Measurement of local IgE in allergic rhinitis and chronic rhinosinusitis with and without nasal polyposis

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14/04/2014

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FOREWORD

This thesis was written within the context of our Master degree in Medicine at Ghent University. It is a composition of statistical analysis on datasets acquired at the Department of Otorhinolaryngology of the University Hospital of Ghent and data we collected in our own experiment.

We compare nasal measurement with different techniques and we discuss their role. Literature research started in September 2012 and was continued until February 2014. Statistical analysis was performed from June 2013 till March 2014. In addition, experiments occurred from November 2013 until March 2014. Later on, we performed the necessary statistical analyses in order to interpret the collected data.

We wish to thank those without whose help this work could not have been accomplished: (1) the patients who participated in the experiments; (2) Prof. Dr. Philippe Gevaert and Dr. Margot Berings for their guidance, assistance and encouragements; last but not least our friends and family who supported us throughout the course of this assignment.

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LIST OF ABBREVIATIONS

AR = Allergic rhinitis
ARIA= Allergic Rhinitis and its Impact on Asthma
BDL= Below detection level
CRS= Chronic rhinosinusitis
CRSsNP= Chronic rhinosinusitis without nasal polyposis
CRSwNP= Chronic rhinosinusitis with nasal polyposis
CSR= Class switch recombination
CUAI= Chronic upper airway inflammation
DF= Dilution factor
EPOS= European Position paper on Rhinosinusitis and Nasal polyps
ESS= Endoscopic sinus surgery
FLC= Free light chains
Gx3 IgE= Grass pollen specific IgE
HDM= House dust mite
IQR= Interquartile range
NAPT= Nasal provocation test
NCA= Neutrophil chemotactic activity
NP= Nasal polyposis
NSA= Nasal secretory activity
RR= Receptor revision
RSV= Respiratory syncytial virus
SAE= Staphylococcus aureus enterotoxin
SPA= Staphylococcal protein A
SPT= Skin prick test
RAST= Radio-Allergo-Sorbent-Test
TSST= Toxic shock syndrome toxin
VAS= Visual analogue scale
WNS= Weight of nasal secretions collected
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1 ABSTRACT

Introduction: Local production of IgE in the respiratory mucosa has been demonstrated in allergic rhinitis (allergen specific IgE) and in chronic rhinosinusitis with nasal polyposis (polyclonal IgE), suggesting a role for local IgE in the pathogenesis of these inflammatory diseases of the upper airway. It has been suggested that the majority of allergen-specific IgE in the peripheral blood is not derived from IgE-secreting cells in the blood, but is produced locally in target organs of allergy such as the nasal mucosa. Considering the importance of local IgE production, it seems to be essential to develop non-invasive, reproducible and easy to perform approaches for measurement of local mucosal IgE production.

Methods: Different datasets with CRS patients (with and without nasal polyps) and AR patients were analyzed to obtain more knowledge concerning local IgE and its measurement techniques. Furthermore, a small experiment was performed: in AR patients and non-allergic control patients, nasal secretions were collected by means of filter discs and Merocels. The purpose of this experiment was to compare and optimize both methods for collection of nasal secretions and measurement of local IgE.

Results:
Measurement of IgE in serum, tissue homogenates and nasal secretions
In a dataset of 112 patients with CRS (with and without NP) levels of total IgE in serum, in tissue and in nasal secretions (collected with Merocels) were significantly correlated ($p<.0001$).

Dynamic measurements of local IgE in nasal secretions collected by means of Merocels
24 patients with CRSwNP and comorbid asthma were treated with omalizumab or placebo. With omalizumab treatment ($n=16$), a significant increase in total IgE in nasal secretions was observed ($p=.041$). No significant change was found in the placebo group ($n=8$). 30 patients with severe NP had a treatment with mepolizumab or placebo. Mepolizumab treatment ($n=20$) did not result in a significant change in total IgE and SAE-IgE in nasal secretions. In the placebo group ($n=10$) a significant increase in total IgE ($p=.028$) and a non-significant increase in SAE-IgE were observed. 47 patients with CRSwNP had a treatment with methylprednisolone, doxycycline or placebo. In the placebo group ($n=18$), a significant change from baseline in total IgE was observed at week 4 ($p=.009$). In the methylprednisolone group ($n=14$) an initial decrease in total...
IgE was followed by an increase in total IgE, however, these changes were not significant. In the doxycycline group (n=14) total IgE levels did not change during treatment.

Local measurements in nasal secretions collected by means of filter discs
Nasal secretions were collected by means of filter discs and different biomarkers of inflammation were measured. The highest total IgE levels were seen in the AR patients (n=12), followed by the allergic CRSwNP patients (n=12). Both in the AR and the allergic CRSwNP group, total IgE levels were significantly higher (resp., \( p = .026 \) and \( p = .017 \)) compared to the control patients (n=12). In the AR patients and the allergic CRSwNP patients, total IgE was BDL in resp. 3 and 2 subjects. In the CRSwNP patients without allergy (n=12) and in the control patients, total IgE was BDL in resp. 7 and 8 subjects. Parallel with total IgE, gx3 IgE was the highest in the AR patients followed by the allergic CRSwNP patients. Levels of Gx3 IgE were BDL in all of the CRSwNP patients without allergy and the control patients. Gx3 IgE was significantly higher in the AR patients in comparison with the control (\( p = .001 \)) and the CRSwNP patients without allergy (\( p = .001 \)).

Comparison of Merocels and filter discs for the measurement of local IgE
In nasal secretions collected with filter discs and with Merocels, total IgE and house dust mite specific (HDM) IgE were significantly higher in AR patients (n=15) compared to control patients (n=15). In the nasal secretions collected with Merocels total IgE and HDM IgE were BDL in resp. 3 and 4 AR patients, with the filter discs in resp. 2 and 6 AR patients. HDM sIgE levels in serum, in the nasal secretions collected by means of Merocels and in nasal secretions collected by means of filter discs were significantly correlated.

**Conclusion:** IgE levels in nasal secretions collected by means of Merocels seem to be a good reflection of local IgE levels in nasal tissue. As collection of nasal secretions by Merocels is a standardized and non-invasive method, it can be used for repeated measurements of local IgE and other markers of mucosal inflammation, in order to study nasal diseases and monitor their treatment. The use of filter discs seems to be a feasible alternative for collection of nasal secretions and measurement of local IgE.
Inleiding: In allergische rhinitis (allergeen-specifiek IgE) en in chronische rhinosinusitis met neuspoliepen (polyklonaal IgE) is lokale IgE productie in de respiratoire mucosa aangetoond. Dit veronderstelt een rol voor lokaal IgE in de pathogenese van deze inflammatoire aandoeningen van de bovenste luchtwegen. Er is gesuggereerd dat de meerderheid van allergeen-specifiek IgE in perifeer bloed niet afgeleid is van IgE-secretierende cellen in het bloed, maar afkomstig is van lokale productie in doelorganen van allergie zoals het neusslijmvlies. Gezien het belang van lokale IgE productie lijkt het essentieel om niet-invasieve, reproduceerbare en eenvoudige benaderingen te ontwikkelen waarmee lokale IgE productie kan gemeten worden.

Methodologie: Verschillende datasets van CRS patiënten (met en zonder neuspoliepen) en AR patiënten werden geanalyseerd om meer inzicht te verwerven in lokaal IgE en zijn meettechnieken. Verder werd ook een klein experiment uitgevoerd: bij AR patiënten en niet-allergische controepatiënten werden neussecreties verzameld door middel van filter discs en Merocels. Het doel was om beide methodes met elkaar te vergelijken en te optimaliseren voor de collectie van neussecreties en lokale IgE meting.

Resultaten:

Meting van IgE in serum, weefselhomogenaten en nasale secreties
In een dataset van 112 patiënten met CRS (met en zonder NP) was er een significante correlatie (p<.0001) tussen de concentraties van totaal IgE in serum, in weefsel en nasale secreties (verzameld met Merocels).

Dynamische metingen van lokaal IgE in neussecreties verzameld met Merocels
24 patiënten met CRSwNP en comorbide astma ondergingen een omalizumab of een placebo behandeling. Bij de behandeling met omalizumab (n=16) werd een aanzienlijke toename van totaal IgE in nasale secreties waargenomen (p=.041). Geen significante wijzigingen werden gevonden in de placebo groep (n=8). 30 patiënten met ernstige NP ondergingen een behandeling met mepolizumab of placebo. Mepolizumab behandeling (n=20) resulteerde niet in een significante verandering in totaal IgE en SAE-IgE in nasale secreties. In de placebo groep (n=10) werd er een significante stijging van totaal IgE (p=.028) en een niet significante toename in SAE-IgE waargenomen. 47 patiënten met CRSwNP ondergingen een behandeling met methylprednisolone, doxycycline of placebo. In de placebo groep (n=18) werd een
significant verandering in totaal IgE ten opzichte van de baseline waargenomen in week 4 ($p=.009$). In de methylprednisolone groep (n=14) werd aanvankelijk een afname van het totaal IgE gezien, gevolgd door een toename, maar dit was niet significant. In de doxycyclinegroep (n=14) veranderden de totale IgE niveaus niet tijdens de behandeling.

