NASAL POLYPOSIS

Advances in diagnosis and management

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chapter 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Chronic rhinosinusitis with nasal polyps

Chronic rhinosinusitis (CRS) is a multifactorial chronic inflammatory disease of the nose and paranasal sinuses and is one of the most common chronic health conditions in the world. CRS has a major impact on the quality of life and in its treatment many different clinicians can be involved such as general physicians, otorhinolaryngologists, pulmonologists, pediatricians, allergists and even sometimes neurosurgeons (1, 2). As a consequence of this all CRS places a large financial burden on society as a whole due to frequent doctor visits, the need for repeated surgery, and prolonged use of medication. Moreover, also indirect costs of CRS are very high due to missed workdays and reduced productivity (3-8).

According to the EPOS position paper CRS in adults is defined as inflammation of the nose and the paranasal sinuses for more than 12 weeks, characterized clinically by two or more symptoms, one of which should be either nasal obstruction or nasal discharge (4). Further symptoms include facial pain or pressure and reduction or loss of smell. This should be combined with either endoscopic signs of nasal polyps, mucopurulent discharge or edema in the middle meatus and/or mucosal changes within the ostiomeatal complex or sinuses on CT scan. CRS can be categorized into two different entities, CRS without nasal polyposis (CRSsNP) and CRS with nasal polyposis (CRSwNP). Nasal polyps are grey benign masses filled with inflammatory material, which originate from the anterior ethmoid are descending into the nasal cavity. The difference between CRSsNP and CRSwNP is based on the presence or absence of polyps, with endoscopic findings and/or CT scanning. Many clinical symptoms overlap in both CRSsNP and CRSwNP. However, patients with CRSsNP have more problems with facial pain compared to patients with CRSwNP, whereas patients with CRSwNP show more nasal discharge and decreased sense of smell (9).

Recent epidemiological CRS studies have shown a worldwide incidence between 5-12% (10-12). For CRSwNP the exact prevalence is more difficult to estimate because of the need for endoscopic evaluation. Using postal questionnaires in Finland a prevalence of CRSwNP was reported of 4.3% (13). Using endoscopy, a prevalence of 2.7%-5.5% have been reported (14, 15). When removing the whole naso-ethmoidal block in cadavers, nasal polyps were found in 5 out of 19 cadavers (16). Nasal polyps are more frequently seen in men than in women, elderly and asthmatics and are uncommon under the age of 20 years (13, 17, 18). In children nasal polyps are very rare and it has been reported that the majority of children with CRSwNP also have cystic fibrosis (CF) (4, 19).

ASSOCIATED FACTORS OF CRSWNP

CRS is associated with allergic rhinitis and asthma (20). The prevalence of allergy in patients with CRSwNP has been reported as high as 64%, whereas other studies report

an incidence of allergy in CRSwNP which is comparable to the patients without CRSwNP (21-23). However, these finding have not always been linked to skin prick test results and there might be a selection bias. Conversely, in patients with allergic rhinitis only 0.5-4.5% have found to have CRSwNP, which is comparable to the normal population. Although total and specific IgE are increased in nasal polyps tissue and are related to eosinophilia and severity of eosinophilic inflammation, allergy to aeroallergens does not seem to play an important role (24). Further studies are needed to explore the exact role of IgE production in CRSwNP.

We know that the prevalence of asthma in patients with CRSwNP goes up to 20-60% (25, 26) The presence of CRSwNP in patients with asthma is associated with a higher severity of asthmatic symptoms and asthma seems to be more difficult to control is these patients (27, 28). Especially patients with late onset asthma and high levels of periostin and eosinophilia, often have CRSwNP (29).

Nasal polyps are present in 36-96% of the patients with nonsteroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease (NERD), formerly known as aspirin intolerance or Samter's Triad (15, 30-32). This is a combination of NSAID intolerance, nasal polyps and asthma (33). These patients are mainly nonatopic and are more difficult to treat. Recurrence rates of CRSwNP after FESS is much higher and also asthma is more difficult to control (34).

CRSwNP in children is mainly thought to be associated with cystic fibrosis (CF). CF is a lethal autosomal recessive disorder that is caused by a mutation in the CFTR gene on chromosome 7 which leads to defective chlorine channels. CF has severe impact of the function of the pancreas, lungs and sinuses by the production of abnormal thick mucus and ciliary malfunction (35). In around 1/3 of the children with CF nasal polyps are present and these polyps are generally difficult to treat (36). The prevalence of CRSsNP in adult patients with CF is 63%, and the prevalence of CRSwNP 25%

PATHOPHYSIOLOGY

The pathophysiology of CRS is very complex and many different factors play a role. Current therapeutic options for CRS are based on the two phenotypes CRSsNP and CRSwNP. However it remains to be confirmed if all patients with a given phenotype do indeed suffer from the same disease. By better understanding the different underlying pathophysiologic mechanisms, different endotypes can be identified. These so called endotypes may lead to new and better treatment options by targeting specific pathological mechanisms in individual patients. This can even imply that treatment could be based on targeting multiple different underlying mechanisms at once in one patient (37). A first example of this approach would be the currently evaluated approach of targeting IL-5 in eosinophilic CRSwNP patients.

Recently there has been a great progress in understanding the pathophysiology of CRS at the local and systemic level that includes environmental, microbial, genetic, and

iatrogenic factors. Early hypotheses considered only exogenous factors like microbes or fungi, whereas today the focus is more on aberrant interactions between these factors and the host immune system in general and the innate response in particular. Bacteria play a significant role in the development of acute rhinosinusitis (ARS) and it has been hypothesized that CRS evolves from ARS. However, the precise role of bacteria in CRS remains unclear. In Caucasian patients with CRS *Staphylococcus aureus* is the most common bacterial pathogen, although in Asian patients the rate of S. aureus is much lower (38). Indeed, different studies have shown that the superantigen of *S. aureus* could be responsible for the local Th2 responses environment in CRSwNP leading to a polyclonal IgE production (39). In addition to a concrete role for S. aureus also a potential role for bacterial biofilms has been hypothesized as biofilms are correlated with more severe disease (40) . However, it is not known if biofilms damage local tissue or that biofilms are better formed on damaged tissue. Similarly, fungi play a role in allergic fungal rhinosinusitis (AFRS) and have been hypothesized to be important for the pathophysiology of CRS in general. Fungi are found in almost all patients and could trigger local eosinophilia as part of the defense mechanism against fungi. As fungi are also found in most healthy individuals we should assume deficits in anti-fungal immunity in patients with CRS (41). However, the ineffectiveness of antifungal treatment made the relevance of fungi in the pathogenesis of CRS more controversial (42). In addition to these biological environmental factors, also physical environmental factors can play a role in CRS. A GALEN study showed that cigarette smoke was associated with having CRSsNP in all parts of Europe, just like occupational pollution (10). The role of environmental factors in CRSwNP remains unclear. Neither smoking nor pollution seem to influence the prevalence of CRSwNP (43).

In addition to the effect of various exogenous environmental agents, we now know that we should also consider the contribution of local immune responses. The first part of the innate immunity is the anatomical barrier composed of mucus, epithelium, and the process of mucociliairy clearance. These aspects act in unison to prevent easy access to the host. The immune barrier hypothesis proposes that mechanical and innate immune barrier defects are present in CRS, resulting in increased exposure to exogenous factors and uncontrolled innate and adaptive immune responses. Indeed genetic defects responsible for cystic fibrosis that affect the hydration state of the mucus, as well as increased mucus production per se will lead to mucociliary dysfunction (44-46). Although that mucociliary dysfunction is present in both forms of CRS, a lower expression of tight junction proteins and an increased susceptibility to exogenous protease degradation suggest that mechanical defects are more common in CRSwNP (47-49).

In addition to direct changes in the barrier function, epithelial cells are also thought to contribute actively to the pathogenesis of CRS at multiple levels. First of all epithelial cells produce antimicrobial compounds like lysozyme, lactoferrin, defensins, and cathelicidins as well as reactive oxygen and nitrogen species that can be upregulated during active infection (48, 49). Traditionally a major role for the detection of potential

pathogens was given to the presence of toll-like receptors on innate and adaptive immune cells (50). Recently, taste receptors have emerged as an interesting new players in the regulation of innate immune defenses (51). Among other cells, ciliated epithelial cells express taste receptors that are able to respond to a variety of bitter products secreted by potential pathogens. This response induces local inflammation, increased mucous clearance, and antimicrobial peptide secretion. Indeed, mutations in the bitter taste receptors have been linked to increased susceptibility to infection in multiple diseases including chronic rhinosinusitis (51).

Another way by which epithelium may contribute to pathogenesis of CRS is through the regulation and activation of innate lymphoid cells. This hypothesis was triggered by a very exciting notion that the recently discovered innate lymphoid cells (ILCs) could be key players in the pathogenesis of CRSwNP and asthma (52, 53). ILCs are related to T cells but do not express the CD3 antigen receptor. Instead ILCs react directly to "danger signals" and produce an array of cytokines that direct ensuing immune responses. ILCs are a family of effector cells that are important for protection against infiltrating pathogens and restoration of tissue integrity. Three major subsets have been defined on the basis of their phenotype and functional similarities to helper T cells. Group 2 ILCs (ILC2s) are known to produce type 2 cytokines, especially IL-5 and IL-13, and are activated by cytokines from epithelial cells such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), which are also associated with type 2 inflammatory responses. ILC2s numbers are highly elevated in nasal polyp tissues contrary to ILC1s and ILC3s numbers that are diminished (52-54). Although the precise roles of ILCs in CRS are still under investigation, it is clear that inhibition of ILC function represents a potential target that could provide novel treatments for CRS.

TREATMENT

Considering the complex pathogenesis of CRS, it is not surprising that effective pharmacotherapy requires a broad approach where most evidence has been accumulated for corticosteroids and antibiotics. With the realization that multiple endotypes of CRSsNP and CRSwNP may exist it is perhaps not surprising that we should even consider tailoring treatment to individual patients. Precision Medicine represents a novel approach in medicine, embracing 4 key features: personalized care based on molecular, immunologic and functional endotyping of the disease, with participation of the patient in the decision-making process of therapeutic actions, and taking into account predictive and preventive aspects of the treatment. Implementation of Precision Medicine into clinical practice may help to achieve to stop the epidemic of allergies and chronic airways diseases we see worldwide (55, 56).

The goal of CRS treatment is to achieve and maintain clinical control so that patients do not have symptoms at all or that the symptoms are not bothersome. If possible this should be combined with a healthy or almost healthy mucosa. According

to the EPOS evidence based guidelines, the management of CRSwNP includes nasal saline irrigation, topical or systemic steroids, and (long-term) antibiotics (4).

Nasal irrigation

Given that in CRS inflammation occurs at the interface of mucosa with the external environment suggestive of a dysfunctional host-environment interaction, it only seems logical to target the exogenous agents acting at this interface that drive the secondary inflammatory mechanisms (57). Nasal saline irrigation is an easy and effective way of cleaning the nose, thereby improving mucus clearance, enhancing ciliary beat activity, removal of antigen, biofilm and inflammatory mediators. It should be used as a supplement to other treatments. The evidence suggests that there is benefit of daily, large-volume (150 ml) saline irrigation with a hypertonic solution when compared with placebo (58). Several additions to the nasal saline irrigation have been investigated and proven effective, such as sodium hypochloride (NaClO), Xylitol and baby shampoo (59-61). Also topical corticosteroid droplets can be combined with nasal saline irrigation for a more effective treatment, by better reaching the sinus.

Corticosteroids

Topical intranasal corticosteroids, in the form of either spray of droplets, are the most common choice of treatment for CRSwNP (62). Corticosteroids act on two different types of intracellular glucocorticoid receptors (GRα and GRβ), which results in promoting anti-inflammatory and repressing pro-inflammatory gene transcription (63). This is how corticosteroid suppress the inflammatory response of the nasal mucosa, thereby improving nasal congestion, facial pain and nasal blockage and also improving drainage of the osteomeatal complex. Based on several randomized controlled trials intranasal corticosteroids are highly effective especially in mild disease (62). They show very few side effects, besides a chance of local irritation of the nose (64).

In more severe disease a short-term course of systemic corticosteroids can be prescribed (65). Short courses of oral corticosteroids in patients with CRSwNP show an improvement in Quality of Life (QoL) (66). Systemic corticosteroids are more effective, but also have a higher risk of side effects, like weight gain, adrenal suppression, osteoporosis and steroid induced diabetes mellitus (64). In general a maximum of three courses a year of oral corticosteroids is considered safe. Also the postoperative use of corticosteroids has a significant effect on the recurrence of polyps (67). Even though there is evidence of effectiveness of corticosteroids, still a substantial number of patients remain refractory to corticosteroid treatment. In general patient with eosinophilic nasal polyps respond better to corticosteroid treatment than neutrophilic polyps (68).

Antibiotics

Even though short-term antibiotics are frequently prescribed, there is no substantial evidence for the effectiveness in CRSwNP. There are two placebo-controlled trials with

short-term course of antitbiotics in CRSwNP. In a double blind, placebo-controlled study by Van Zele et al, a 1-week-course of methylprednisolone was compared to 3 weeks of doxycycline and placebo in patients with CRSwNP. This study showed a significant effect of oral methylprednisolone and doxycycline on the polyp size, nasal symptoms, and mucosal and systemic markers of inflammation (69). Another study by Schalek et al compared oral anti-staphylococcal antibiotics to placebo after endoscopic sinus surgery. No significant results were obtained for the antibiotic group for symptom-specific and endoscopic scores, as well as quality of life (70). Studies on long-term use of antibiotics in CRSwNP demonstrate some effect on polyp size and patient symptoms without proper quality of life data.

Therefore the role of antibiotics in the treatment of CRSwNP seems to be small. Only in acute exacerbations short-term antibiotics can be effective, if combined with a bacterial culture from the middle meatus. For treatment with long-term antibiotics (macrolides) there are data supporting a moderate effect, but one should always be aware of the potential risk of developing bacterial resistance (71).

Biologicals

When patients with CRSwNP are refractory to current medical treatments, there are very limited treatment options available beyond FESS. Therefore there is a need for new and better medical treatments focusing on the underlying pathophysiological mechanisms of CRSwNP. The development of biologicals is rapidly progressing over the last years and several studies have been performed which reported good results in patients with allergic diseases and asthma (72). For CRSwNP several studies have been performed with different antibodies, like omalizumab (anti-IgE), reslizumab (anti-IL-5) and dupilimab (anti-IL-4 receptor alpha and interfering with both IL-4 and IL-13 pathways) (73-75). All these studies have shown a positive clinical effects and show very limited side effects.

Sinus surgery

If patients do not respond to optimal medical therapy, currently no other therapies are available which means that the polyps have to be surgically removed (76). History of surgical treatment for CRSwNP goes as far back as the time of Hippocrates around 400 BC. Hippocrates is not only known as the 'father of medicine', also as the 'father of rhinology'. He first described a surgical method for removing nasal polyps by pulling a rough sponge on a string through the nasal canal. He also used a hot iron passed through the nostrils to cauterize polyps (77). In the years after, polypectomy has further evolved and nowadays functional endoscopic sinus surgery (FESS) is the technique of choice in sinus surgery. The goal of FESS is to restore normal ventilation and mucus drainage of the paranasal sinuses and to resect irreversibly changed mucosa. Overall FESS is a frequently performed and safe procedure and there are data suggesting there is only a 1% chance on major complications and 5-6 % on minor complications.

The efficacy of FESS is demonstrated in several studies but there still is a revision rate of around 20% (78). Therefore nasal irrigation in combination with nasal steroids is a very important part of postoperative management of CRSwNP (79).

Even though the link between rhinosinusitis and asthma is well established it is interesting to note that FESS has been shown to improve lower airway and reduce medication use for asthma (80). Recently studies in the UK have shown that patients with a surgical intervention for rhinosinusitis five years after the start of the disease had lower Sino-nasal Outcome Test-22 (SNOT-22) QoL scores, greater post-operative healthcare needs, and had a significantly higher prevalence of asthma, than patients treated at earlier time points (81).

AIM AND OUTLINE OF THE THESIS

The general aim of this thesis is to analyze and thereby optimize both existing and new treatments for CRSwNP and to improve our current knowledge about the pathophysiologic mechanisms of CRSwNP.

According to European guidelines current medical treatment of CRS is mostly based on the clinical differentiation of two phenotypes, CRSsNP and CRSwNP(4). Both these phenotypes are likely to have different subtypes (or endotypes) based on the existence of several underlying conditions. These include cystic fibrosis, aspirin-exacerbated respiratory disease (AERD), and also different comorbidities like asthma and allergies. Besides topical and oral steroids, current medical treatment options for CRSwNP exist of treating these different phenotypes with for example intranasal and oral corticosteroids, long-term antibiotics, antileukotrienes, Xolair and nasal saline irrigation. If patients with CRSwNP are refractory to these treatments, there are very limited treatment options available beyond FESS. Even though there is a proven efficacy of FESS, there still is a revision rate of around 20% (78). Because this recurrence rate of CRSwNP after FESS is so high, there is a need for new and better treatments which focus more on the underlying pathophysiologic mechanism as a target for the treatment of CRSwNP.

Just like in severe eosinophilic asthma, most nasal polyps in Caucasians are characterized by prominent local eosinophilic inflammation and high IL-5 concentration as well (82). IL-5 appears to have a key role in the pathogenesis of nasal polyposis. Consequently, IL-5 could be a major target for personalized therapeutic intervention. Previous studies with small numbers of patients have shown that anti-IL-5 treatments, such as mepolizumab (Glaxo Smith Kline), can successfully reduce nasal polyp size (73, 83).

In a clinical randomized double-blind placebo controlled trial in **Chapter 2** we assessed the safety and efficacy of mepolizumab in the treatment of severe bilateral nasal polyposis.

When maximal medical treatment fails, FESS is the technique of choice in sinus surgery in patients with CRSwNP. The extent of FESS in the treatment of CRSwNP can vary from polypectomy and infundibulotomy, to opening all the sinuses. In the last few decades surgeons tend to use a more custom approach based on the extent of the disease and comorbidities (84). The specific goal of FESS in patients with CRSwNP is not only to resect irreversibly changed mucosa, but also to re-establish normal ventilation and mucus drainage from the sinuses. This extensive approach improves postoperative drug delivery to the sinuses (85). In the last decades surgical techniques in FESS have been refined and new instruments are introduced. Besides traditional instruments, such as the cutting and non-cutting Blakesley forceps, nowadays the microdebrider (shaver) is widely used (86). This is a powered rotary shaving device, which can resect tissue very precisely while minimizing mucosal trauma. Because the microdebrider supplies continuous suction there always is a bloodless surgical field while operating. This may improve safety because of the increased visibility. But along with the use of powered instrumentation there are reports of higher incidence of serious complications, for example cerebrospinal fluid leaks or orbital injuries.
Even though the use of the microdebrider (Shaver) is well known in FESS, there is a lack of evidence from comparative studies focusing on operating time, blood-loss and user friendless between traditional techniques and the microdebrider. In **Chapter 3** the use of the microdebrider is compared to conventional instruments in FESS for patients with CRSwNP.

Corticosteroids are the most common choice of treatment for CRSwNP (62). Corticosteroids are able to suppress the inflammatory response of the nasal mucosa and suppress the productions of pro-anti-inflammatory mediators, cell chemotactic factors and adhesion molecules. They thereby improve nasal congestion, facial pain and nasal blockage and also drainage of the osteomeatal complex (87). Corticosteroids are included in the initial treatment of CRS, but also can be used preoperatively, intraoperatively and postoperatively in patients with CRS undergoing FESS. About the role of corticosteroids in FESS there are several randomized controlled trails, but they report conflicting results. It is not clear what the exact benefits of perioperative use of corticosteroids are regarding postoperative pain, symptoms and wound healing. Furthermore we know that the recurrence rate of CRSwNP after FESS is high and goes up to 15-20 % in adults (78). The exact influence of postoperative use of corticosteroids on the recurrence rate is not known.

Therefore in **Chapter 4** we performed a systematic review and meta-analysis of randomized controlled trials. The aim of this study was to systematically review all existing evidence on the role of corticosteroids in patients undergoing FESS. We determined whether preoperative corticosteroids affect operative parameters, intraoperative corticosteroids reduce pain and postoperative corticosteroids affect symptom scores, endoscopic scores and recurrence rates.

Although CRSwNP is rare in children it has a major impact on the QoL and therefore a thorough treatment is needed (88). In adults with CRSwNP FESS is considered to be the treatment of choice when maximum medical treatment fails. Several studies have shown that FESS in adults with CRSwNP is effective and safe with a revision rate of 20%. (78) In children with CRSwNP on the other hand surgical success rates are not known. There are a few studies describing results of FESS in children, but they mainly focus on CRSsNP and data are very limited (89-91). Moreover safety of FESS has only been established in children with CRSsNP in small case series (92, 93). Furthermore in previous literature is described that most children with CRSwNP also have CF (94). There are several studies that show positive outcome after FESS in children with CF, but CF still is a chronic disease of mucociliary transport where even after FESS problems like infections or nasal polyps can recur. Because of this there might be a more negative attitude to perform FESS in children with CF.

Therefore in **Chapter 5** we assessed the long-term results of FESS in children with CRSwNP with CF and without CF and determined outcome, symptoms, quality of life and complications.

There is not much known about the etiology of CRSwNP. We know that sinonasal epithelial cells play an important role in the immune response as a passive physical barrier, but the potential role as an active participant in the regulation of local immune responses in patients with CRSwNP is not well explored. In allergic airway disease on the other hand, there is an established awareness of the role of epithelial cells as an active participant in the regulation of local immune responses (95). Epithelial cells are able to detect and respond to environmental signals through a wide variety of receptors. The exact role of epithelial cells from nasal polyps in the pathophysiology of CRSwNP and their involvement in the innate defense against microbes or as a passive target for local inflammation, is relatively poorly explored. **Chapter 6** explores the potential contribution of nasal epithelial cells to the pathophysiology of CRSwNP. We performed micro-array expression profiling on epithelial cells from CRSwNP patients and healthy controls to investigate the role of polyp epithelium in the pathogenesis of CRSwNP.

CRSwNP is a chronic disease with a high prevalence estimated more than 10% in Europe and the United States (10, 11). Thereby it results in high costs for society as a whole mainly because of the need for repeated surgery. There are no good data available about the natural course of CRSwNP.

Suggested is that the prevalence of CRSwNP increases with age with the highest prevalence around the sixth decade of life and the lowest prevalence up to 40 years old (14, 96). Reliable data on the exact prevalence of CRSwNP in different age groups are very rare, because of the difficulties in selecting a representative group of

the population and also due to the fact that most studies only used questionnaires and no endoscopic evaluation, thereby missing asymptomatic patients.

Therefore in Chapter 7 we adopted FESS as an objective sign of active/uncontrolled disease and measured the time between first and final surgical intervention with a follow-up of 10 years. We determined the active disease duration of CRSwNP by looking at the relation between age, total number of times of sinus surgery and age at the time of the first operation ever.

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chapter 2

REDUCED NEED FOR SURGERY IN SEVERE NASAL POLYPOSIS WITH MEPOLIZUMAB: RANDOMISED TRIAL

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ABSTRACT

Background

Patients with eosinophilic nasal polyposis frequently require surgery, and recurrence rates are high.

Objective

To assess the efficacy and safety of mepolizumab vs placebo for severe bilateral nasal polyposis.

Methods

This randomised, double-blind, placebo-controlled trial recruited patients aged 18–70 years with recurrent nasal polyposis requiring surgery. Patients received intravenous mepolizumab 750 mg or placebo every 4 weeks for a total of six doses, in addition to daily topical corticosteroid treatment. The primary endpoint was the number of patients no longer requiring surgery at Week 25, based on a composite endpoint of endoscopic nasal polyp score and nasal polyposis severity visual analogue scale (VAS) score. Secondary endpoints included change in nasal polyposis severity VAS score, endoscopic nasal polyp score, improvement in individual VAS symptoms (rhinorrhoea, mucus in throat, nasal blockage, and sense of smell), patient-reported outcomes (PRO), and safety.

Results

105 patients received mepolizumab (n=54) or placebo (n=51). A significantly greater proportion of patients in the mepolizumab group compared with the placebo group no longer required surgery at Week 25 (16[30%] vs 5[10%], respectively; P=0.006). There was a significant improvement in nasal polyposis severity VAS score, endoscopic nasal polyp score, all individual VAS symptom scores, and sino-nasal outcome test [SNOT]-22 PRO score in the mepolizumab group compared with placebo. Mepolizumab's safety profile was comparable to placebo.

Conclusion

In patients with recurrent nasal polyposis on topical corticosteroids who required surgery, mepolizumab treatment led to a greater reduction in the need for surgery and a greater improvement in symptoms than placebo.

INTRODUCTION

Chronic rhinosinusitis is common, with a prevalence of 11% in Europe (1). It can be categorized into two phenotypes based on results from nasal endoscopy and computed tomography: chronic rhinosinusitis with nasal polyps and chronic rhinosinusitis without nasal polyps (2). Up to 4% of the general population is estimated to be affected by nasal polyps (3). Nasal polyps often have a negative impact on numerous aspects of quality of life (QoL), including physical health, general health, social functioning, sleep, and mental health (4), and can lead to workplace absenteeism (5). Symptoms experienced by patients with nasal polyposis include nasal blockage, loss of smell, rhinorrhoea, as well as symptoms derived from lower airway involvement (4).

Current treatment options for patients with nasal polyposis are limited to intranasal and oral corticosteroids, long-term antibiotics, and surgery (6–8). Intranasal corticosteroids are usually the initial treatment option for nasal polyps, with good results for patients with mild disease (9). Short-term courses of systemic corticosteroids are reserved for more severe cases (10). While symptoms can be controlled medically in some patients, surgery is often required (7). Surgery can range from a simple polypectomy to full removal of polypoid mucosal tissue from sinuses (7). The recurrence rate is impactful for patients and repeated surgery is often required; one study found that 15% of patients had 4–6 procedures within an 8-year period (11). Furthermore, long-term follow-up and treatment with topical corticosteroids is usually still required postsurgery (12). There is also a proportion of patients for whom surgery and/or oral corticosteroids fail to achieve disease control.

Interleukin (IL)-5 is the critical factor that promotes eosinophil development and survival (13,14). Mepolizumab, an IL-5 antibody, is under investigation as treatment for nasal polyposis (15). Mepolizumab reduces blood and tissue eosinophil counts (16,17), and is approved for the treatment of severe eosinophilic asthma. Both severe eosinophilic asthma and nasal polyposis are characterized by prominent local eosinophilic inflammation (18). IL-5 appears to have a key role in the pathogenesis of nasal polyposis: 1) nasal polyps are associated with IL-5 expression (19-22); 2) the expression of IL-5 within nasal polyp tissue has been associated with asthma comorbidity (23); 3) eosinophilic inflammation is associated with polyp recurrence after surgery (24). Consequently, IL-5 is a major target for therapeutic intervention, and previous studies with small numbers of patients have shown that inhibiting IL-5 with anti-IL-5 treatments such as mepolizumab, can successfully reduce nasal polyp size (15,25).

The aim of this study was to build on previous studies and determine if mepolizumab treatment could reduce the need for surgery in patients with severe recurrent bilateral nasal polyposis. This was assessed using a novel composite endpoint of polyp size and symptom severity.

METHODS

Study design and oversight

This was a 1:1 randomised, double-blind, placebo-controlled, multicentre study (NCT01362244, EudraCT: 2008-003772-21, GSK study ID: 111782). Six centres across three countries (Belgium, the Netherlands, and United Kingdom) took part in the study between May 2009 and December 2014. Further details of the investigators and methods can be found in the Supplementary Appendix. Initially the study comprised two phases: a treatment phase and a follow-up extension phase. The extension phase was subsequently removed from the protocol due to few patients enrolling into this phase. Although patients were required to have been receiving intranasal steroids for at least 3 months prior to study entry, to standardise treatment prior to randomisation, patients received intranasal steroids (fluticasone propionate [1 mg/mL], two sprays [50 µg per spray]in the morning into each nostril daily [Flixonase aqueous nasal spray, GSK, UK]) for 10–14 days (run-in period). Patients were then randomised to receive 750 mg mepolizumab or placebo by intravenous (IV) infusion every 4 weeks (Weeks 1, 5, 9, 13, 17, and 21), for 6 doses. Intranasal steroids were continued during the study at the same dose and regimen as used during the run-in period. See Supplementary Appendix for randomization and blinding details.

Mepolizumab and placebo were identical in appearance and administered by a staff member who was blinded to the infusion content, unaware of the study randomization, and independent of the study protocol and outcomes. An un-blinded pharmacist dispensed the study drugs. The protocol was approved by local ethics committees, and the study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from participants prior to the start of any procedures. The protocol for the analysis is available from the GSK Clinical Study Register (GSK study ID: 111782).

Patients

Patients were screened for eligibility prior to (within 28 days) commencing the run-in-period. Patients were 18 to 70 years of age with severe recurrent bilateral nasal polyposis who required surgery according to the predefined criteria of endoscopic nasal polyp score of ≥3 in one nostril (and a minimum score of two on the other side) and a visual analogue scale (VAS) nasal symptom score >7 (Supplementary Table 1). Patients were also required to be eligible for surgery as a result of being refractory to standard of care steroid therapy (received intranasal steroids for ≥3 months and/or received a short course of oral steroids) at the time of enrollment, and to have undergone at least one previous nasal polyp removal surgery. Exclusion criteria included the requirement for continuous high-dose oral corticosteroids, treatment with other biologics in the past 12 months, or asthma exacerbations requiring hospitalisation within 4 weeks of screening.

Endpoints

The primary endpoint was the number of patients who no longer met the criteria for requiring surgery 4 weeks after the final dose (Week 25) based on endoscopic nasal polyposis scores and nasal polyposis severity VAS scores (Supplementary Table 2). Endoscopic nasal polyposis score was assessed by endoscopy for each nostril separately and using the highest unilateral score. Each nostril was scored from 0–4 (0=no polyps, 4=large polyps causing almost complete nasal obstruction) according to the criteria in Supplementary Table 3. Nasal polyposis severity VAS scores were assessed by asking patients to indicate on a VAS (0–10 cm) the severity of their nasal polyposis, considering how troublesome each of the following symptoms were: rhinorrhoea, mucus in the throat, nasal blockage and loss of smell (see Supplementary Appendix for details on the analysis).

Secondary endpoints were the number of patients who met the criteria for requiring polyposis surgery at each time point, change in nasal polyposis severity VAS score from baseline to Week 25, change in endoscopic nasal polyp score from baseline to Week 25, individual symptom VAS scores (rhinorrhoea, mucus in the throat, nasal blockage, loss of smell), patient-reported outcomes (Sino-nasal outcome test [SNOT-22] questionnaire, and the EuroQual 5-dimensions [EQ-5D] questionnaire), peak nasal inspiratory flow (PnIF), olfaction testing (performed using the Sniffin' Sticks Screening-12 test), lung function assessments (forced expiratory volume in 1 second [FEV_1], forced vital capacity [FVC], and peak expiratory flow rate [PEFR]), blood eosinophil counts, and pharmacokinetics (PK). In the Sniffin' Sticks Screening-12 test, patients are presented with 12 different odours in turn and asked to identify the source from a list of four options. Patients were blindfolded and performed the test for each nostril separately (with the other blocked with tape). Scores range from 0–12 for each nostril.

Safety assessment included adverse events (AEs), vital signs, electrocardiogram (ECG) testing, and clinical laboratory testing.

Statistical analysis

Efficacy was analysed in the intent-to-treat (ITT) and per-protocol (PP) populations; PK and safety were analysed in the safety population (patients who received at least one dose of study drug). The maximum sample size was 110, with an unblinded sample size re-estimation conducted using "predictive power" when 46 patients completed the study (after applying the stopping rules for efficacy and futility) (26). The predictive power calculation suggested a sample size of 50 patients per treatment arm. For the analysis of the primary endpoint, patients were classified as responders or non-responders at Week 25 based on the endoscopic nasal polyposis score and nasal polyposis severity VAS score; patients with missing data at Week 25 were considered non-responders. Fisher's exact test was used for the primary endpoint analysis. Additional post-hoc analyses were performed on change from baseline in endoscopic

polyp scores and blood eosinophil counts. Endoscopic nasal polyp scores and pharmacodynamic data (PnIF, olfaction, FEV_1, FVC, and PEFR) were analysed using ordinal logistic regression; other secondary endpoints were analysed using mixed effects modelling.

Role of the funding source

GSK, in collaboration with the academic clinical investigators, provided input on the design and conduct of the study; oversaw the collection, management and statistical analysis of data; contributed to the interpretation of the data and the preparation, review and submission of the manuscript. All authors had roles in the conception, design, and interpretation of the analysis. All authors participated in the development of the manuscript and had access to the data from the study. The decision to submit for publication was that of the authors alone. The final decision on manuscript submission was made by the authors. The sponsors did not have the right to veto publication.

