than 30 years of age were 3.31 times more likely to have UCAR than those 30 years or older ($p = 0.003$). Based on serum vitamin D, those with a low serum vitamin D level had a 3.86-fold increased probability of having UCAR ($p = 0.001$). Similarly, patients with the permanent form and those with the moderate to severe form were 3.11 ($p = 0.006$) and 4.31 ($p = 0.001$) times more likely to have UCAR than those with the intermittent and mild forms, respectively.

Discussion

More than half (60.1%) of the patients interviewed in this study had a VAS score indicating poor control of AR. This frequency is similar to 60% reported in a multicenter study performed in non-asthmatic patients with AR symptoms in Italy (7) and 62% in another study in Tunisia (8). A higher frequency (71.7%) than ours was previously reported in France (6). On the contrary, the multinational study conducted in Egypt, Turkey and 3 countries of the Persian Gulf (Saudi Arabia, United Arab Emirates and Kuwait) reported an overall frequency of UCAR of 33% after assessment with the Rhinitis Control Assessment Test (RCAT). However, the frequency was higher in Egypt (55.6%) than in Turkey (27.9%) and in the 3 Persian Gulf countries combined (30.5%) (16). In China, an investigation in 250 AR patients prospectively assessed the frequency of UCAR using the Allergic Rhinitis Control Test (ARCT) at enrollment and then every 15 days after treatment and intensification of treatment in case of poor control. At enrollment, the incidence of UCAR was 99.2% before decreasing to 66% at 15 days, 29.2% at 30 days, 11.2% at 45 days, 3.6% at 60 days and 3.2% at 75 days after treatment (17). In Thai children, the Control of Allergic Rhinitis and Asthma Test (CARAT) showed a frequency of 28.2% in a hospital setting (18). In addition, a survey conducted in 5 European countries (Germany, Spain, France, Italy, and the United Kingdom) revealed, based on physicians' assessment, poor control of nasal AR symptoms in 18% and good control in 45.4% of patients regardless of the drug used (19). In Bousquet et al.'s study (20) on severe chronic upper respiratory disease, the incidence of UCAR after two weeks of treatment was 18% in patients treated based on physician's choice and 10.3% in those treated based on ARIA guidelines. Finally, in the AIMES survey conducted in 5 Middle Eastern countries (Egypt, Iran, Lebanon, Saudi Arabia, and the United Arab Emirates), 15% of respondents felt that their AR symptoms were poorly controlled compared to 40% whose symptoms were completely or well controlled despite taking medication to treat the symptoms (21). Several factors may contribute to the variability of UCAR frequency, including the type of study (cross-sectional vs. clinical trials), the type of instrument used to assess AR control, the

TABLE 2 Factors associated with uncontrolled allergic rhinitis.

Variables	Univariate analysis			Multivariate analysis		
	Crude OR	CI 95%	*P*	Adjusted OR	CI 95%	*P*
Age (< 30 years)	2,52	1,29–4,91	0,007	3,31	1,49–7,36	0,003
Vitamin D level (abnormal)	2,44	1,25–4,74	0,009	3,86	1,72–8,68	0,001
Number of people in the bedroom (> 2)	2,27	1,08–4,77	0,031	1,92	0,79–4,62	0,148
Allergic conjunctivitis	2,24	1,16–4,33	0,017	2,15	0,99–4,66	0,053
Persistent allergic rhinitis	3,72	1,83–7,65	< 0,001	3,11	1,39–6,98	0,006
Moderate to severe allergic rhinitis	3,61	1,67–7,78	0,001	4,31	1,77–10,49	0,001

characteristics of the study population, current or previous treatment, compliance with treatment, the level of knowledge and perception of the disease by the study population, and environmental factors. Ultimately, although the impact of treatment on AR control was not assessed in the present study, there is ample evidence to show that AR remains uncontrolled in a substantial number of patients despite well conducted treatment according to therapeutic guidelines (22). Despite the variability in the frequency of UCAR across studies and countries, the preceding data agree on the high frequency of UCAR.

We observed a significantly higher frequency of patients under 30 years of age among those with UCAR than those with CAR. Patients in this age group were 3.31 times more likely to have UCAR than those aged 30 years and older. *One possible explanation for this association is the lack, delayed or lack, refusal, delayed or inadequate treatment in young patients. In addition, medication high cost and the lack of health insurance prevent for most patients prevent them from being adequately treated.* Age is an important factor not only in awareness, but also in control of AR. In contrast, such an association was not found in the Italian multicenter study by Gani et al. (7). Other previous studies have described a strong association between allergic sensitization, asthma and rhinitis in children, adolescents, and young adults (23, 24). A separate analysis in the present series did not show a difference in the proportions of polysensitized between young (63.2%) and elderly (58.4%) subjects, $p = 0.55$. Elsewhere, investigations on allergic sensitization in different age groups consistently showed a biphasic trend of prevalence with age, with an initial increase until early adulthood and then a decrease (25, 26). Surprisingly, the prevalence of AR follows the same pattern (27, 28). This may suggest that patient's age plays a significant role in AR control. This hypothesis was tested in a prospective Korean study in which the clinical features of young (mean age: 28.9 ± 5.9 years) and elderly (mean age: 70.8 ± 5.4 years) AR patients were assessed before and after 4 weeks of treatment according to ARIA guidelines. Comparison of the Total Symptom Score (TSS), RCAT and VAS scores revealed that the therapeutic response was more favorable in young than in elderly patients on all assessment scales (29).