Lokale metingen in neussecreties verzameld door middel van filter discs
Neussecreties werden verzameld door middel van filter discs en verschillende ontstekingsparameters werden gemeten. De hoogste totale IgE niveaus werden waargenomen bij AR patiënten (n=12), gevolgd door CRSwNP allergische patiënten (n=12). Zowel in de AR groep als in de allergische CRSwNP groep waren de totale IgE concentraties significant hoger (resp. $p=.026$ en $p=.017$) vergeleken met controlepatiënten (n=12). In de AR groep en de allergische CRSwNP groep was totaal IgE onder de detectielimiet (BDL) in resp. 3 en 2 patiënten. In CRSwNP patiënten zonder allergie (n=12) en de controlepatiënten was totaal IgE BDL in resp. 7 en 8 patiënten. Parallel aan totaal IgE was Gx3 IgE het hoogst bij de AR patiënten, gevolgd door de allergische CRSwNP patiënten. Gx3 IgE concentraties waren BDL bij alle CRSwNP patiënten zonder allergie en bij alle controlepatiënten. Gx3 IgE was significant hoger bij de AR patiënten in vergelijking met de controlegroep ($p=.001$) en de CRSwNP patiënten zonder allergie ($p=.001$).

Vergelijking tussen Merocels en filter discs voor het meten van lokaal IgE
In neussecreties verzameld door middel van filter discs en Merocels waren totaal IgE en huisstofmijt specifiek IgE (HDM sIgE) significant hoger bij AR patiënten (n=15) in vergelijking met controlepatiënten (n=15). In neussecreties verzameld met Merocels waren totaal IgE en HDM sIgE BDL in resp. 3 en 4 AR patiënten, met de filter discs in resp. 2 en 6 AR patiënten. Significante correlaties werden gevonden tussen HDM sIgE concentraties in serum, in neussecreties verzameld met Merocels en in neussecreties verzameld met filter discs.

Conclusie: IgE niveaus in neussecreties verzameld met Merocels lijken een goede weerspiegeling te zijn van deze in nasaal weefsel. De collectie van nasale secreties met Merocels is een gestandaardiseerde en niet invasieve methode die gebruikt kan worden voor herhaaldelijke metingen van lokaal IgE en andere mucosale inflammatie parameters om zo nasale ziektes te bestuderen en hun behandeling op te volgen. Het gebruik van filter discs lijkt een haalbaar alternatief.
2 INTRODUCTION

2.1 Chronic upper airway inflammation

Chronic upper airway inflammation can be divided into two major clinical entities: rhinitis and rhinosinusitis. Rhinitis and sinusitis usually coexist [1]. The Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines define rhinitis as an ‘inflammation of the lining of the nose which is characterized by nasal symptoms including anterior or posterior rhinorrhoea, sneezing, nasal blockage and/or itching of the nose being present during two or more consecutive days for more than 1 h on most days’ [2]. Allergic rhinitis (AR) is an IgE-mediated inflammation of the nose after allergen exposure such as house dust mite (HDM) and pollen from grass and trees [2,3]. Many nonallergic conditions can cause nasal symptoms which mimic allergic rhinitis: infections, physical agents, hormonal imbalance, the use of certain drugs and anatomical anomalies [4]. According to the European Position Paper on Rhinosinusitis and Nasal Polyps 2012 (EPOS 2012) [1], rhinosinusitis in adults is defined as an ‘inflammation of the nose and the paranasal sinuses, characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), ± facial pain/pressure , ± reduction or loss of smell and either endoscopic signs of: nasal polyps, and/or mucopurulent discharge primarily from middle meatus and/or oedema/mucosal obstruction primarily in middle meatus and/or CT changes: mucosal changes within the ostiomeatal complex and/or sinuses.’ Acute rhinosinusitis (ARS) in adults is defined as a sudden onset of two or more symptoms as mentioned above for less than 12 weeks. If the problem is recurrent, there must be symptom free intervals to meet the definition. Chronic rhinosinusitis (CRS) in adults is defined as a presence of two or more symptoms as listed above for 12 or more weeks [1]. CRS can be divided into a group with and without endoscopic evidence of nasal polyps [1,5,6]. Chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by bilateral, endoscopically visualized nasal polyps in the middle meatus. In chronic rhinosinusitis without nasal polyps (CRSsNP) no visible polyps in the middle meatus can be visualized [1]. Nasal polyps are edematous masses protruding from the nasal and paranasal mucosa which can lead to an obstruction of the nasal cavities [7,8].
2.1.1 Allergic rhinitis

2.1.1.1 Classification
AR can be divided into ‘intermittent’ and ‘persistent’. Intermittent means that the symptoms are present less than 4 days a week or for less than 4 consecutive weeks. Persistent means that the symptoms are present more than 4 days a week and for more than 4 consecutive weeks. The severity of AR can be classified as mild or moderate/severe [2,9]. The classification in severity involves the symptomatology, but also includes the impairment on quality of life (appendix 4).

2.1.1.2 Epidemiology
AR is a common condition affecting 10-20% of the adult population worldwide [10]. In some countries up to 50% of the adolescents register with symptoms of AR [2]. The prevalence has increased during the past 50 years and is still increasing, especially in those countries where there was a low or mediate prevalence. Recent increase in the prevalence cannot be due to a change in gene pool [2]. Rhinitis and allergic diseases are taken more seriously and the European Union has developed programs to understand, manage and prevent allergic diseases in a better way [2,11].

2.1.1.3 Comorbidity

2.1.1.3.1 Asthma
AR and asthma, both allergen allergic diseases, often coexist [12]. AR and asthma have a corresponding etiology and show familial and intra-individual connection [2]. Rhinitis is a significant risk factor for adult-onset asthma in both atopic and nonatopic subjects [2]. Over 80% of asthmatics have rhinitis and 10-40% of patients with rhinitis have asthma [2]. So the majority of patients with asthma have rhinitis symptoms suggesting the concept of one airway one disease [13,14]. How more severe the rhinitis, the more severe the asthma will be [2]. However, not all the patients with rhinitis have asthma and there are differences between the diseases [13,14]. The prevalence of asthma in subjects without rhinitis is usually less than 2% [15-17].

2.1.1.3.2 Allergy
Other allergic diseases are common for patients with AR. The complex indoor environment may contribute to an increasing prevalence of atopic diseases. It is proven that multiple indoor allergens have an additional effect on atopic comorbidities [18].
2.1.1.3.3 Chronic rhinosinusitis

Inflammation of the nasal mucosal in AR predisposes the atopic individual to develop CRS. Both diseases show an increasing prevalence and are frequently associated [2]. The association with asthma is stronger in those patients reporting both CRS and AR. There is an increased prevalence of AR in patients with CRS, but the role of allergy in CRS remains unclear [19-21]. IgE-mediated mechanisms, responsible for nasal inflammation, are a main factor in the development of acute and/or chronic sinus disease [22,23]. In acute and chronic rhinosinusitis a high prevalence of sensitization to inhalant allergens is found and these patients seem to experience more sinus related symptoms [2].

2.1.1.4 Diagnosis

The diagnosis of AR is usually easy, but is still underdiagnosed. Many patients do not perceive their symptoms as an impairment in daily life. Normally the diagnosis of AR is based on a questionnaire, examination and skin tests to common aeroallergens [2]. In most studies, the diagnosis of allergy was based on the presence of a positive skin prick test and/or serum specific IgE determinations. This indicates atopy but may not be sufficient to diagnose AR, particularly the persistent form [2]. In a study from Tschopp et al. [24] the skin prick test had the best positive predictive value for the epidemiologic diagnosis of AR compared to Phadiatop or total serum IgE. AR can also be defined by a positive RAST (Radio-Allergo-Sorbent-Test) [1].

2.1.1.5 Pathophysiology

2.1.1.5.1 IgE-dependent mechanisms

Allergy is caused by a continuous overproduction of IgE to environmental antigens. This involves different antigens such as indoor or outdoor allergens, foods and other antigens [25]. Rhinitis is associated with increased levels of total serum IgE [26,27]. Allergen-specific IgE binds to receptors on the membranes of mast cells and basophils. Mast cell accumulation in the airway mucosa is an important pathophysiologic event in AR. Inhaled allergens are captured in the mucosa of nose and/or lungs. The accumulation of receptor-bound IgE molecules on exposure to specific allergen results in the production of inflammatory mediators (histamine, leukotriene and others) responsible for the allergic response [28]. The link between this IgE-mediated reaction and rhinitis has been confirmed by the effect of an anti-IgE monoclonal antibody [29-
2.1.1.5.2 Role of IgE and the inflammatory infiltrate
AR is characterized by an inflammatory infiltrate and the release of mediators is responsible for the symptoms. Eosinophils, mast cells, T-cells, macrophages, fibroblasts and other cells form the inflammatory infiltrate of the nasal mucosa in patients with AR [32-34]. IgE has several pathophysiologic roles such as antigen presentation, increased mast cell survival, defense against viruses, bacteria, fungi and parasites and mucosal homeostasis. IgE interacts with B-cells, T-cells, mast cells, eosinophils and basophils. Cross linking of IgE (bound to the surface of mast cells), the subsequent degranulation and the release of Th2 cytokines lead to recruitment of inflammatory cells [2]. Th2-cells drive the synthesis of IgE and the recruitment, maturation, survival and effector function of accessory cells such as eosinophils, basophils and mast cells [35].