RESULTS

A total of 107 patients were randomised to receive mepolizumab (n=54) or placebo (n=53) (Figure 1). The first patient was enrolled on 12 May 2009 and the last patient completed on 5 December 2014. Two randomised patients withdrew prior to treatment. One patient randomised to receive mepolizumab received a first dose of placebo in error and was withdrawn; this patient was assigned to the placebo group for safety analyses and mepolizumab group for efficacy analyses (ITT). The ITT population therefore included 105 patients who received at least one dose of mepolizumab or placebo (mepolizumab, 54; placebo, 51). The PP population excluded five patients from the mepolizumab group, as they did not require surgery after the run-in period (n=1), repeated cigarette smoking during the study (n=1), received placebo as their first dose and then withdrew (n=1), or received placebo as their second dose and withdrew (n=2). Patient demographics and characteristics were well balanced between the treatment groups (Table 1). The majority of patients had a history of asthma in both groups; all patients with asthma had mild or moderate disease (Table 1).

A significantly greater proportion of patients in the mepolizumab group no longer met the criteria for requiring surgery compared with placebo at Week 25 (ITT, 16 (30%) vs 5 (10%); P=0.006) (Figure 2A, Supplementary Table 4). This effect was observed from Week 9 and maintained until Week 25.

There was a significant improvement in nasal polyposis severity VAS score in the mepolizumab group compared with placebo, with a treatment difference in favour of mepolizumab of -1.8 at Week 25 (ITT, 95% confidence intervals [CI], -2.9 to -0.8; P=0.001) (Figure 2B, Supplementary Table 4). The probability of having a reduction in endoscopic nasal polyp score was significantly higher in the mepolizumab group

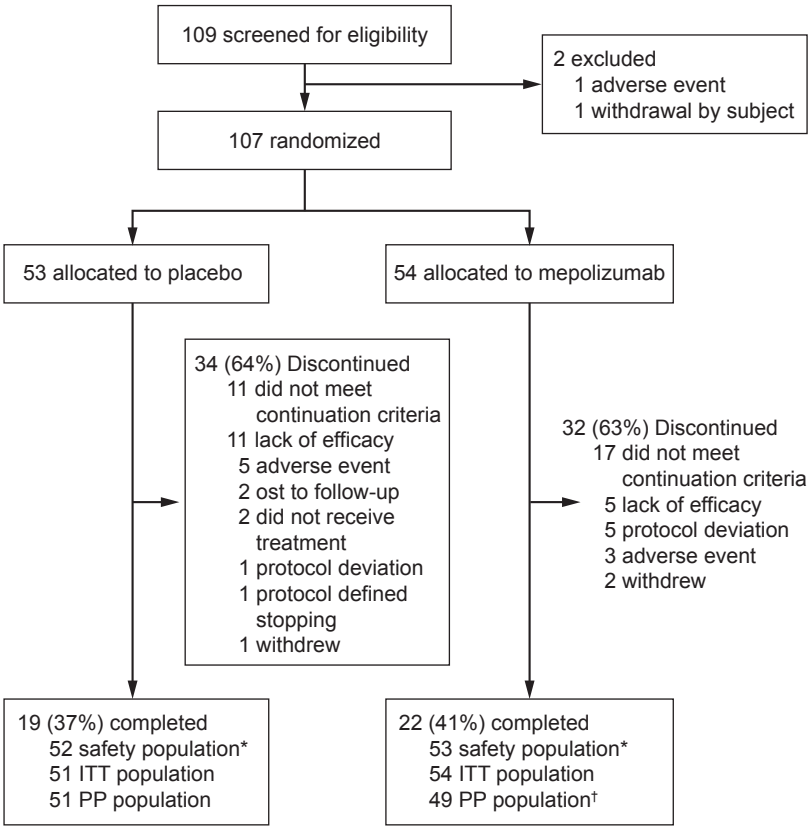

Figure 1. Patient flow through the study. *One patient randomised to the mepolizumab group received placebo in error; this patient is included in the safety population for placebo; †Protocol deviations were incorrect treatment/dose (n=3), did not meet inclusion criteria (n=1), and smoked during the study (n=1); ‡Prior to a protocol amendment that discontinued recruitment to the extension study, patients were given the option to enter the extension study. Patients were eligible to enter the extension if they were judged to no longer have a requirement for surgery according to the criteria defined in Supplementary Table 2. Results from the extension phase are not presented due to low patient numbers. ITT, intent to treat; PP, per protocol.

Table 1. Patient baseline demographics and characteristics (ITT population)

Demographics	Placebo	Mepolizumab 750 mg IV
Age in years, Mean (SD)	50 (10)	51 (11)
Sex, n (%)		
Female	17 (33)	13 (24)
Male	34 (67)	41 (76)
BMI (kg/m^2), Mean (SD)	25.1 (3.0)	26.1 (2.7)

Table 1. (continued)

Demographics	Placebo	Mepolizumab 750 mg IV
Height (cm), Mean (SD)	175 (9)	176 (9)
Weight (kg), Mean (SD)	77.2 (13.1)	81.1 (10.7)
Ethnicity, n (%)		
Hispanic or Latino	0	0
Not Hispanic or Latino	51 (100)	54 (100)
Race, n (%)		
Asian: Central/South Asian heritage	0	2 (4)
Asian: Japanese/East Asian or South East Asian heritage	1 (2)	0
White	50 (98)	52 (96)
Baseline symptom severity VAS symptom score*		
Nasal polyposis, LS Mean (95% CI)	8.55 (7.88–9.23)	8.50 (7.84–9.16)
Rhinorrhoea, LS Mean (95% CI)	6.19 (5.50–6.87)	6.24 (5.57–6.91)
Mucus in throat, LS Mean (95% CI)	6.27 (5.57–6.96)	6.01 (5.34–6.69)
Nasal blockage, LS Mean (95% CI)	8.01 (7.32–8.69)	7.90 (7.23–8.56)
Loss of smell, LS Mean (95% CI)	9.10 (8.45–9.75)	9.06 (8.43–9.69)
Baseline total endoscopic nasal polyp score[†], Mean (SD)	6.31 (0.88)	6.28 (0.88)
Baseline SNOT-22 score[‡], Mean (SD)	49.5 (19.0)	51.5 (17.0)
Baseline EQ-5D		
Index score[‡‡], Mean (SD)	0.84 (0.20)	0.88 (0.15)
VAS score[§], Mean (SD)	67.74 (19.65)	73.30 (17.07)
Baseline PnIF, L/min, Mean (SD)	102 (65)	101 (67)
History of asthma[¶], yes, n (%)	38 (75)	44 (81)
Baseline lung function		
FEV_1, L, Mean (SD)	3.27 (0.97)	3.15 (0.98)
FVC, L, Mean (SD)	4.45 (1.09)	4.46 (1.14)
PEFR, L/min, Mean (SD)	474 (156)	461 (140)

*0–10 cm VAS scale: 0=not troublesome, 10=worst possible; [†]Post-hoc analysis; [‡]SNOT-22 scores range from 0–110, lower scores imply less severe symptoms; [‡‡]Mean score of five questions, a score of 1 indicates full health and lower scores indicate poorer health; [§]0–100 mm VAS scale: 0=worst imaginable health state, 100=best imaginable health state. [¶]All patients with asthma had mild or moderate disease.
BMI, body mass index; CI, confidence interval; FEV_1, forced expiratory volume in 1 second; PEFR, peak expiratory flow rate; FVC, forced vital capacity; ITT, intent to treat; IV, intravenous; LS, least squares; PnIF, peak nasal inspiratory flow; SNOT, Sino-nasal outcome test; VAS, visual analogue scale.

than the placebo group from Week 9 (odds ratio [OR] mepolizumab vs placebo, 5.6; 95% CI, 1.2 to 26.6; P=0.031); and remained higher at Week 25 (OR, 6.6; 95% CI, 1.3 to 34.5; P=0.025). Similarly, a post-hoc analysis of the mean change from baseline in total endoscopic nasal polyp score showed a significant difference between placebo and mepolizumab groups from Week 9 (Figure 2C) to Week 25. In total, 27 (50%) patients receiving mepolizumab and 14 (27%) patients receiving placebo improved by ≥1 point in total endoscopic nasal polyp score (Supplementary Table 4). A post-hoc analysis of patients in the mepolizumab group found there was no association between baseline eosinophil counts and achieving a ≥1 point improvement in endoscopic nasal polyp score at Week 25 (Supplementary Figure 1); however, it is important to note that numbers were small in this analysis (n=28 had a ≥1 point improvement in total endoscopic nasal polyp score vs n=14 with a <1 point improvement).

Mean individual symptom VAS scores (rhinorrhoea, mucus in the throat, nasal blockage, loss of smell), adjusted for baseline, visit, treatment group, and visit x treatment group interaction, were significantly improved in the mepolizumab group compared with placebo at Week 25 (ITT population; Figure 3A–D and Supplementary

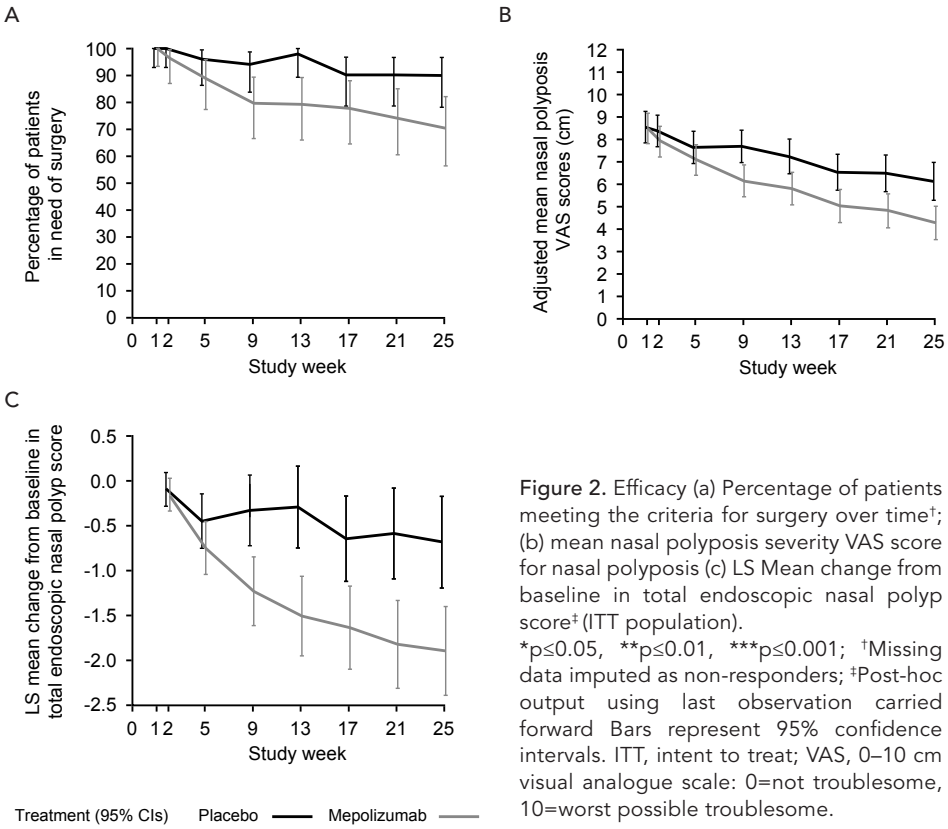

Figure 2. Efficacy (a) Percentage of patients meeting the criteria for surgery over time†; (b) mean nasal polyposis severity VAS score for nasal polyposis (c) LS Mean change from baseline in total endoscopic nasal polyp score‡ (ITT population).
*p≤0.05, **p≤0.01, ***p≤0.001; †Missing data imputed as non-responders; ‡Post-hoc output using last observation carried forward Bars represent 95% confidence intervals. ITT, intent to treat; VAS, 0–10 cm visual analogue scale: 0=not troublesome, 10=worst possible troublesome.

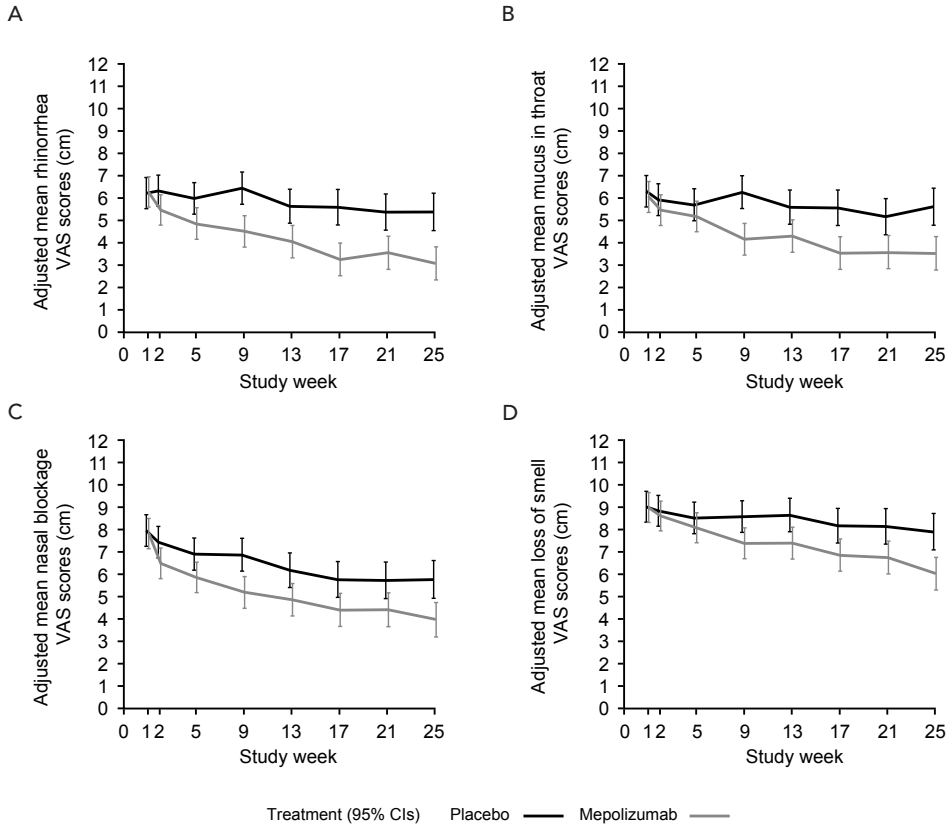

Figure 3. Adjusted† mean individual symptom VAS scores over time for (A) rhinorrhoea, (B) mucus in throat, (c) nasal blockage, and (d) loss of smell (ITT population). Bars represent 95% confidence intervals.
*p≤0.05, **p≤0.01, ***p≤0.001; †adjusted for baseline, visit, treatment group, and visit x treatment group interaction; ITT, intent to treat; VAS, 0–10 cm visual analogue scale: 0=not troublesome, 10=worst possible troublesome.

Table 5). Statistically significant differences were first observed at Week 5 for rhinorrhoea and nasal blockage and Week 9 for mucus and loss of smell; all remained statistically significantly different through Week 25 (ITT population; Supplementary Table 6).

The improvement in mean SNOT-22 questionnaire scores was significantly greater in the mepolizumab group compared with placebo (Supplementary Figure 2). SNOT-22 scores improved between Week 0 and Week 25 in the placebo (Week 1, 49.5 [SD, 19.0]; Week 25, 38.2 [24.5]) and mepolizumab (Week 1, 51.5 [17.0]; Week 25, 28.8 [22.0]) groups. At Week 25, the score for the mepolizumab group was statistically significantly lower than the placebo group (Supplementary Table 5).

There were no differences between the mepolizumab and placebo groups in the EQ-5D index scores at Week 25, which assessed general QoL not specific to nasal

polyposis. Both groups showed increases in mean EQ-5D VAS symptom score at Week 25 compared with Week 0; at Week 25, scores were higher in the mepolizumab group compared with the placebo group (Supplementary Table 5).

At Week 25, least squares (LS) mean PnIF was statistically significantly higher for the mepolizumab group compared with placebo (mean difference 26.7 [95% CI, 3.1 to 50.2]; P=0.027; Supplementary Table 5 and 7). For olfaction, there was a numerical but not statistically significant difference between mepolizumab and placebo groups in favour of mepolizumab (mean difference 0.7 [95% CI, -0.5 to 1.9]; p=0.233) at Week 25. For lung function (FEV_1, FVC, and PEFR), there were no statistically significant differences between mepolizumab and placebo groups at Week 25 (Supplementary Table 5), although there were significant differences at other time points in favour of mepolizumab over placebo in FVC and PEFR (Supplementary Table 7). A post-hoc analysis demonstrated that blood eosinophil counts decreased from a geometric mean (SD log) of 500 cells/μL (0.712) at baseline to 50 cells/μL (1.134) at Week 25 in the mepolizumab group. Decreases were not seen in the placebo group (Supplementary Table 8). PK results were as expected for mepolizumab 750 mg IV (Supplementary Table 9).

The overall incidence of treatment-emergent AEs, AEs considered drug related by the investigator, and AEs leading to treatment discontinuation were similar between the treatment groups (Table 2). Details of AEs leading to treatment discontinuation are presented in Supplementary Table 10. The most frequently reported AEs were headache and nasopharyngitis, both reported by more patients in the placebo group than the mepolizumab group. Of the remaining AEs with >5% incidence in either treatment group, oropharyngeal pain, back pain, influenza, and pyrexia were the only events reported by more patients in the mepolizumab group compared with the placebo group. There were

Table 2. Summary of treatment-emergent adverse events reported in >5% of patients in either treatment group (safety population)

AE type	Number (%) of patients	
	Placebo	Mepolizumab 750 mg IV
All AEs		
On treatment	42 (81)	40 (75)
Drug related[1]	3 (6)	5 (9)
Led to discontinuation of study drug or withdrawal from the study	6 (12)	3 (6)
SAEs		
Nonfatal	0	0
Fatal	0	0

Table 2. (continued)

AE type	Number (%) of patients	
	Placebo	Mepolizumab 750 mg IV
Any on-treatment AE		
Headache	20 (38)	13 (25)
Nasopharyngitis	12 (23)	10 (19)
Oropharyngeal pain	4 (8)	6 (11)
Back pain	0	5 (9)
Influenza	2 (4)	4 (8)
Arthralgia	3 (6)	3 (6)
Pyrexia	1 (2)	3 (6)
Dyspnea	4 (8)	2 (4)
Nausea	4 (8)	2 (4)
Asthma	3 (6)	2 (4)
Cough	3 (6)	2 (4)
Ear pain	5 (10)	1 (2)
Epistaxis	3 (6)	1 (2)
Fatigue	4 (8)	1 (2)
Insomnia	3 (6)	0
Rhinorrhoea	3 (6)	0
Sinus headache	3 (6)	0

AE, adverse event; SAE, serious adverse event; [1]Investigators assessment of causality.

no serious AEs reported during the study. In general, clinical laboratory evaluations and vital signs were similar between groups with no notable trends identified; 11 patients (placebo, 9; mepolizumab, 2) had abnormal clinical chemistry values and 6 patients (placebo, 3; mepolizumab, 3) had vital sign abnormalities at any point during the study, but these were not reported as AEs. There was also a decrease in mean leukocyte counts in the mepolizumab group from 7.16 x 10^9/L pre-dose to 5.90 x 10^9/L at Week 9, which was sustained over the course of treatment.

DISCUSSION

This randomised, double-blind, placebo-controlled study aimed to determine whether mepolizumab treatment could reduce the need for surgery in patients with severe, recurrent bilateral nasal polyps on topical corticosteroid therapy. Based on a composite endpoint of reductions in endoscopic nasal polyposis score and nasal

polyposis severity VAS score, the study demonstrated a statistically significant reduction in the proportion of patients who met the criteria for requiring surgery 4 weeks after the last dose at Week 25 in the mepolizumab group compared with placebo. This was supported by clinically significant improvements in symptoms and QoL–related SNOT-22 scores in the mepolizumab group compared with placebo. Of note, statistically significant improvements in efficacy outcomes were observed 9 weeks after starting treatment with mepolizumab. The combination of these findings suggests that mepolizumab has a beneficial effect on nasal polyposis and may reduce the need for surgery in patients with refractory nasal polyposis.

The primary goal of treatment for nasal polyps is to achieve and maintain clinical control through a reduction in polyp size and growth, thereby improving symptoms and maintaining a healthy or almost healthy nasal mucosa with local treatment only (8). Previous studies have shown that systemic steroids improve nasal-related symptom scores for a short time (8,10). However, their use is limited by the potential long-term side effects of systemic steroids (27). The current study showed that mepolizumab treatment led to significant improvements in symptom scores (rhinorrhoea, mucus in throat, nasal blockage, loss of smell) during treatment. Efficacy results from this study were consistent with results from the smaller study investigating mepolizumab in the treatment of nasal polyposis (15). Improvements in endoscopic nasal polyp scores were also comparable to those shown in an initial study using the anti-IL-4/13 therapy dupilumab (30). Loss of smell was shown to be improved by dupilumab compared with baseline and placebo using the UPSIT (30), and mepolizumab significantly improved olfaction compared with placebo measured by VAS score in the current and the former study (15). As different olfaction tests (Sniffin' Sticks Screening-12 test for mepolizumab vs. University of Pennsylvania Smell Identification Test smell test for dupilumab) (30) were used in the studies, comparisons of objective tests are difficult and a head-to-head study is required. Furthermore, safety results were similar between the placebo and mepolizumab treatment groups, as seen in previous nasal polyposis (15) and asthma studies (16,28).

As an IL-5 specific antibody, mepolizumab selectively and effectively inhibits eosinophilic inflammation. In the majority of patients, nasal polyposis is characterized by local eosinophilic inflammation and high production of IL-5 (29,31). The expression of IL-5 and high levels of markers for T2 disease, such as immunoglobulin E and eosinophil cationic protein, were also associated with an increased likelihood of asthma comorbidity (23). In the mepolizumab group, there was a 10-fold reduction in eosinophil counts at Week 25 which was accompanied by nasal polyp symptom improvements and reduced need for surgery. This reduction is consistent with results from the previous smaller study investigating mepolizumab in the treatment of nasal polyposis, and studies investigating the use of mepolizumab in the treatment of severe eosinophilic asthma (15,16,28). Although there was a reduction in eosinophil counts in the mepolizumab group, no improvements were seen in lung function outcomes. This

is likely due to patients with asthma in this study having mild or moderate disease, rather than severe eosinophilic asthma.

In the current study, all patients were followed up 4 weeks after the final dose. Preliminary results from the discontinued follow-up stage suggested a sustained effect, but low patient numbers prevented conclusions from being made ; similar results from the previous smaller mepolizumab study also suggested a sustained effect until 8 months posttreatment in the responder subgroup (15). Further research with higher numbers of patients is required to fully assess the duration of mepolizumab efficacy posttreatment. As nasal polyposis is not characterized by high production of IL-5 and eosinophilia in all patients (31) and not all patients respond to mepolizumab, additional future research should be designed to detect potential biomarkers that could be used to identify patients with nasal polyposis who are most likely to respond to mepolizumab treatment (24). In this study, with low patient numbers, post-hoc analysis demonstrated that baseline blood eosinophil counts did not impact the responder rate and could not be used to identify responders.

One limitation of this study is the length of treatment. It is possible that efficacy could be increased with a longer treatment period, as clinical improvements were continuing at the end of this 6-month study. For example, two patients were classified as responders for the first time at Week 25. Additionally, the time taken to recruit participants was longer than expected, given that only up to 4% of the population has nasal polyposis (8). This issue will have to be noted and addressed for recruitment of future studies. It should also be noted that in this study a dose of 750 mg mepolizumab IV was administered every 4 weeks as this was the only dose available at the start of the study. As a result of new evidence (16,32), a dose of 100 mg mepolizumab subcutaneously every 4 weeks has been approved for the add-on maintenance treatment of adult patients with severe eosinophilic asthma (33) and will be used in future studies.

In conclusion, this study showed that mepolizumab significantly reduced the number of patients who met the criteria for needing surgery and improved nasal polyp score and symptoms compared with placebo in patients with severe bilateral nasal polyposis treated by topical corticosteroids. Mepolizumab treatment therefore has the potential to improve the QoL of and reduce surgery-associated burden for patients with nasal polyposis.

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DECLARATION OF INTERESTS

CB received fees from GSK during the conduct of the study. VJL has received speaker or consulting fees from Merck, Crucell (Johnson & Johnson), NeilMed, Vitaccess and Vifor. GKS's department received fees for patient recruitment from GSK during the conduct of the study and has also received speaker or consulting fees from ALK Abelló and Meda. SN has received grants for research from AstraZeneca and is a NICE clinical expert for omalizumab and mepolizumab technology appraisals. SRD received a grant and consulting fees from GSK during the conduct of the study, received grants from Merck and ALK and speaker fees or consulting fees from Circassia, Merck, ALK, Boehringer Ingelheim, Medical Update, Anergis, and Allergy Therapeutics. HHK has received speaker fees and conference attendance support from GSK. WJF's department received support from GSK for costs associated with conducting the study. ARS, DA, RPM and ACM are all employees of GSK and own stock in GSK. JG is a contractor employed by Parasol working solely for GSK. PG and MEC have no conflicts of interest to declare.

AUTHORS CONTRIBUTIONS

Conception and design: CB, ARS, VJL, GKS, PG, SN, SRD, MEC, JG, DA, RPM, WJF; Acquisition of data: CB, ARS, VJL, GKS, PG, SN, SRD, MEC, HHK, JG, DA, RPM, WJF; Data analysis and interpretation: CB, ARS, VJL, GKS, PG, SN, SRD, MEC, HHK, JG, DA, ACM, RPM, WJF. All authors were involved in development of all stages of the manuscript, revising it critically for important intellectual content. All authors provided final approval of the version submitted for publication. All authors are accountable for the accuracy and integrity of the work.

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SUPPLEMENTARY APPENDIX

Supplementary methods

Study design and patients

The present study was a randomized, double-blind, placebo-controlled, multicenter study (NCT01362244). A randomization schedule was generated prior to the start of the study using validated internal software. Patients were randomised using the GSK IVRS system, RAMOS. Site staff called the RAMOS system to register the patient on the system and allocated a randomisation number. The randomisation schedule utilised by the RAMOS system was generated by the GSK study statistician prior to the start of the study using validated internal software. A centre-based randomisation schedule was used, with blocking (block size 4). The randomisation was not stratified for any covariate. The patients and treating doctors were blinded to treatment. A site third-party unblinded pharmacist dispensed the investigational product. Blinding was strictly maintained until all data had been collected and cleaned and Database Freeze had been declared, hence site staff (except for the unblinded pharmacist), GSK study staff (except for the independent statistician that analysed the interim data) and bioanalytical staff (placebo subjects were not assayed for PK concentrations) had no access to the random codes until after completion of the study.

For inclusion, patients must have been diagnosed with severe bilateral nasal polyps requiring surgery. Requirement for surgery was assessed using the endoscopic nasal polyposis score ≥3 and symptom score >7 on a visual analog scale (VAS) (Supplementary Table 1). Patients were also required to be eligible for surgery, to have undergone at least one previous nasal polyp removal surgery and considered to be refractory to steroids (still met the criteria for requiring surgery after continuous intranasal steroids for at least 3 months and/or received a short course of oral steroids). Patients with concurrent asthma must be maintained on ≤10 mg/day of oral corticosteroids (prednisolone or the equivalent). Additionally, they must not have had an asthma exacerbation requiring hospital admission within 4 weeks of screening. Treatment with an immunotherapy within the previous 12 months also excluded patients from the study.

Patients had a 10 to 14 day run-in period with low-dose intranasal steroids prior to the first dose. This was required to prevent over treatment with steroids and standardize treatment prior to study entry. Patients were instructed on administration of intranasal steroids and daily administration was documented using a diary card. At the end of the run-in period, patients were assessed for eligibility into the trial using the criteria described in Supplementary Table 1. Daily treatment with low-dose intranasal steroids continued throughout the study.

Endpoints

Primary endpoint

The primary endpoint was a composite endpoint of the number of patients who no longer met the criteria for requiring surgery at the end of the treatment period (Week 25), based on assessment of nasal polyposis using the endoscopic nasal polyposis score and nasal polyposis severity VAS score (Supplementary Table 2).

Secondary endpoints

- The change in individual nasal polyposis VAS symptom scores were determined for the severity of four nasal polyposis symptoms (rhinorrhea, mucus in the throat, nasal blockage, and loss of smell). Subjects were asked to indicate on a VAS (0 to 10 cm) the severity of individual symptoms. Higher scores indicated more troublesome symptoms.
- Each nostril was assessed for polyps and graded to determine endoscopic nasal polyp score.
- Sino-nasal outcome test (SNOT-22) questionnaires were completed. Patients were asked to rate the severity of their condition on each of 22 items using a 0—5 rating system. Higher scores indicated more severe symptoms.
- EuroQual 5-dimensions (EQ-5D) questionnaires were performed. The first part of the questionnaire assessed mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The second part asked the subjects to rate how good or bad their own health was that day on a scale of 0 to 100 (with a score of 100 being the best state of health)
- The following clinical pharmacodynamics assessments were performed: peak nasal inspiratory flow (PnIF) and lung function assessments (forced expiratory volume in 1 second [FEV_1], forced vital capacity [FVC], and peak expiratory flow rate [PEFR]).
- Olfaction tests were performed using the Sniffin' Sticks Screening-12 to assess each subject's sense of smell. Each nostril was assessed separately.
- Pharmacokinetic (PK) samples were obtained at time points outlined in the following assessments section. The predose PK sample could be taken at any point before dosing.
- Systemic exposure-clinical outcome relationship and anti-mepolizumab antibody testing.

Safety endpoints

Safety endpoints were the monitoring of treatment-emergent adverse events (AEs), vital signs, electrocardiogram (ECG) testing, immunogenicity, and clinical laboratory testing. The investigator or site staff were responsible for detecting, documenting, and reporting events that met the definition of an AE or SAE. SAEs were defined as any untoward medical occurrence that resulted in death, was life-threatening, required hospitalization, resulted in disability/incapacity, or was a congenital anomaly/birth defect.

Assessments

Assessments of endoscopic nasal polyposis scores, nasal polyposis severity VAS scores, individual VAS symptom scores, lung function, olfaction, nasal secretions, PnIF, pharmacodynamic blood samples, clinical tests, vital signs, ECGs, and AEs were performed on Weeks 1, 2, 5, 9, 13, 17, 21, and 25. SNOT-22 and EQ-5D questionnaires were performed on Weeks 1 and 25.

Safety monitoring occurred during the intravenous infusion and for 1 hour at the end of the infusion. SAE/AEs, vital signs, and ECG were assessed at each treatment visit (Weeks 1, 2, 5, 9, 13, 17, 21 and 25). Blood was taken for clinical laboratory tests and pharmacodynamics assessments at each treatment visit (Weeks 1, 2, 5, 9, 13, 17, 21, and 25). Immunogenicity was assessed at Weeks 1, 5, 13, and 25. PK was assessed at Weeks 1, 2, 5, 9, 13, and 25.

Analysis of individual symptom VAS scores

Individual mean symptom scores were adjusted for baseline, visit, treatment group, and visit x treatment group interaction. The adjusted means are calculated by correcting the raw values to the overall mean baseline score of the two treatment groups using a statistical model. For example, if the baseline score for rhinorrhoea was higher in the mepolizumab group than the placebo group, the Week 52 values would be adjusted according to the baseline mean of both groups. If the baseline scores were identical in both the mepolizumab and placebo groups, then the raw and adjusted means would be identical.

Supplementary figures

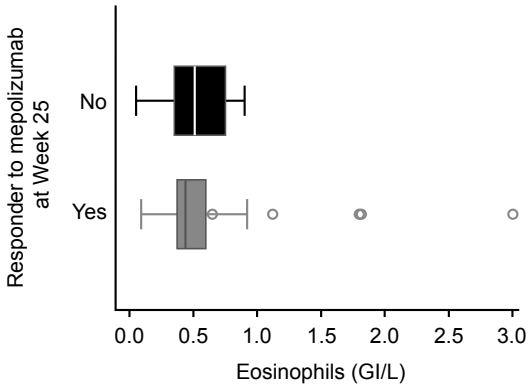

Figure E1. Patients in the mepolizumab group achieving a ≥1 point improvement in total endoscopic nasal polyp score at Week 25 according to baseline eosinophil count (ITT population)*.
*Post-hoc analysis.

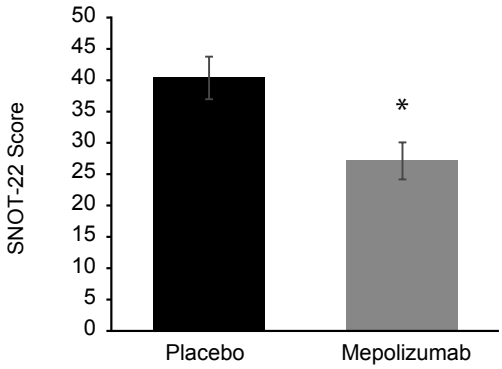

Figure E2. Adjusted means for SNOT-22 results at Week 25 (ITT population).
*P=0.005. Missing data not imputed; bars represent standard error. ITT, intent to treat; SNOT, Sino-nasal outcome test.