The association between AR and serum vitamin D level remains a controversial topic in light of conflicting results from different studies summarized in meta-analyses and reviews (30, 31). In the present study, however, we evaluated the association between vitamin D and the level of AR control in a cross-sectional manner. It is important to note that this aspect has been very rarely investigated. There were significantly more patients with low serum vitamin D levels among patients with UCAR than among those with CAR. The probability of having RANC was 3.86 times higher for patients with low serum vitamin D than for those with normal serum levels. A similar observation was made in two prospective studies evaluating the effect of vitamin D supplementation on the severity of AR. Kalsotra et al. evaluated the symptoms in two groups of patients with AR before and 4 weeks after administering oral vitamin D in combination with intranasal steroid sprays to one group and vitamin D alone to another group. After treatment, total nasal symptoms scores (TNSS) were significantly lower in both groups compared with pre-treatment scores, indicating an improvement in rhinitis symptoms and thus a progression towards control of AR (32). In another similar investigation, Modh et al. (33) evaluated two groups of 21 patients with AR and compared TNSS before and after routine antiallergic treatment and daily vitamin D supplementation for 21 days in one group and routine treatment only in the other. There was a significant post-treatment reduction in TNSS scores in both groups, but the reduction was significantly pronounced in the routine treatment only group. Similar results were reported in one more study including 35 cases and 33 controls with AR with similar serum vitamin D deficiency and nasal symptom severity scores. Eight weeks after treatment of cases with vitamin D plus a common anti-allergic (cetirizine) and controls with the common anti-allergic only, there was a significant increase in serum vitamin D levels in cases compared to controls in whom the level remained unchanged. There was also a significant difference between the nasal symptom scores of the two groups, mainly due to a significant reduction in scores in the cases (34). In summary, our observation and those of the studies listed above suggest that vitamin D deficiency is associated with poor control of AR.

It is noteworthy mentioning that there is a paucity of investigations on the association between control and severity as well as persistent or intermittent nature of AR. In the current series, poor control of AR was also independently associated with persistence and moderate to severe AR. This contrasts with findings from the Italian series where poor control of AR was not associated with disease duration (7). It is also important to underline that such an association described in a few studies was the result of confusion between poor control, severity, and response to AR treatment, probably stemming from the erroneous assumption that moderate to severe disease is uncontrolled. Indeed, in asthma, for example, where the relationship between severity and control has been extensively studied, it has been shown that the likelihood of a patient being controlled is not dependent on the severity of the disease before treatment (35, 36). Since the concept of control implies that patients are adequately treated beforehand (23), it cannot be excluded that the association found in the present study is rather a reflection of one or more of the factors such as lack of treatment, noncompliance in all its forms, application of a treatment regimen different from the ARIA guidelines, and treatment resistance.

Despite the fact that this study is multicentric and the first to systematically analyze the determinants of UCAR in the DRC, it has a number of limitations. First, the hospital-based, cross-sectional nature of the investigation and the small sample size (given the high prevalence of the disease in this setting) limit the generalizability of the results, and warrant the need for a larger, prospective study. *While we acknowledge that it would have been ideal to conduct a population-based study, it is also important to keep in mind that data from well conducted hospital-based studies are important as they may provide the first line of information*

about hospital utilization and basic epidemiologic measures needed for strategy planning, resource prioritization and allocation, and development of prevention, diagnosis, and management programs. In setting such as the DRC where population-based are difficult to conduct mainly due to limited funding, hospital data have become more valuable resources for studying epidemiology of diseases. Second, the study population included some patients who were not adequately treated. As mentioned previously, including them may have influenced the reported results. Beyond these limitations, however, the present study has the merit of having investigated variations in serum vitamin D levels in relation to AR control using the reference tool and of having provided data suggesting that patients with UCAR are candidates for vitamin D supplementation. In addition, it has the merit of being considered as a first, to our knowledge, in sub-Saharan Africa addressing this issue.

In conclusion, this study shows that UCAR is frequent in the hospital environment of Kinshasa as previously reported. Age less than 30 years, vitamin D deficiency, permanent and moderate to severe nature of AR emerged as factors associated with UCAR in this series.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The Biomedical Ethics Committee of the School of Public Health of the University of Kinshasa. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

PKK: is the first author of this manuscript; designed the study, collected the data, interpreted the results and drafted the manuscript. HKK collected the data and reviewed the manuscript. JTK collected the data and reviewed the manuscript. PBM analyzed the data and reviewed the manuscript. DTN, PWH and JMK designed the study and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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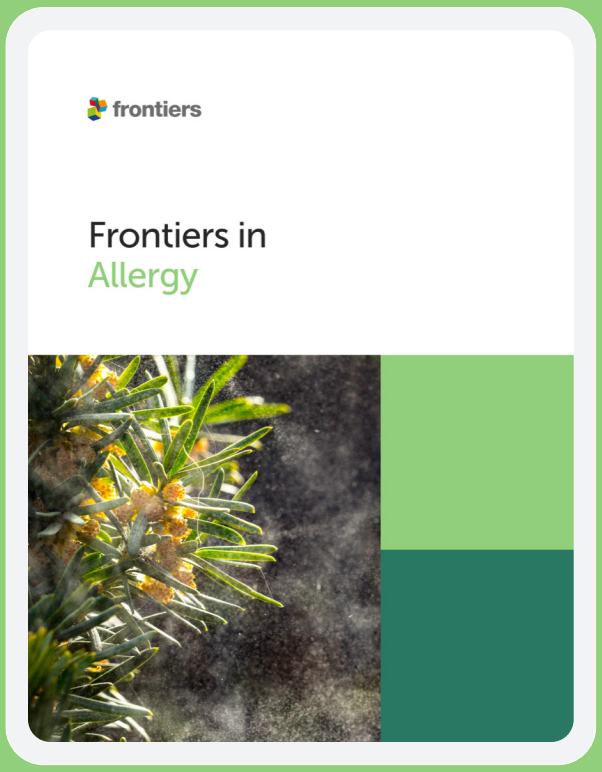

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