2.1.1.6 Treatment
Standard treatment for IgE-mediated allergic diseases such as AR are antihistamines. Histamine, one of the major effector cells of allergic reaction, is released by mast cells and basophils [2].
2.1.2 Chronic rhinosinusitis with nasal polyposis

2.1.2.1 Epidemiology

There is a lack of valid epidemiological studies for CRSwNP. In a survey based on endoscopy, Sködve et al. [36] reported a NP prevalence of 2.7% in Sweden. In Finland, Hedman et al. [37] found in a postal questionnaire survey that 4.3% of the adult population answered positively to the question as to whether polyps had been found in their nose. Klossek et al. [38] reported a NP prevalence of 2.1% in France by using a disease-specific questionnaire. NP can be found in all races and becomes more prevalent with higher age [38-41]. The average age of onset is approximately 42 years. NP is uncommon under the age of 20 and in general more frequently found in men than in women [1].

2.1.2.2 Diagnosis

2.1.2.2.1 Symptoms

Diagnostic tests are available to verify the clinical symptoms and signs of rhinosinusitis. Nonetheless, for the majority of patients, the diagnosis is made in primary care based on symptoms alone. Symptoms remain the mainstay of diagnosis in primary care. Symptoms are mainly the same in acute and chronic rhinosinusitis with and without nasal polyposis, but symptom pattern and intensity may vary. Acute forms of infections have usually more distinct and often more severe symptoms. [1]

2.1.2.2.2 Examination

2.1.2.2.2.1 Anterior rhinoscopy and nasal endoscopy

Anterior rhinoscopy is the first examination in a patient, but alone it has a limited value [1]. The diagnosis of CRS is based on nasal endoscopy. Compared to anterior rhinoscopy, nasal endoscopy provides significantly better illumination and visualization. This allows to obtain detailed information of the nasal cavities [1].

2.1.2.2.2 Imaging

The plain sinus x-ray features a low cost and availability, but its usefulness is limited for diagnosing rhinosinusitis due to underestimation of bony and soft tissue pathology compared to computed tomography (CT) and magnetic resonance imaging (MRI) [1,42]. CT scanning is first choice for the paranasal sinuses due to optimal display of air bone and soft tissue, but it should not be regarded as the primary step in the diagnosis [1].
2.1.2.3 **Pathophysiology**

2.1.2.3.1 *Nasal polyps*

Polyps are infiltrated with inflammatory cells, mainly eosinophils. The local Th2 polarization observed in these polyps is characterized by high levels of eosinophilic markers, IL-5 and local polyclonal IgE [7]. IL-5 is the key driver of eosinophilic differentiation and survival. It is assumed that, through release of toxic products, eosinophils lead to tissue damage and growth of polyps. IL-5 inhibition is a potential novel therapeutic approach in patients with severe eosinophilic nasal polyposis [43].

2.1.2.3.2 *Local IgE*

In NP homogenates, the level of total or specific IgE is independent of the atopic status of the patient [1,44-46]. Local IgE in NP is the result of two types of IgE production: systemic allergic IgE formation and a local polyclonal IgE formation. The local polyclonal IgE production is located in the stroma and the epithelium of nasal polyps [7]. Local polyclonal IgE formation occurs mainly in the subgroup of polyps containing Staphylococcus Aureus enterotoxin-specific IgE [46,47]. High total IgE concentrations can also be associated with an increase of the soluble low affinity-IgE receptor sCD23. Membrane-bound CD23 normally binds to IgE, which leads to a downregulation of IgE production. sCD23 can capture IgE and prevents binding to his receptor [46].

2.1.2.3.3 *Inflammatory triggers*

Parasites, fungi, viruses and bacteria can stimulate polyclonal lymphocyte responses and thereby obliterate the specific immune system, resulting in a deficiency of specificity of antibodies. Two polyclonal stimuli can trigger B-lymphocyte proliferation and differentiation in the absence of antigen: bacterial products and T-cells activated by a third-party antigen. Activated T-cells stimulate B-cells in a non-cognate fashion via CD40 ligand and cytokine production [44]. IgE correlates with the presence of Staphylococcus aureus enterotoxins (SAE) [1,7,44,45]. Gevaert et al. demonstrated in about half of the examined polyp specimens polyclonal IgE formation specific to SAE and a high prevalence of asthma [45]. Stimulation of polyclonal IgE synthesis could be a way for S.aureus to colonize the nasal mucosa and devoid the allergic response [47]. Up to 73% of S.aureus strain isolates, produces one or more enterotoxins [45]. These enterotoxins may act as superantigens [44,45,47,48] (appendix 1). There are two prominent IgE positive areas in NP: the follicular
structures (CD20 B cells and CD3 T cells) and the diffuse lymphoid accumulations (CD38 plasma cells and CD3 T cells) [44,47]. Biotinylated SAE superantigens bind to these areas and directly affect the activation of B cells and plasma cells. This activation leads to isotype switching and synthesis of IgE [45]. These superantigens can also interact with the T cell receptor (TCR), resulting in a polyclonal T-cell activation with a Th2 cytokine polarization. [1,45]. Key markers of Th2 inflammation are increased in NP [47]. These cytokines favour IgE production indirectly by triggering B cell class switching towards IgE production [1,45]. Each enterotoxin can only bind to a certain subset of TCR Vβ-chains [45]. The T-cell receptor Vβ repertoire corresponds to the specific IgE in the polyp tissue [45]. SAE can exert direct effects on eosinophil proliferation and survival which possibly results in a severe eosinophilic inflammation [44,45,48]. Beside the enterotoxins, Staphylococcal protein A (SPA) as well as the toxic shock syndrome toxin (TSST-1) from Staphylococcus aureus, possess B-cell superantigen moieties which induces polyclonal IgE synthesis [44].

2.1.2.3.4 Local receptor revision and class switch recombination
Evidence has been found for local receptor revision (RR), class switch recombination (CSR) and B-cell differentiation into IgE-secreting plasma cells in NP. NP is associated with an increase of IL-4. IL-4 stimulates the synthesis of ε-germline gene transcript (GLT), which has a significant role in the class switch recombination (CSR) to IgE. Gevaert et al. [47] detected Iε-Cγ switch circle transcript in NP. B-cells and plasma cells are enhanced in NP, as well as the ratio of plasma cells to B cells [47].

2.1.2.4 Comorbidity
2.1.2.4.1 Allergy
To date, there is no clear evidence for a direct relationship between allergy and rhinosinusitis [49,50]. Increased local IgE synthesis can reinforce allergic reactivity by triggering mast cell degranulation. However, when the IgE formation is extreme it can suppress specific reactivity by flooding the high affinity IgE receptors (Fce receptors) on mast cells in the tissue through polyclonal IgE. This polyclonal IgE would neutralize any effect of specific IgEs to environmental allergens [47,48]. This could explain why the IgE level is independent of the atopic status of the patient [44]. Some studies show an increase of atopy markers in CRS patients [1]. Between 10 to 54% of subjects with CRSwNP have allergy. On the other hand, the prevalence of NP in AR patients has been reported between 0,5 to 4,5%, which is comparable with the normal
2.1.2.4.2 **Asthma**

CRSwNP is a multifactorial disease that is frequently associated with asthma [46,51]. The group that coexists with asthma is particularly characterized with tissue eosinophilia and high local IgE levels. In mucosal tissues, mRNA for the $\varepsilon$-chain of IgE was associated with a significant proportion of B cells [44,47]. This strengthens the hypothesis of local IgE synthesis [44]. Asthma is described by 26% of patients with CRSwNP, compared to 6% of controls [1,38,39]. There is a direct correlation between NP, bronchial hyperreactivity and asthma [51]. Asthmatic patients with CRSwNP have more nasal symptoms and are associated with high levels of local IL-5 and specific IgE against SAE [1,45,52].

2.1.2.5 **Medical treatment options**

The figure in appendix 2 represents the management of CRSwNP for ENT-specialists as proposed by EPOS 2012 [8].

2.1.2.5.1 **Corticosteroids**

Treatment with methylprednisolone results in a reduction of systemic or local free light chains concentrations and can be related to its potent systemic anti-inflammatory action [43]. Methylprednisolone has a short but dramatic effect on polyp size and symptoms [1].

2.1.2.5.2 **Antibiotics**

Antibiotics are a therapeutic option because Staphylococcus aureus plays a role in the pathophysiology of CRSwNP. Doxycycline is an anti-staphylococcal antibiotic that can be used [1]. A RCT showed a significant effect of oral methylprednisolone and doxycycline on size of nasal polyps, nasal symptoms and mucosal and systemic markers of inflammation [53].

2.1.2.5.3 **Anti-IgE**

The local production of IgE is functional and has a role in the regulation of chronic inflammation. Antagonizing IgE antibodies is a relevant strategy. Omalizumab is a recombinant DNA-derived humanized IgG1k monoclonal antibody. It reduces serum and tissue IgE-levels and is a treatment option for patients with allergic and non-allergic NP [1,48]. Omalizumab binds selectively free human circulating IgE and
inhibits the binding of IgE to the high-affinity IgE receptor, decreasing free IgE levels. Omazilumab also leads to a decrease in IgE receptors on mast cells, basophils, and dendritic cells [48].