Supplementary tables

Table E1. Assessment of need for surgery (at screening/baseline)*

Nasal polyposis severity VAS score	Is the subject eligible for entry into the treatment period?			
	Endoscopic nasal polyp score			
	0	1	2	≥3
≤3	No	No	No	No
>3 to ≥7	No	No	No	No
>7	No	No	No	Yes

*Assessment parameters based on consensus of study investigators.
VAS, visual analog scale.

Table E2. Assessment of continuing need for surgery (primary endpoint)*

Nasal polyposis severity VAS score	Is the subject no longer in need of surgery?			
	Endoscopic nasal polyp score			
	0	1	2	≥3
≤3	Yes	Yes	Yes	No
>3 to ≥7	Yes	Yes	Yes	No
>7	Yes	Yes	No	No

*Assessment parameters based on consensus of study investigators.
VAS, visual analog scale.

Table E3. Endoscopic nasal scoring criteria

Polyp score	Polyp size
0	No polyps
1	Small polyps in the middle meatus not reaching below the inferior border of the middle concha
2	Polyps reaching below the lower border of the middle turbinate
3	Large polyps reaching the lower border of the inferior turbinate or polyps medial to the middle concha
4	Large polyps causing almost complete congestion/obstruction of the inferior meatus

Table E4. Efficacy results for composite primary endpoint and its individual components at Week 25 (ITT population)

Week 25	Placebo	Mepolizumab
Patients who no longer met the criteria for requiring polyposis surgery, n (%)*	**n=51** 5 (10)	**n=54** 16 (30)
Patients who still met the criteria for requiring polyposis surgery, n (%)	**n=51** 46 (90)	**n=54** 38 (70)
Worst affected endoscopic nasal polyp score, n (%)†	**n=31**	**n=42**
0	1 (3)	3 (7)
1	2 (6)	7 (17)
2	4 (13)	7 (17)
≥3	24 (77)	25 (60)
Improvement by ≥1 point in total endoscopic polyp score, n(%)†	**n=51** 14 (27)	**n=54** 27 (50)
Nasal polyposis severity VAS score, mean (95% CI)‡	**n=31** 6.1 (5.3–7.0)	**n=42** 4.3 (3.6–5.0)

*Missing values were classified as nonresponders; †Post-hoc analysis; ‡0–10 cm VAS scale response to "How troublesome are your symptoms of nasal polyposis?"; 0=not troublesome, 10=worst possible; Results based on a consensus between surgeons.
CI, confidence interval; ITT, intent to treat; VAS, visual analog scale

Table E5. Efficacy results for selected secondary endpoints at Week 25 (ITT population)

Week 25	Placebo, LS mean (95% CI)	Mepolizumab, LS mean (95% CI)	Treatment difference vs placebo (95% CI)	P-value
Symptom severity VAS score*	n=31	n=42		
Rhinorrhea	5.4 (4.5, 6.2)	3.0 (2.3, 3.8)	-2.3 (-3.4, -1.2)	<0.001
Mucus in throat	5.6 (4.8, 6.4)	3.5 (2.8, 4.2)	-2.1 (-3.2, -1.0)	<0.001
Nasal blockage	5.8 (5.0, 6.6)	4.0 (3.3, 4.8)	-1.8 (-2.9, -0.7)	0.002
Loss of smell	8.0 (7.2, 8.7)	6.1 (5.4, 6.8)	-1.9 (-2.9, -0.9)	<0.001
SNOT-22 score**	n=32	n=42		
	40.4 (33.6, 47.1)	27.1 (21.2, 33.0)	-13.2 (-22.2, -4.2)	0.005
EQ-5D Index score†	n=32	n=41		
	0.91 (0.86, 0.95)	0.91 (0.87, 0.95)	0.00 (-0.06, 0.07)	0.891
EQ-5D VAS score††	n=32	n=42		
	75.5 (70.2, 80.7)	81.1 (76.5, 85.7)	5.7 (-1.3, 12.7)	0.111
PnIF	n=32	n=42		
	110.4 (92.9, 127.8)	137.0 (121.2, 152.9)	26.7 (3.1, 50.2)	0.027

Table E5. (continued)

Week 25	Placebo, LS mean (95% CI)	Mepolizumab, LS mean (95% CI)	Treatment difference vs placebo (95% CI)	P-value
Olfaction testing, mean of two nostrils‡	n=32 3.7 (2.8, 4.6)	n=41 4.4 (3.6, 5.2)	0.7 (-0.5, 1.9)	0.233
Lung function	n=32	n=42		
FEV_1, L	3.18 (3.05, 3.32)	3.35 (3.23, 3.47)	0.16 (-0.02, 0.34)	0.077
FVC, L	4.41 (4.25, 4.57)	4.59 (4.45, 4.74)	0.18 (-0.03, 0.40)	0.094
PEFR, L/min	467 (437, 497)	481 (454, 508)	14.1 (-25.8, 54.0)	0.484

*0–10 cm VAS scale: 0=not troublesome, 10=worst possible troublesome; **SNOT-22 scores range from 0–110, lower scores imply less severe symptoms; †Weighted mean score of five questions, a score of 1 indicates full health and lower scores indicate poorer health; ††0–100 mm VAS scale: 0=worst imaginable health state, 100=best imaginable health state; ‡Scores can range from 0–12, with lower scores indicating a worse sense of smell.
CI, confidence interval; EQ-5D, EuroQual 5-dimensions; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; ITT, intent to treat; LS, least squares; PEFR, peak expiratory flow rate; PnIF, peak nasal inspiratory flow; SNOT, Sino-nasal outcome test; VAS visual analog scale

Table E6. Individual symptom VAS scores over the course of 25 weeks (ITT population)

	Week 1	Week 2	Week 5	Week 9	Week 13	Week 17	Week 21	Week 25
Rhinorrhea								
Treatment difference (95% CI)	0.05 (-0.90, 1.01)	-0.86 (-1.82, 0.10)	-1.15 (-2.11, -0.18)	-1.94 (-2.93, -0.95)	-1.59 (-2.62, -0.55)	-2.35 (-3.40, -1.29)	-1.83 (-2.91, -0.75)	-2.31 (-3.41, -1.21)
P-value	0.912	0.078	0.020	<0.001	0.003	<0.001	0.001	<0.001
Mucus in throat								
Treatment difference (95% CI)	-0.25 (-1.22, 0.72)	-0.47 (-1.44, 0.51)	-0.52 (-1.50, 0.45)	-2.11 (-3.11, -1.11)	-1.29 (-2.33, -0.25)	-2.04 (-3.10, -0.98)	-1.58 (-2.66, -0.50)	-2.09 (-3.19, -1.00)
P-value	0.608	0.347	0.293	<0.001	0.015	<0.001	0.004	<0.001
Nasal blockage								
Treatment difference (95% CI)	-0.11 (-1.07, 0.85)	-0.93 (-1.89, 0.03)	-1.03 (-2.00, -0.06)	-1.67 (-2.66, -0.68)	-1.31 (-2.35, -0.28)	-1.36 (-2.42, -0.29)	-1.31 (-2.39, -0.23)	-1.77 (-2.87, -0.67)
P-value	0.823	0.057	0.037	0.001	0.013	0.012	0.018	0.002
Loss of smell								
Treatment difference (95% CI)	-0.04 (-0.94, 0.87)	-0.23 (-1.13, 0.68)	-0.45 (-1.36, 0.46)	-1.19 (-2.13, -0.26)	-1.26 (-2.23, -0.29)	-1.32 (-2.31, -0.33)	-1.40 (-2.41, -0.39)	-1.88 (-2.91, -0.85)
P-value	0.935	0.625	0.336	0.012	0.011	0.009	0.007	<0.001

0–10 cm VAS scale: 0=not troublesome, 10=worst possible troublesome. Treatment difference = Mepolizumab score – Placebo score.
CI, confidence interval; ITT, intent to treat; VAS, visual analog scale.

Table E7. Mepolizumab vs placebo treatment differences in PD over the course of 25 weeks (ITT population)

	Week 2	Week 5	Week 9	Week 13	Week 17	Week 21	Week 25
PnIF (L/min)							
Treatment difference Mepo vs placebo	21.16	16.89	7.20	27.71	15.40	29.51	26.65
Standard error	7.40	7.62	9.97	10.30	9.64	11.77	11.83
P-value	0.005	0.029	0.472	0.009	0.114	0.014	0.027
Olfaction score							
Treatment difference Mepo vs placebo	0.09	1.14	0.79	0.73	0.38	0.65	0.71
Standard error	0.35	0.42	0.43	0.48	0.54	0.63	0.59
P-value	0.790	0.008	0.066	0.127	0.481	0.308	0.233
FEV_1 (L)							
Treatment difference Mepo vs placebo	0.04	0.05	0.09	0.17	0.21	0.23	0.16
Standard error	0.07	0.08	0.10	0.09	0.11	0.10	0.09
P-value	0.567	0.495	0.365	0.058	0.056	0.028	0.077
FVC (L)							
Treatment difference Mepo vs placebo	0.06	0.07	0.06	0.2	0.22	0.28	0.18
Standard error	0.08	0.08	0.10	0.10	0.12	0.11	0.11
P-value	0.486	0.384	0.546	0.050	0.061	0.016	0.094
PEFR (L/min)							
Treatment difference Mepo vs placebo	5.11	14.55	8.91	23.24	38.16	38.72	14.13
Standard error	12.61	14.59	17.99	17.11	19.39	18.79	20.10
P-value	0.686	0.321	0.622	0.178	0.052	0.042	0.484

FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; ITT, intent to treat; L, liter; mepo, mepolizumab; min, minute; PD, pharmacodynamics; PEFR, peak expiratory flow rate; PnIF, peak nasal inspiratory flow.

Table E8. Blood eosinophil counts over the course of 25 weeks (ITT population, post-hoc analysis)

	Week 1	Week 2	Week 5	Week 9	Week 13	Week 17	Week 21	Week 25
Mepolizumab, n	53	54	54	53	45	45	42	54
Geometric mean, cells/μL (SD log)	500 (0.71)	80 (0.76)	40 (0.83)	40 (0.84)	30 (0.90)	30 (0.99)	30 (0.72)	50 (1.13)
Median ratio to baseline (min, max)	-	0.16 (0.0, 1.2)	0.08 (0.0, 1.2)	0.07 (0.0, 0.8)	0.07 (0.0, 3.1)	0.07 (0.0, 0.8)	0.09 (0.0, 1.0)	0.10 (0.0, 1.3)
Placebo, n	51	51	49	48	41	35	34	45
Geometric mean, cells/μL (SD log)	470 (0.57)	450 (0.62)	450 (0.53)	450 (0.63)	450 (0.73)	380 (0.58)	390 (0.65)	380 (0.52)
Median ratio to baseline (min, max)	-	1.00 (0.5, 1.5)	1.00 (0.6, 2.1)	0.98 (0.5, 2.4)	0.96 (0.1, 3.0)	1.00 (0.4, 1.8)	0.94 (0.4, 2.4)	0.93 (0.4, 1.8)

ITT, intent to treat; min, minimum; max, maximum. Geometric mean n values presented.

Table E9. Final Population PK Model Derived Summary Parameters (ITT population)

Label	Estimate (SE)
Clearance (L/day)	0.22 (0.01)
Volume of Distribution at Steady-State (L)	7.10 (0.32)
Cmax (µg/mL)	193.22 (7.85)
Cmax (µg/mL) SS	268.40 (10.37)
AUC(0-inf) (µg.day/mL)	3456.69 (152.11)
Cav(0-inf) (µg/mL)	123.45 (5.43)
Half-life (Alpha) (days)	1.55 (0.41)
Half-life (Beta) (days)	24.13 (1.03)

AUC, area under curve; L, liter; mL, milliliter; SE, standard error; SS, steady state; µg, microgram.

Table E10. Adverse events leading to treatment discontinuation

Age/gender	AE (preferred term)	Onset (study day)	Maximum intensity	Duration (days)	Outcome	Relation to study drug
Placebo						
46/M	Pneumonia/pneumonie	148	Moderate	58	Recovered/ resolved	No
31/F	Eosinophilic pneumonia/eosinophilic pneumonia only left side	74	Moderate	201	Recovered/ resolved	No
32/M	Cellulitis orbital/orbital (right) infection with cellulitis	56	Mild	27	Recovered/ resolved	No
62/M	Lower respiratory tract infection/chest infection	160	Moderate	242	Recovered/ resolved	No
43/M	Syncope patient fainted	28	Mild	0	Recovered/ resolved	No
54/F	Anosmia/loss of sense of smell	1	Severe	89	Recovered/ resolved	No
Mepolizumab						
53/F	Toxic skin eruption/rash, toxic eruption	31	Moderate	22	Recovered/ resolved	Yes
63/M	Rash/rash	95	Severe	115	Recovered/ resolved	Yes
NA/M	Hematuria/hematuria	NA	Mild	NA	Recovered/ resolved	No
70/F	Hematuria/hematuria	55	Moderate	86	Recovered/ resolved	No
Not assigned						
NA/M	Hematuria/hematuria	NA	NA	NA	Recovered/ resolved	No

F, female; M, male; NA, not available.

chapter 3

THE MICRODEBRIDER, A STEP FORWARD OR AN EXPENSIVE GADGET?

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ABSTRACT

Background

Although the use of the microdebrider (shaver) is well known in endoscopic sinus surgery (FESS), there is lack of evidence from comparative studies focussing on the difference in operating time, intra-operative blood loss and user-friendliness between the microdebrider and traditional operating techniques. In this study we compared the use of the microdebrider to conventional instruments in FESS in these areas.

Methods

A prospective randomised double blind controlled trial in 60 patients with chronic rhinosinusitis with nasal polyposis (CRSwNP) undergoing bilateral FESS. Each subject received FESS using only traditional instruments (Blakseley forceps) on one side and the other side with the additional use of the microdebrider, this way serving as their own control. The primary outcome was operation time, intra-operative blood loss and user friendliness and secondly safety and postoperative healing with a follow-up period at different time points up to three months postoperative.

Results

We found a 37% longer operating time when operating without a microdebrider. This difference was highly significant. The microdebrider scored significantly higher on every different parameter of user friendliness, except on the preparation of the instrument needed before surgery. For estimated blood loss during surgery we found no differences. Also there was no significant difference in postoperative healing at any point of time.

Conclusions

This study demonstrates that operating patients with CRSwNP with the microdebrider is efficient and that the microdebrider at the same time is safe and easy to use.

INTRODUCTION

Chronic rhinosinusitis (CRSsNP) and chronic rhinosinusitis with nasal polyposis (CRSwNP) are one of the most common health problems these days. The GALEN European health survey reported a prevalence of CRS in Europe around 11%. (1) Besides that CRS also has a major impact on the quality of patient's lives. (2) Patients who have CRSwNP frequently need endoscopic surgery, when conservative treatment with topical and oral steroids fails. At the moment approximately 700 sinus surgeries per year per 1 million persons are performed. (3)

Although polypectomy has already been described since Hippocrates around 400 BC, nowadays Functional Endoscopic Sinus Surgery (FESS) is the technique of choice in sinus surgery. The goal of endoscopic surgery is to re-establish normal ventilation and mucus drainage from the sinuses and to resect irreversibly changed mucosa. FESS is considered a safe procedure and meta-analysis of data on complication rates suggest that major complications occur in about 1% and minor complications in about 5-6% of the cases.(4) Several studies have demonstrated the efficacy of FESS for CRSwNP. (5;6) However like in all fields of surgery surgical techniques have been further refined and with the ongoing evolution in the field of endoscopic mechanisms, new devices are introduced frequently.

Originally in FESS only the non-cutting Blakesley forceps was used based on the principle of grabbing the polyp and tearing it off. But its use was thought to result in a lot of mucosal trauma and surgical bed scarring. Therefore one of the first innovations was the cutting forceps, which has been claimed to result in less trauma of the mucosa and therefore better wound healing. In 1992 the thought of further minimizing mucosal trauma led to the introduction of the microdebrider (shaver) by Setliff. (7) The microdebrider is a powered rotary shaving device, which originally was used in arthroscopic surgery. It consists of a small rotating blade protected by a blunt end, which can resect tissue that is suctioned into the opening. Because the microdebrider resects tissue very precisely, it minimizes mucosal trauma, which is considered to result in faster healing, compared to traditional instruments. Without the need for removal, the microdebrider supplies continuous suction, enabling the surgeon to maintain a bloodless field while operating. This may improve safety, because the visibility is very important to identify the anatomy is FESS and as well could safe precious operating time. On the other hand with the use of powered instrumentation there are reports published showing a higher incidence of serious complications, like cerebrospinal fluid leak and orbital injury. (8;9)

Furthermore, the use of the microdebrider could bring along higher costs, because blades are disposable so they stay very sharp and sometimes more than one blade is needed in one operation.(10)

Although the use of the microdebrider is well known, conflicting results are found in literature whether the microdebrider technique is superior, equal or inferior to

the traditional techniques. While there are many papers on this subject, there are very few comparative studies and most of them are case reports or retrospective studies instead of randomized controlled trials.(11) Besides that most studies include very mixed groups of patients.

The first study that compared traditional techniques with powered instrumentation was conducted in 1996. This was a retrospective, non-blinded study of 250 patients, which reported faster healing with less crusting, and to have less bleeding, synechia formation, lateralization of the middle turbinate, and ostial reoclusion than the traditional group.

There have been only two prospective randomized controlled trials focusing on the healing time after the use of the microdebrider in patients undergoing bilateral FESS.(12;13)

These two studies found no difference in synechia formation, patency of middle meatal antrostomy and open access tot the ethmoid, and therefore no major advantages compared to standard instruments.(12;13) In the study of Sauer et al. the authors even concluded that operating time needed for the microdebrider was significantly longer than with traditional instruments.(13) In all studies until now the difference in operating time and therefore cost effectiveness, intra-operative blood loss and post-operative pain, have only been scarcely investigated.

Therefore we designed this prospective randomized double-blind (concerning the postoperative outcomes) controlled trial to compare the use of the microdebrider to the use of conventional instruments only in FESS. The present paper will first focus on the per-operative parameters and the efficacy of the different operating techniques, evaluating operating time, estimated intra-operative blood loss and user friendliness of the different instruments, and second at the safety and difference in post-operative healing.

MATERIALS AND METHODS

Patients

In this mono-centre prospective double-blind randomized controlled trial conducted in the Academic Medical Centre in Amsterdam, the Netherlands, we included 60 patients (120 sides) with bilateral CRSwNP (35 man, 25 women). All participants were over 18 years and the age distribution was between 18 and 72 years old. All patients are diagnosed with strictly symmetrical disease of the paranasal sinuses and had to give written informed consent. Because this study was conducted in a university referral centre, almost all patients were operated one or several times on the sinuses before. This protocol was approved by the local ethics committee. The inclusion and exclusion criteria are shown in Table 1.

Table 1. In- and exclusion criteria

Inclusion criteria	Exclusion criteria
Male or female aged ≥18 years	Patients with any serious or unstable disease
Patients diagnosed with CRSwNP with indication for FESS	Any structural nasal abnormalities (other then polyps), e.g. severe nasal septum deviation
Written informed consent	Rhinosurgery during past 6 weeks
Symmetrical disease conformed by CT scanning	Females who are pregnant
Operation done by ENT-surgeon or experienced resident, as judged by ENT-surgeon supervising resident	Inability to follow instructions within protocol or to attend clinical visits

Randomisation

The subjects who fulfilled the randomisation criteria were randomized to receive FESS using only traditional instruments (straight and curved cutting and blunt Blakesley forceps) on one side and the other side with additional use of the microdebrider, this way serving as their own control. The side on which the microdebrider was used was randomized using an internet-based randomisation program with a 1:1 ratio.

Study design

We performed preoperative assessment including clinical and ENT history, a full ENT examination, CT scans of the sinuses, nasal assessment and evaluation of symptoms. The nasal assessment was done directly preoperative by rigid endoscopy by the surgeon himself before randomisation was revealed. Then the surgery was performed using traditional instruments on both sides and on top of that it was allowed to use the microdebrider on the side indicated by randomisation. Postoperatively all patients were treated identical including the use of a topical steroid spray and nasal washing with saline. A few patients required extra treatment with prednisone or antibiotics. We registered the use of medication during the whole period of follow up.

Follow-up visits where performed at 1, 2, 4 and 6 weeks, and 3 months postoperatively. During these visits nasal assessment and evaluation of symptoms was done as described later. Furthermore the adverse events were evaluated during the whole study period. (14)

Endoscopic evaluation

The endoscopic examination included a polyp score for each side separately and scoring of nasal discharge, evidence of oedema, scarring, crust formation for each side in a standardised way per location (nasal cavity, ethmoïd region, region of the infundibulum) according to the Lund-Kennedy scoring system (Table 2). (15)

If preoperative the nose was so obstructed by polyps that the different regions could not be properly scored, we scored 2 points (= maximum score) for oedema and the rest -2, meaning this score could not be evaluated. We calculated the mean value of every endoscopic finding separately and compared the means from the microdebrider side with the traditional side.

Besides that the total endoscopic score for each instrument at different time points was calculated. We added up all the different mean scores (oedema, nasal discharge, synechiae and crusting) at 4 different time points and compared again the microdebrider with the traditional side.

Evaluation of symptoms

Evaluation of subjective symptoms was done by validated questionnaires, using pain scores and lateralised symptom scores by means of visual analogue symptom scales (VAS) and a modified CSS form to determine symptoms on both sides (Table 3). (15)

The symptom score using VAS included symptoms of nasal blockage, nasal discharge, sense of smell, headache and purulent discharge for each side with a maximum score of 100. Furthermore we calculated a total symptom score by adding up all the separate scores for each side with a maximum score of 500. The modified CSS score for lateralised symptoms included headache, nasal blockage and secretions with a maximum score of 4 for each side separately. The total CSS score was calculated by adding up all the different score with a maximum of 12 points.

Surgical procedure

The surgery was performed under general anaesthesia and with traditional instruments on both sides and on top of that it was allowed to use the microdebrider on the side indicated by randomisation. At the side randomised to use traditional instruments only these instruments were used. At the side randomised to use the microdebrider on top of the traditional instruments, the microdebrider was used for the major

Table 2. Healing score as evaluated by nasal endoscope

Characteristics		Left	Right
Nasal Discharge	(0=none, 1=clear, 2=thick, purulent discharge)	0-2	0-2
Oedema	(0= absent, 1=mild, 2= severe)	0-2	0-2
Scarring (synechiae)	(0=absent, 1=mild, 2=severe)	0-2	0-2
Crusting	(0=absent, 1=mild, 2=severe)	0-2	0-2
Total endoscopic score:		0-8	0-8
Polyps (residual) scored for each side of the nose	0=absent, 1=within middle meatus, 2=beyond middle meatus, 3=completely obstructing nose	0-3	0-3

Table 3. Symptom score (VAS) and Modified CSS score

VAS score	Left	Right
Nasal blockage	0-100	0-100
Nasal discharge	0-100	0-100
Sense of smell	0-100	0-100
Headache/pain	0-100	0-100
Purulent discharge	0-100	0-100
Total symptom score:	0-500	0-500
CSS score	**Left**	**Right**
Headache/pain	0-4	0-4
Nasal blockage	0-4	0-4
Secretions	0-4	0-4
Total CSS score:	0-12	0-12

part of the operation. It was used to remove the polyps, diseased mucosa and part of the bony partitions between the ethmoidal cells. When necessary conventional instruments were used, e.g. to cut thicker bone fragments. All the surgeries were performed by an ENT specialist (5 different in total) together with a resident (5 different in total). The resident always operated the same amount of time on both sides and the operation was always finished by the ENT specialist himself. The surgeon always started operating on the left side. During the surgery 0° and 30° microdebrider blades from Medtronic were used, depending on the anatomic situation. On both sides we performed standard surgical procedures including uncinectomy, anterior and posterior ethmoidectomy, draf IIa or sphenoidectomy if necessary. If there was any reason for the surgeon during the operation to deviate from the protocol it was registered in the file. Operating time (in minutes) on the first randomised side was measured when the surgeon started operating and ended when the nasal packaging has achieved haemostatic acceptability on that side.

Blood loss

Intra-operative blood-loss (in ml) was measured on the left (starting side) at the end of the surgery by measuring the fluid in the suction bags. A small merocel packing was put in the left ethmoid tp prevent spill over of blood loss when surgery was performed on the right side. All rinsing fluids was accounted for and deducted from the from the total amount of fluids removed by suction during operation. Subsequently the other side was operated with the same measurements at the end.

Questionnaire for the surgeon

Furthermore to determine the user-friendliness of the different instruments for the operating surgeon, a questionnaire was completed by the surgeon directly after the operation. This questionnaire contained the following parameters: general use, preparation, reach thoroughness, versatility and handiness. Most of the time the ENT specialist filled in this form, but if the resident operated the major part of the surgery, he did.

Statistical analysis

The estimated number of patients needed to show a relevant and significant difference for surgical time comparing the microdebrider side to the conventional side was 60 based on a reduction of 25% in surgical time. In the analysis, we first tested whether our data were normally distributed. If that was the case, we used the Student's *t*-test to evaluate statistically significant differences. Significance level was set at $p < 0.05$. If the data were not normally distributed we used the Wilcoxon Signed Rank test.

Over the difference in symptoms score over time after surgery we performed a repeated measurements analysis ANOVA for all different variables.

RESULTS

Patients

Patients were male in 58% (n= 35) and female in 42% (n= 25). The mean age of all patients was 48 years (range 18 to 72 years). Almost all patients had previous sinus surgery (85%). Some even had several operations, with a mean of 2.1 operations per patient.

Operative data

We found a 37% longer operating time at the side with only traditional instruments (41 [inter quartile range (IQ) range 28-49] minutes; $p<0.001$) than on the microdebrider side (30 [IQ range 22-39] minutes) (Table 4). This difference was highly significant.

For the estimated blood loss during surgery, no significant difference was found. It showed a total blood loss (median) in the microdebrider group of 100 [IQ range 43-244] and in the traditional group of 100 too [IQ range 50-180] (p=0.94) (Table 4).

Table 4. Operating time and Blood loss

	Microdebrider median [IQ range]	Traditional median [IQ range]	Z	Asymp. Sig. (2-tailed)
Operating time (min)	30 [22;39]	41 [28;49]	-5,285	<0.001
Blood loss (ml)	100 [43;244]	100 [50;180]	-0,76	0.94

User friendliness

The user-friendliness of the two different instruments was analyzed for all the separate questions (Table 5).

These results show that there is a significant difference between all different parameters scored in favour of the microdebrider, except the preparation, which scores significantly higher for the traditional instruments. We observed no differences between the scores of the 5 different residents and 5 different specialists who filled out the forms.

Protocol deviation

In 11 cases (19%) the surgeon felt the need to deviate from the protocol by using the microdebrider for a short period on the traditional side. These were all cases where the surgeon felt that he/she was safer using the microdebrider, mostly in cases where polyps were growing in the frontal recess.

Subjective symptoms

The average total symptom score (VAS) showed an improvement after surgery on the microdebrider side as well as the side with only traditional instrument from a mean score of 260 before surgery in both groups to a final score of 103,9 on the microdebrider side and 103,6 on the traditional side at 3 months. We found no significant differences in total symptom scores after surgery at any point in time (Fig. 1). Furthermore repeated measurements analyses showed no significant differences between microdebrider and traditional side over time.

While analyzing the individual lateralised symptoms scores of nasal blockage, nasal discharge, sense of smell, pain/headache and purulent discharge, we found significantly more purulent discharge at 4 weeks after surgery from the nose on the traditional side (17,4) than on the microdebrider side (14,4) (p=0,02). Furthermore there was a insignificant tendency at 2 weeks postoperative towards more pain on

Table 5. User-friendliness of the different instruments

	Microdebrider Median [inter quartile range]	Traditional Median [inter quartile range]	Z	Asymp. Sig. (2-tailed)
General Use	8 [8;9]	7 [6;8]	-4,650	0,00
Preparation	7 [7;8]	9 [8;9]	-3,000	0,003
Reach	8 [7;9]	7 [6.25;8]	-2,631	0,009
Thoroughness	9 [8;10]	8 [7;9]	-2,669	0,008
Versatility	9 [8;9]	8 [7;8]	-2,953	0,003
Handiness	9 [8;9]	8 [7;8]	-5,428	0,000

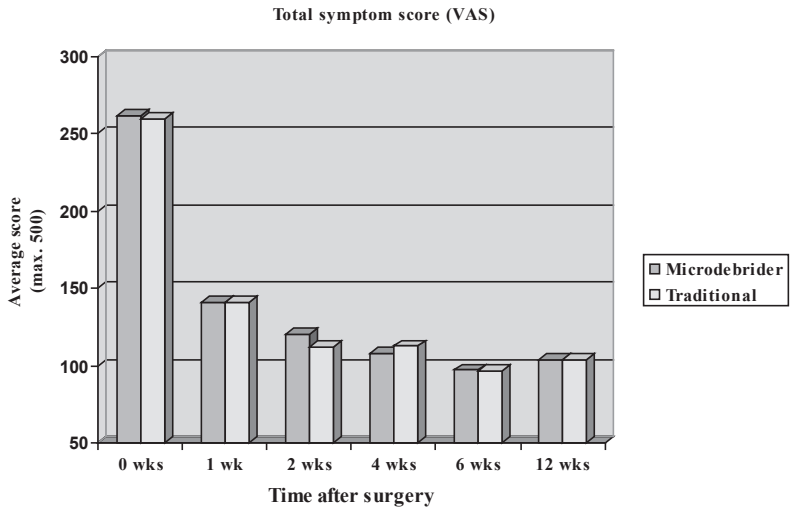

Figure 1. Total symptom score (VAS).

the microdebrider side (16,4 compared with 13,6) (p=0,09). Otherwise no statistical significant differences were found.

We observed an improvement in CSS scores over time, with an mean score difference of 4,9 (preoperatively 7,4 to 2,5 three months postoperatively) points on the microdebrider side and 5,2 (preoperatively 7,2 to 2,0 three months postoperatively) on the side of the nose operated with only traditional instruments. We found no significant differences between total CSS scores of the different groups after surgery at any of the points in time and also in the repeated measurements over time were no significant differences measured (Fig. 2).

Besides that we analyzed all the separate subjective, lateralised CSS scores (headache/pain, nasal blockage and nasal secretions) between the microdebrider - and traditional side and here we also didn't find any statistic significant differences.

Nasal assessment

We see an improvement of the total endoscopic score over time, both in the microdebrider group and in the traditional group. There is a total endoscopic score at 2 weeks after surgery of 2,4 on the microdebrider side and 2,2 on the traditional side and this score goes down to a score of 1,4 in the microdebrider group and 1,6 in the traditional group at 12 weeks after surgery. We found no significant differences in total endoscopic scores between the microdebrider and traditional instruments at any point in time after surgery and also no differences while analyzing repeated measurements over time.

Furthermore we analyzed all the detailed endoscopic scores (nasal discharge, oedema, scarring and crusting) for each different side at all time points after surgery. Here we didn't find any significant differences.

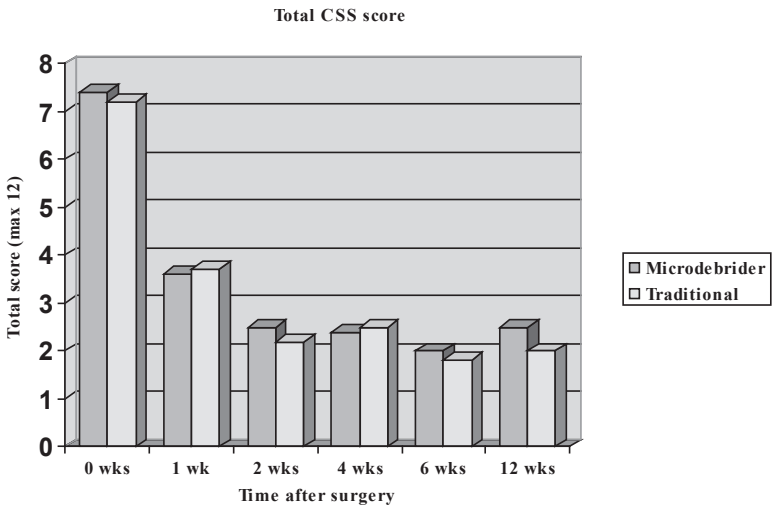

Figure 2. Total CSS score at different points in time after surgery.

Recurrence of polyps / cobblestones

Endoscopic examination showed no difference in final recurrence rate after 3 months between the microdebrider and the traditional side. We found a rate of 30% in both groups, however most of these recurred polyps were classified as very small or even cobblestoned mucosa.

In between time at 4 weeks after surgery we did find a significant (p=0,04) higher recurrence rate of polyps and cobblestones on the traditional operation side (27%) compared to the microdebrider side (10%).

Adverse Events

In 56 patients a total of 8 minor adverse events occurred. Most of these were small postoperative bleedings only needing an extra Merocell tampon. We found no difference in level of adverse events between the microdebrider and traditional instruments. In one patient the anterior ethmoidal artery was damaged, resulting in a preseptal hematoma which resolved by itself without any residual damage. This happened on the side were only traditional instruments were used. There were no cases of any serious adverse events, CSF leak, loss of vision, meningitis or death.