2.1.2.5.4 Anti-IL-5
Mepolizumab is a humanized anti-IL-5 monoclonal antibody and forms a potential novel therapeutic approach for patients with severe eosinophilic NP. Mepolizumab achieved a statistically significant reduction in NP size for at least 1 month after dosing in 12 of patients in a study of Gevaert et al. [43].

2.2 Measurement of local IgE
Local IgE can be measured in nasal tissue homogenates and secretions [44,48]. Different sorts of cells (expelled epithelial cells and immunocompetent cells), plasma exudation and mucus can be found in nasal secretions. Interesting is the fact that nasal fluid contains inflammatory mediators reflecting the activity within the nasal mucosa. Therefore it is possible to detect specific inflammatory cells, inflammatory mediators and cytokines with biochemical analyzes and to follow the evolution of nasal diseases [54]. Earlier, blown secretions were the only techniques to investigate nasal secretion, but the obtained quantities of secretions were extremely variable [55]. Several other techniques like the brush method, nasal scraping [56], biopsies [57] and the imprint technique are explored and described. The standardization for these techniques has improved the quality of analysis, but they are painful for the patient and traumatizing for the nasal mucosa [54]. Microsuction [58], aspiration [59] and nasal lavage were designed to collect nasal secretion with the least discomfort and the best reproducibility [54]. Aspiration and nasal lavage have been described as the most reliable methods for nasal secretion sampling [60]. These techniques require the cooperation from the patient, which may be a problem with children or comatose patients. Another disadvantage is the fact that the collected quantity of material can fluctuate significantly [54]. Alternative methods for sampling of nasal secretions are the use of swabs and sponges [54,61]. The use of pre-humidified gauze [62], filter discs [63] and sinus packs (Merocels) has been described. Measurement of local IgE in the collected material (tissue and/or nasal secretions) can be done by means of the UniCAP system [44,48].
2.2.1 Merocels

Merocels have already been used for several different purposes. They are mostly used in nasal surgery, epistaxis management, endoscopic sinus surgery (ESS), transseptal suture, and septoplasty to control bleeding [54]. Merocels are also considered as a non-invasive, atraumatic, and reproducible technique for the collection of nasal secretions in order to do biochemical analyses [54]. Examples of use of Merocels for monitoring local inflammation, for monitoring medical and surgical treatment are the following: In a study of Watelet et al. [64] Merocels were used to follow different phases of wound repair by detecting changes in the secretion of growth factors. In another study Merocel samples were collected to determine IgA titers to respiratory syncytial virus (RSV) fusion and attachment glycoproteins [65]. Gevaert et al. [48] measured ECP, IL-5, sCD23, sIL-5Rα, total IgE and tryptase in nasal secretions collected with Merocels from one sample. Klimet et al. [61] used Merocels for monitoring the therapeutic efficacy of immunotherapy in AR by detecting specific cell activation markers like ECP or tryptase. In a twelve-year follow-up study after endoscopic sinus surgery (ESS) in patients with CRSwNP, nasal secretions were collected by placing Merocels in the nasal cavities to measure different proteins (IL-5, IL-5Rα, TGF-β1, MPO, ECP, total IgE and specific IgE antibodies) [66]. In a study from Cassona et al. [67] Merocels appeared to be well tolerated by patients and showed the lowest percentage of cytological alteration of ciliated cells compared to Clauden, two-fingered glove pack with gauzes inside and Lyofoam.

2.2.2 Filter discs

A filter disc is a small round piece of paper, punched out from a commercially available synthetic filter card, placed into the nose against the nasal septum. Filter discs are the collection system the most comparable to Merocels. It is a non-invasive, relatively simple, quantitative technique that can be removed atraumatically [68]. Filter discs are smaller than Merocels and give less stimulation of the nasal mucosa. In a study from Naclerio et al. [68] filter discs were used to observe the effects of histamine, methacholine, ipratropium bromide and atropine on the nasal mucosa. Baumann et al. [69] used the filter disc method to detect cytokine levels in AR patients. In another study with AR patients, filter discs were used to investigate the role of IL-31 [70]. Filter discs have also been used to differentiate congestive hyperresponsiveness in AR by applying nasal bradykinin unilateral [71]. The paper filter method was
already used in the context of HDM allergy to measure neutrophil chemotactic activity (NCA) and histamine content in nasal secretions [72]. Howarth et al. used an allergen solution on the surface of a small filter disc to induce local allergen provocation in order to stimulate inflammatory cells such as eosinophils, mastcells and neutrophils [73]. Lien Calus et al. [66], collected nasal secretions before and 15 minutes after NAPT, by placement of filter discs in the nose, to measure total IgE, total IgG, specific IgG₄ and tryptase concentrations.

2.2.3 Tissue
Tissue extraction is the most reliable local measurement technique and thereby the golden standard. It is an invasive procedure, but can inform directly about a range of mucosal tissue cellular events [73]. Gevaert et al. [44] obtained fresh tissue strips from nasal polyps during routine septal and sinus surgery to determine concentrations of total IgE and IgE antibodies to inhalant allergens and S. aureus enterotoxins. In a study from Lien Calus et al. [66] nasal tissue, namely NP tissue from CRSwNP patients, was collected during endoscopic sinus surgery (ESS) to measure different proteins (IL-5, IL-5Ra, TGF-β1, MPO, ECP, total IgE and specific IgE antibodies).

2.3 Aims of the study
Several datasets were analyzed to obtain more knowledge concerning local IgE in AR patients and CRSwNP patients. Further, we wanted to demonstrate the validity of local IgE measurements on nasal secretions collected with Merocels and illustrate the role of filter discs as a possible alternative. The experiment was performed in order to optimize the process of collection of nasal secretions by using Merocels and filter discs and to make a comparison between them.
3 MATERIALS AND METHODS

3.1 Analyzed datasets
Data of 112 study subjects with CRS (with and without NP) collected at the Department of Otorhinolaryngology of the University Hospital of Ghent were used to compare IgE measurement by means of Merocel, tissue and serum [74]. Data of a study with omalizumab [48] were used to analyze differences in total IgE concentration when subjects underwent a placebo or an omalizumab treatment. A randomized, double-blind, placebo-controlled study of allergic and nonallergic patients with NP and comorbid asthma (n= 24) was conducted. Subjects received 4 to 8 (subcutaneous) doses of omalizumab (n= 16) or placebo (n= 8). The primary end point was a reduction in total nasal endoscopic polyp scores after 16 weeks. The study was conducted at the Department of Otorhinolaryngology of the University Hospitals of Ghent (n= 20 patients) and Leuven (n= 4 patients), Belgium. A dataset of thirty patients with severe NP (grade 3 or 4 or recurrent after surgery), refractory to corticosteroid therapy, was used to measure the differences in total IgE after a placebo or mepolizumab treatment. Patients were randomized in a double-blind fashion to receive either 2 single intravenous injections (28 days apart) of 750 mg of mepolizumab (n= 20) or placebo (n= 10). Change from baseline in NP score was assessed monthly until 1 month after the last dose (week 8). Computed tomographic scans were also performed at week 8. The objective of this study was to investigate the therapeutic potential of inhibiting IL-5 with a humanized mAb as treatment for severe NP. This study was also conducted at the Department of Otorhinolaryngology of the University Hospital in Ghent. At last, data were used to analyze differences in total IgE concentration over the time during 6 visits (at baseline, week 1, week 2, week 4, week 8, week 12), in placebo (n= 18), in doxycycline (n= 14) and in methylprednisolone (n= 14) treated groups. In a double-blind, placebo-controlled, multicenter trial, 47 participants with bilateral NP were randomly assigned to receive either methylprednisolone in decreasing doses (32–8 mg once daily), doxycycline (200 mg on the first day, followed by 100 mg once daily) or placebo for 20 days. Participants were followed for 12 weeks. Patients were assessed for nasal peak inspiratory flow and symptoms and by nasal endoscopy. Markers of inflammation such as eosinophilic cationic protein (ECP), IL-5, myeloperoxidase, matrix metalloproteinase 9, and IgE
were measured in nasal secretions. Concentrations of eosinophils, ECP, and soluble IL-5 receptor α were measured in peripheral blood samples. The original endpoint of this study was to evaluate the effects of oral glucocorticoids and doxycycline on symptoms and objective clinical and biological parameters in patients with CRSwNP. To illustrate the role of filter discs for local measurements, different biomarkers (before nasal allergen provocation) were analyzed (total IgE, gx3 IgE, IgG, IgG4 and tryptase) based on a dataset [66] with 12 grass pollen allergic CRSwNP patients. The patients underwent a nasal allergen provocation test (NAPT) with grass pollen and were compared to 12 grass pollen AR patients and 12 control patients. Further, VAS scores of different symptoms, serum and nasal secretions were collected before and after NAPT. A skin prick test was performed on all the patients for different common aeroallergens to diagnose grass pollen allergy. The primary endpoint of this study was the NAPT. The study was conducted at the Department of Otorhinolaryngology of the University Hospitals of Ghent.

3.2 Experiment

3.2.1 Patient recruitment

15 patients with AR to house dust mite (HDM) and 15 control patients were recruited from the Department of Otorhinolaryngology at the Ghent University Hospital. All patients submitted a skin prick test (SPT) for 8 different common aeroallergens. Patients with HDM sensitive AR were selected based on symptoms and a positive SPT for HDM (Derm. Pteronyssinus ± Derm. Farina). The control subjects had a negative SPT and were free of acute nose or sinus disease at the moment of sampling, no chronic nasal or sinus complaints were reported in their history. The local ethical committee on human experimentation of the institution (Ghent University Hospital, Belgium) had given its approval for this project, and each participant gave an informed consent before enrolment.

3.2.2 Skin prick testing

A skin prick test (SPT) was performed in control and patient groups to demonstrate an allergic response to a specific allergen (figure 1). Controls were performed with Histamindihydrochloride 0,1% (positive control) and NaCl 0.9% (negative control). Eight allergens were selected: Grasses, Trees, Artemesia vulgaris, Dermatophagoides pteronyssinus, Dermatophagoides farina, Cat, Dog and Alternaria alternate. The SPT
was carried out on the inner forearm 2-3 cm from the wrist and the antecubital fossae. The location of each allergen was coded with a marker pen to identify the allergens to be tested with a distance between two SPTs (≥ 2 cm) to avoid false-positive reactions due to direct contamination of a nearby test or secondary to an axon reflex [75]. A drop of the allergen solution was placed on the skin in identical order for each tested subject and immediately pricked through the drop using the tip of a lancet. Positive and negative controls were first measured. After 15 minutes the largest diameter of the wheal of each particular test was measured. The result was considered as positive when the wheal had a diameter of ≥ 3 mm.

![Image](image.jpg)

**Figure 1: SPT procedures.** (A) Allergen solutions. (B) Lancets. (C) Prick testing with lancet through a drop of allergen extract. (D) Waiting for 15 minutes. (E) Measuring the largest diameter of the wheal of each particular test.

### 3.2.3 Blood testing

All the control and AR patients had a blood test to measure the concentration of total IgE and HDM sIgE. Blood was collected by performing a standard venipuncture (Serum gel separator tubes Terumo Venosafe, Ref VF-109SDK, figure 2) and allowed to clot for 15-30 minutes. After that the blood was centrifuged for 15 minutes at 1500g at 4°C. Serum was stored in aliquots at -20°C until further analysis with the UniCAP system (Pharmacia & Upjohn, Uppsala, Sweden).
3.2.4 Procedure for collection of nasal secretions

3.2.4.1 Sampling of nasal secretions

Filter paper discs (figure 3 C) were punched from filter cards (Shandon, Pittsburgh, PA) using a 10-mm punch. The filter cards are made from a blend of 100% cotton and cellulose fibers and have excellent absorbent properties. Before the experiments, the Merocels and filter discs were placed separately in pairs in Falcon tubes (Blue Max Jr. 15-ml polypropylene conical tube, 17×120 mm style FALCON, Becton Dickinson Lab-ware, Franklin Lakes, N.J., figure 3 A) and weighed (figure 4 A), coded and registered. Filter discs were first placed in both nasal cavities under direct visualization using a surgical headlight, a speculum and bayonet forceps (figure 3 A) in control and patient groups for 5 minutes (figure 4 B) and afterwards placed back in the respective Falcon tube. Secondly, Merocels went through the same procedure, but for 10 minutes (figure 4 D). The filter discs and Merocels were inserted on the floor of the cavity between the septum and inferior turbinate (appendix 3, figure 4 C).
Figure 4: Sampling of nasal secretions. (A) Filter discs in Falcon tube weighed before insertion. (B) Placing filter discs in the nasal cavities for 5 minutes. (C) Position of the filter disc in the nasal cavity. (D) Merocels went through the same procedure, but were placed in the nasal cavities for 10 minutes.

3.2.4.2 Processing of nasal secretions

The quantity of secretions collected was determined by comparing the weight of Merocels and filter discs with the Falcon tube before and after insertion using the same balance (A&D Instruments Ltd., type HR-120, Japan) (figure 4 A and figure 5 A). The difference corresponds to the weight of nasal secretion collected (WNS). The Merocels and filter discs were washed with 0.9% NaCl solution (Merck Eurolab, Belgium, figure 5 B) and incubated for two hours at 4°C (figure 5 C). The volume of 0.9% added NaCl solution was measured by a formula based on a dilution factor (DF) and the WNS:

\[
\text{volume (ml)} = (\text{DF} \times \text{WNS}) - \text{WNS}
\]

In previous experiments [54] with fixed amount of 0.9% NaCl solution for dilution (e.g. 3 ml), the amount of added NaCl was sometimes very high in comparison with the amount of nasal secretion that was collected (weight difference). Therefore, it is likely that some of the measurements were BDL due to excessive dilution. We chose to use fixed DFs: 10 for the Merocels and 20 for the filter discs. With this method it is unlikely that measurements are BDL due to excessive dilution. The filter discs and Merocels were placed into the shaft of a syringe (Plastipak 5 ml, Becton-Dickinson S.A., Cno. De Valdeoliva S/N, Madrid) and all the fluid was pushed out of it by moving the piston of the syringe (figure 5 D). After that, the falcon tubes, with syringe and Merocels or syringe and filter discs, were
placed in the centrifuge at 1500g/15 minutes at 4°C (figure 5 E). The supernatants were divided in aliquots (500 µL) and stored at or below -20°C (figure 5 F). Total IgE and HDM sIgE concentrations in nasal secretions were measured by the UniCAP system.

Figure 5: Processing of nasal secretions. (A) Weighing filter discs in Falcon tubes after insertion in the nasal cavities. (B) Washing with 0.9% NaCl solution. (C) Incubating for two hours at 4°C. (D) Filter discs placed into the shaft of a syringe and pushing all the fluid out of it. (E) Centrifuging at 1500g/15 minutes at 4°C. (F) Dividing the supernatants in aliquots (500 µL) and storing at or below -20°C. The same procedure was applied for the Merocels.

3.3 Literature

Particularly PubMed was used to study literature. Other used search engines were Web of Science and Google Scholar. Combinations of the following keywords were used: allergic rhinitis, chronic rhinosinusitis, nasal polyps, IgE, nasal secretion, treatment, nasal irrigations, corticosteroids, antibiotics, anti-IgE, anti-IL-5, surgery, skin prick test, nasal swabs, Merocel, filter disc, filter paper, tissue, diagnosis, pathophysiology, Staphylococcus aureus, superantigens, inflammation, immunology, cytokines, IL-5. A selection of articles was made by evaluating their abstract.
3.4 **Statistical analysis**

All statistics were performed using the SPSS 21.0 software. Data are presented as median and interquartile range (IQR). Statistical significance was assessed using two-tailed tests and was defined as p<0.05. Graphics were made by MedCalc 12.7.0.0 software. Non-parametric test were used because the populations were not normally distributed as assessed by Shapiro-Wilk tests. Correlations between continuous data were determined by the Spearman correlation coefficient (R_s). A Wilcoxon Signed-Rank test was executed to determine changes from baseline in inflammatory parameters in different treatment groups. A Friedman test was carried out to determine differences in the concentration of inflammatory parameters over the time in different treatment groups. Pairwise comparisons were performed with a Bonferroni correction for multiple comparisons. A Kruskal-Wallis test was done to determine differences in inflammatory parameters between more than two disease groups. Comparisons between two disease groups were performed by the Mann-Whitney U test for continuous data.
4 RESULTS

4.1 Correlations between Merocel, tissue and serum total IgE measurements

An assessment of the relationship between total IgE measured in nasal secretions collected with Merocel, tissue and serum for 112 patients with CRS (with and without NP) was made (table 1). Preliminary analysis showed the relationship to be monotonic, as assessed by visual inspection of scatterplots. A positive correlation between total IgE in tissue and serum (figure 6 A), Merocel nasal secretions and serum (figure 6 B), Merocel nasal secretions and tissue (figure 6 C) has been found (resp. $r_s=.623$, $p<.0001$; $r_s=.645$, $p<.0001$ and $r_s=.595$, $p<.0001$). Total IgE in Merocel nasal secretions had the lowest value (Mdn= 4.65 kU/L). Tissue and serum total IgE measurements were comparable (resp. Mdn= 50.16 kU/L and 50.18 kU/L).

Figure 6: Correlations between tissue, serum and Merocel nasal secretions. Scatterplots on a logarithmic scale showing a correlation in the measurement of total IgE between (A) tissue and serum (B) Merocel nasal secretions and serum and (C) Merocel nasal secretions and tissue. $R_s$: Spearman’s rank correlation coefficient; $p$: significance level.
Table 1: Descriptives of total IgE measurement by means of Merocel, tissue and serum

<table>
<thead>
<tr>
<th>Total IgE (kU/L)</th>
<th>Merocel</th>
<th>Tissue</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>100</td>
<td>81</td>
<td>108</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>4.65 (2.23 – 13.69)</td>
<td>50.16 (10.34 – 240.76)</td>
<td>50.18 (17.40 – 141.75)</td>
</tr>
</tbody>
</table>

Legends: IQR = Interquartile range.

4.2 Dynamic local IgE measurements by means of Merocels

4.2.1 Comparison omalizumab versus placebo

Differences in total IgE concentrations (at week 18), in nasal secretions collected with Merocels (table 2), were analyzed in 24 subjects with CRSwNP and comorbid asthma who had a placebo (n=8) or an omalizumab (n=16) treatment (figure 7). Total IgE decreased not significantly after placebo treatment ($p=0.686$). In contrary, there was a significant increase after omalizumab treatment ($p=0.041$).