DISCUSSION

This study shows for the first time that using the microdebrider is significantly time saving and more easy to use compared to using only traditional techniques in FESS for patients with CRSwNP. We performed this study to primarily look at the efficacy of the different operating techniques, evaluating operating time, estimated intra-operative

blood loss and user friendliness of the different instruments, and secondly at the safety and difference in post-operative healing.

In our study we found a clear significant difference in operating time, showing that operating with the microdebrider on top of the traditional instruments is faster than without. This can be explained because operating with the microdebrider improves visualization and does not require placing the instrument back into the nose every time the tissue is resected.

A recent study from Sauer showed the opposite result: a significant higher average operating time for the microdebrider compared to the traditional side. (13) A limitation of the Sauer study was the fact that the microdebrider side was operated only with the microdebrider, not with the microdebrider on top of traditional instruments, which we think is a more realistic approach.

Furthermore in contrast to other studies, this study showed no difference in blood loss between the microdebrider and traditional instruments. In 1996 Krouse and Christmass described a tendency of decreased bleeding on the microdebrider side. (10) However, this was a retrospective study were they analyzed the data of 250 patients who underwent surgery with the microdebrider and 225 patients with traditional instruments.

The fact that we found no decrease in blood loss on the microdebrider side despite the shorter surgical time might be because the microdebrider removes the mucosa in smaller pieces resulting in higher blood loss per cm^3 removed tissue. There was a potential spillover of blood loss on the second (right) surgical side. Because the side at which the microdebrider was used was randomized this potential spillover is also random and thus can be neglected in the comparison.

This study is the first to focus on the user friendliness of the different instruments. We found a significant difference in the advantage of the microdebrider between all parameters, except the preparation of the instrument. This means that it is easier to operated with a microdebrider than without in patients with CRSwNP. Only the preparation before using the microdebrider is more work than with the traditional instruments, although this will only take a few minutes.

Earlier claimed theoretical advantages of the microdebrider on postoperative healing time and symptom scores were not confirmed by our study. We did not find any significant differences in post operative healing time at all visits. Furthermore we found no significant differences in the subjective total symptom scores. We did see an improvement of total symptom score between one week postoperative and 3 months, but this was the same for both operating techniques. Although the study is rather small, post hoc analysis reveals that also with much larger numbers (>500 patients per group) significancy would not have been reached.

We did observe a significant difference in the recurrence rate of polyps at 4 weeks postoperative. On the microdebrider side were significantly less residual polyps than on the traditional side. At the 3 months follow up visit this difference was disappeared. This

finding can be explained because the microdebrider can resect mucosa more precisely, therefore leaving less sick mucosa behind, resulting in less polyps directly postoperative. After a few weeks this advantage will be decreased because of the additional use of steroids and nasal washing. However a very likely explanation could be the multiple testing that was done and this result has to be confirmed in other studies.

The literature on potential complications when operating with powered instruments is limited. There is no available literature indicating an increased complication rate with the use of powered instruments, but in a few case reports potentially dangerous complications are described to occur at a higher rate when operating with the microdebrider. (8;16)

In this study we found no difference between complication rate between the microdebrider and traditional instruments. Besides that there was no difference between the severity in adverse events between both groups. Because in our study not only experienced surgeons but also surgeons in training operated with the microdebrider, we can conclude that the use of the microdebrider is as safe as traditional instruments and can be used by both very experienced as more inexperienced surgeons, as long as in areas of risk the surgeon will operate with more caution.

In 11 cases (19%) in this study the experienced surgeon deviated from the protocol by using the microdebrider also on the traditional side. These were all cases where the surgeon felt that he/she was safer using the microdebrider, mostly in cases where polyps were growing in the frontal recess. To make sure that these deviations do not interfere with our results, operating time was recalculated after excluding the cases were deviation took place. Even after excluding these cases operating time was much shorter with the additional use of the microdebrider than without (p= 0,001). This supports our overall results.

A strength of this study is that it is prospective and comparing traditional instruments on one side to the microdebrider on top of traditional instruments on the other side, in contrast to most studies performed before. Besides that not only very experienced ENT specialists operated, but also ENT surgeons on training. This makes the setting more realistic and fair to compare both techniques.

A limitation of our article is the short period of follow-up, which is only 3 months. Maybe if we analyze the subjects for a longer period of time, there would be differences in final outcome between the microdebrider and traditional instruments.

However, the choice for this 3 month period is made because a longer follow up would mainly evaluate the natural evolution of the disease itself, rather than the effects of the two different sets of instruments.

Secondly, when calculating the operating time at the microdebrider side, we didn't measure the exact time needed to install and remove the microdebrider. To prove that this would make no difference afterwards we added 3 minutes to the operating time at the microdebrider side and repeated the statistics again to look for significance. Even with these 3 minutes extra time, there was a significance level found of p= 0,001.

CONCLUSION

This study demonstrates that operating patients with CRSwNP with the microdebrider on top of traditional instruments is very time efficient. Besides that the microdebrider is safe and easy to use showed by the significant higher user-friendliness as evaluated by every surgeon after the operation.

Furthermore there is no difference in intra-operative blood loss and post-operative pain or healing time. Our results are encouraging, showing that it could be worthwhile to invest in a microdebrider, saving operating time and meanwhile having good results.

We suggest in the future a analysis of the cost-effectiveness of the use of the microdebrider should be performed, taking into account all the different aspects of the use of the microdebrider such as shorter operating time, extra cost of the disposable blades and general costs of healthcare around the operation.

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chapter 4

ROLE OF CORTICOSTEROIDS IN FUNCTIONAL ENDOSCOPIC SINUS SURGERY – A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Background

The aim of our study is to systematically review the existing evidence on the role of corticosteroids in patients undergoing functional endoscopic sinus surgery (FESS).

Methodology

Systematic search of MEDLINE (1950- 2014), EMBASE (1980-2014), metaRegister, Cochrane Library and ISI conference proceedings was carried out.

Results

Eighteen randomised controlled trials with 1309 patients were included. Use of local and/or systemic corticosteroids
with FESS was reported in four categories; operative, anaesthesia related, post-operative outcomes and risk of recurrence. Meta-analysis for operative outcomes demonstrated that, mean operative time (MD -10.70 minutes; 95% CI -15.86, -5.55; P <0.0001) and mean estimated blood loss (MD -28.32 mls; 95% CI -40.93, -15.72; P <0.0001) was significantly lower; and surgical field quality (MD -0.81; 95% CI -1.32, -0.30; P = 0.002) was significantly better in corticosteroid group. Meta-analysis showed that post-operative endoscopic scores (SMD -0.39; 95% CI -0.60, -0.17; P = 0.0004) were significantly better in corticosteroid group compared to no corticosteroid group. There was no increase in risk of sinusitis (RR 0.64; 95% CI 0.32, 1.30; P = 0.22) between use of corticosteroids and no corticosteroids; There was no significant difference in recurrence risk of chronic rhinosinusitis (CRS) in mixed population studies (RR 0.77; 95% CI 0.35, 1.70; P = 0.52) between the two groups but analysis of studies reporting on chronic rhinosinusitis with nasal polyps (CRSwNP) (RR 0.64;95% CI 0.45,0.91;P=0.01) showed significant difference in favour of the corticosteroid group.

Conclusion

Pre-operative use of local and/or systemic corticosteroids in FESS, results in significantly reduced blood loss, shorter operative time and improved surgical field quality. Studies are limited on the intra-operative use of corticosteroids to reduce postoperative pain. Postoperative corticosteroids improve postoperative endoscopic scores in CRS and recurrence rates in cases of CRSwNP.

INTRODUCTION

Chronic rhinosinusitis (CRS) is a common disabling condition resulting in significant healthcare cost and loss in productivity. The prevalence rate of CRS have been quoted from 5.5% in South America, 10.9% in Europe to about 16% in America (1-3). CRS (including CRS with nasal polyps(CRSwNP)) is defined by European position paper on rhinosinusitis and nasal polyps (EPOS 2012), as "inflammation of the nose and the paranasal sinuses characterised 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 polyps and/ or mucopurulent discharge primarily from middle meatus and/ or; oedema/mucosal obstruction primarily in middle meatus, and/or CT changes showing mucosal changes within the osteomeatal complex and/or sinuses"(4). Rhinosinusitis (RS) can be acute when symptoms or signs subside within 12 weeks and chronic (CRS) if these persist for more than 12 weeks (4). CRS can be with or without nasal polyps (CRSwNP, CRSsNP) and affects 2-16 % (5,6) and 2-3% (4,7) of the population, respectively. CRS is considered as a multifactorial disease. Environmental factors include pollution, smoking, fungus, bacterial and viral infections. Host factors can be general factors like immune deficiencies and genetic factors, and local host factors causing persistent focal inflammation within the ostiomeatal complex (8). Initial therapy for CRS includes nasal saline irrigation, topical and systemic corticosteroids, and in cases of CRSsNP potentially long term antibiotics followed by surgical intervention in unresponsive patients (4,6). Corticosteroids reduce nasal mucosal inflammation and therefore increase drainage of infected mucosal secretions and aid the healing process.

Patients who fail to respond to medical therapy are considered for functional endoscopic sinus surgery (FESS), which is one of the most common surgical procedures performed (5,9). Endoscopic sinus surgery was described by Stammberger (10) in 1985 and Kennedy (11) coined the term FESS to highlight its surgical philosophy of mucosal sparing. About 80% of patients have successful outcome but 20% patients suffer from relapse of sinusitis or complications warranting further surgical intervention (12).

Corticosteroids have been used preoperatively, intraoperatively and postoperatively in FESS for rhinosinusitis. FESS creates a conduit for topical steroids to reach the deeper part of the sinus cavity and act on the mucosa which was previously inaccessible. Intranasal corticosteroids are therefore often included in postoperative treatment regimens. Both local and systemic corticosteroids have also been used preoperatively to reduce inflammation and intraoperative bleeding, thereby improving surgical field (13,14). It has also been shown that asthmatic patients who are given corticosteroids preoperatively have low incidence of pulmonary complications in the perioperative time period (15). Corticosteroids have also been postulated in pain control when used intraoperatively (16). There are several randomised controlled trials evaluating the role of corticosteroids in FESS, however, these studies have reported conflicting results.

The aim of our study was to systematically review the existing evidence on the role of corticosteroids in patients with CRS undergoing FESS. The aim was to determine whether preoperative corticosteroids affect operative parameters; intra-operative corticosteroids reduce surgical pain; and postoperative corticosteroids affect patient's symptom scores, endoscopic appearance and recurrence rates.

MATERIALS AND METHODS

Data sources and Literature search

We conducted systematic searches for randomised controlled trials (RCTs). There were no language, publication year or publication status restrictions. The date of the last search was 20.09.2014. We searched MEDLINE, EMBASE, Web of science, metaRegister, Cochrane Library and ISI conference proceedings. A combination of MeSH and text words were used to generate two subsets of citations, one including studies of endoscopic surgery ('endoscopic sinus surgery', 'endoscopic polypectomy', 'FESS', 'functional endoscopic sinus surgery') and the second including corticosteroids ('corticosteroids', 'steroids', 'corticoids', 'dexamethasone', 'fluticasone', 'budesonide', 'mometasone", "prednisone", "prednisolone", "beclomethasone", "triamcinolone"). These subsets were combined using 'AND' to generate a subset of citations relevant to our research question. The reference lists of all known primary and review articles were hand searched to identify cited articles not captured by electronic searches. The searches were conducted independently by VP and JP.

Study selection

Two review authors (VP and JP) performed data selection and extraction based on predetermined criteria. Studies were selected in a two-stage process. Firstly, the titles and abstracts from the electronic searches were scrutinized and full manuscripts of all citations that were likely to meet the predefined selection criteria were obtained. Final inclusion or exclusion decisions were made on examination of the full manuscripts. In cases of duplicate publication, the most recent or complete versions were selected. We documented our justification for the exclusion of studies.

Data extraction

Two reviewers (JP and VP) completed data extraction. Study characteristics and participant features were extracted from each study regarding: characteristics of trials - setting, design, method of data analysis; participants - study population, number of participants; type of intervention: dose, route of administration, duration of treatment, follow-up and outcomes. Inconsistencies between reviewer's data were resolved through discussion with a third reviewer (SB) until a consensus was reached. After identifying the studies where additional data were needed, a request was sent by

means of electronic mail to the corresponding author of each study. If no response was received, a second request was sent 2 weeks later by means of electronic mail.

Data Synthesis

Inclusion and exclusion criteria

Studies were selected if the target population underwent FESS, and were exposed to corticosteroids and compared with either placebo or no corticosteroids. Only RCTs were included. Trials which included participants of any age, who had any co-morbidity including asthma and aspirin sensitivity, allergic or non-allergic, followed for any duration and CRS with and without polyps were included. Studies were excluded if the patients had taken corticosteroids in the absence of FESS.

Outcomes assessed

The outcomes were assessed under four categories. Operative outcomes, anaesthetic related outcomes, post-operative outcomes and risk of recurrence. Operative outcomes included estimated blood loss (EBL), surgical field quality and operative time. Postoperative outcomes included symptoms score (subjective improvement), endoscopic score (objective improvement) and risk of sinusitis.

Assessment of risk of bias in included studies

We assessed the methodological quality of the included studies and carried out the assessment of risk of bias taking into consideration: method of randomisation; allocation concealment; blinding; incomplete outcome data; selective outcome reporting; and other sources of bias (17). We used the Cochrane 'Risk of bias' tool in RevMan 5.1,which involved describing each of these domains as reported in the trial and then assigning a judgement about the adequacy of each entry as low, high or unclear risk of bias (18). We presented this information in a 'Risk of bias' graph and summary.

Statistical analysis

Meta-analysis was performed in line with recommendations from the Cochrane collaboration and the quality of reporting of meta-analyses (QUORUM) guidelines (19,20). From each study, dichotomous outcome data were summarised in 2 x 2 tables by two reviewers (VP, JP). The results were pooled and expressed as risk ratios (RR). Continuous variables were analyzed using mean differences (MD), with 95% confidence intervals (CIs) (21). The results were pooled using either a fixed effect (22) or random effect model as appropriate (21). For symptoms scores, the measurements used were sino-nasal outcome test score (SNOT 21) by Rotenberg et al. (0-120) (23) and Jorrisen et al. (12). used their own score (0-50). Results for endoscopic scores were derived from four studies; Cote et al. (24) and Rotenberg et al. (23) used Lund-Kennedy endoscopic

score (LKES score; range 0-12 in one nasal cavity) (25); Chang et al. (26) used Philpott-Javer score (range 0-40) (27) and Jorissen et al. (12) used their own scoring system combining inflammation, oedema and polyps (range 0-6). We used standardised mean difference as a summary statistic in this meta-analysis because the included studies assessed the same outcome but measured it in a variety of ways, to standardise the results of the studies to a uniform scale before they could be combined.

Heterogeneity of the exposure effects was evaluated statistically using the I2 statistic to quantify heterogeneity across studies (28). A I2 value of >50% was taken as evidence of substantial heterogeneity and in such cases a random effect model was used. A chi-squared test for heterogeneity was also performed and the p-values are presented.

When only medians were available, these were used as estimates of means (29,30). When a study failed to present a standard deviation (SD), this statistic was either calculated from the standard error of the mean, 95% CI, t value or interquartile range (29). Some studies provide only ranges, in such instances the SD was estimated using the formula total range/4 (30). Statistical analyses were performed using RevMan 5 software.

RESULTS

Study selection

Of the 307 citations identified by the search, 39 were selected after initial screening. Following examination of the full manuscripts of these 39 studies, 21 more were excluded; 2 studies compared different corticosteroids (31,32), 4 studies were cohort studies with no comparison group (33-36), 4 were non-randomised comparative studies (37-40), 1 study compared two different doses of a steroid (41), 5 studies did not use FESS as surgical technique (42-46), 3 studies reported incomparable outcomes (47-49) and 2 were review articles (50,51) (Figure 1).

Eighteen studies satisfied the selection criteria and were included in this review (12-14,23,24,26,52-63). In total 1309 patients were included in this review. Four studies had an intrapatient control design in which one side of the nasal cavity was compared with the other side (n=182) (24,59,60,62). These studies were included in the meta-analysis and the two groups treated as independent, and then sensitivity analysis was performed excluding these studies to determine the robustness of the results. The remaining 1127 patients were randomised to the steroid group of 607 patients and 520 controls. Sample size per study varied across the trials and ranged from 19 to 162 participants. Use of corticosteroids with FESS was reported for four categories; operative outcomes, anaesthesia related, post-operative outcomes and risk of recurrence. Operative outcomes were reported by three studies (13,14,55); anaesthetic outcomes were reported by one study (58); post-operative outcomes were reported by ten studies (12,23,24,26,54,55,57,59,61,62), and risk of recurrence was

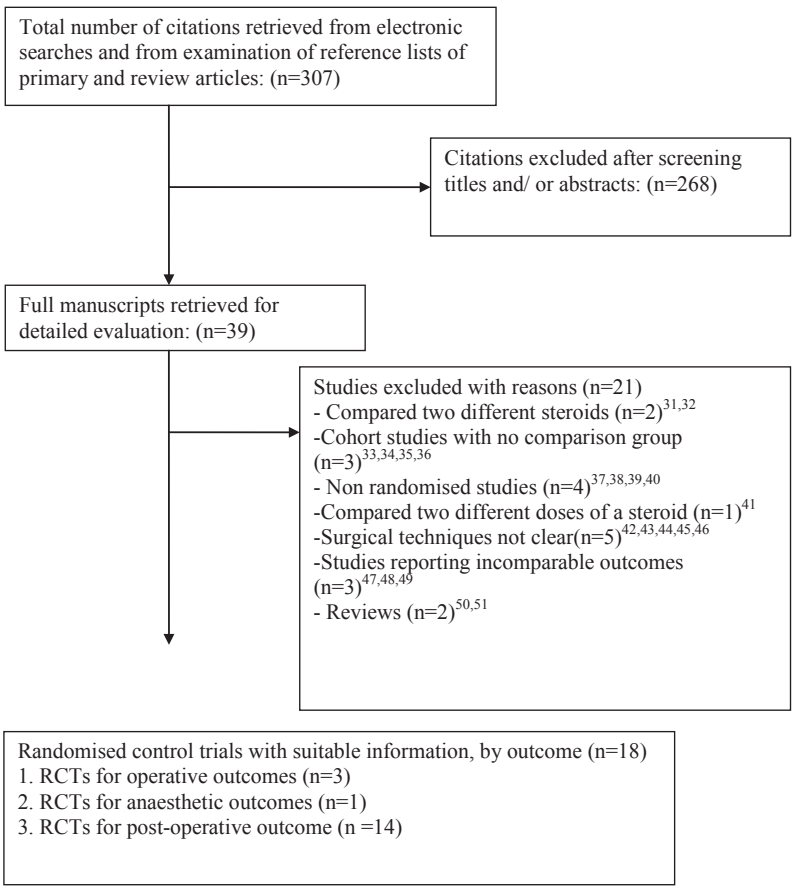


Figure1. Consort diagram - Study selection process.

reported by six studies (52,53,56,59,60,63). One RCT reported both on operative and post-operative outcomes, therefore it was included in both categories (55). Albu et al., reported on patients with and without polyps (14); data from this study is included in the meta-analysis as Albu et al. (1) and Albu et al. (2). Albu et al. (1) represent data of patients with CRSwNP and Albu et al. (2) represent data of patients with CRSsNP. In our attempt to get more information about studies with inadequate data, we received no response from the relevant authors (13,24,53,55).

Study characteristics

A description of the included studies is summarised in Table 1. Risk of bias from the included studies is represented in Figures 2 and 3. Our judgements about each risk of bias item, presented as percentages across all included studies, are shown in Figure 2, and for each risk of bias item for each included study in Figure 3. Generally, included

Table 1. Summary of the clinical and pathological findings of the patients enrolled in this survey

Author/ Number in intervention and control group	Inclusion Criteria	Exclusion Criteria	Intervention Protocol	Control Protocol	Follow-up	Outcomes
Jorrisen et al[12] 2009 Intervention -46 Controls-45	Age ≥18 years Bilateral nasal polyposis and/or CRS, as diagnosed by history, nasal endoscopy and CT-scan. Failure to conventional medical treatment or contra-indications to medical treatment and required FESS for their disease.	Patients who had undergone sinus surgery in the last 5 years and those with surgical contra-indications, primary ciliary dyskinesia, asthma requiring inhalant CS treatment, aspirin hypersensitivity, immunodeficiency or cystic fibrosis. Patients who received systemic and topical CS within 4 weeks, IM CS within 3 months, antihistamines or leukotriene receptor antagonists within 10 days, nasal decongestants within 24 hours, and nasal sodium cromolyn, atropine or ipratropium bromide, or antifungals within 1 week of screening. Patients with contra-indications for intranasal or oral use of CS or hypersensitivity to the study drugs. Pregnant or breast feeding women.	Oral Betamethasone 2 mg for 7 days, followed by topical MFNS 200µg b.i.d for 6 months after FESS.	Matched placebo tablets and intranasal spray for 7 days and 6 months, respectively.	6 months	Total Endoscopic Scores Combination Endoscopic Scores Total Symptom Scores Rescue Medications Adverse Events
Sieskiewick et al[13] 2006 Intervention -18 Controls-18	Age-20-65 Yrs CRSwNP	Uncompensated arterial hypertension, hemostatic disorders, diabetes, Deviated nasal septum, inferior turbinate hypertrophy	30 mgs prednisolone daily 5 days before the operation.	Without steroids	100%	Total mean blood loss Operative field Surgical operative time

Table 1. (continued)

Author/ Number in intervention and control group	Inclusion Criteria	Exclusion Criteria	Intervention Protocol	Control Protocol	Follow-up	Outcomes
Albu et al [14] 2010 Intervention-35 Controls-35	CRSsNP and CRSwNP(Grade 1 polyps)	Coagulation disorders, hypertension, cardiac disorders, unilateral sinus disorders, unstable asthma, regular use of decongestant within one month of surgery, simultaneous septal or inferior turbinate surgery, odontogenic sinusitis, extra mucosal mycotic sinusitis, NSAID intolerance, diabetes, history of intra and extranasal sinus procedure, CRS with grade 2 and 3 polyps.	MF 200 µg (2 sprays in each nostril 2 times a day) for 4 weeks preoperative.	Placebo spray same dose for 4 weeks.	100%	Operative data- Duration of surgery Surgical field Estimated blood loss
Rotenberg et al[23] 2011 Intervention -20 Controls-21	Age > 18 years, Diagnosis of CRSwP as per the American Academy of Otolaryngology guidelines, A Samter's triad phenotype, Failure of a minimum of 6 months of standard medical management prior to ESS, and severe disease as documented by a minimum LKES of 8.	Smokers, revision sinus surgery, CS for conditions other than CRSwP, and patients with diseases that were relative or absolute contraindications for CS use.	Saline irrigation mixed with 2 ml of 0.5-mg/ml budesonide per bottle of Sinus-Rinse, using half of the solution twice daily (for a total of 1000 lg budesonide daily).	saline irrigation	6 months	SNOT 21 Score LM CT Scan Score LKES ACTH Ranges IOP.

Table 1. (continued)

Author/ Number in intervention and control group	Inclusion Criteria	Exclusion Criteria	Intervention Protocol	Control Protocol	Follow-up	Outcomes
Cote et al[24] 2010 Intervention -19 Controls-19	Patients with CRS with polyposis refractory to medical treatment	Ineligible for informed consent, unwilling or unable to comply with post operative visits necessary for data collection, intolerance to triamcinalone.	Randomised cavity - 2 ml of 40mg/ml triamcinolone impregnated dressing.	2 ml of normal saline solution-impregnated dressing.	6 months	LKES Score POSE Score
Chang et al[26] 2011 Intervention -16 Controls-16	Age 17 to 80 years Bilateral chronic rhinosinusitis with or without polyposis, failed maximal medical treatment, and required bilateral FESS for the treatment of CRS.	Unilateral sinus disease, Bleeding disorder; Genetic disorder such as cystic fibrosis, Immunosuppressed, or Undergoing surgery for excision of a tumour	budesonide-soaked Merocel pack	Non medicated Merocel Pack	4 weeks	Endoscopic Grading of postoperative Mucosal Healing. Biopsy Grading Scores
Bross-Sariano et al[52] 2004 Intervention -108 Controls-54	Sinonasal polyposis	Not documented	Steroid group-saline lavage then Group I-FP 400 µg /day(54 patients) Group II-beclomethasone dipropionate 600 µg/day(54 patients)	Saline lavage	12 months	Relapse rate of Polyps or Infection rate

Table 1. (continued)

Author/ Number in intervention and control group	Inclusion Criteria	Exclusion Criteria	Intervention Protocol	Control Protocol	Follow-up	Outcomes
Dijkastra et al[53] 2004 Intervention -106 Controls-56	Age > 18 years Nasal polyps or CRS requiring treatment. Abnormality on CT Scan confirming diagnosis.	Use of systemic CS or other medication influencing nasal mucosa during study. Significant anatomical abnormalities persisting after FESS +- Septoplasty History of acetylsalicylic acid intolerance, Pregnancy, Cystic fibrosis, serious concurrent disease Rhinosurgery in past 6 weeks.	FPANS 800 µg BD (53 cases) FPANS 400 µg BD (53 cases).	Placebo BD	1 year	Symptom reduction after FESS Relapse rate Effect of CS in post operative period.
Rowe Jones et al[54] 2005 Intervention -55 Controls-54	Patient with symptoms of CRS received 3 weeks of Fluticasone propionate, 100 µg (2 spray). If failed, they were considered for FESS. Patients with 4 episodes per year of acute, recurrent RS of at least 10 days duration and a persistent CT scan score of at least 3, excluding an isolated polypoidal opacity within a sinus.CT scan changes had to be present at least 4 weeks after an acute infection.	Pregnant women; age > 60 years or <16 years; patients taking regular oral steroids; patients taking > 1500 µg of inhaled CS per day; patients with antro-choanal or isolated polyps; patients requiring combined external approach and ESS patients requiring frontal sinus ostioplasty procedures; patients who had undergone sinus surgery within the last 12 months; patients with mucocoeles; patients with tumours.	FPANS 200 µg b.d.	Placebo spray comprised all constituents of standard FPANS spray, excluding fluticasone propionate.	5 years	Visual analogue score Total sum of all 6 visual analogue scores, Endoscopic polyp, oedema, discharge scores, Nasal mucociliary clearance time Olfactory detection threshold values Nasal volumes. Rescue medication Failures rate

Table 1. (continued)

Author/ Number in intervention and control group	Inclusion Criteria	Exclusion Criteria	Intervention Protocol	Control Protocol	Follow-up	Outcomes
Wright et al[55] 2007 Intervention -11 Controls-15	Age-18 yrs old, CRSwNP, Failed or refused full medical treatment.	Immunocompromised status and mucociliary disorders, allergic fungal sinusitis.	30 mg prednisalone 5 days preoperatively and 9 days post operatively.	Placebo tablets	6 mths	Duration of surgery Difficulty of surgery Estimated blood loss Endoscopic score (LKES & POES) Symptom severity Questionnaire score
Stjarne et al[56] 2009 Intervention -79 Controls-80	Age-18 years or older Bilateral nasal polyps.	Polypectomy within the previous 6 months; unhealed nasal surgery or trauma; > 5 previous polypectomies; or ongoing concurrent nasal infection, rhinitis medicamentosa, hereditary mucociliary dysfunction, nasal structural abnormalities, or an idiosyncratic reaction to CS. Active or latent pulmonary tuberculosis; other significant medical conditions that could interfere with evaluations (eg, cystic fibrosis); or a history of hypersensitivity to the study medication, Women who are Pregnant, lactating, or not using an adequate prophylactic measure.	Mometason furoate, 200 µg once daily.	Matching placebo spray	6 months +-7 days	Rate of relapse Time to relapse Side Effects

Table 1. (continued)

Author/ Number in intervention and control group	Inclusion Criteria	Exclusion Criteria	Intervention Protocol	Control Protocol	Follow-up	Outcomes
Ehnhage et al[57] 2009 Intervention -30 Contols-38	Age 18 years Bilateral nasal polyps and Asthma	Unfit for GA; Polypectomy in last 6 months; Illness or medication that may interfere with the study; Idiosyncratic reaction to CS; Prohibited medication within wash-out period; Participated in clinical trial within 30 days Pregnant or lactating women; Women of child bearing potential not using adequate anti-contraceptive method Study personnel or patients related to study personnel; Aspirin Senstivity	Fluticasone proprionate nasal drops 400 µg b.i.d for 10 weeks	Matched placebo	14 weeks	Nasal symptoms scores. Asthma symptoms score. Peak expiratory flow rate Need of b2-agonists. Peak nasal inspiratory flow (PNIF) Butanol threshold test for olfactory function. Nasal endoscopy. Pulmonary function and bronchial histamine sensitivity.
Al-Qudah et al[58] 2010 Intervention -32 Controls-30	All patients undergoing elective FESS with ASA1/2.	Patients under 16 yrs, Previous systemic CS treatment for > 3 months at any time or within 1 month before randomization. Grade 3 nasal polyps.	8 mg of i/v dexamethasone sodium phosphate preoperatively.	2ml of i/v saline solution	24 Hours	Postoperative pain score- PACU – 6 and 24- Hours. Patients needing rescue anesthesia.

Table 1. (continued)

Author/ Number in intervention and control group	Inclusion Criteria	Exclusion Criteria	Intervention Protocol	Control Protocol	Follow-up	Outcomes
Murr et al [59] 2011 Intervention -38 Controls-38	Adult patients with or without nasal polyps scheduled to undergo primary or revision FESS, and in whom placement of the sinus stents was deemed to be both feasible and medically appropriate	Patients were excluded if they had known history of intolerance to corticosteroids, an oral steroid–dependent condition, a history of immune deficiency, insulin-dependent diabetes, or allergic fungal sinusitis.	Bio-absorbable drug-eluting sinus stents releasing a total dose of 370 µg of MF is blended into the polymer structure of polylactide-co-glycolide, which releases the MF by diffusion in a controlled fashion over approximately 30 days.	Non-eluting control stent.	45 days	Endoscopic Scores Recurrence rates Adhesion rates. Medial Turbinate Lateralization rates.
Marple et al[60] 2011 Intervention -105 Controls-105	CRS with or without nasal polyps	Patients were excluded for a known history of immune deficiency, insulin-dependent diabetes, allergy or intolerance to corticosteroids, oral steroid-dependent condition, clinical evidence of acute bacterial sinusitis, or clinical evidence of invasive fungal sinusitis. Ocular exclusionary criteria included history or diagnosis of glaucoma or ocular hypertension,closed angle, presence of cataracts grade +3 or higher, or presence of posterior subcapsular cataract.	Propel sinus implant which contains 370 µg mometasone furoate, embedded in a polymer matrix that provides local, controlled release of the drug over 30 days.	Non-drug-releasing implant	30 days	Recurrence rates Adhesion rates. Medial Turbinate Lateralization rates. Intraocular pressure increase.

Table 1. (continued)

Author/ Number in intervention and control group	Inclusion Criteria	Exclusion Criteria	Intervention Protocol	Control Protocol	Follow-up	Outcomes
Rudmik et al [61] 2012 Intervention -18 Controls-18	Age ≥18 years old Chronic rhinosinusitis persistent symptoms despite medical management undergoing minimum bilateral ESS .	Nasal polyposis , uncorrectable coagulopathy; emergency surgical procedures; and presence of a systemic inflammatory disease.	Sinu-Foam with dexamethasone mixture .4 mL of dexamethasone (4 mg/mL) in 4 mL of sterile water.	Sinu-Foam syringes with placebo mixture (8 mL of sterile water)	3 months	Endoscopic Score.
Jin et al[62] 2012 Intervention -20 Controls-20	CRSwNP failing medical treatment and undergoing FESS	Revision Endoscopic Sinus Surgery Cases. Immunosuppressed Patients	2 ml of 40mg/ ml triamcinolone impregnated dressing.(for a week)	2 ml of normal saline solution-impregnated dressing.(for a week)	3 months	LKES Score
Passali et al[63] 2003 Intervention -33 Controls-40	Patient with Grade 3 CRSwNP refractory to medical treatment	No exclusion criterion mentioned in the paper	Mometasone Nasal Sprays-200micrograms for 30 days	No specific Treatment	3-6 yrs	Recurrence rates Rhinomanometry results

Mometasone Furoate (MF), Post Anaesthetic Care Unit (PACU), Lund-Kennedy endoscopic score (LKES), Perioperative Sinus Endoscopy Scores POSE, Fluticasone Propionate Aqueous Nasal Spray (FPANS), Sino-Nasal Outcome Test Score (SNOT 21),Lund Mackay CT Scan Score (LM Score), Adernocorticotropic Harmone (ACTH). Intra Optic Pressure (IOP); GA general anesthesia; CS corticosteroids

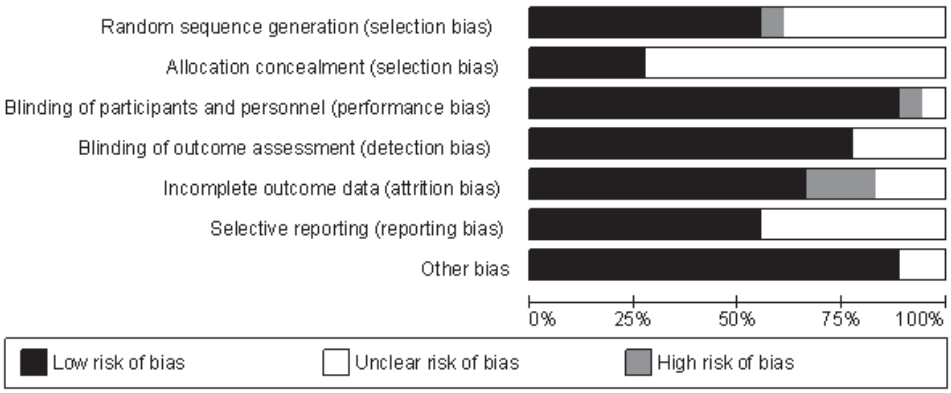


Figure 2. Risk of bias graph: each risk of bias item presented as percentages across all included studies.

studies had low risk of bias for method of randomisation and blinding, medium risk of bias for incomplete outcome data and selective reporting and unclear risk of bias for allocation concealment.