Table 2: Descriptives of total IgE measurements at baseline and after treatment with placebo or omalizumab in nasal secretions collected with Merocels.

<table>
<thead>
<tr>
<th>Total IgE (kU/L)</th>
<th>Placebo (n= 8)</th>
<th>Omalizumab (n= 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>88.98 (26.44 – 146.99)</td>
<td>20.17 (9.31 – 56.63)</td>
</tr>
<tr>
<td>Week 18</td>
<td>74.85 (13.72 – 205.18)</td>
<td>72.375 (15.24 – 165.27)</td>
</tr>
</tbody>
</table>

Legends: IQR = Interquartile range.
4.2.2 Comparison mepolizumab versus placebo

Analyzing differences in total IgE and SAE-IgE concentrations (at week 12), in nasal secretions collected with Merocels (table 3) in 30 patients with severe NP who had a placebo (n= 10) or a mepolizumab (n= 20) treatment, revealed a significant increase in total IgE after placebo treatment ($p= .028$). After mepolizumab treatment no significant differences were noted ($p= .199$; figure 8 A). For SAE-IgE, there were no significant differences after placebo and mepolizumab treatment (resp. $p= .173$ and $p= .327$; figure 8 B).

![Figure 8](image)

Figure 8: Comparison of change in total IgE (A) and SAE-IgE concentrations (B) in placebo and mepolizumab treated groups in nasal secretions collected with Merocels. SAE-IgE: *Staphylococcus aureus* enterotoxin-specific IgE. All measurements are marked with a black open circle.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n= 10)</th>
<th>Mepolizumab (n= 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total IgE (kU/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>21.94 (10.85 - 177.73)</td>
<td>20.76 (6.64 - 95.93)</td>
</tr>
<tr>
<td>week 12</td>
<td>63.28 (14.38 - 273.77)</td>
<td>10.58 (4.34 - 29.00)</td>
</tr>
<tr>
<td><strong>SAE-IgE (kU/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>4.79 (2.32 - 15.20)</td>
<td>6.94 (4.77 - 17.57)</td>
</tr>
<tr>
<td>week 12</td>
<td>12.63 (9.04 - 20.23)</td>
<td>7.45 (3.72 - 15.89)</td>
</tr>
</tbody>
</table>

Legends: SAE-IgE: *Staphylococcus aureus* enterotoxin-specific IgE; IQR: Interquartile range.
4.2.3 Comparison doxycycline, methylprednisolone and placebo

Study of total IgE concentrations (change from baseline) during 6 visits (figure 9), in nasal secretions collected with Merocels in 47 CRSwNP patients (table 4), showed significant differences in the placebo group (n=18; \( p=.003 \)). Post-hoc analysis revealed significant differences at week 4 (\( p=.009 \)). In the methylprednisolone group (n= 14) an initial decrease in total IgE was followed by an increase in total IgE, however, these changes were not significant (\( p=.130 \)). In the doxycycline group (n=14) total IgE levels did not change during treatment (\( p=.412 \)).

Figure 9: Total IgE in nasal secretions after treatment with methylprednisolone (solid triangles), doxycycline (solid squares) or placebo (solid circles) in nasal secretions collected with Merocels.

Table 4: Descriptives of total IgE change from baseline in placebo, doxycycline and methylprednisolone treated groups in nasal secretions collected with Merocels.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=18)</th>
<th>Doxycycline (n=14)</th>
<th>Methylprednisolone (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total IgE (kU/L) change from baseline</strong></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>week 1</td>
<td>0.62 (-2.98 - 23.88)</td>
<td>-0.96 (-11.68 - 0.00)</td>
<td>-0.25 (-27.32 - 12.62)</td>
</tr>
<tr>
<td>week 2</td>
<td>5.91 (-0.60 - 49.44)</td>
<td>0.00 (-8.01 - 27.37)</td>
<td>-11.85 (-36.29 - 0.96)</td>
</tr>
<tr>
<td>week 4</td>
<td>29.81 (4.93 - 67.91)</td>
<td>0.00 (-8.01 - 1.28)</td>
<td>-3.92 (-57.96 - 4.01)</td>
</tr>
<tr>
<td>week 8</td>
<td>6.98 (0.00 - 155.30)</td>
<td>0.00 (-4.14 - 0.00)</td>
<td>1.76 (-18.76 - 21.83)</td>
</tr>
<tr>
<td>week 12</td>
<td>29.74 (0.00 - 124.57)</td>
<td>0.00 (0.00 -11.52)</td>
<td>10.06 (0.00 - 34.63)</td>
</tr>
</tbody>
</table>

Legends: IQR= Interquartile range.
4.3 Local measurements by means of filter discs

Filter discs are an alternative option for the collection of nasal secretions. Analyzes on a dataset with 12 control patients, 12 grass pollen allergic CRSwNP patients and 12 grass pollen AR patients (table 5) revealed that the group of patients with AR had the highest value of total IgE (Mdn= 43.13 kU/l), followed by the patients with CRSwNP with allergy (Mdn= 23.00 kU/l), those with CRSwNP without allergy (Mdn= BDL) and the control group (Mdn= BDL) in nasal secretions collected with filter discs (figure 10 A). Total IgE concentrations were significantly different between the disease groups ($p=.005$). Post-hoc analysis revealed significant differences between the control and AR group ($p=.026$) and the control and CRSwNP with allergy group ($p=.017$). There were no significant differences between the AR group and CRSwNP with allergy group ($p=1.00$). In the control group, the measurement of total IgE was BDL in 8 subjects (n=11). In the AR group and CRSwNP with allergy group, total IgE was BDL in resp. 3 (n=12) and 2 (n=12) subjects. In the CRSwNP without allergy group, 7 subjects (n=12) were BDL. Parallel with total IgE, gx3 IgE was the highest in the AR group (Mdn= 14.11 kU/l), followed by the patients with CRSwNP with allergy (Mdn= 3.53 kU/l), those with CRSwNP without allergy (Mdn= BDL) and the control group (Mdn= BDL) (figure 10 B). Gx3 IgE (kU/l) was significantly different between the disease groups ($p<0.05$). Post-hoc analysis revealed that gx3 IgE was significant higher in the AR group in comparison with the control group ($p=.001$) and the CRSwNP without allergy group ($p=.001$). Total IgG and IgG4 concentrations were the highest in the control group (resp. Mdn= 256.17 mg/l and 16.75 mg/l), followed by the CRSwNP group (resp. Mdn= 212.01 mg/l and 13.11 mg/l), AR group (resp. Mdn= 182.62 mg/l and 12.87 mg/l) and the CRSwNP with allergy to grass pollen group (resp. Mdn= 173.50 mg/l and 11.34 mg/l), but the differences were not significant (resp. $p=.837$ and $p=.994$; figure 10 C and D). Finally, in the control group and CRSwNP with and without allergy group, tryptase was not measurable in the majority of patients (Mdn= BDL). In the AR group, tryptase was measurable in 7 out of 12 patients (Mdn= 33.67 µg/l), but the differences were not significant ($p=.057$; figure 10 E).
Table 5: Inflammatory measurements in nasal secretions collected with filter discs in control patients, grass pollen sensitive AR patients, CRSwNP patients and CRSwNP with grass pollen allergy patients.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>AR</th>
<th>CRSwNP</th>
<th>CRSwNP with allergy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total IgE (kU/l)</strong></td>
<td>8 BDL; 1.65 (1.65-2.82)</td>
<td>3 BDL; 43.13 (4.01-72.26)</td>
<td>7 BDL; 1.65 (1.65-33.19)</td>
<td>2 BDL; 23.00 (7.11-111.67)</td>
</tr>
<tr>
<td><strong>gx3 IgE (kU/l)</strong></td>
<td>All BDL</td>
<td>3 BDL; 14.11 (2.95-24.02)</td>
<td>All BDL</td>
<td>5 BDL; 3.53 (1.66-7.26)</td>
</tr>
<tr>
<td><strong>Total IgG (mg/l)</strong></td>
<td>256.17 (193.12-425.45)</td>
<td>182.62 (87.62-290.74)</td>
<td>1 BDL; 212.01 (60.22-581.42)</td>
<td>2 BDL; 173.50 (36.96-702.39)</td>
</tr>
<tr>
<td><strong>Tryptase (µg/l)</strong></td>
<td>10/12 BDL</td>
<td>5 BDL; 33.68 (8.28-78.32)</td>
<td>8 BDL; 8.28 (8.28-13.04)</td>
<td>9 BDL; 8.28 (8.28-14.62)</td>
</tr>
</tbody>
</table>

Legends: Gx3 IgE: IgE to mix of grass pollen; IQR: Interquartile range; BDL: below detection level.
Figure 10: Comparison of inflammatory measurements in nasal secretions collected with filter discs. Total IgE concentrations (A), g3 IgE concentrations (B), total IgG concentrations (C), IgG4 concentrations (D) and tryptase concentrations (E) in control patients, grass pollen sensitive AR patients, chronic rhinosinusitis with NP without allergy patients (CRSwNP) and chronic rhinosinusitis with NP with allergy patients (CRSwNPwA). All measurements are marked with a black open circle and significant differences are marked with a line.

4.4 Comparison of Merocels and filter discs for the measurement of local IgE (experiment)

4.4.1 Demographic and clinical features

15 control patients and 15 HDM sensitive AR patients were recruited in order to analyze total IgE and HDM sIgE concentrations in serum and nasal secretions collected with both Merocels and filter discs. In the AR group, 13 patients were poly-sensitized and had a positive SPT for one or more of the other tested allergens. Two control patients had a SPT with a HDM wheal of 2 mm. Three patients had comorbid asthma. Three AR patients used oral antihistamines during the days prior to sampling of the nasal secretions (in these patients, SPT was performed on another occasion) (table 6).

Table 6: Baseline demographic and clinical characteristics of control patients and HDM sensitive AR patients.

<table>
<thead>
<tr>
<th></th>
<th>Control (n= 15)</th>
<th>AR (n= 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr), median (IQR)</td>
<td>25.00 (23.25-33.00)</td>
<td>24.00 (23.00-25.00)</td>
</tr>
<tr>
<td>Men/Women, n/n</td>
<td>4/11</td>
<td>6/9</td>
</tr>
<tr>
<td>Asthma, n</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>HDM (SPT+), n</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Other allergies (SPT+), n</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Antihistaminica use (last 3 days), n</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Legends: yr: years; IQR: Interquartile range; n: numbers; HDM: House dust mite; SPT: Skin prick test.
4.4.2 Total IgE and HDM sIgE are increased locally in nasal secretions in AR patients

Total IgE and HDM sIgE concentrations, in control patients and HDM sensitive AR patients, in nasal secretions (Merocels and filter discs) and serum are shown in table 7 and illustrated in figure 11. For each AR patient, the individual weight of the collected nasal secretions, the added NaCl volume, serum and nasal secretion measurements (raw and final data) are pointed out in table 8. Three control patients had a slightly increased concentration of HDM sIgE in serum. Total IgE and HDM sIgE in nasal secretions collected with Merocels were BDL in resp. 3 and 4 AR patients, with filter discs resp. 2 and 6 AR patients were BDL. Total IgE and HDM sIgE were significantly increased in HDM sensitive AR patients in serum and nasal secretions collected with both Merocels and filter discs (table 7). Significant positive correlations between HDM sIgE measured in Merocel nasal secretions and filter disc nasal secretions ($r_s=0.753$, $p=.001$, figure 12 A), Merocel nasal secretions and serum ($r_s=0.569$, $p=.027$, figure 12 B) and filter disc nasal secretions and serum ($r_s=0.749$, $p=.001$, figure 12 C) were found in the AR patients.

Table 7: Total IgE and HDM sIgE in nasal secretions (Merocels and filter discs) and serum in control patients and HDM sensitive AR patients.

<table>
<thead>
<tr>
<th>Nasal Secretions</th>
<th>Control (n= 15)</th>
<th>Control vs AR</th>
<th>AR (n= 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Merocel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IgE (kU/L)</td>
<td>12 BDL</td>
<td><strong>&lt;0.001</strong></td>
<td>3 BDL; 10.50 (2.38-51.98)</td>
</tr>
<tr>
<td>HDM sIgE (kU/L)</td>
<td>All BDL</td>
<td><strong>&lt;0.001</strong></td>
<td>4 BDL; 4.60 (1.25 - 12.15)</td>
</tr>
<tr>
<td><strong>Filter disc</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IgE (kU/L)</td>
<td>11 BDL; 2.00 (2.00 - 5.60)</td>
<td><strong>0.002</strong></td>
<td>2 BDL; 7.80 (4.45 - 30.90)</td>
</tr>
<tr>
<td>HDM sIgE (kU/L)</td>
<td>All BDL</td>
<td><strong>0.001</strong></td>
<td>6 BDL; 4.00 (2.00 - 13.75)</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IgE (kU/L)</td>
<td>11.75 (9.96 -66.00)</td>
<td><strong>0.001</strong></td>
<td>164.00 (57.50 - 233.75)</td>
</tr>
<tr>
<td>HDM sIgE (kU/L)</td>
<td>12 BDL</td>
<td><strong>&lt;0.001</strong></td>
<td>27.30 (8.62 - 35.18)</td>
</tr>
</tbody>
</table>

Legends: Differences between groups were analyzed for each parameter by a Mann-Whitney U Test. All numbers are represented as median (IQR). BDL= Below detection level.
Figure 11: Comparison of total IgE and HDM sIgE concentrations in serum (E and F) and nasal secretions collected with Merocels (A and B) and filter discs (C and D) in control patients and AR patients. Total IgE and HDM sIgE are significantly increased in HDM sensitive AR patients with all the different measurement techniques. All measurements are marked with a black open circle.
Figure 12: Correlations between HDM sIgE concentrations in serum and in nasal secretions collected with Merocels and filter discs. Scatterplots displaying the correlation between HDM sIgE levels in Merocel and filter disc nasal secretions (A), in Merocel nasal secretions and serum (B) and in filter disc nasal secretions and serum in HDM sensitive AR patients (C). Rs: Spearman's rank correlation coefficient; p: significance level.
Table 8: Total IgE and HDM sIgE measurements in HDM sensitive AR patients in serum and nasal secretions (filter disc and Merocel).

<table>
<thead>
<tr>
<th>AR patients (n=15)</th>
<th>Serum</th>
<th>Filter disc (DF 20)</th>
<th>Merocel (DF 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total IgE (kU/l)</td>
<td>HDM sIgE (kU/l)</td>
<td>WNS (g)</td>
</tr>
<tr>
<td><strong>Patient 1</strong></td>
<td>472.00</td>
<td>45.80</td>
<td>0.078</td>
</tr>
<tr>
<td><strong>Patient 2</strong></td>
<td>595.00</td>
<td>82.80</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Patient 3</strong></td>
<td>65.50</td>
<td>7.67</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Patient 4</strong></td>
<td>307.00</td>
<td>33.60</td>
<td>0.081</td>
</tr>
<tr>
<td><strong>Patient 5</strong></td>
<td>134.00</td>
<td>36.10</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Patient 6</strong></td>
<td>26.65</td>
<td>10.70</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Patient 7</strong></td>
<td>166.50</td>
<td>33.00</td>
<td>0.076</td>
</tr>
<tr>
<td><strong>Patient 8</strong></td>
<td>194.00</td>
<td>20.20</td>
<td>0.063</td>
</tr>
<tr>
<td><strong>Patient 9</strong></td>
<td>62.00</td>
<td>8.28</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Patient 10</strong></td>
<td>56.00</td>
<td>2.87</td>
<td>0.041</td>
</tr>
<tr>
<td><strong>Patient 11</strong></td>
<td>164.00</td>
<td>29.10</td>
<td>0.037</td>
</tr>
<tr>
<td><strong>Patient 12</strong></td>
<td>197.00</td>
<td>35.70</td>
<td>0.056</td>
</tr>
<tr>
<td><strong>Patient 13</strong></td>
<td>28.40</td>
<td>3.42</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Patient 14</strong></td>
<td>28.30</td>
<td>9.63</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Patient 15</strong></td>
<td>246.00</td>
<td>27.30</td>
<td>0.028</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>164.00</td>
<td>27.30</td>
<td>0.081</td>
</tr>
</tbody>
</table>

Legends: WNS: weight of nasal secretion collected, DF: dilution factor, /: below detection level, volume= (DF x WNS) – WNS.
5 DISCUSSION

Local production of IgE in the respiratory mucosa has been demonstrated in AR (allergen specific IgE) and in CRSwNP (polyclonal IgE), suggesting a role for local IgE in the pathogenesis of these inflammatory diseases of the upper airway. It has been suggested that the majority of allergen-specific IgE in the peripheral blood is not derived from IgE-secreting cells in the blood, but is produced locally in target organs of allergy such as the nasal mucosa. Considering the importance of local IgE production, it seems to be essential to develop non-invasive, reproducible and easy to perform approaches for measurement of local mucosal IgE production.

5.1 Merocels for local IgE measurement

Tissue IgE is only partially related to IgE concentrations in serum [1,45]. We identified a significant positive correlation between total IgE measured by means of Merocels, serum and tissue in 112 patients with CRS (with and without NP). Nevertheless, total IgE concentrations measured with Merocels were much lower than those measured in serum or tissue. We already know that there is an initial increase in total IgE concentration due to omalizumab. Omalizumab binds selectively free IgE [48] and forms more slowly eliminating IgE complexes [76]. Besides free IgE, complexed IgE is also included in the measurements, as a result total IgE concentration increases shortly after commencing omalizumab therapy. This phenomenon is also demonstrated in nasal secretions collected with Merocels. We found a significant increase in total IgE after omalizumab treatment in patients with CRSwNP and comorbid asthma at week 18. Patients with CRSwNP and comorbid asthma are particularly characterized with high local IgE levels [48]. We also found high baseline values of total IgE in this group. Recent research demonstrated that omalizumab was equally efficacious in both allergic and non-allergic patients (SPT negative) with NP and asthma [48]. This strengthens the hypothesis that blood tests and skin prick tests are misleading us and that local IgE is more important. IL-5 seems to play a key role in the chemotaxis, activation, and survival of eosinophils [77,78]. Bachert et al. [46] found an association between levels of both total and specific IgE and eosinophilic infiltration in NP. These findings were unrelated to skin prick test results. There is an increasing evidence that Staphylococcus aureus–derived enterotoxins stimulate eosinophilic inflammation through production of TH2
cytokines and local IgE formation [79]. In vitro studies indicate that SAE induced cytokine release tends to be pro-inflammatory and Th2 skewed, promoting IL-4 and IL-5 [80,81]. In our results, no significant differences were found in total IgE and SAE-IgE concentrations after mepolizumab (anti-IL5) treatment for patients with severe NP.