Outcomes

1. Operative outcomes in response to pre-operative corticosteroids

1.1 Operating time

Data addressing this comparison were available from three studies, Sieskiewicz et al. (13), Albu et al. (14) and Wright et al. (55). Data from Wright et al. (55) could not be included because the SD could not be calculated. Albu et al. (14) used mometasone furoate nasal sprays for 4 weeks whereas Sieskiewicz et al. (13) used 30 mgs prednisalone for five days preoperatively. Pooling the results of the remaining two studies (13,14) showed that, mean operative time was significantly lower in the steroid group compared to the non steroid group (MD -10.70 minutes; 95% CI -15.86, -5.55; $P < 0.0001$; Figure 4A). I2 was 19%, suggesting insufficient evidence of any significant heterogeneity ($\chi 2 = 2.47$, $P = 0.29$).

A subgroup analysis was done according to population group, which showed that in CRSwNP patients there was significant difference favouring steroid group (MD -13.93 minutes; 95% CI -21.02, -6.85; $P = 0.0001$; Figure 4A). I2 was 0%, suggesting insufficient evidence of any significant heterogeneity ($\chi 2 = 0.78$, $P = 0.38$). CRSsNP did not show statistically significant difference between the two groups (MD -7.07 minutes; 95% CI -14.58, -0.44; $P = 0.07$; Figure 4A).

As Albu et al. (14) used local steroids and Sieskiewicz et al. (13) used systemic steroids we undertook a subgroup analysis looking at different modes of delivery. This showed a significant difference in favour of corticosteroids both local (MD -10.58 minutes;

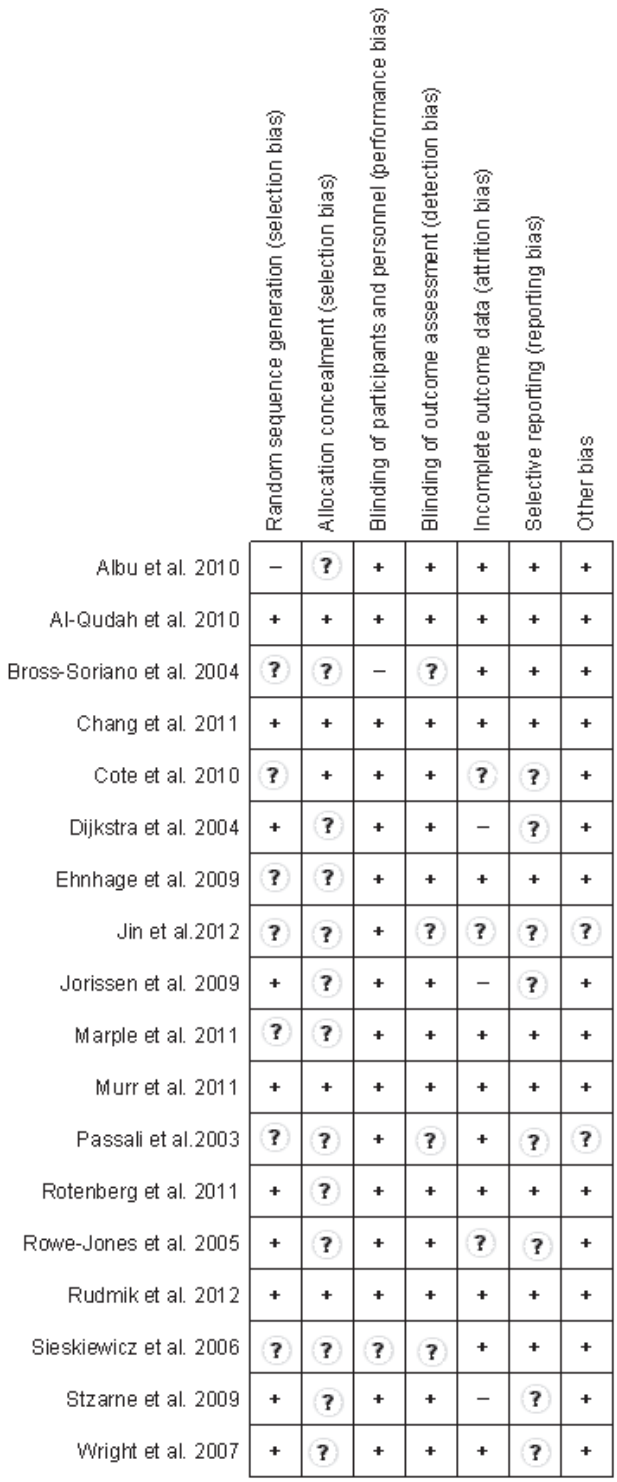
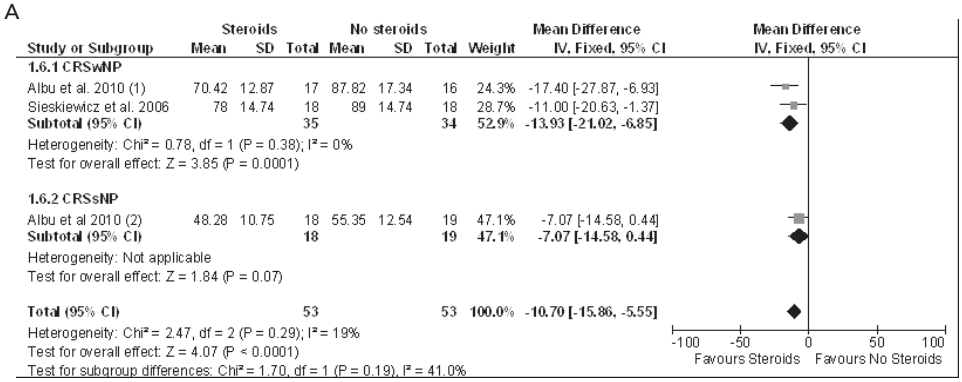


Figure 3. 'Risk of bias' summary: Each risk of bias item for each included study.

A



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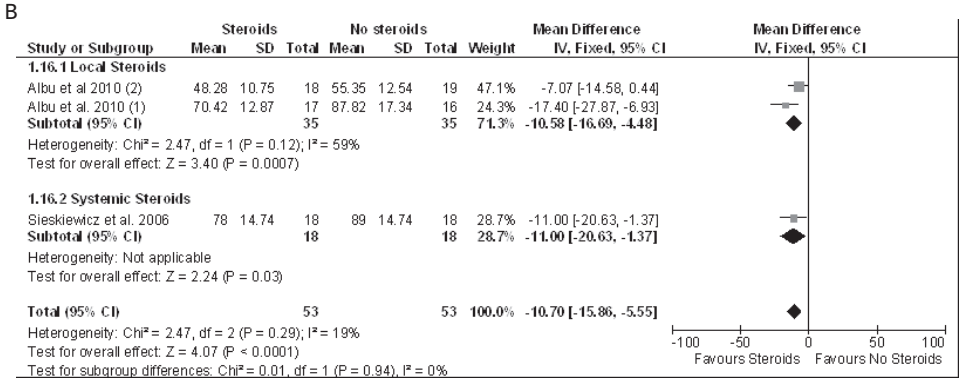

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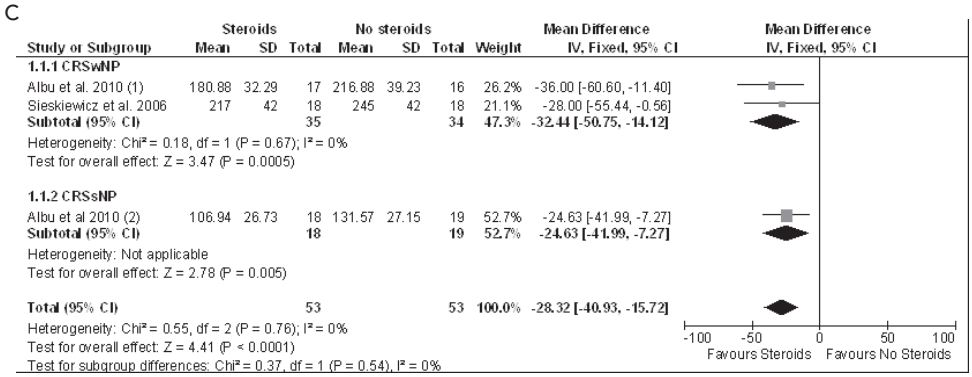
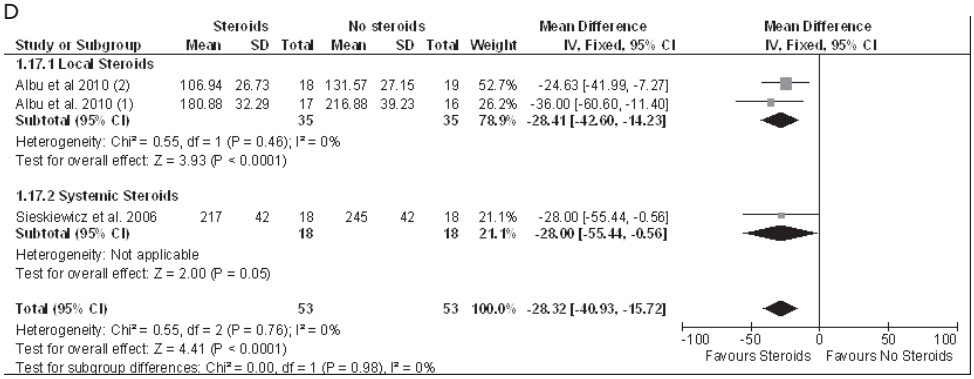


Figure 4. Forest plot of comparison - Operative outcomes. (A) Forest plot of comparison: Steroids versus No steroids. Outcome: 1.1 Operative time.Subgroup Analysis- Population Groups. (B) Forest plot of comparison: Steroids versus No steroids. Outcome: 1.1 Operative time. Subgroup Analysis-Mode of Drug Delivery. (C) Forest plot of comparison: Steroids versus No steroids Outcome: 1.2 Estimated blood loss. Subgroup Analysis- Population Group ►

D



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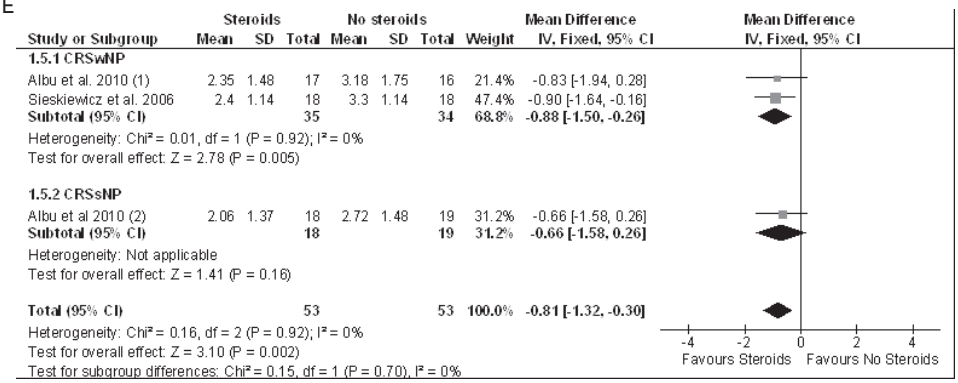

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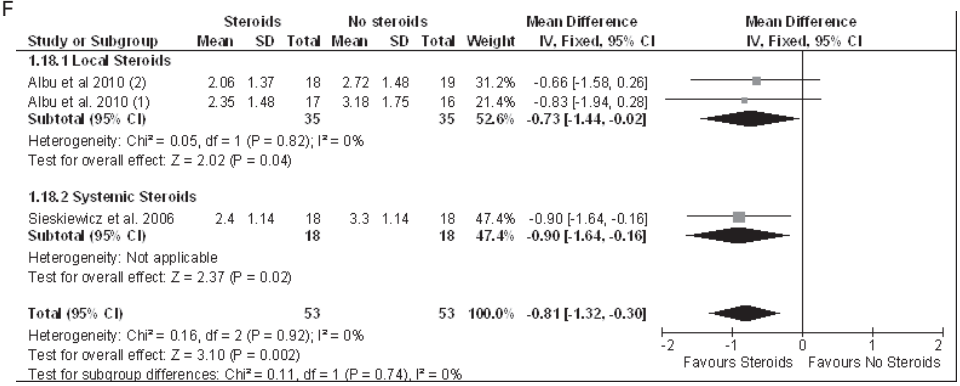

► Figure 4. (continued)
(D) Forest plot of comparison: Steroids versus No steroids. Outcome: 1.2 Estimated blood loss. SubgroupAnalysis-Mode of Drug Delivery. (E) Forest plot of comparison: Steroids versus No steroids. Outcome: 1.3 -Surgical field quality. Subgroup Analysis- Population Groups. (F) Forest plot of comparison: Steroids versus No steroids. Outcome: 1.3 -Surgical field quality. Subgroup Analysis-Mode of Drug Delivery.

95% CI -16.69, -4.48; P = 0.0007; Figure 4B) and systemic (MD -11.00 minutes; 95% CI -20.63, -1.37; P = 0.03; Figure 4B). In local corticosteroid subgroup analysis, I2 was 59%, suggesting significant heterogeneity ($\chi 2 = 2.47$, P = 0.12).

1.2 Estimated blood loss (EBL)

Data addressing this comparison were available from three studies, Sieskiewicz et al. (13), Albu et al. (14) and Wright et al. (55). Data from Wright et al. (55) could not be included because the SD could not be calculated. Albu et al. (14) used mometasone furoate nasal sprays for 4 weeks whereas Sieskiewicz et al. (13) used 30 mgs prednisalone for five days preoperatively. Pooling of results from the remaining two studies (13,14) showed that, mean EBL was significantly lower in the steroid group compared to the non steroid group (MD -28.32 mls; 95% CI -40.93, -15.72; P < 0.0001; Figure 4C). I2 was 0%, suggesting no significant heterogeneity ($\chi 2 = 0.55$, P = 0.76).

A subgroup analysis was done according to population group, which showed significant difference favouring the steroid group in both CRSwNP patients (MD-32.44 mls; 95% CI -50.75, -14.12; P = 0.0005; Figure 4C) and CRSsNP patients (MD -24.63 mls; 95% CI -41.99, -7.27; P = 0.005; Figure 4C). In CRSwNP subgroup analysis, I2 was 0%, suggesting no significant heterogeneity ($\chi 2 = 0.18$, P = 0.67).

As Albu et al. (14) used local steroids and Sieskiewicz et al. (13) used systemic steroids we undertook a subgroup analysis looking at different modes of delivery. This showed a significant difference in favour of corticosteroids both local (MD -28.41 mls; 95% CI -42.60, -14.23; P <0.0001; Figure 4D) and systemic (MD -28.00 minutes;95% CI -55.44, -0.56; P = 0.05; Figure 4D). In local corticosteroid subgroup analysis, I2 was 0%, suggesting insignificant evidence of heterogeneity ($\chi 2 = 0.55$, P = 0.46).

1.3 Surgical field quality

Data addressing this comparison were available from two studies, Sieskiewicz et al. (13) and Albu et al. (14). Both these studies used Boezaart grading system to measure surgical field quality. Albu et al. (14) used mometasone furoate nasal sprays for 4 weeks whereas Sieskiewicz et al. (13) used 30 mgs prednisalone for five days preoperatively. Pooling of the results of these showed that, surgical field quality was significantly better in the steroid group as compared to no steroid group (MD -0.81; 95% CI -1.32, -0.30; P = 0.002; Figure 4E). I2 was 0%, suggesting no significant heterogeneity ($\chi 2 = 0.16$, P = 0.92).

A subgroup analysis was done according to population group, which showed significant difference favouring steroid group in CRSwNP patients (MD -0.88; 95% CI -1.50, -0.26; P = 0.005; Figure m4E) but not in CRSsNP patients (MD -0.66;95% CI -1.58, 0.26; P = 0.16; Figure 4F). In CRSwNP subgroup analysis, I2 was 0%, suggesting no significant heterogeneity ($\chi 2 = 0.01$, P = 0.92).

As Albu et al. (14) used local steroids and Sieskiewicz et al. (13) used systemic steroids we undertook a subgroup analysis looking at different mode of delivery. This

showed a significant difference in favour of corticosteroids both local (MD -0.73;95% CI -1.44, -0.02; P = 0.04; Figure 4F) and systemic (MD -0.90; 95% CI -1.64, -0.16; P = 0.02; Figure 4F). In local corticosteroid subgroup analysis, I2 was 0%, suggesting insignificant evidence of heterogeneity ($\chi 2 = 0.05$,P = 0.82).

2. Anaesthetic outcomes in response to intra-operative corticosteroids

This was reported by Al-Qudah (58). They used 8 mg dexamethasone intravenously in the steroid group. Analysis of data showed that there was no significant difference in post operative pain score at 6 hours postoperatively (p = 0.45) and 24 hours postoperatively (p = 0.17) in the steroid group as compared to the non steroid group.

3. Post-operative outcomes in response to corticosteroids

Postoperative outcomes in the form of symptom score and endoscopic score were reported by twelve studies (12,23,24,26,53- 57,59,61,62). Data from Rowe-Jones et al. could not be pooled in the meta-analysis as their data were not homogenous with other studies and SD could not be calculated (54). Individual subjective symptom outcomes mainly, congestion, sense of smell and rhinorrhoea were reported in two studies Stjarne et al. and Enhange et al. but the data could not be pooled for meta-analysis (56,57).

3.1 Symptom score

Even though postoperative symptom outcomes were reported by seven studies (12,23,53-57) data from only two studies could be pooled for the meta-analysis. Jorrisen et al. (12) used oral betamethasone 2 mg for 7 days, followed by topical mometasone furoate 200µg twice daily and Rotenberg et al. (23) used topical budesonide 1000 µg daily. Data from Rowe-Jones et al. could not be pooled as their data was not homogenous with other studies (54). They reported that overall visual analogue score, endoscopic polyp score and total nasal volume were significantly better in the steroid group at 5 years. Data from Dijkstra et al. and Wright et al. could not be included because the SD could not be calculated (53,55). Dijkstra et al. reported no significant difference in total symptom score between the steroid group and control group (53). Individual subjective symptom outcomes mainly, congestion, sense of smell and rhinorrhoea, were reported by Stjarne et al., Enhange et al. and Wright et al., but could not be pooled for meta-analysis (55-57). Wright et al. concluded that there was no treatment effect on subjective symptoms noted between corticosteroids compared with placebo (55). Stjarne et al. reported no significant difference in baseline to end of treatment scores for nasal congestion and subjective sense of smell between the steroid and placebo group (56). Similarly, Enhange et al. also reported that there were no statistically significant differences in the changes in all these nasal parameters between the steroid and the placebo group after undergoing FESS (57). Pooling of data from the remaining two studies (12,23) showed that there was no significant difference in mean post operative symptom score between the steroid

group compared to the non steroid group (SMD -0.01; 95% CI -0.36, 0.33; P = 0.94:). I2 was 0%, suggesting no significant heterogeneity ($\chi 2$ = 0.36, P = 0.55).

3.2 Endoscopic score

Data addressing this comparison were available from eight studies (12,23,24,26,55, 59,61,62). Jorrisen et al. (12) used oral betamethasone 2 mg for 7 days, followed by topical mometasone furoate sprays, Rotenberg et al. (23) used topical budesonide 1000 µg daily, Cote et al. (24) used triamcinolone impregnated packs, Chang et al. (26) used budesonide impregnated packs, Murr et al. (59) and Rudmik et al. (61) used mometasone furoate eluding stents, and Jin et al. (62) used sinufoam with dexamethasone dressing. Data from Wright et al. could not be included because the SD could not be calculated (55). Pooling of data from the remaining seven studies (12,23,24,26,59,61,62) showed that there was significant difference in mean post operative endoscopic scores between the steroid group as compared to no steroid group (MD -0.39; 95% CI -0.60, m-0.17; P = 0.0004; Figure 5A). I2 was 0%, suggesting no significant heterogeneity ($\chi 2$ = 4.64, P = 0.59).

A subgroup analysis was performed to assess the results according mto the population group. Three studies reported data from mixed population, CRSwNP and CRSsNP (12,26,59), one study reported data from CRSsNP patients (61) whereas three other studies showed data from CRSwNP (23,24,62). No significant difference between steroid and no corticosteroids were found in the CRSsNP group (SMD 0.12; 95% CI - 0.52, 0.76; Figure 5A). Analysis of studies reporting on CRSwNP showed significant difference between steroid and no steroid groups (SMD -0.62; 95% CI -0.99, -0.24; P = 0.001; Figure 5A). I2 was 0%, suggesting no significant heterogeneity, ($\chi 2$ = 0.16, P = 0.92). Analysis of data from the mixed population group also showed significant difference between the steroid and no steroid groups (SMD -0.36; 95% CI -0.64, -0.08; P = 0.01; Figure 5A). I2 was 0%, suggesting no significant heterogeneity, ($\chi 2$ = 0.58, P = 0.75).

3.3 Risk of sinusitis

Risk of sinusitis as an adverse event associated with the use of corticosteroids was reported by four studies (12,52,54,60). Cote et al. (24) used triamcinolone impregnated packs, Bross-Sariano et al. (52) used fluticasone or beclomethasone spray, Rowe-Jones et al. (54) used fluticasone sprays, and Marple et al. (60) used mometasone furoate releasing stents. Pooling of the results showed no significant difference between use of corticosteroids and no corticosteroids (RR 0.64; 95% CI 0.32, 1.30; P = 0.22; Figure 5B). I2 was 0%, suggesting no significant heterogeneity ($\chi 2$ = 2.01, P = 0.57).

4. Recurrence risk

Risk of recurrence was reported by six studies (52,53,56,59,60,63). Bross- Sariano et al. (52) used fluticasone or beclomethasone spray, Dijkstra et al. (53) used fluticasone nasal sprays, Stjarne et al. (56) and Passali et al. (63) used mometasone furoate nasal sprays whereas Murr et al. (59) and Marple et al. (60) used mometasone furoate

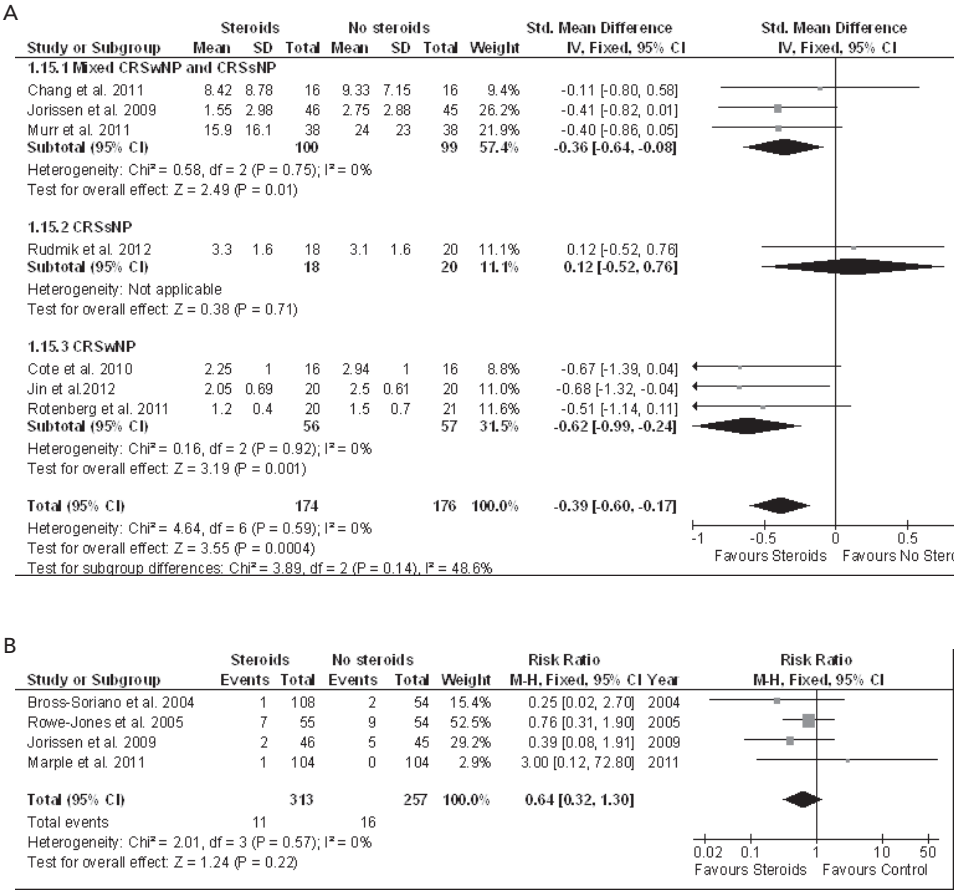

Figure 5. Forest plot of comparison – Post-operative outcomes (A) Forest plot of comparison: Steroids versus No steroids. Outcome: 3.2 Post operative endoscopic score. (B) Forest plot of comparison: Steroids versus No steroids. Outcome: 3.4 Risk of infection (Sinusitis).

eluding stents. Pooling of results of these studies showed no significant difference between use of corticosteroids and no corticosteroids (RR 0.72; 95% CI 0.48, 1.08; P = 0.11; Figure 6). I2 was 66%, suggesting significant heterogeneity ($\chi 2$ = 14.85, P = 0.01). A subgroup analysis was performed to assess the results according to the population group. Three studies reported data from mixed population, CRSwNP and CRSsNP (54,60,61) whereas three other studies showed data from CRSwNP (53,57,64). No significant difference between steroid and no corticosteroids were found in the mixed population group (RR 0.77; 95% CI 0.35, 1.70; P = 0.52; Figure 6). I2 was 71%, suggesting significant heterogeneity, ($\chi 2$ = 6.86, P = 0.03). Analysis of studies reporting on CRSwNP showed significant difference between steroid and no steroid groups (RR 0.64; 95% CI 0.45, 0.91; P = 0.01; Figure 6). I2 was 30%, suggesting no significant heterogeneity, ($\chi 2$ = 2.86, P = 0.24).

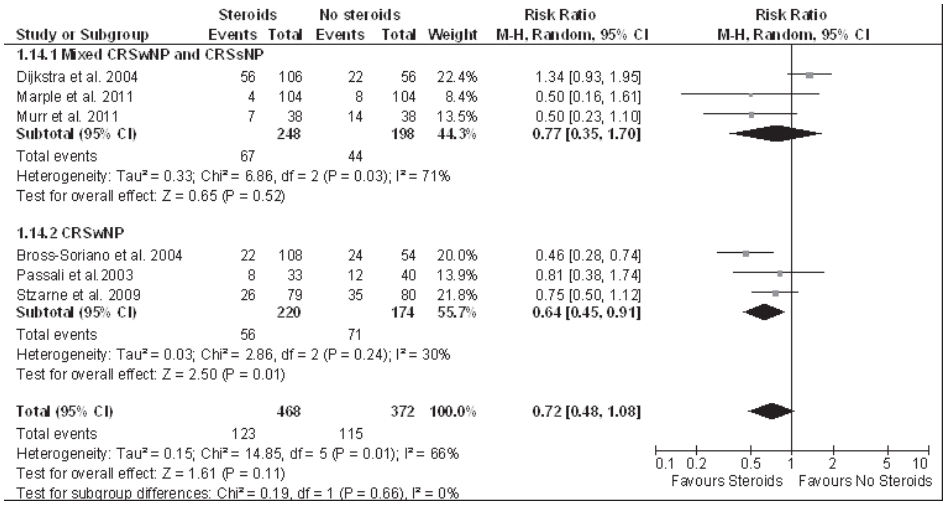

Figure 6. Forest plot of comparison-Recurrence Risk.

DISCUSSION

Principal findings of the review

This systematic review and meta-analysis of randomised controlled trials for operative outcomes demonstrated that operative time and estimated blood loss were significantly lower, and surgical field quality was significantly better in the local and/ or systemic steroid group compared to the non steroid group. These results were based on two studies, Albu et al. (14) used local steroids and Sieskiewicz et al. (13) used systemic steroids. In relation to anaesthetic outcomes in response to intra-operative corticosteroids there was no significant difference in post operative pain scores between the two groups. For post-operative outcomes in response to the corticosteroids there was no significant difference in symptom scores but endoscopic scores were better for the steroid group between the two groups. The use of corticosteroids was not associated with an increased risk of sinusitis. There was no significant difference in the recurrence risk between those given corticosteroids and controls in mixed population group, but subgroup analysis showed favourable results for steroid use in cases of CRSwNP.

Strengths of the review

CRS is an inflammatory disease and therefore, corticosteroids have long been utilized in its management due to their potent anti-inflammatory properties. Patients who fail to respond to medical therapy are considered for FESS. FESS differs from traditional, radical and less physiological drainage procedures as it restores mucociliary clearance pathways and ventilation by opening the osteomeatal complex and is customized to

disease extent. Corticosteroids have been indicated in FESS for various reasons. Our review included studies reporting use of corticosteroids on the operative outcome, anaesthetic related outcome, postoperative outcome and recurrence risk when used with FESS.

An important factor affecting the success of FESS is a clean surgical field (64). Poor endoscopic view secondary to bleeding is associated with increased operative time, complications and even cessation of surgery (64,65). Preoperative corticosteroid treatment has been proposed to minimise bleeding and improve surgical field (66,67). Corticosteroid reduce intra operative bleeding by not just their anti-inflammatory effect but also have a positive effect on regulation of vascular tone. Various mechanisms explaining this positive effect of corticosteroids on the vascular tone have been proposed (68). These include potentiation of action of other α adrenergic agonists like norepinephrine at the receptor level. Our meta-analysis for operative outcomes including operative time, EBL and surgical field quality showed significant benefit from the use of preoperative corticosteroids, both systemic (13) and topical (14). Even though these studies varied in definitions of CRS (CRSsNP and CRSwNP), timing and commencement of corticosteroids, and type, volume and route of administration of corticosteroids, the benefit was seen consistently in all three studies. Though we could not include the data from Wright et al. in our meta-analysis, these authors also concluded that patients who were not given pre-operative corticosteroids showed a higher percentage of severely inflamed mucosa and were associated with technically more difficult surgery (55).

Patients after FESS may experience pain which might prevent them from returning to normal daily activities (69). Corticosteroids due to their potent anti-inflammatory effect have been proposed in the management of acute surgical and postoperative pain control (16). In this respect one study was found to assess the outcome of intra-operative corticosteroid in reducing pain after FESS (58). This study did not show any benefit of using intraoperative steroid as a tool to reduce post operative pain.

Comparison with other studies

Due to the anti-inflammatory effects and excellent safety profile, topical nasal corticosteroids have become a common treatment modality for CRS (70). A previous systematic review on use of topical corticosteroids following FESS reporting a significant improvement in symptoms, endoscopic appearance and delay in polyp recurrence, recommended the use of nasal corticosteroids after FESS (70). However, these authors did not perform a meta-analysis and summarized their recommendations based on individual studies. Subgroup analysis from a Cochrane review (71) on use of corticosteroids in CRS based on two studies showed benefit of steroid on symptom scores who had sinus surgery (12,36). However, the study by Lavigne et al. had to be excluded from our study as it recruited patients with failed FESS, and therefore does not fulfil the inclusion criteria.

Recent EPOS 2012 systematic review on the role of corticosteroids in postoperative treatment for adults with CRS recommended, topical corticosteroids for patients with CRSsNP; and both topical and oral corticosteroids in patients with CRSwNP (4). This document, in a subgroup analysis showed that only patients with prior surgery for CRSsNP had symptom improvement but there was no improvement for those patients without surgery. Similarly, in CRSwNP, patients with sinus surgery responded to topical steroid greater than patients without sinus surgery in polyp size reduction but improvement in symptoms and nasal airflow was not statistically different between the two subgroups. The meta-analysis in the EPOS 2012 document incorporates studies which include patients who have had a history of sinus surgery including polypectomy. Whereas in our meta-analysis all patients underwent FESS. Our meta-analysis showed no significant benefit with the use of corticosteroids in post-operative symptom outcomes.