5.2 **Filter discs for local IgE measurement**

We found total IgE, measured in nasal secretions with filter discs, in 9 out of 12 grass pollen sensitive AR patients and in 10 out of 12 grass pollen allergic CRSwNP patients. Gx3 IgE was measurable in 9 out of 12 grass pollen sensitive AR patients and 7 out of 12 grass pollen allergic CRSwNP patients. IgE in CRSwNP is polyclonal and allergen-specific IgE forms only a small fraction of the total IgE concentration. In contrast, a big fraction of total IgE in nasal secretions in AR patients is allergen-specific, resulting in sensitization of resident mast cells for immediate hypersensitivity on exposure to allergen [66]. Total IgE and Gx3 IgE were not significantly different in the AR group compared to the CRSwNP with allergy group. Although IgE antibodies characterize the response to allergens, it also includes other parameters like total IgG, IgG4 and tryptase [82]. A model [82] suggests that a route to IgE production is a switch from IgM to IgG to IgG4 inside germinal centers with subsequent switch from IgG4 to IgE production in the periphery. Zhang et al. found evidence that the switch to IgE could occur sequentially through IgG4 [83]. IgG can inhibit histamine release and IgG4 antibodies can block IgE receptor-facilitated allergen binding to B cells and diminishes activation of mast cells and basophils [82,84]. Total IgG and IgG4 concentrations were measurable with the filter discs, but there were no significant differences between the different disease groups. Mucosal IgE in NP tissue is functional by activating mast cells [85]. Tryptase is a marker of mast cell activation, but no significant differences were found.
5.3 Comparison Merocels versus filter discs

The datasets mentioned above were collected for other primary endpoints than local IgE measurements. In those studies, local IgE concentrations were mostly determined to research trends and to function as a possible activity predictor. In our experiment, data were collected to investigate the presence of total IgE and HDM sIgE in nasal secretions collected with Merocels and filter discs in control patients and AR patients and to analyze differences between those groups. Further, we wanted to make a comparison between the Merocel and the filter disc technique.

AR patients had highly increased total IgE and HDM sIgE concentrations in serum and in nasal secretions collected with both Merocels and filter discs. HDM sIgE levels in serum and in nasal secretions collected by means of Merocels and filter discs were significantly correlated. Together, these observations support the validity of Merocels and filter discs for local IgE measurements. In contrast with our expectations, filter discs showed relatively good results. Total IgE concentrations in nasal secretions collected with Merocels and filter discs were BDL in resp. 3 and 2 out of 15 AR patients. HDM sIgE was BDL in 6 out of 15 AR patients, in nasal secretions collected with filter discs, whereas in nasal secretions collected with Merocels HDM sIgE was BDL in 4 AR patients. At this point, we can conclude that we can be optimistic about the use of filter discs in clinical trials.

The Merocel technique is highly reproducible, as shown in both in vitro models and humans. Unlike with filter discs, sufficient amounts of secretions can be collected with Merocels to measure several mediators from one sample [54]. However, there are also limitations to be kept in mind. Merocels are larger and can cause local irritation and stimulation of the nasal mucosa. Stimulation of the mucosa may vary from time to time, which makes Merocels probably less reproducible in comparison with filter discs. For these reasons, Merocels are not recommended for repeated measurements on the same day and in nasal provocation tests. Insertion of the Merocel into the nose may be difficult and uncomfortable in patients with an anterior septal deviation, a narrow vestibulum nasi or severe NP [54]. Filter discs are easier to manipulate and its placement in the nasal cavity is simple. In a study from Kristiansen et al., filter discs were used to detect serum IgA, IgM and IgG antibody activity. The secretory IgA concentrations of multiple samples from one person were stable, which proved reproducibility [63]. Filter discs also have disadvantages. They are not
commercialized and thereby not medically approved. Another disadvantage is the fact that filter discs require a higher dilution factor. As a result, total IgE and HDM sIgE concentrations, which are BDL in nasal secretions collected with filter discs, are sometimes just above the detection limit in nasal secretions collected with Merocels (table 8, patient 13 and 14). With both Merocels and filter discs, a clear differentiation and comparative analysis between nasal sides is possible. On the other hand, no clear distinction can be made between nasal secretion coming from the septum, the turbinates, the sinus cavities or local glands with both Merocels and filter discs [54]. The amount of nasal secretions depends on several factors. As an example, in upper respiratory tract infections watery secretions are an early symptom [86]. As mentioned before, Merocels can give a variable stimulation of the nasal mucosa and thereby induce a natural dilution of the nasal secretion. Heikkinen et al. [87] postulated that the interpretation of substances present in nasal specimens is difficult because of the unknown dilution in these samples.

In summary, we can conclude that IgE levels in nasal secretions collected by means of Merocels seem to be a good reflection of local IgE levels in nasal tissue. As collection of nasal secretions by Merocels is a standardized and non-invasive method, it can be used for repeated measurements of local IgE and other markers of mucosal inflammation, in order to study nasal diseases and monitor their treatment. In the experiment, Merocels performed slightly better than filter discs. The use of filter discs seems to be a feasible alternative for collection of nasal secretions and measurement of local IgE. Both Merocels and filter discs have advantages and disadvantages and both methods will probably have their specific applications in future experiments and clinical trials. Strengths and weaknesses of tissue, Merocels and filter discs are summarized in table 9.
<table>
<thead>
<tr>
<th></th>
<th>Tissue</th>
<th>Merocels</th>
<th>Filter discs</th>
<th>Tissue</th>
<th>Merocels</th>
<th>Filter discs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strengths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Golden standard</td>
<td></td>
<td>Highly reproducible</td>
<td>Probably more reproducible compared to Merocels</td>
<td></td>
<td>Very invasive</td>
<td>Local irritation and stimulation</td>
</tr>
<tr>
<td>Superior local IgE measurements</td>
<td></td>
<td>Sufficient amounts of secretions</td>
<td>Less discomforting and local irritation</td>
<td></td>
<td>Can not be used several times on the same day</td>
<td>Not recommended to use them several times on the same day</td>
</tr>
<tr>
<td>Clear differentiation between nasal sides</td>
<td></td>
<td>Clear differentiation between nasal sides</td>
<td>Clear differentiation between nasal sides</td>
<td></td>
<td>No clear distinction between different origins</td>
<td>No clear distinction between different origins</td>
</tr>
<tr>
<td>Informs directly about a range of mucosal tissue cellular events</td>
<td></td>
<td>Standardized and non-invasive</td>
<td>Non-invasive</td>
<td></td>
<td>Unknown dilution</td>
<td>Unknown dilution</td>
</tr>
<tr>
<td>Slightly better local IgE measurements compared to filter discs</td>
<td></td>
<td>Easy to manipulate and simple placement</td>
<td>Expensive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allow qualitative measurements</td>
<td></td>
<td>Allow qualitative measurements</td>
<td></td>
<td></td>
<td>Sometimes difficult and uncomfortable insertion</td>
<td></td>
</tr>
<tr>
<td>Can be used for follow-up in clinical trials</td>
<td></td>
<td>Can be used for follow-up in clinical trials and in nasal provocation tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good reflection of local IgE levels in nasal tissue</td>
<td></td>
<td>Good reflection of local IgE levels in nasal tissue</td>
<td></td>
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<td></td>
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<tr>
<td>Lower dilution factor needed</td>
<td></td>
<td></td>
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<tr>
<td><strong>Weaknesses</strong></td>
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6 REFERENCES


Calus L. Diagnostic and therapeutic consequences of mucosal lymphoid aggregates and local IgE in chronic rhinosinusitis with nasal polyposis [Doctoral thesis]. Belgium: Ghent University; 2013 Jul.


83. Zhang K, Mills FC, Saxon A. Switch circles from IL-4-directed epsilon class switching from human B lymphocytes. Evidence for direct, sequential, and multiple step sequential switch from mu to epsilon Ig heavy chain gene. J. Immunol. 1994 Apr. 1;152(7):3427–35.


APPENDICES

Appendix 1: Overview of the ‘superantigen hypothesis’ of CRS.
Retrieved from [1].

Appendix 2: Management scheme for adults with CRS with NP for ENT specialists based on the EPOS guidelines.
Retrieved from [8].
Appendix 3: Position of a Merocel inside the nasal cavity between the inferior turbinate and the septum.
Retrieved from [54].

Appendix 4: Classification of allergic rhinitis according to ARIA
Retrieved from [10].

Classification of allergic rhinitis according to ARIA.

1. Intermittent means that the symptoms are present
   < 4 days a week
   Or for < 4 consecutive weeks
2. Persistent means that the symptoms are present
   More than 4 days a week
   And for more than 4 consecutive weeks
3. Mild means that none of the following items are present:
   Sleep disturbance
   Impairment of daily activities, leisure and/or sport
   Impairment of school or work
   Symptoms present but not troublesome
4. Moderate/severe means that one or more of the following items are present:
   Sleep disturbance
   Impairment of daily activities, leisure and/or sport
   Impairment of school or work
   Troublesome symptoms