It has been postulated that, use of corticosteroids in the immediate post operative period may increase the risk of sinusitis (32). Our meta analysis from four studies which used local corticosteroids, showed that there was no evidence of increased risk of sinusitis with steroid use in postoperative period. We acknowledge that rare adverse events are possibly not detected in RCTs. However, they were extremely low and there was no difference in adverse events between the study groups and control groups in any trial.

Limitations of the review

Limitations of our systematic review include potential biasesin the review process regarding the eligibility criteria and data analyses. The inclusion of trials studying mixed populations of polyps and non-polyps patients possibly brings heterogeneity. We decided to include trials with mixed populations in patients with CRS with or without polyps, since this is in line with the definition of CRS by the European Position Paper 2012 (4). We also included four trial which used a paired intrapatient design, but treating the two groups as independent. Sensitivity analysis omitting these trials showed that the pooled results remained consistent. Trials required data imputation where standard deviations were missing and we conducted data imputation, as guided by the Cochrane Handbook for Systematic Reviews of Intervention (28). The majority of these studies were limited to small sample size and adopted different symptom and endoscopic scores. Clinical diversity, including variability in the agents used, dose, route, duration and the delivery methods, led to heterogeneity in the studies included in this review. We tried to overcome this risk of heterogeneity by doing a subgroup analysis where data was available but this was not possible to do in all comparisons. It is difficult to select between topical or oral steroid use in preoperative cases due to limited studies and data available for comparison. Although both mode of delivery showed better outcomes in the steroid group. Our review even though it had significant heterogeneity in some outcomes, has

attempted to bring the existing evidence together and represents the best evidence on this subject available.

Clinical implications of the review

Our systematic review and meta-analysis supports the use of pre-operative corticosteroids prior to FESS. Based on current existing evidence it statistically reduces operative time and blood loss and significantly improves surgical field quality. Whether this statistical difference reflects in clinical setting remains open to debate. Studies in relation to anaesthetic outcomes in response to intra-operative corticosteroids during FESS are limited with no significant benefit in post operative pain score and rescue analgesic requirement. More studies are required to assess the benefit of corticosteroids in this respect. Postoperative use of corticosteroids following FESS is not associated with any significant improvement in symptom scores but it is associated with better endoscopic scores in CRSwNP. Use of corticosteroids was not associated with increased risk of sinusitis, which is reassuring. There was no significant difference in the recurrence risk shown in mixed population studies of CRS, CRSwNP showed favourable results towards the steroid use. However, these results need to be interpreted with caution because these studies were limited to small sample sizes and adopted different symptom and endoscopic scores and reported a small number of bleeding, infection and recurrence events.

CONCLUSIONS

Preoperative use of local and/or systemic corticosteroids in FESS, results in significantly reduced blood loss, shorter operative time and improved surgical field quality. Studies are limited on intraoperative use of corticosteroids to reduce post operative pain. There is no significant benefit seen with the use of postoperative corticosteroids following FESS in improving symptom scores. Corticosteroids improve postoperative endoscopic scores. Risk of recurrence is reduced by postoperative corticosteroids in CRSwNP although this role is unclear in CRSsNP patients. Well conducted large RCTs are required using, standardised inclusion criteria, specified dose, duration and route of corticosteroids, validated subjective and objective outcome measures, including reporting on long term recurrence rates and complications.

KEY POINTS

Pre-operative use of local and systemic corticosteroids in FESS, results in significantly reduced blood loss, shorter operative time and improved surgical field quality.

Studies are limited on intra-operative use of corticosteroids to reduce post operative pain.

The limited data available do not point to significant benefit with the use of post-operative corticosteroids following FESS in improving symptom scores.

Corticosteroids improve postoperative endoscopic scores. Risk of recurrence is reduced by postoperative corticosteroids although this role is unclear in CRSsNP patients.

Well-conducted large RCTs are required using, standardised inclusion criteria; specified dose, duration and route of corticosteroids; validated subjective and objective outcome measures; including reporting on long term recurrence rates and complications.

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chapter 5

LONG-TERM RESULTS OF FUNCTIONAL ENDOSCOPIC SINUS SURGERY IN CHILDREN WITH CHRONIC RHINOSINUSITIS WITH NASAL POLYPS

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ABSTRACT

Background

Chronic rhinosinusitis with nasal polyps (CRSwNP) is rare in children and has a major impact on Quality of Life (QoL). Functional endoscopic sinus surgery (FESS) has proven to be an effective treatment, but it is still unclear what long term outcomes are in children with CRSwNP. The objective of this study was to assess long term results of FESS in children with CRSwNP.

Methods

We performed a combined prospective and retrospective study. A QoL questionnaire was send to all children with CRSwNP who received FESS between the year 2000-2010. Almost half of these children also filled in this questionnaire preoperatively.

Results

44 Children underwent FESS. From 18 patients we also prospectively collected preoperative QoL questionnaires. The mean follow-up period was 4.0 years (± 2.9). The mean age at surgery was 13 years (±2.9). Of these children 9 had CF (25%) and 10 children asthma (28%). R-SOM scores showed a significant improvement both in general symptoms as well as several different domains when comparing pre- and postoperative questionnaires (p=0.04). Only 14% (5) of the patients needed a subsequent intervention. In children with CF this was 33% (3/9).

Conclusion

This study demonstrates that long term results of FESS in children with CRSwNP are good. QoL has improved significantly, especially in nasal symptoms, showing that FESS is a good treatment in children with CRSwNP. Furthermore even children with CF show good results.

INTRODUCTION

CRS with nasal polyps (CRSwNP) is rare in children and has a major impact on the Quality of Life (QoL) of paediatric patients and their parents.(1) Because of the physical and psychological consequences of CRSwNP in children a thorough treatment is needed.

In adults with CRSwNP functional endoscopic sinus surgery (FESS) is considered to be the treatment of choice when maximum medical treatment fails.(2;3) Several studies have shown that most patients benefit from this approach and that there is a revision rate of 20%. (4;5) In children with CRSwNP on the other hand, surgical success rates are not known.

Until now there are some studies published describing the results of FESS in children, but they mainly focus on the results in children with CRS without nasal polyps (CRSsNP) and they report contradictory outcomes.(6-13) A meta-analysis performed by Hebert and Bent showed positive outcome in 88.7% of 832 children with CRSsNP who underwent FESS with an average follow up of 3.7 years. (8) Also several studies indicate that there is significant improvement in QoL after FESS in children with CRSsNP. (6;11) Besides, overall safety of FESS in children with CRSsNP has been established in some case series.(14;15)

Up to now, all studies on results of paediatric FESS included mainly children with CRSsNP. Only a small number of children that were included had CRSwNP. The largest study describes the results of FESS in 51 children with CRSwNP. (16) The main objective was to determine appropriate duration of postoperative evaluation and they concluded that it should be performed for 4 years up to an age of at least 12 years. Interestingly, they found that CT images at 1 year after surgery were rated as unchanged or worsened in approximately half of the patients. However, at 4 years after operation nearly all patients were rated as improved or better on CT images. A long term retrospective study was performed by Siedek et al. who focused on the prognostic factors in FESS.(7) In total 115 children were included and response rate was 64% (73/115). Of these 73 children with a mean age of 12 years, 39.1% had CRSwNP, 51.3% had CRSsNP, 5.2% had a maxillary cyst and 4.3% an antrochoanal polyp. They reported a 76% improvement at 5.4 years after FESS and several negative prognostic factors were found, including: cystic fibrosis, asthma, nasal polyps, allergies, previous surgery and smoking.

Furthermore it has been reported that the majority of children with CRSwNP has cystic fibrosis (CF). (17)

Even though several studies addressed positive outcomes after FESS in children with CF, usually length of follow-up is limited. (18-21) CF is still a chronic disease of mucociliary transport and even after successful surgery problems like infections or nasal polyps can recur. Because exact results of FESS in children with CF are not known, it can lead to a more negative attitude of surgeons to perform FESS in children with CF.

The main objective of this study was to assess long-term results of FESS in children with CRSwNP with and without CF and to determine outcome, symptoms, quality of life and complications.

MATERIAL AND METHODS

Patients

We included 44 children with bilateral CRSwNP (aged 6-18 years) undergoing FESS between the year 2000 and 2010 in the Department of Otorhinolaryngology of the Academic Medical Centre in Amsterdam, the Netherlands. Approval to sent questionnaires to the participants was obtained from the local ethical committee. Children with antrochoanal polyps or inverted papillomas were excluded from the study.

Study design

A QoL questionnaire was send to all (44) children who received FESS because of CRSwNP. Herefore we used the Rhinosinusitis Outcome Measure (RSOM-31). Almost half (18) of these children also filled in this questionnaire preoperatively. We first performed a prospective analysis in which we compared the preoperative results to the postoperative results to look at possible improvement after FESS in children.

Secondly we performed a retrospective analysis of the QoL at long term follow up after FESS in all children. We calculated the mean post-operative R-SOM score for the whole group and when analyzing these findings we compared results of children with CF or asthma to children without CF or asthma.

Furthermore we distinguished 4 subgroups according to mean R-SOM score points to make the analysis easier. Group I (0-1 points) consisted of patients with no or little symptoms, group II (2 points) little to moderate symptoms, group III (3 points) moderate to severe symptoms and group IV (4 points) severe to extreme symptoms. Besides this we separately analysed the specific nasal domain of the R-SOM score, consisting of the first 6 questions.

Moreover we performed a retrospective analysis of medical records of the children, including: age, sex, medical history, allergies, asthma, CF (sweattest and/or genetic tests), smoking, presenting symptoms, use of medication (steroids), CT scan, type of surgery, prior operations, complications and need for revision surgery.

Surgery performed

FESS was performed in all children after failure of optimal medical treatment consisting minimally of topical corticosteroids and saline irrigation for at least three months and sometimes also including oral antibiotics and systemic corticosteroids. All children had a preoperative CT scan. The extent of the surgery was tailored to the extent of the disease and always consisted of infundibulotomy and partial ethmoidectomy and sometimes included more extended ethmoidectomy and/or sphenoidectomy. In

most cases a microdebrider was used during surgery.(22) Frontal sinus surgery was not performed in this patient group.

All operative details and complications were recorded. Postoperatively patients received topical corticosteroid treatment and saline irrigation. Furthermore we recorded the need for revision surgery.

Postal questionnaires

Questionnaires were filled in preoperatively by part of the patients (18/44). Most of the time the patients were old enough to fill out the questionnaires themselves, but sometimes there was assistance needed from the parents and/or the physician. The same questionnaires were sent out to all 44 patients postoperatively and consisted of several items. We measured quality of life (QoL) with the RSOM-31 questionnaire. (23) The RSOM-31 assesses 6 nasal symptoms (congestions, rhinorrhea, sneezing, hyposmia, postnasal discharge and thick nasal debris) and 25 other symptoms summarized in several domains: non-nasal, sleep-disorders, emotional symptoms, practical problems and general symptoms with a symptom score from 1-5. Furthermore we asked every patient the question how nasal symptoms are now compared to before surgery on a 5 point scale (1=much better, 5=much worse).

Statistical analysis

Statistical analysis was performed in SPSS and a p value ≤ 0.05 was considered significant. We compared the pre- and postoperative scores using the Mann-Whitney U test. In the retrospective analysis RSOM-31 scores were compared using the paired *t*-test if there was a normal distribution or otherwise the Mann-Whitney U test.

RESULTS

Patient characteristics

In total 44 children underwent FESS due to CRSwNP. From 18 of these patients we prospectively collected preoperative QoL questionnaires. Response rate of postoperative questionnaires was 82% (36 out of 44) and these 36 children (16 boys, 20 girls) constitute the final study group. Mean follow-up period of the whole group was 4.0 years (1-12 years). The follow-up period in children with CRWwNP without CF was 3.0 years (1-9 years) and in children with CF 6.0 years (3-12 years). Mean age at time of surgery for the whole group was 13 years old (± 2.9).

Main preoperative symptoms were anosmia (100%), nasal congestion (95%) and rhinorrhea (83%). About half of the children experienced headache (49%) and cough (44%).

Of these 36 children who underwent FESS because of CRSwNP, 9 children had CF (25%) and 10 children asthma (28%). Other predisposing factors are shown were allergy (25%) which was tested using a skin prick test, smoking (6%) and aspirine intolerance (3%).

Outcome of FESS

Eighty-six percent of the patients had a positive outcome after FESS. In total 14 % (5) of the children needed a revision at time of follow up. Of the children with CF 3/9 (33 %) had a revision and of the children without CF only 2/27 (7%). Revision surgery in children with CF was performed at 2, 3 and 7 years after primary surgery and in children without CF 2 and 4 years later. Peri-operatively no complications occurred.

Postoperatively of all the patients 66.7% (24/36) still uses intranasal steroids at time of follow-up.

Prospective results (n=18)

R-SOM scores showed a significant improvement both in general symptoms as well as several different domains when comparing pre- and postoperative questionnaires. In total 77% of the patients showed improvement in RSOM scores at long term follow-up.

Mean total pre-operative RSOM score was 43.4 and mean total postoperative score in was 31.3 (p=0.04). There was no significant difference in outcome between male and female patients. If we look at CF patients in this group (n=4), we could not find a difference in reduction of total RSOM score because the number of patients was too small.

Furthermore when analyzing the total RSOM scores of the different domains, nasal symptoms (p<0.01), sleep (p=0.04) and practical functioning (p=0.004) showed significant improvement. The total mean RSOM scores and domain scores are shown in table 1. In figure 1 RSOM scores (n=18) are compared.

Furthermore it was found that from the subset of nasal specific RSOM scores (6 parameters) 4 showed a significant improvement (p<0.05) in the whole group: blocked nose, rhinorrhea, anosmia and post nasal drip (PND). Of children with CF the same parameters improved. (Fig.2)

Table 1. Mean pre- and postoperative RSOM scores compared (n=18)

	Preoperative score mean (±SD)	Postoperative score mean (±SD)	95% Confidence Interval		Sig. (p-value)
Total score	43.4 (18.4)	31.3 (21.8)	-23.6	-0.5	0.04
Mean Item score	1.4 (0.6)	1.0 (0.7)	-0.7	-0.1	0.04
Nasal item score	2.8 (0.6)	1.7 (1.2)	0.7	1.5	0.0001
Eye item score	0.6 (1.0)	0.4 (0.8)	-0.5	0.7	0.73
Sleep item score	2.1 (1.8)	1.1 (1.1)	0.01	1.9	0.04
Ear item score	0.7 (0.9)	0.3 (0.7)	-0.03	0.8	0.07
General symptoms	1.4 (1.0)	1.1 (0.9)	-0.3	0.8	0.30
Practical problems	1.9 (1.2)	1.0 (1.2)	0.3	1.5	0.004
Emotional consequences	1.2 (1.3)	0.7 (1.1)	-0.3	1.3	0.21

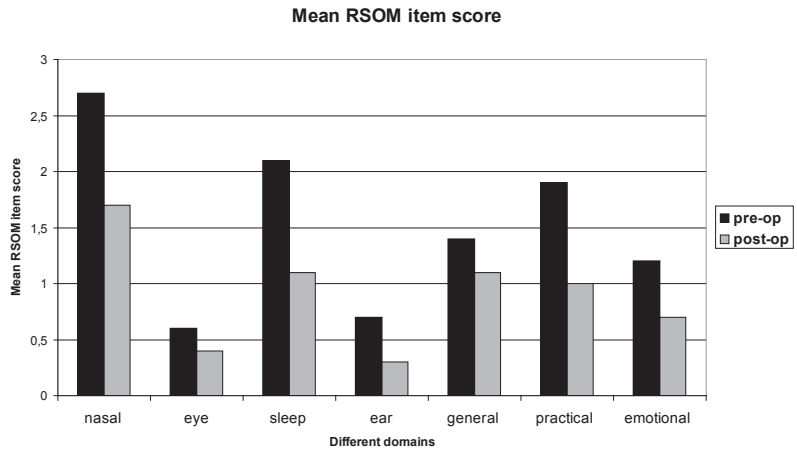


Figure 1. Changes in different domains of pre- and postoperative RSOM item scores (n=18).

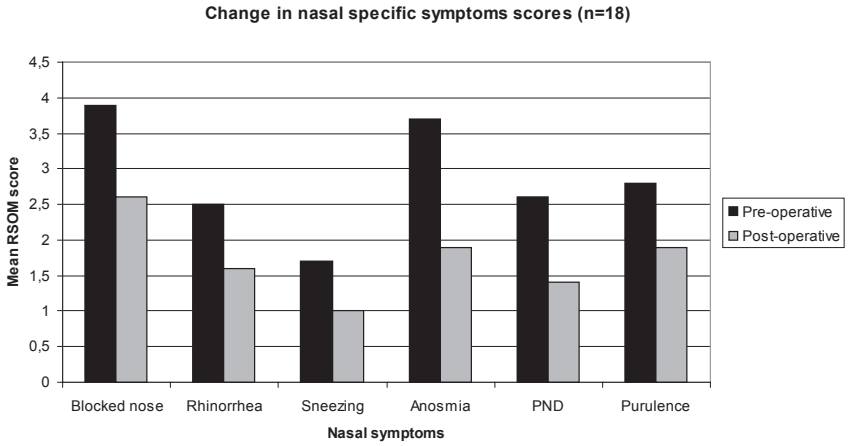


Figure 2. Change in nasal specific symptom scores (R-SOM) analysed in different groups (n=18).

Retrospective results: whole group after FESS (n=36)

Using a 5 point scale, a total of 78% of the patients reported overall improvement after FESS (score 1-2). In the CF group 75% of patients reported that their overall nasal symptoms are much better. There are only 2 patients with CF who reported the nasal symptoms being the same as before surgery and none of them reports the symptoms being worse. (Fig.3)

The mean RSOM item score of the whole postoperative patient group (n=36) was 1.16 (SD± 0.87). The mean postoperative RSOM item score of the prospective group (n=18) was 1.01 (SD±0.70). For the children with CF (n=9) the mean post-operative

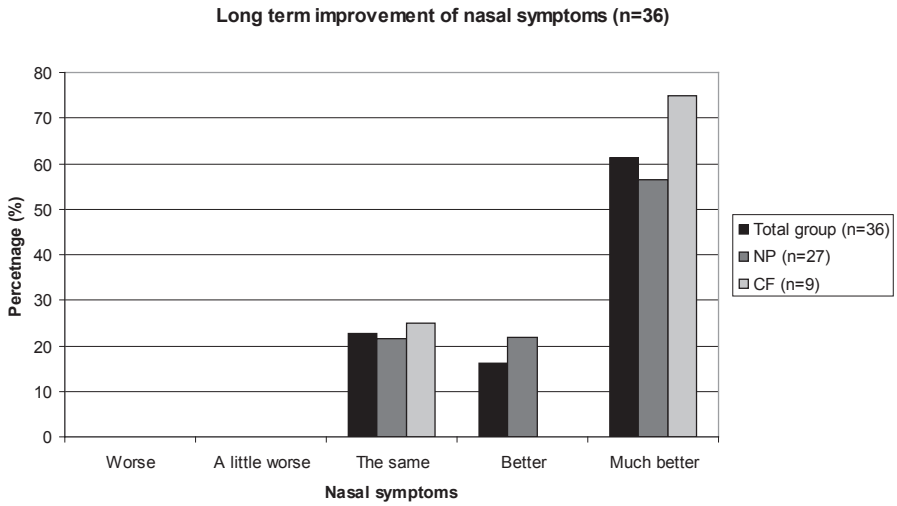


Figure 3. Long term improvement of nasal symptoms by self assessment after FESS (n=36).

R-SOM score was 0.91 (SD± 0.99) and children without CF (n=27) 1.25 (SD±0.83). There was no significant difference between these postoperative outcomes (p=0.35).

Furthermore when analyzing the difference in outcome of mean nasal item RSOM score between children with and without asthma, we find a significant better score in children without asthma (mean item score=1.5) than in children with asthma (mean item score=2.3) (p=0.048).

If we look at the separate subgroups we created, 67% of the whole group belonged to subgroup I (no-little symptoms) and 25 % to subgroup II (little- moderate symptoms). Only 8% had moderate to severe symptoms (subgroup III) and 0% was in subgroup IV (severe to extreme symptoms). (Tbl.2 and Fig.4 A)

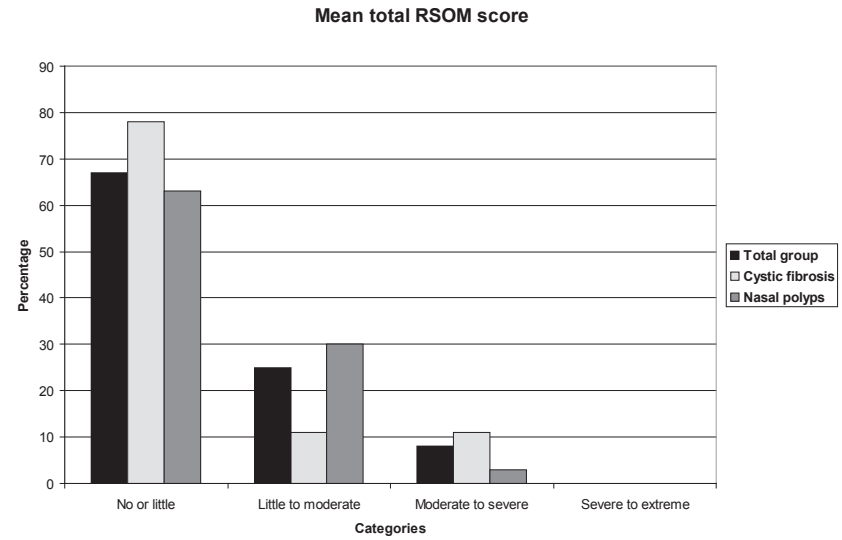
The children with CF showed very good results compared to the whole group at follow-up. Out of the 8 children with CF, 7 (78%) were postoperatively in the group with no or little nasal symptoms.

These are the results of the specific nasal postoperative RSOM scores. (Tbl 3+Fig4 B)

Table 2. Mean postoperative RSOM scores graded in four subgroups (n=36)

Group	Mean RSOM score	Level of symptoms	All children (n=36)	CF (n=9)	NP (n=27)
I	0 and 1	No or little	24 (67%)	7 (78%)	17 (63%)
II	2	Little to moderate	9 (25 %)	1 (11%)	8 (30%)
III	3	Moderate to severe	3 (8%)	1 (11%)	3 (7%)
IV	4 and 5	Severe to extreme	0	0	0

A



B

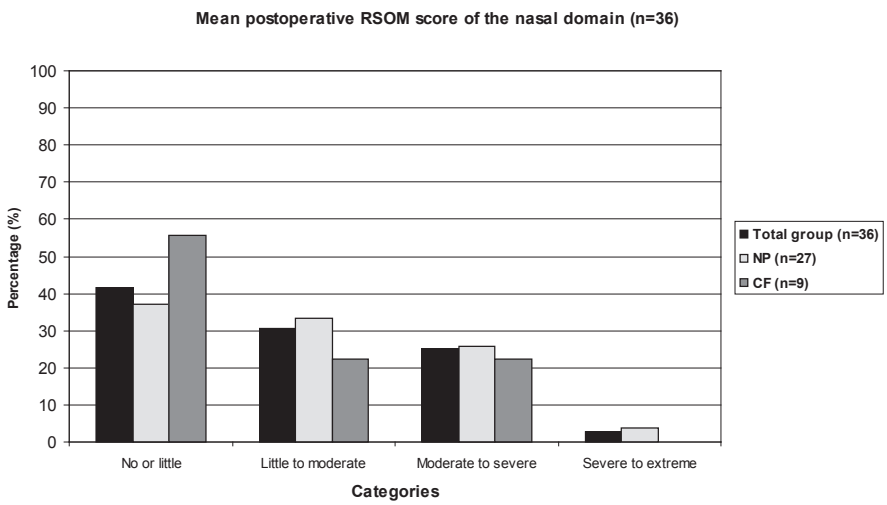


Figure 4. (A) Mean postoperative total R-SOM score (n=36). (B) Mean postoperative R-SOM score of the nasal domain (n=36).

Table 3. Mean postoperative RSOM scores of the nasal domain (n=36)

Group	Mean nasal RSOM score	Level of symptoms	All children (n=36)	CF (n=9)	NP (n=27)
I	0 and 1	No or little	15 (42%)	5 (56%)	10 (37%)
II	2	Little to moderate	11 (30%)	2 (22%)	9 (33%)
III	3	Moderate to severe	9 (25%)	2 (22%)	7 (26%)
IV	4 and 5	Severe to extreme	1 (3%)	0	1 (4%)

DISCUSSION

CRSwNP has a severe impact on QoL of adults and paediatric patients and can be difficult to treat.(1) The consensus is that surgical intervention should be considered in paediatric patients with CRSwNP when maximum medical therapy has failed. (9) Interestingly the data on paediatric FESS in patients with CRSwNP are very limited. This study shows that FESS is a very effective and safe treatment in children with CRSwNP.

Our study shows significant improvement in QoL at long term follow up after FESS in children with CRSwNP. In total 78% of the patients reported an overall improvement in RSOM score over time.

In our prospective analysis RSOM scores showed significant improvement both in general symptoms as well as several different domains. Comparable results were found using the postoperative RSOM questionnaire showing that around 75% of the children with CRSwNP were in the group with no or little or little to moderate symptoms at long term follow-up. Moreover from the subset of nasal specific RSOM scores, 4 out of 6 parameters showed significant improvement ($p<0.05$) postoperatively. These results are comparable to the study from Siedek et al.(7)

Furthermore if we look at re-intervention rate, there was a revision surgery rate of 14% (5) in the whole group. We found that CF is a predictor for revision surgery. In the group of children with CF 3 out of 9 CF patients (33 %) needed another operation after primary FESS and of the children without CF only 2 out of 27 (7%). This means that CF might be a predictor for revision surgery and these children should be carefully monitored by an ENT specialist.

In contrary to common beliefs, in our group of children with CRSwNP who underwent FESS was only a small percentage of children with CF (25%). We see that children with CF, although they have more recurrences, have good improvement of symptoms after FESS comparable to the rest of the group. Out of 9 children with CF, 6 (78%) were postoperatively in the group with no or little nasal symptoms. Because our measurements were subjective using questionnaires, there could be a bias in answers from children with CF. It could be that the standard of health in a child with CF is generally different than in 'non' CF-children. This could mean that nasal symptoms in children with CF can seem less important to them compared to their general health and therefore they might fill in a lower score on the RSOM questionnaire.

Asthma is commonly associated with CRSwNP in the paediatric population and therefore may influence FESS outcomes. Prevalence of asthma in children with CRSwNP is much higher than in the normal population. In our study the prevalence of asthma was 28% compared to around 10% in normal childhood population.(24;25)

When analyzing our results we found a difference in outcome between children with and without asthma. Mean postoperative nasal RSOM score in asthma patients was significantly higher than in children without asthma ($p=0.048$). However, there

is lack of good randomized controlled studies describing the relationship between asthma in children and CRSwNP.

The strength of our study is the long follow-up period of approximately 4 years and the partly prospectively collected data. A limitation of this study is that not all data are prospectively collected. We only have preoperative questionnaires from half the patient group. Besides that our outcomes of surgery were measured by subjective questionnaires, not physical examination and therefore could be biased. Besides that, also other factors that have not been assessed in this study, like socioeconomic factors and patient expectations, could have played a role. Nevertheless, the promising results in this study show benefits of FESS for the treatment of children with CRSwNP even in children with CF.

CONCLUSION

We can conclude that long term results of FESS in children with CRSwNP are good. Overall QoL has improved significantly for the whole group, especially in the domain of nasal symptoms, showing that FESS is also a very good treatment in children with CRSwNP. Furthermore even children with CF show good results at long term follow-up, besides the fact that 33% of the children with CF needed revision surgery in the long term. In literature until now was very little evidence at all about the efficacy of FESS specifically in children with CRSwNP, therefore we advice that more prospective studies should be performed to see if our results of FESS in children with CRSwNP are justified.

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chapter 6

NOVEL ROLES FOR NASAL EPITHELIUM IN THE PATHOGENESIS OF CHRONIC RHINOSINUSITIS WITH NASAL POLYPS

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Submitted

ABSTRACT

Background

Airway epithelial cells have a well-accepted role in the regulation of local inflammatory processes in allergic and innate defence responses. However, their role in the pathophysiology of chronic rhinosinusitis with nasal polyps (CRSwNP) is unclear.

Objective

To investigate whether potential differences in the mRNA expression profile of nasal epithelia from healthy individuals and from CRSwNP patients would shed new light on disease mechanisms.

Methods

Primary epithelial cells from nasal polyps of 24 affected individuals and from middle turbinates of 9 healthy controls were obtained using magnetic beat assisted isolation and were used for expression profiling using the Human Genome U133 Plus 2.0 Genechip Array.

Results

Multiple gene probes corresponding to 27 genes showed an aberrant expression profile in polyp epithelial cells compared to healthy controls. Most of these genes are linked to pathogenic mechanisms seen in neoplasm formation, including changes in cell-cell adhesion, metabolic processes, cell cycle control, and differentiation. Remarkably, our data additionally suggest a role for maternally expressed genes in the pathogenesis of CRSwNP and reveal two distinct states of polyp epithelium that could not be linked to the presence or absence of atopy in patients or to the level of eosinophilia or neutrophilia of the polyp.

Conclusions

Our data suggest new roles for nasal epithelium in the pathogenesis of CRSwNP.

INTRODUCTION

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a multifactorial chronic inflammatory disease that is characterized by neoplasms in the nasal cavity that most often originate from the paranasal sinuses (1). It is a relative common disease with a prevalence of 4-5% in the general population with typical symptoms comprising nasal congestion, rhinorrhea, hyposmia, and facial pressure. Although the aetiology of CRSwNP is largely unknown, in people with asthma prevalence goes up to 6-15% and nasal polyps also share typical histological features. Polyps seem to resemble the bronchial mucosa of asthmatic patients with epithelial damage, goblet cell hyperplasia, thickening of the basement membrane, accumulations of extracellular matrix, fibrosis, and eosinophil-dominated inflammation (2). Given the active contribution of epithelium to the regulation of local inflammatory responses (3) in this manuscript we have explored the potential contribution of polyp epithelium to the pathogenesis of CRSwNP using micro-array expression profiling.

The potential contribution of nasal epithelium to the pathophysiology of CRSwNP has not been extensively explored, although expression of toll-like receptors and other innate immune response factors is well documented (4). In allergic airway disease there is an established awareness of the role of epithelial cells as an active participant in the regulation of local immune responses (5,6). Epithelial cells are able to detect and respond to environmental signals through a wide variety of receptors, while epithelial integrity is still considered an important aspect in maintaining local homeostasis (7). In addition to epithelial involvement in CRSwNP, it has been suggested that the interaction between nasal epithelium and myofibroblasts in CRSwNP could resemble the interaction between bronchial epithelium and smooth muscle cells (8). In asthma the mutual and reciprocal activation within this epithelial mesenchymal trophic unit is thought to contribute to the pathophysiology of the disease. Indeed this link may help to explain the increased prevalence of CRswNP in asthma patients (1). Other processes through which epithelium may directly or indirectly affect CRSwNP would be in the interaction with bacterial biofilms and orchestrations of (innate) immune responses (6).

Previously, we have investigated the role of nasal epithelium in allergic rhinitis to investigate intrinsic differences in expression profile that could contribute to the pathology of disease (9). Using similar approach in this study we identified affected genes in CRSwNP related to epithelial integrity, neoplasm formation, and glucose metabolism. Moreover we detected a novel epithelial dichotomy in CRSwNP that is not related to atopic status or eosinophilic versus neutrophilic inflammation level of the polyp.

METHODS

Study design

This study was reviewed and approved by the medical ethical committee of the Academic Medical Center (06/062) and all participants signed informed consent.

We included 24 CRSwNP patients and 9 healthy controls (Table 1) that were generally healthy and did not have any auto-immune disorders or other relevant comorbidities (e.g. aspirine intolerance) that could affect outcome measures. EP^{3}OS criteria were used for inclusion of CRSwNP patients, while ARIA and GINA guidelines were used to establish allergic and asthma status. Atopy status was determined using the recommended GA^{2}LEN panel of the 20 most common aeroallergens. The CRSwNP patients were operated because of severe symptoms of their disease while controls had pituitary adenomas requiring endoscopic surgery and none of the participants had used steroids four weeks prior to surgery.

Primary epithelial cell culture

Primary cells were obtained by digesting nasal biopsies or nasal polyps from the participants with 0.5 mg/mL collagenase 4 (Worthington Biochemical Corp., Lakewood, NJ, USA) for 1 hour in Hanks' balanced salt solution (Sigma-Aldrich, Zwijndrecht, the Netherlands). Epithelial cells were isolated using an anti-EpCAM microbead assisted cell isolation procedure (Miltenyi Biotec, Bergisch Gladbach, Germany) and grown in T25 cultured with BEGM in fully humidified air containing 5% CO2 at 37°C to 80% confluence within 2 weeks.

RNA extraction

Total RNA from each sample was extracted using Trizol (Life Technologies, Inc., Gaitersburg, MD, USA), followed by purification by nucleospin RNA II (Machery-Nagel, Düren, Germany). The RNA concentration was measured on the nanodrop ND-1000 (NanoDrop Technologies inc., Wilmington, DE, USA) and RNA quality was checked on the Agilent 2100 bio-analyzer (Agilent Technologies, Palo Alto, CA, USA).

Microarray data analysis and statistics

Human Genome U133 Plus 2.0 Genechip Array (Affymetrix inc., Santa Clara, CA, USA) was used in the analysis of the different expression patterns in diseased and healthy nasal epithelium. Technical handling of microarray experiments was performed at the MicroArray Department (MAD) of the University of Amsterdam and array images were acquired using a GeneChip Scanner 3000 (Affymetrix) and analyzed with Affymetrix GeneChip® Operating Software (Affymetrix).

Table 1. Demographics

	Total number	Allergy	Asthma	Aspirin intolerance	Mean age (years)	Gender (M/F)
Healthy	9	0	0	0	54	7/2
CRSwNP	24	10	12	0	45	15/9

The images and raw data passed manufacturers recommended quality criteria. GeneSpring v13 (Agilent technologies, Santa Clara, CA, USA) expression console was used to extract expression values, perform statistic testing, and analyse data. Expression levels were calculated using robust multi-array average (RMA) algorithm. Differences in expression were determined using unpaired statistical *t* tests with correction for multiple testing with a false discovery rate of 0.05 using the Westfall-Young procedure.

Immunohistochemistry

Section of snap frozen biopsies were stained for eosinophils (Clone BMK13 at 0.05 µg/mL, Monosan, Uden, the Netherlands) and neutrophils (Elastase at 2.2 µg/mL, DACO, Glastrup, Denmark) using Brightvision (Immunologic, Duiven, the Netherlands) as per manufacturer's instructions. All sections were examined by two independent observers blinded to the experimental conditions. The numbers of positively stained cells were counted in the lamina propria (per mm^2) at a final magnification of 200x. Statistical significance was determined with the Mann-Whitney U-test.

RESULTS

Deregulated expression of epithelial cancer related genes dominate the intrinsic differences between epithelia of healthy controls and CRSwNP patients

In total we identified 35 probe sets with a statistical significant different expression pattern (Table 2) between the concha epithelium of healthy individuals and the epithelium covering nasal polyps of patient with CRSwNP. These probe sets correspond to 27 different genes as the genes for *calbindin 1* (CALB1), *cyclin-dependent kinase inhibitor 1C* (CDKN1C), *iodothyronine deiodinase 2* (DIO2), *keratin 10* (KRT10), *lipopolysaccharide-induced TNF factor* (LITAF) and *junctional adhesion molecule 3* (JAM3) are represented by multiple probes. The fold change for the probes belonging to each individual gene showed a conserved change of expression. The probes for CALB1 (-4.08 and -2.64 fold), JAM3 (-2.62 and -1.42 fold), KRT10 (-1.70 fold), and DIO2 (-1.43 and -1.51 fold) are all down-regulated in polyposis versus healthy controls, whereas in contrast the 4 probes representing CDKN1C (+1.58, +1.79, +1.83, and +1.96 fold) and the 2 probes representing LITAF (+1.33 and +1.37 fold) where all up-regulated. A selection of genes was used to validate the microarray expression data by correlating the expression level detected in the microarray with the expression level of an independent real time PCR of random samples. Indeed table 3 shows a high level of correspondence in both data sets.

Eighteen of 27 deregulated genes form a shortest connection network (Figure 1). The most prominent of these genes is the maternally expressed tumour suppressor gene CDKN1C that previously has been linked to colon polyp formation (10). Indeed,

Table 2. Expression differences between epithelium from CRSwNP patients and healthy controls showing probe identity number (ID), fold change (FC), gene name, and chromosome location. N/A refers to not assigned probes

Probe ID	Gene	FC	Gene name	Location
230835_at	KRTDAP	-7.49	keratinocyte differentiation-associated protein	chr19q13.12
206642_at	DSG1	-5.56	desmoglein 1	chr18q12.1
205625_s_at	CALB1	-4.08	calbindin 1, 28kDa	chr8q21.3
206004_at	TGM3	-4.05	transglutaminase 3	chr20q11.2
220225_at	IRX4	-3.21	iroquois homeobox 4	chr5p15.3
214536_at	SLURP1	-2.79	secreted LY6/PLAUR domain containing 1	chr8q24.3
205626_s_at	CALB1	-2.64	calbindin 1, 28kDa	chr8q21.3
212813_at	JAM3	-2.63	junctional adhesion molecule 3	chr11q25
221328_at	CLDN17	-2.56	claudin 17	chr21q22.11
231733_at	CARD18	-2.43	caspase recruitment domain family, member 18	chr11q22.3
212915_at	PDZRN3	-1.87	PDZ domain containing ring finger 3	chr3p13
217564_s_at	CPS1	-1.86	carbamoyl-phosphate synthase 1, mitochondrial	chr2q35
205637_s_at	SH3GL3	-1.84	SH3-domain GRB2-like 3	chr15q24
212730_at	SYNM	-1.83	synemin, intermediate filament protein	chr15q26.3
231148_at	IGFL2	-1.81	IGF-like family member 2	chr19q13.32
238022_at	CRNDE	-1.79	colorectal neoplasia differentially expressed	chr16q12.2
207023_x_at	KRT10	-1.70	keratin 10	chr17q21
213287_s_at	KRT10	-1.70	keratin 10	chr17q21
231240_at	DIO2	-1.51	deiodinase, iodothyronine, type II	chr14q24.2
242157_at	CHD9	-1.49	chromodomain helicase DNA binding protein 9	chr16q12.2
1555773_at	BPIFC	-1.46	BPI fold containing family C	chr22q12.3
227491_at	ELOVL6	-1.45	ELOVL fatty acid elongase 6	chr4q25
203700_s_at	DIO2	-1.43	deiodinase, iodothyronine, type II	chr14q24.2-3
231720_s_at	JAM3	-1.42	junctional adhesion molecule 3	chr11q25
244722_at	N/A	1.27	Not Assigned	N/A
232417_x_at	ZDHHC11	1.28	zinc finger, DHHC-type containing 11	chr5p15.33

Table 2. (continued)

Probe ID	Gene	FC	Gene name	Location
200704_at	LITAF	1.33	lipopolysaccharide-induced TNF factor	chr16p13.13
200706_s_at	LITAF	1.37	lipopolysaccharide-induced TNF factor	chr16p13.13
238348_x_at	N/A	1.42	Not Assigned	N/A
202755_s_at	GPC1	1.44	glypican 1	chr2q35-37
209522_s_at	CRAT	1.56	carnitine O-acetyltransferase	chr9q34.1
216894_x_at	CDKN1C	1.58	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	chr11p15.5
213182_x_at	CDKN1C	1.79	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	chr11p15.5
219534_x_at	CDKN1C	1.83	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	chr11p15.5
226403_at	TMC4	1.84	transmembrane channel-like 4	chr19q13.42
206088_at	LRRC37A3	1.90	leucine rich repeat containing 37, member A3	chr17q24.1
213348_at	CDKN1C	1.96	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	chr11p15.5
228742_at	N/A	1.98	Not Assigned	N/A

a substantial number of the affected genes have been linked to epithelial neoplasms and related biological processes. The deregulated expression of KRTDAP (*keratinocyte differentiation-associated protein*, -7.49 fold), DSG1 (*desmoglein 1*, -5.56 fold), TGM3 (*transglutaminase 3*, -4.05 fold), CLDN17 (*claudin 17*, -2.56 fold), JAM3 (-2.03 fold), SYNM (*synemin*, -1.83 fold), and KRT10 (keratin 10, -1.70 fold) shows changes in cell adhesion/ ultra-structural complexes (11-17). The deregulation of ELOVL6 (*Elongation of Very Long Chain Fatty Acid Elongase 6*, -1.45 fold), CRAT (*Carnitine acetyltransferase*, +1.56 fold),

Table 3. Correlation coefficients showing high correspondence between microarray and RT-PCR expression levels

Gene	Correlation	p-value
CALB1	0.900	0.019
TGM3	0.913	0.015
DSG1	0.972	0.003
JAM3	0.946	0.008
SLURP1	0.988	0.001
KRTDAP	0.989	0.001

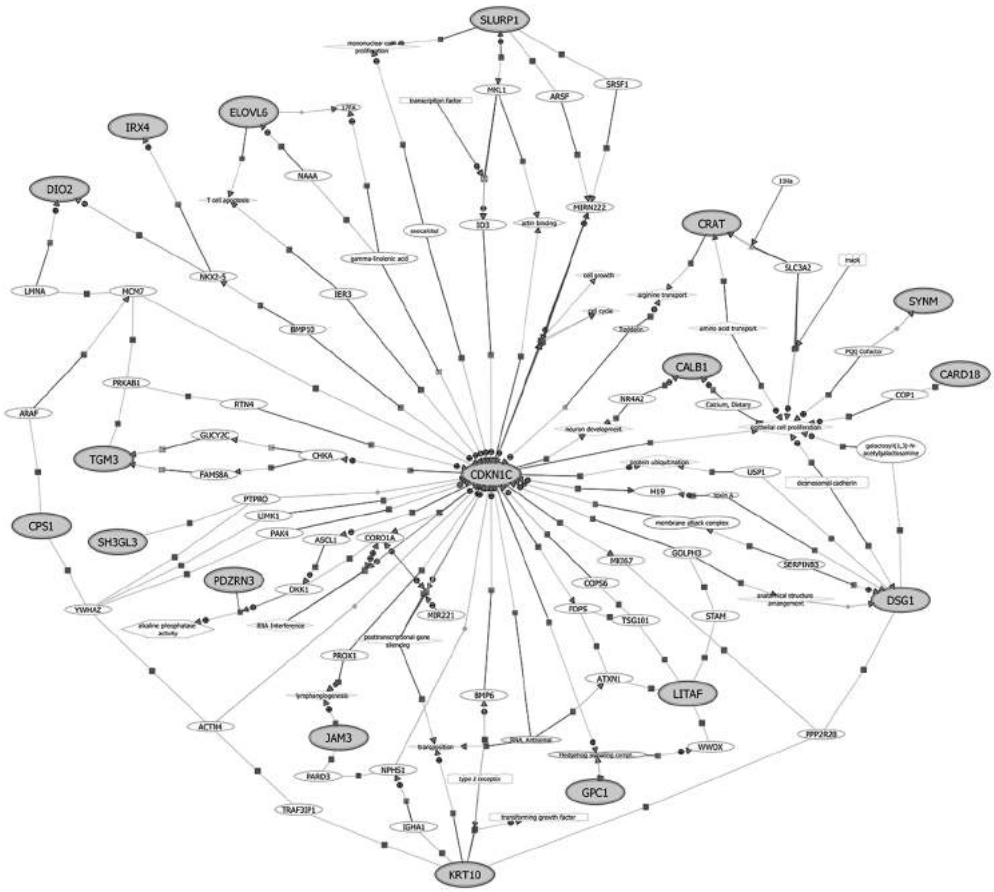

Figure 1. Shortest connect network of genes that are differentially expressed between healthy epithelium and polyp epithelium.

CRNDE (*colorectal neoplasia differentially expressed*, -1.79 fold), IGFL2 (*IGF-like family member 2*, -1.81 fold) point towards changes in insulin, glucose, and lipid metabolism (18-21). Other proteins within our deregulated gene set that also have been previously reported to be affected in epithelial cancers include CHD9 (*chromodomain helicase DNA binding protein 9, -1.49 fold*), IRX4 (*iroquois homeobox 4*, -3.21 fold), SLURP1 (*secreted LY6/PLAUR domain containing 1*, -2.79 fold), CARD18 (*caspase recruitment domain family member 18*, -2.43 fold), CPS1 (*mitochondrial carbamoyl-phosphate synthase 1*, -1.86), DIO2 (-1.47 fold), and GPC1 (*glypican 1*, +1.44 fold) (22-28).

Two distinct epithelial expression profiles in nasal polyps

A more detailed representation using hierarchical clustering shows individual expression levels of 35 probes in healthy turbinates and nasal polyps. In addition

to the overall differences of up- and down-regulated genes, this analysis suggests that epithelium of nasal polyps can be divided into two clusters as suggested by the tree cluster structure for the different polyps (Figure 2). The differences between the two expression clusters in CRSwNP seem largely dominated, but not uniquely, by expression of KRTDAP that has previously been reported to be involved in squamous cell differentiation and stratification of epithelia (11).

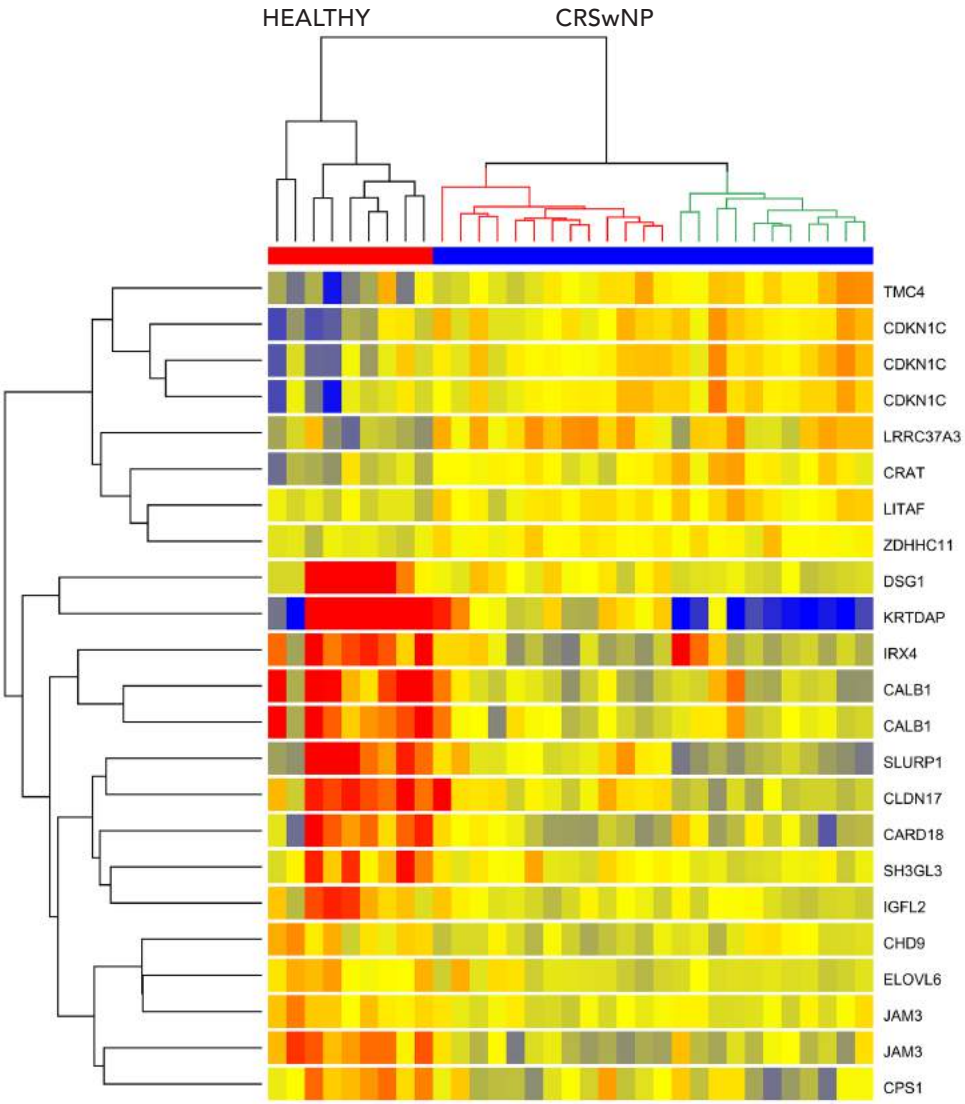

Figure 2. Hierarchical clustering (for probes on the left hand side and for individuals on the top) of the differentially expressed genes between healthy epithelium (black bar) and polyps epithelium (grey bar) showing relative expression (high in red and low in blue). Within the CRSwNP group the two sub-clusters are indicated by the red en green colouring of the dendrogram.

To explore the differences between these potential groups of polyps we compared the expression profiles between these two groups. Indeed, in addition to the differences suggested by expression profiles of KRTDAP, the direct comparison of expression profiles of both groups of polyps reveals 77 probes that correspond to 68 genes that are differentially expressed (Table 4A/4B). The expression profile strengthens the notion of two types of differentiated epithelia, as in addition to KRTDAP we now also detect differential expression of the structural protein CNFN (*cornifelin*), SPRR1A (*small proline-rich protein 1A*), and SBSN (*suprabasin*) that together with HES5 (*hairy and enhancer of split 5*), RRAD (*Ras-related associated with diabetes*), SDR9C7 (*short chain dehydrogenase/reductase 9C7*, ASCL2 (*achaete-scute complex homolog 2*), GLTP (*glycolipid transfer protein*), S100A8 (*S100 calcium binding protein A8*), SAG (*S-antigen*), NR3C2 (*nuclear receptor subfamily 3, member C2*), BIRC3 (*baculoviral IAP repeat containing 3*), GAD1 (*glutamate decarboxylase 1*), ATP7B (*ATPase 7B*), TSPAN1 (*tetraspanin 1*), and NEDD9 (*neural expressed, developmental down-regulated 9*) are typically deregulated in squamous cell epithelial carcinoma's (29-44).

Gene set enrichment analysis furthermore identifies deregulated expression of TNFSF12-13 (*TNF superfamily, member 12-13*), TNFRSF10C (*TNF receptor superfamily, member 10C*), and BIRC3 to TWEAK- (wikipathway 2036, p=0.0018) and TRAIL-mediated apoptosis (wikipathway 1772, p=0.0065), as important differences between the two types of epithelia. When we further examine the genes that are most affected we note deregulation of LYNX1 (*Ly-6/neurotoxin-like protein 1* or *SLURP2*) and SLC44A4 (*solute carrier family 44, member 4* or CTL4) that, like the previously identified SLURP1, are involved in acetylcholine-mediated neurogenic inflammation (45,46). These two processes (apoptosis and neurogenic inflammation) are indeed often affected in different forms of carcinomas. Their relevance is further supported by representatives of these processes that feature in a shortest connect pathway of all affected genes (Figure 3) centred around S100A8 and IFI44 (*interferon-induced protein 44*). The differences in expression of these genes and processes, however, are not related to differences in level of inflammation (Figure 4) for eosinophils ($p = 0.143$) or neutrophils ($p = 0.126$), nor in prevalence of atopic comorbidities ($p = 0.616$) between the two polyp groups.

DISCUSSION

In this manuscript we provide a detailed overview of intrinsic expression differences between CRSwNP epithelium and healthy control epithelium. The outcome shows that most of these differences are related to changes in ultra-structural organisation, differentiation state, and processes linked to (epithelial) neoplasm formation. In addition to these processes, the data also identified an acetylcholine-centred inflammatory process as potential pathological mechanism in CRSwNP and also suggest a role for epigenetical expression regulation. Moreover, at the epithelial level CRSwNP polyps seem to divide into two groups that are not linked to the presence or absence of allergy or the level of inflammation (Figure 4).

Table 4 A and B. Expression differences between two types epithelium from nasal polyps showing probe identity number (ID), fold change (FC), gene name, and chromosome location. N/A refers to not assigned probes

Table 4 A.

Probe ID	FC	Gene	Gene name	Location
219975_x_at	-3.28	OLAH	oleoyl-ACP hydrolase	chr10p13
233126_s_at	-3.09	OLAH	oleoyl-ACP hydrolase	chr10p13
222945_x_at	-2.72	OLAH	oleoyl-ACP hydrolase	chr10p13
205597_at	-2.72	SLC44A4	solute carrier family 44, member 4	chr6p21.3
225496_s_at	-2.53	SYTL2	synaptotagmin-like 2	chr11q14
221011_s_at	-2.47	LBH	limb bud and heart development	chr2p23.1
1555203_s_at	-2.43	SLC44A4	solute carrier family 44, member 4	chr6p21.3
221523_s_at	-2.42	RRAGD	Ras-related GTP binding D	chr6q15-q16
232914_s_at	-2.32	SYTL2	synaptotagmin-like 2	chr11q14
203892_at	-2.30	WFDC2	WAP four-disulfide core domain 2	chr20q13.12
211163_s_at	-2.28	TNFRSF10C	TNF receptor superfamily, member 10c	chr8p22-p21
214453_s_at	-2.23	IFI44	interferon-induced protein 44	chr1p31.1
218885_s_at	-2.19	GALNT12	N-acetylgalactosaminyltransferase 12	chr9q22.33
206222_at	-2.16	TNFRSF10C	TNF receptor superfamily, member 10c	chr8p22-p21
221524_s_at	-2.10	RRAGD	Ras-related GTP binding D	chr6q15-q16
223551_at	-2.04	PKIB	protein kinase inhibitor beta	chr6q22.31
223423_at	-1.97	GPR160	G protein-coupled receptor 160	chr3q26.2-q27
235911_at	-1.95	MFI2	melanoma associated antigen p97	chr3q28-q29
205259_at	-1.88	NR3C2	nuclear receptor subfamily 3, member C2	chr4q31.1
210538_s_at	-1.74	BIRC3	baculoviral IAP repeat containing 3	chr11q22
212503_s_at	-1.72	DIP2C	DIP2 disco-interacting protein 2 homolog C	chr10p15.3
212686_at	-1.71	PPM1H	protein phosphatase, Mg/Mn dependent 1H	chr12q14.1
205278_at	-1.70	GAD1	glutamate decarboxylase 1	chr2q31
223784_at	-1.70	TMEM27	transmembrane protein 27	chrXp22

Table 4 A. (continued)

Probe ID	FC	Gene	Gene name	Location
234689_at	-1.69	PTCHD4	patched domain containing 4	chr6p12.3
227909_at	-1.62	LINC086-87	long intergenic non-protein coding RNA 86-87	chrXq26.3
204671_s_at	-1.60	ANKRD6	ankyrin repeat domain 6	chr6q14.2-q16.1
204624_at	-1.59	ATP7B	ATPase, Cu++ transporting, beta polypeptide	chr13q14.3
202161_at	-1.56	PKN1	protein kinase N1	chr19p13.12
209114_at	-1.56	TSPAN1	tetraspanin 1	chr1p34.1
209499_x_at	-1.55	TNFSF12-13	TNF superfamily, member 12-13	chr17p13-p13.1
242931_at	-1.55	LONRF3	LON peptidase N-term domain and ring finger 3	chrXq24
214667_s_at	-1.54	TP53I11	tumor protein p53 inducible protein 11	chr11p11.2
202150_s_at	-1.53	NEDD9	neural expressed, developmental down-regul. 9	chr6p25-p24
207949_s_at	-1.49	ICA1	islet cell autoantigen 1, 69kDa	chr7p22
203332_s_at	-1.49	INPP5D	inositol polyphosphate-5-phosphatase	chr2q37.1
212325_at	-1.49	LIMCH1	LIM and calponin homology domains 1	chr4p13
236656_s_at	-1.47	N/A	uncharacterized LOC100288911	chr2p22.3
244486_at	-1.47	N/A	Not Assigned	N/A
209500_x_at	-1.42	TNFSF12-13	TNF superfamily, member 12-13	chr17p13-p13.1
225548_at	-1.38	SHROOM3	shroom family member 3	chr4q21.1
204276_at	-1.38	TK2	thymidine kinase 2, mitochondrial	chr16q22-23
205298_s_at	-1.38	BTN2A2	butyrophilin, subfamily 2, member A2	chr6p22.1
219952_s_at	-1.37	MCOLN1	mucolipin 1	chr19p13.2
229253_at	-1.36	THEM4	thioesterase superfamily member 4	chr1q21
213045_at	-1.34	MAST3	microtubule associated ser/thr kinase 3	chr19p13.11
212745_s_at	-1.31	BBS4	Bardet-Biedl syndrome 4	chr15q22.3-q23
241353_s_at	-1.29	N/A	Not Assigned	N/A
224998_at	-1.28	CMTM4	CKLF-like MARVEL domain containing 4	chr16q21-q22.1
209166_s_at	-1.28	MAN2B1	mannosidase, alpha, class 2B, member 1	chr19cen-q13.1

Table 4 B.

Probe ID	FC	Gene	Gene name	Location
230835_at	3.78	KRTDAP	keratinocyte differentiation-associated protein	chr19q13.12
1554179_s_at	3.45	LYNX1	Ly6/neurotoxin 1	chr8q24.3
203691_at	2.72	PI3	peptidase inhibitor 3, skin-derived	chr20q13.12
224329_s_at	2.70	CNFN	cornifelin	chr19q13.2
41469_at	2.57	PI3	peptidase inhibitor 3, skin-derived	chr20q13.12
235272_at	2.33	SBSN	suprabasin	chr19q13.13
1554253_a_at	2.27	CERS3	ceramide synthase 3	chr15q26.3
204803_s_at	2.18	RRAD	Ras-related associated with diabetes	chr16q22
206517_at	2.08	CDH16	cadherin 16, KSP-cadherin	chr16q22.1
204802_at	2.07	RRAD	Ras-related associated with diabetes	chr16q22
1553077_at	1.94	SDR9C7	short chain dehydrogenase/ reductase 9C 7	chr12q13.3
213796_at	1.91	SPRR1A	small proline-rich protein 1A	chr1q21-22
214536_at	1.79	SLURP1	secreted LY6/PLAUR domain containing 1	chr8q24.3
214549_x_at	1.75	SPRR1A	small proline-rich protein 1A	chr1q21-22
1570005_at	1.58	N/A	Not Assigned	N/A
227854_at	1.50	N/A	Not Assigned	N/A
229215_at	1.49	ASCL2	achaete-scute complex homolog 2	chr11p15.5
226177_at	1.48	GLTP	glycolipid transfer protein	chr12q24.11
243388_at	1.48	N/A	Not Assigned	N/A
202917_s_at	1.48	S100A8	S100 calcium binding protein A8	chr1q21
239230_at	1.48	HES5	hairy and enhancer of split 5 (Drosophila)	chr1p36.32
1555019_at	1.46	CDHR1	cadherin-related family member 1	chr10q23.1
211076_x_at	1.37	ATN1	atrophin 1	chr12p13.31
1564658_at	1.33	NAT16	N-acetyltransferase 16 (GCN5-related, putative)	chr7q22.1
206671_at	1.26	SAG	S-antigen; retina and pineal gland (arrestin)	chr2q37.1
234761_at	1.25	N/A	Not Assigned	N/A
1556753_s_at	1.23	N/A	Not Assigned	N/A
1554044_a_at	1.21	MRAP	melanocortin 2 receptor accessory protein	chr21q22.1

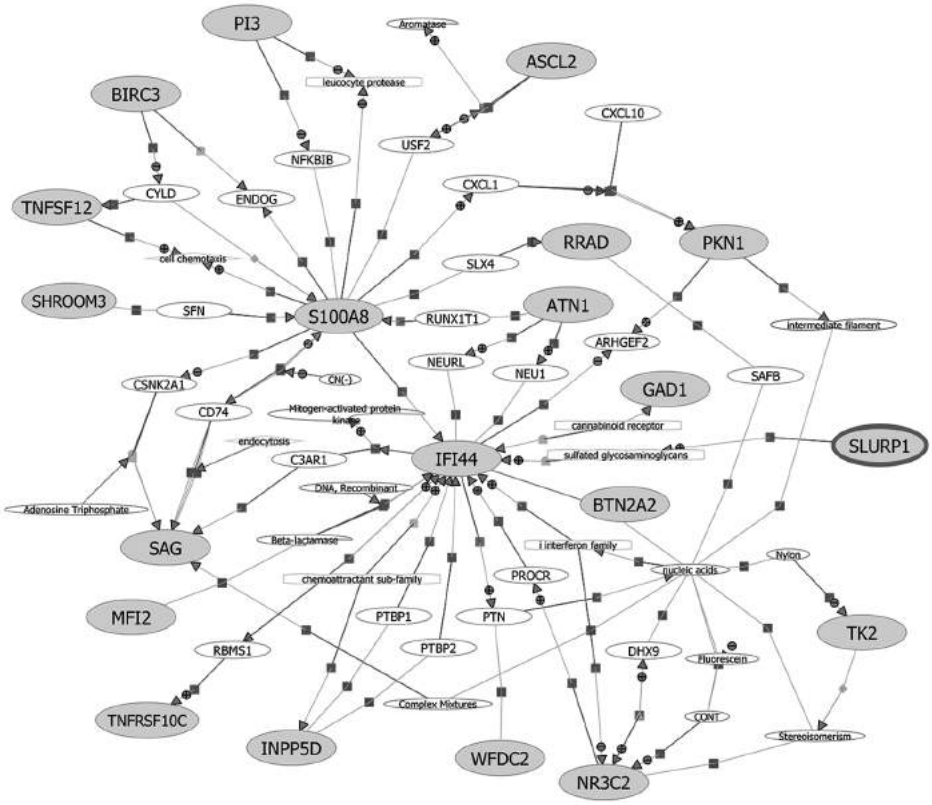

Figure 3. Shortest connect network of genes that are differentially expressed between the two types of polyp epithelium.

Pathological concepts in CRSwNP

The deregulated expression of DSG1, JAM3, TGM3, SYNM, and CLDN17 in our *ex vivo* isolated and cultured CRSwNP primary nasal epithelial cells suggests that the epithelial integrity defects seen *in vivo* are not solely a consequence of the diseased state in CRSwNP, but also reflects cell intrinsic differences in the expression of proteins related to cell-cell contacts. In allergic disease intrinsic barrier dysfunction is thought to directly contribute to the pathophysiology, as it would facilitate access of allergen. To what extend the changes in adhesion/tight junction proteins play a direct role in the pathology of CRSwNP remains to be explored. On one hand changes in the composition of these adhesion structures could affect their functionality or these changes are part of a compensatory mechanism that tries to counteract the negative effect local inflammatory processes may have on epithelial integrity. On the other hand the loose epithelial structure may in contrast even help to drain local inflammation and mitigates its effect.

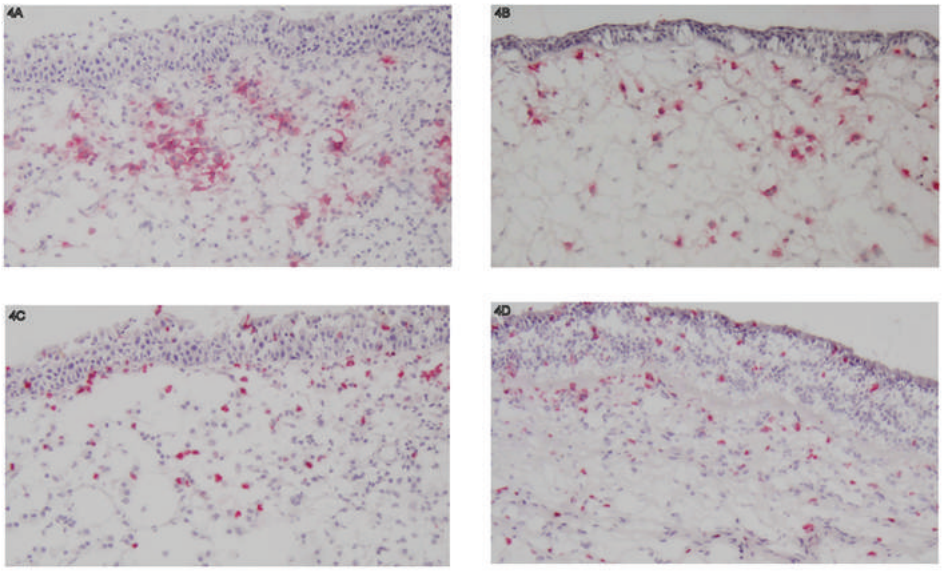

Figure 4. Representative immunohistochemical photographs showing similar levels of eosinophils (A and B) and neutrophils (C and D) between the two groups of CRSwNP patients (A and C versus B and D).

Although the nasal polyps we have studied in this manuscript are not malignant per se, it is perhaps not surprising that we see deregulated genes in our analysis that are involved in neoplasm formation. In addition to the changes in adhesion molecules genes we have discussed earlier, ELOVL6, CHD9, ASCL2, CRNDE, IGFL2, IRX4, SLURP1, CARD18, CPS1, DIO2, GPC1 and CDKN1C have been linked to cellular transformation, mostly in carcinomas (10,18-20,22-28). That some of these genes are found in malignant tumours does not contradict the largely benign nature of CRSwNP, as the distinction between benign and malignant does not rely on the activation of growth or cell cycle related genes per se, but more on the ease of migration of cancer cells away from the primary site of induction or infiltration into lower tissue levels. An additional aspect that is shared between different cancers are changes in glucose utilisation. In this so called Warburg effect, the Krebs cycle is partly shut down to facilitate de novo synthesis of aminoacids, nucleotides, and lipids (47). Indeed, a substantial number of the deregulated genes (ELOVL6, DIO2, IGFL2, CALB1) have been linked to insulin signalling and CRNDE is even considered to be a regulator of the Warburg effect itself (20,48-51).

The most interesting of the deregulated genes might well be the maternally expressed tumour suppressor gene CDKN1C. Deregulated expression of this gene has been linked to multiple forms of cancer, and mutations in this gene underlie Beckwith-Wiedemann Syndrome (10). In addition to the many growth and mental

defects in affected patients the syndrome also includes abundant polyp formation in the colon, the urinary tract, or occasionally in the oral cavity (52-54). The reason for these pleiotropic effects of CDKN1C deregulation is that this protein normally negatively regulates cell proliferation by inhibiting cyclin and cyclin-depedent-kinase complexes in the G1 phase of the cell cycle (55). This central function also seems reflected in the shortest connect network we have observed for the genes that are deregulated in the epithelia of nasal polyps where CDKN1C is seen to connect these genes (Figure 1). Growth regulatory genes often are associated with imprinted or epigenetic forms of transcription regulation and this also applies to CDKN1C (55). The gene copy is only transcribed from the maternally inherited chromosome in a dedicated locus on chromosome 11 (chr11p15.5) that is home to the archetypes of epigenetically regulated and growth affecting gene IGF2 (*insulin growth factor 2*) and the non-coding RNA H19 (55). Indeed also the deregulated gene ASCL2 (*achaete-scute complex homolog 2*) is located in this region and also this gene has been reported to be imprinted and affected in intestinal neoplasms (56). Importantly, the possible involvement of pathological processes regulated by imprinted genes will affect interpretation of genomic studies that investigate CRSwNP as such studies will only consider the presence or absence of specific SNPs (single nucleotide polymorphisms) and not whether or not the genes associated with the SNPs are expressed.

Neurogenic inflammation

Although neurogenic inflammation has been studied in relationship to inflammation and neoplasm formation, it has not been extensively considered in CRSwNP. Neurogenic inflammation centres around acetylcholine that traditionally has been investigated in the interaction of neurons with down-stream targets like smooth muscle cells, macrophages, and other inflammatory cells (57). Recently it has become clear that also epithelial cells produce and respond to acetylcholine and that this could play a role in neoplasm formation (58). In this respect it was remarkable that we were initially able to detect a deregulated expression of SLURP1 in the epithelia of CRSwNP patients. SLURP1 acts as a potential negative regulator of acetylcholine receptor mediated signalling due to its high affinity for this receptor. Indeed, this epithelial form of neurogenic inflammation is also under investigation as a basis for the epithelial transformation induced by nicotine contained within cigarette smoke, as nicotine is a ligand for the acetylcholine receptor. Interestingly, SLURP1 can also mitigate the effects of nicotine by again preventing activation of the receptor (68). We should stress that none of our patients or healthy volunteers are smokers/have smoked but these observation should be taken as an indication that neurogenic inflammation can have a profound effect on neoplasm formation. When we compared the expression profiles of both types of nasal polyps we noted that also LYNX1 (or SLURP2 as it was previously known as) and SLC44A4 highlight the link of CRSwNP with neurogenic inflammation. LYNX1 belongs to the same family of proteins and

has a similar function as SLURP1. Indeed, both these soluble mediators are able to bind to the acetylcholine receptor, whereas SLC44A4 is a acetylcholine transporter that seems specific for acetylcholine secretion (46,59). The precise role of epithelial-centred neurogenic inflammation is not yet fully established. One aspect seems to focus on signalling between adjacent epithelial cells and inflammatory cells in the nasal cavity (58). However given the potential to influence mucus glands and smooth muscle cells in the lower airways it might well be that this mechanism could be responsible for activation of mucus production and of smooth muscle actin-positive myo-fibroblasts in nasal polyps and it this way provides a mechanistic link with asthma remodelling (8). It should be noted that mutations in CFTR (cystic fibrosis transmembrane conductance regulator), the gene affected in cystic fibrosis also affect neurogenic acetylcholine signalling, which might even offer a possible explanation for the frequent occurrence of nasal polyps in cystic fibrosis patients (60).

The link between epithelium and neuronal processes we have observed in our data set may be part of a more general mechanism. Activation of the TRPV1 receptor on neurons by capsaicin could be part of the mechanism by which capsaicin is able to suppress symptoms in idiopathic rhinitis and CRSwNP (61,62). However the recently described deregulated expression of this receptor on epithelial cells of asthmatics suggests a potential involvement of an epithelial centred pathway. As TRPV1/capsaicin also effects acetylcholine signalling it would be interesting to explore this link further (63).

Additional considerations

Even though the CRSwNP patients included in our study display a mix of varying allergic and/or asthmatic co-morbidities, none of the epithelial deregulated genes show a direct link to allergy. This suggests that at least from an epithelial perspective, allergy is an epiphenomenon and that the epithelial differences we have previously observed in allergic rhinitis do not play a common role in CRSwNP (9).

Cause or effect remains a difficult issue when trying to interpret the differences between the epithelia of CRSwNP patients and health controls. In active disease the nasal epithelium will be exposed to many inflammatory triggers that could affect the expression profile. While on one hand these effects could play an important role in the pathological conditions of CRSwNP, on the other hand these differences could just point to innocent bystander effects. We have tried to mitigate some of these aspects by limited culturing of epithelial cells rather than analysing the expression profile *in situ* or directly after isolation. Although, this approach also allows us to obtain a pure epithelial population, the culture procedure itself may have undocumented effects on the expression profile, and furthermore these effects may differ between diseased and healthy epithelium. The advantage of this approach of being able to look at stably transmitted differences induced by CRSwNP in airway epithelial cells, we feel would outweigh these disadvantages. We are somewhat reassured that the deregulated genes

we have identified can be linked to processes that previously have been hypothesized to have some role in the pathophysiology of the disease (1). The re-affirmation of older hypotheses also gives credence to observations that seem to point to a potential involvement of neurogenic inflammation in the pathophysiology of CRSwNP.

Our expression data furthermore shows that at epithelial level nasal polyps could come in two distinct groups. If true, this would have large implications for the interpretation of any genetic study that has tried to investigate CRSwNP and perhaps these studies should be revisited with this new insight. Also other clues suggest that correct interpretation of genome wide association studies might be hampered, as also epigenetical mechanisms could be part of the pathogenesis of CRSwNP. This conclusion stems from the observation that a major deregulated gene (CDKN1C) is expressed only from the maternal gene copy and not from the paternal copy (55).

The differences in epithelial expression profile come from our specific patient population. All of our CRSwNP patients have recurrent disease, despite optimal medical treatment, have had multiple previous surgeries, and would be as far removed from primary polyposis as can be imagined. Although these patients with recurrent disease need our help the most, it may well be that the initial changes that would lead to the formation of nasal polyps could be different from the differences we have discussed here.

In conclusion, our data identified differences in expression profile between epithelia of CRSwNP patients and that of healthy controls. Although these differences might not necessarily be the cause of the disease, they do point towards new processes that could play a role in the pathophysiology and open new avenues for future research.

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chapter 7

CHRONIC RHINOSINUSITIS WITH NASAL POLYPS APPEARS TO BE A "SELF-LIMITING DISEASE" LASTING APPROXIMATELY ELEVEN YEARS

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ABSTRACT

Not much is known about the influence of age and time on the results of sinus surgery (FESS) for chronic rhinosinusitis with nasal polyps (CRSwNP). We therefore used FESS as an objective sign of active disease and measured time between first and last surgical interventions in a follow-up of 10 years. We determined relation between age, the total number of times of sinus surgery and age at time of the first operation ever and calculated the mean interval time between first and last operation. We found no relation between age and total number of surgical procedures. When we compared age at time of the first surgery to age at time of last surgery, we found a mean time interval of 11.1 years. We can conclude that the total number of sinus surgery procedures in patients' lifetimes seems to be independent of age. We think that CRSwNP is a self-limiting disease.

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a chronic disease with a prevalence of 4-5% that results in high costs for society as a whole, mainly because of the need for repeated surgical interventions (1, 2). The prevalence of CRSwNP seems to increase with age, with the highest prevalence rate being seen in about the sixth decade of life (3, 4). We were not able to find any data about the natural course of the disease. We therefore used sinus surgery (FESS) as an objective sign of active disease and measured the time between the first and the last surgical interventions in a follow-up of 10 years.

In this study we sent a questionnaire to all adult patients who received FESS for CRSwNP in the AMC in Amsterdam between 2000 and 2005. We recorded their medical histories, including their complete sinonasal history with number of FESS procedures performed.

Questionnaires were returned by 151 out of 225 patients (103 men, 48 women; response rate 67%). The mean total number of sinus surgery procedures during the patients' lifetimes was 3.4 (SD±3.0). At the time of the questionnaire the mean time after the last surgery was 9.9 years (SD± 3.4).

When we compared the age at the time of the first surgery to the age at time of last surgery, we found a linear relationship with a mean time interval of 11.1 years (SD± 11.1, range 0-52 years), a positive Pearson correlation of r = 0.69 (p= 0.01) and a 95% Confidence Interval of 9.32-12.90. This means that there would seem to be a standard active disease duration.

The table below shows the interval between the first and last procedures as compared with patient age at the time of the questionnaire (Figure 1). We find no statistical differences between the age groups (p=0.66).

Our study shows for the first time that the active disease duration based on the need for FESS in patients with CRSwNP is relatively constant at about 11 years, regardless of the age of onset. We hope this finding will hearten all CRSwNP patients requiring revision surgery and asking their doctors whether this awful disease will ever stop.

The limitations of our study were our decision to adopt the need for FESS as a sign of objective disease. Ideally, these results would be compared with nasal symptom scores and nasal endoscopy. Hopkins et al. showed recently that delayed surgical intervention for CRSwNP is associated with a higher need for post-operative health care than when patients undergo FESS in the first 12 months after diagnosis (5). If we combine this finding with our results, we are justified in informing our patients that CRSwNP is a self-limiting disease but that early surgical intervention would seem to improve quality of life during the period when they are suffering from the disease.

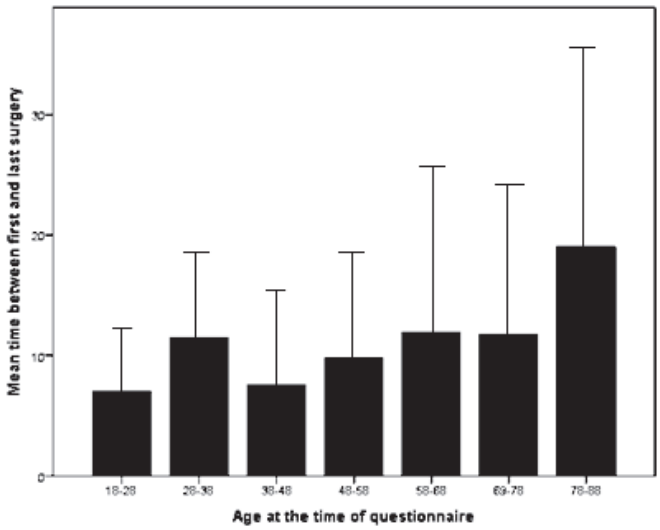

Figure 1. Interval between the first and last procedures as compared with patient age at the time of the questionnaire.

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chapter 8

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

General discussion and future perspectives

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a chronic disease which has a significant negative impact on the quality of life (QoL) and brings along high costs because of high medical resource usage and high societal costs (1, 2). Despite decades of research on the pathophysiology and treatment of CRSwNP, still a group of patients remain symptomatic under current state-of-the-art medical and surgical treatment (3). In order to keep improving our treatment for patients with CRSwNP, we need to critically asses our current therapeutic options and gain more insight in the pathophysiology of CRSwNP. This thesis has investigated multiple aspects of this serious disease to try and better the life of our patients.

Nasal polyposis during a lifetime

The prevalence of CRSwNP is estimated around 10% in Europe and the United States, however it is difficult to estimate because of the need for endoscopic evaluation (2, 4). Suggested is that the prevalence of CRSwNP increases with age with the highest prevalence rate seen around the sixth decade of life and the lowest prevalence up to 40 years old (5, 6). In children CRSwNP is rare and is thought to be associated with cystic fibrosis (CF). We were not able to identify any data about the natural course of the disease and it was always thought that CRSwNP is a lifetime chronic disease. CRSwNP can be treated and controlled with medication and surgery, however there is still a revision rate of around 20% in adults and surgical success rates in children with CRSwNP are not exactly known (3, 7). Therefore a frequently asked question of patients with recalcitrant CRSwNP is if and when their disease will finally stop. Therefore we wanted to know if CRSwNP has a standard active period of disease duration and extinguishes by itself.

In chapter 7 we showed for the first time that the active disease duration of CRSwNP in patients is relatively constant at about eleven years, regardless of the age of onset. This was determined based on the need for FESS as an objective measurement of active disease and could be a first indication that CRSwNP is a self-limiting disease.

Many studies have looked at prognostic factors for failure of FESS, and it is thought that age could influence the objective outcomes of FESS, showing better endoscopic outcomes in elderly patients (8). These results could be influenced by our findings that the activity of CRSwNP decreases when patients get older. A strength of our study is the wide variety of different ages at time of surgery, thereby presenting the results of a very representative mixed group. A limitation of this study was our decision to adopt the need for FESS as a sign of active disease. Ideally, these results would be compared with nasal symptom scores and nasal endoscopy scores before and after surgery. Another potential limitation could be the tertiary setting which could be a group of more severe patients with potential a worse outcome. A recent study also in tertiary care showed that 40% of the patients were uncontrolled and only 20% really were

controlled (9). Furthermore, our results should be validated in the future in a larger cohort of patients with CRSwNP.

Recently Hopkins et al. showed that delayed surgical intervention for CRSwNP is associated with a higher need for postoperative care than when patients undergo FESS in the first 12 months after diagnosis (10). Combining this knowledge to our findings, in the future it would be very interesting to try to indicate the ideal timing of surgery in comparison to optimal medical treatment in patients with CRSwNP. Thereby our study group designed a randomized-controlled trial to investigate whether two regularly applied treatment strategies, namely FESS in addition to drug treatment or drug treatment alone, differ in generic and disease-specific QoL and to establish the presumed superiority of FESS. Also a comparison to cost-effectiveness will be made (11). Hereby we hope to find out which patients exactly to operate and when, to improve the outcome of FESS and reduce recurrence rates.

In children CRSwNP has a severe impact on the quality of life of both children and their parents and can be very difficult to treat (12). The consensus is that surgical intervention should be considered in paediatric patients with CRSwNP when maximum medical therapy has failed (13).

In **chapter 5** we showed that FESS is a very effective and safe treatment in children with CRSwNP even in children with CF. Overall QoL has significantly improved in 78% of the patients at long-term follow-up, especially in the domain of nasal symptoms. In total 14% of the children needed a revision and there were no complications.

Interestingly until now data on paediatric FESS in children with CRSwNP are very limited. There are a few studies describing result of FESS in children, but they mainly focus on CRSsNP and report contradictory outcomes (14-16).

Our study shows significant improvement in QoL at long-term follow-up after FESS in children with CRSwNP. These result are comparable to the study from Siedek et al which consisted of 59 (51.3%) children with CRSsNP and 45 (39.1%) of children with CRSwNP (14).

Asthma is commonly associated with CRSwNP in the paediatric population and therefore may influence FESS outcomes. The prevalence of asthma is much higher in children with CRSwNP than in the normal population. In our study the prevalence of asthma was 28% compared to 10% in normal childhood population (17). When analyzing our results we detected a difference in outcome between children with and without asthma. Mean postoperative nasal RSOM score in asthma patients was significantly higher than in children without asthma ($p=0.048$).

Furthermore in our group of children with CRSwNP who underwent FESS, there was only a small percentage with CF (25%). This was in contrast to an earlier report were the majority of the children with CRSwNP has cystic fibrosis (18). This could mean that the percentage of children with CRSwNP who also have CF, might be lower than we previously thought. We demonstrated in chapter 2 that CF is a predictor for revision surgery. In total 33% of the children with CF needed revision surgery and only 7% of

the children without CF. However even though children with CF have more recurrences, long-term improvement of symptoms after FESS are good and comparable to children without CF. Quality of life has significantly improved, especially nasal symptoms. Other studies have reported also positive outcomes after FESS in children with CRSwNP with CF, but usually length of follow-up was very limited (19-22). If sinus surgery can reduce the need for antibiotics in children with CF remains controversial (23). Because CF is a chronic disease where mucociliary transport is impaired, even after successful surgery children can keep having nasal infections or recurrence of nasal polyps. Therefore there is a more negative attitude of surgeons towards FESS in these children. Our results indicate that sinus surgery in these children is able to improve the QoL in the long-term. Aaneas et al showed an additional purpose of performing extensive FESS in selected patients with CRSwNP and CF. They suggested that extensive sinus surgery combined with intensive follow-up can eradicate pathogenic bacteria from the CF sinuses which theoretically should reduce the frequency of lung infections and thereby pulmonary morbidity (24).

With the emergence of drugs that specifically target the mutations in children with CF, good results have been obtained. Studies with Ivacaftor for example, which targets defective chloride channels, show very good results by improving sinonasal pathology and symptoms of sinonasal disease (25). The development of these new drugs could mean that in the future children with CF no longer develop sinonasal pathology and therefore treatment by an ENT surgeon is no longer needed. However this could take many more years. In the meantime we need to make sure that children with CRSwNP are optimally treated.

Therefore the advice is to treat children with CF not differently than children without CF, but keep in mind that children with CF are more likely to need revision surgery in time. Children with CRSwNP with CF should always be carefully monitored by an ENT specialist.

Pathophysiology and the role of nasal epithelial cells in CRSwNP

The pathophysiology of CRSwNP is very complex and involves many different factors. We know that airways epithelial cells play a well-accepted role in the regulation of local inflammatory processes and innate defense responses. Whether epithelial cells from nasal polyps play a role in the pathophysiology beyond their involvement in the innate immune defense against microbes or as a passive target for local inflammation, is relatively poorly explored.

In chapter 6 we performed expression profiling on epithelial cells from CRSwNP patients and healthy controls. We showed that 27 genes were significantly different between healthy individuals and patients with CRSwNP. Many of these genes could be linked to pathogenic mechanisms in neoplasm formation and cell cycle control. Hereby we have contributed to the notion that epithelium could play a substantial role in the formation of nasal polyps.

There have been many genomics related studies looking at the contribution of individual genes in CRS (26-28). This type of research is relatively easy to perform as DNA of all cell types is the same, even though expression profiles will be different between cell types. A relatively small contribution of individual genes was found, which is not so strange as multiple genes are likely to interact. Moreover it might well be that CRSwNP encompasses potential different endotypes, so that not all patients suffer from the same genetic disease. To circumvent these issues we opted for expression profiling of nasal epithelial cells. This means more work as you need to do expression profiling for this single cell type as whole tissue expression profiling runs into the problem of potential difference in cellular makeup of the tissue. To reduce all possible relevant factors that could influence our results, we included no patients with any auto-immune disorder or other relevant comorbidities, and allergic and asthma status was established in all patients. It still remains very difficult to interpret differences between healthy epithelial cells and epithelia of CRSwNP, because in active disease the epithelium is exposed to many inflammatory triggers that could affect the expression profile. Therefore we have tried to mitigate some of these aspects by limited culturing of the epithelial cells rather than analyzing the expression profile *in situ* or directly after isolation. This approach may have undocumented effects on the expression profile, although this allows us to obtain pure epithelial cells. The advantage of this approach is that we are able to look at stable differences in epithelial cells whereby we can get a summary view of what has gone wrong in the cell.

Our data discovered at least one process that is nearly impossible to find by genomics only: the involvement of epigenetically regulated genes. We detected two genes that are only expressed from the maternal inherited chromosome and not from the paternal copy, CDKN1C and ASCL2. The most interesting of these genes is probably the deregulated tumour suppressor gene CDKN1C (29). This gene has been linked to multiple forms of cancer, and mutations in this gene underlie Beckwith-Wiedemann Syndrome which is characterized by abundant colon polyp formation (30). The possible involvement of pathological processes regulated by imprinted genes will affect interpretation of genomic studies that investigate CRSwNP as such studies will only consider the presence or absence of specific single nucleotide polymorphisms (SNPs) and not whether or not the genes with the SNPs are expressed.

Another process that seems important in the pathogenesis of CRSwNP is neurogenic inflammation. Neurogenic inflammation has been studied in relationship to inflammation and neoplasm formation, but it has not yet been considered in CRSwNP. Neurogenic inflammation centers around acetylcholine that traditionally has been investigated in the interaction of neurons with down-stream targets like smooth muscle cells, macrophages and other inflammatory cells (31). Recently it has become clear that also epithelial cells produce and respond to acetylcholine and that this could play a role in neoplasm formation (32, 33). In this respect it was remarkable that we

were initially able to detect a deregulated expression of SLURP1 in the epithelia of patients with CRSwNP. SLURP1 acts as a potential negative regulator of acetylcholine receptor mediated signalling due to its high affinity for this receptor (32). Besides SLURP, also LYNX1 (or SLURP2 as it was previously known) and SLC44A4 were deregulated thereby highlighting the link with neurogenic inflammation. The precise role of epithelial-centred neurogenic inflammation is not yet fully established, but one aspect seems to focus on signalling between adjacent epithelial cells and inflammatory cells in the nasal cavity. The link between epithelium and neuronal processes we have observed in our data set may be part of a more general mechanism. Activation of the TRPV1 receptor on neurons by capsaicin could be part of the mechanism by which capsaicin is able to suppress symptoms in idiopathic rhinitis and CRSwNP (34, 35). However the recently described deregulated expression of this receptor on epithelial cells of asthmatics suggests a potential involvement of an epithelial centred pathway (36). As TRPV1/capsaicin also effects acetylcholine signalling it would be interesting to explore this link further.

Our data shows two types of epithelium in patients with CRSwNP and this would be hard to find with genomics as well. These two different types of epithelia cannot be linked to any differences in atopic state of the patient or the level of inflammation. This shows that allergy seems to be an epiphenomenon and that the epithelial differences we have previously observed in allergic rhinitis do not play a common role in CRSwNP (37, 38).

Given the recent discovery of innate lymphoid cells (ILCs) as potential new players in the pathogenesis of CRSwNP, perhaps we would have expected to find differences for cytokines important for ILC2 development (TSLP, IL25, IL33) (39). Type 2 innate lymphoid cells (ILC2s) secrete type 2 cytokines which protect against parasites but also are able to contribute to a variety of inflammatory airway diseases. In patients with CRSwNP highly elevated levels of ILC2s were found in nasal polyps tissue (39). It was shown that indirect collaboration of the epithelium with ILC2s in patients with CRSwNP results in shaping of type 2 immunity in the nose (39). However there was no real difference found at baseline between healthy and disease. The exact role of ILCs is still under investigation, but this could be very interesting as potential treatment target in the future.

For the future we would have to look further into the differences and new processes we have detected. For example it would be interesting to investigate other cells like fibroblasts. In addition to epithelial involvement in CRSwNP, it has been suggested that the interaction between nasal epithelium and the myofibroblasts could resemble the interaction between bronchial epithelium and smooth muscle cells (40, 41). This link might help explain the higher prevalence of CRSwNP in asthmatic patients (42, 43). Preliminary data show that nasal epithelial cell conditioned medium (ECCM) stimulates fibroblasts and that diseased fibroblasts seem to be stimulated more strongly. Furthermore recombinant SLURP is able to activate fibroblasts in vitro which

is interesting given the potential role of neurogenic inflammation in the pathogenesis of CRSwNP.

In conclusion, it still remains difficult to determine cause and effect in the interpretation of differences between epithelia of healthy individuals and patients with CRSwNP, however they do seem to point towards new processes that play a role in the pathophysiology of CRSwNP.

Advances in surgical tools for CRSwNP

Different operating technique and tools in FESS have evolved over the years and nowadays surgeons tend to choose a more custom approach based on the extent of the disease and comorbidities (44). In FESS surgical techniques have been refined and new instruments introduced. Besides traditional instruments, such as the cutting and non-cutting Blakesley forceps, nowadays the microdebrider (shaver) is widely used. The microdebrider was introduced in FESS in 1992 and is a powered rotary shaving device, which originally was used in arthroscopic surgery (45).

Even though the microdebrider is well known, there was lack of evidence from comparative studies focusing on operating time, blood-loss, and user friendliness between traditional instruments and the microdebrider. In chapter 3 we demonstrated that operating patients with CRSwNP with the microdebrider on top of traditional instruments is safe and very time efficient. Operating with the microdebrider was quicker mainly because it improves visualization due to the continuous suctioning and thereby no need to repeatedly exchange instruments in the nose each time. Furthermore we found that the user-friendliness of the microdebrider is very high, which means surgeons find it easier to operate patients with CRSwNP with the microdebrider than without. Sauer et al previously showed a significant higher operating time when a microdebrider was used (46). An essential difference with our study is that in the study from Sauer et al only the microdebrider was used, instead of the microdebrider on top of traditional instruments. Therefore making it sometimes harder to perform certain maneuvers like removing cells from the lamina papyracea or skull base, which is not very realistic. This could explain why in this study a longer operating time was found on the side were only the microdebrider could be used.

In our study we did not find any difference in intraoperative blood loss. In a retrospective study from 1996 however, a reduction of blood-loss was found when using the microdebrider (47). This might be because the microdebrider removes the mucosa in smaller pieces resulting in higher blood-loss per cm^3, which is then compensated by the shorter operating time when the microdebrider was used. Earlier claimed advantages of the microdebrider on postoperative healing were not confirmed by our study. There was no difference found in recurrence rate with a follow-up of 3 months. In a recent study performed by Tirello et al., a significant lower recurrence of CRSwNP with traditional instruments compared to the microdebrider was found,

however they found a higher incidence of synechia formation with a follow-up of 13 months (48). A limitation of this study was that only one instrument was used on each side, and that the same side of the nose was always operated with traditional instrument and the other side with the microdebrider. This could also influence the outcome, because when the surgeon is right handed, the right side of the nose sometimes can be more difficult to reach.

Literature on complications of the use of powered instruments in FESS is limited. There is no available literature indicating an increased complication rate, besides some case reports describing potentially dangerous complications (49, 50). In our study no difference was found in complications rate between traditional instrument and the additional use of the microdebrider. Because in our study the microdebrider was used by both experienced surgeons as well as surgeons in training (under supervision), we can conclude that the microdebrider is safe to use.

Since the introduction of the microdebrider in FESS, modifications like different sizes and angles have been made so that the microdebrider could be integrated in different types of surgery as well, like larynx surgery, turbinate reduction, supraglottoplasty and choanal atresia repair. Similar advantages of the microdebrider have been found comparable to the use of the microdebrider in FESS. For example in larynx surgery nowadays the microdebrider is widely used and studies have shown that the use of the shaver requires less operating time than the CO2 laser and therefore can be cost saving (51, 52). In turbinate reduction no differences were found in postoperative healing between the microdebrider and traditional instruments (53). These results confirm our thoughts on the advantages of the microdebrider in FESS.

In conclusion, these results are encouraging and show that it could be worthwhile to invest in a microdebrider, saving operating time and retaining good results. The use of the microdebrider could also bring along higher costs, because blades are disposable and sometimes more than one blade is needed per operation (47). Therefore in the future to optimize surgical management more randomised controlled trials are needed, to evaluate the effectiveness and extensiveness of surgical treatment. An analysis of the cost-effectiveness of the use of the microdebrider should be performed, taking into account all different aspects such as operating time, cost of the disposable blades and general costs of healthcare around the operation.

Perioperative medical management of CRSwNP

Local and systemic corticosteroids are included in the initial treatment of CRSwNP to reduce inflammation, but also can be used preoperatively, intraoperatively and postoperatively in patients with CRSwNP undergoing FESS. About the role of corticosteroids in FESS there are several studies, but they report conflicting results about their perioperative role in the improvement of symptoms and polyp scores. Currently, the choice what to use is very doctor specific, and depends more on their personal preferences rather than evidence.

In **chapter 4** we performed a systematic review and meta-analysis of randomised controlled trials for operative outcomes which demonstrated that operation time and estimated blood loss were significantly lower, and surgical field quality was significantly better in the local and/or systemic steroid group compared to the non-steroid group (54, 55). There was no difference in postoperative pain scores when intraoperative corticosteroids were used. The postoperative use of corticosteroids was shown to significantly improve endoscopic scores, but there was no significant difference in symptom scores when corticosteroids were used (56-59). The use of corticosteroids was not associated with an increased risk of sinusitis. There was a reduced recurrence rate when postoperative corticosteroids were used in patients with CRSwNP, however this role is unclear in patients with CRSsNP (56).

Because of their anti-inflammatory effect, topical corticosteroids are used in the treatment of CRS (60). A recent Cochrane review reported a beneficial effect of intranasal corticosteroids compared to placebo or no treatment on symptoms and nasal blockage especially in patients with CRSwNP (61). When the effects were assessed of the different types of intranasal corticosteroids in patients with CRS there was insufficient evidence found that one steroid is more effective than another, nor that effectiveness of a spray differs from that of an aerosol (62). Another Cochrane review showed that when a short course of systemic corticosteroids is used as an adjunct therapy to intranasal corticosteroids there might be an improvement in symptom severity, polyp size and condition of the sinuses when assessed using CT scans (63). However, it seems unclear whether these beneficial effects of oral corticosteroids are sustained beyond the short follow-up period (up to 30 days) as there are no longer follow-up data available. When we specifically look at the use of corticosteroids after FESS, we find a previous systematic review on the use of topical corticosteroids after FESS which reports a significant improvement of symptoms, endoscopic scores and lower recurrence rate (60). However, these authors only summarized the results of different studies and did not perform a meta-analysis. In the recent EPOS 2012 systematic review on the role of corticosteroids after FESS, topical corticosteroids were recommended for patients with CRSsNP and both topical and systemic corticosteroids for patients with CRSwNP (13). In this review patients with CRSwNP with previous sinus surgery responded better to topical steroid treatment than without sinus surgery in polyp size reduction, but there was no difference in improvement in symptoms between the two subgroups. In contrast to our review where we included only patients who underwent FESS, here were also patients included with just a history of polypectomy which might influence this result positively.

Besides steroids, perioperative management can include the use of antibiotics. A Cochrane review from 2016 reported very little evidence that systemic antibiotics are effective in patient with CRS (64). There was only moderate quality evidence of a modest improvement in disease-specific quality of life in adults with CRSsNP receiving 3 months of macrolides (64). Three months after the end of the treatment

this effect was gone. On the use of preoperative antibiotics is very limited evidence. The current consensus is that in patients with acute infection prior to surgery, preoperative antibiotics can be useful by reducing inflammation and thereby improving the surgical field (13, 65). Experts' opinion on the postoperative use of antibiotics is that 7-14 days is advised, however the evidence for this choice is very limited (13, 65). Both amoxicillin-clavulanate and macrolides have shown improvement in endoscopy scores, but especially in patients with CRSsNP (66, 67). Only in patients with CF specific antibiotics (tobramycin) play a role in the treatment of bacterial rhinosinusitis and postoperative outcomes of FESS (68).

Even though our review included some potential biases, like the inclusion of a very mixed group of patients with both CRSsNP and CRSwNP and a wide variability in agents used, dose, route and duration, we have attempted to bring together the best evidence on this subject available. Based on current available evidence preoperative use of corticosteroids is advised to reduce operative time and blood loss. There are limited studies on the use of intraoperative corticosteroids regarding symptom scores, but there seems to be no benefit. More studies should be performed to assess this further in the future. Postoperative use of corticosteroids is advised to improve endoscopic scores and reduce recurrence rate in CRSwNP. However, most studies are limited to small sample size and for the future there are large RCTs required which better analyze the long term outcomes and recurrence rates.

New treatments for severe bilateral CRSwNP

When patients with CRSwNP are refractory to current medical treatments, there are very limited treatment options available beyond FESS. Therefore there is a need for new and better medical treatments focusing on the underlying pathophysiological mechanisms of CRSwNP. Considering the complex pathogenesis of CRSwNP, it is not surprising that a broad approach can be effective. With the realization that CRSwNP consists of different endotypes, a tailored treatment should be considered. These endotypes can be defined by distinct pathophysiologic mechanisms that correspond with different biomarkers and could help us identify new treatment targets.

The development of biologicals is progressing over the last years and several studies have been performed which reported good results in patients with allergic diseases and asthma (69). For CRSwNP several studies have been performed with different antibodies, like anti-IgE, anti-IL-5 and anti-IL-4 receptor alpha (70-72). All these studies have shown positive clinical effects and show very limited side effects.

Given the fact that in Europe and the US most polyps are characterized by a Th2 inflammatory pattern with cytokines IL-4, IL-5, IL-13, eosinophils and IgE involved, these Th2 cytokines could be targets for therapeutic intervention. Mepolizumab, a humanized anti-interleukin (IL)-5 antibody, is as new treatment of nasal polyposis. Mepolizumab reduces eosinophil counts and is approved for the treatment of severe

eosinophilic asthma (73). A randomized double-blind trial in 2011 demonstrated the efficacy of mepolizumab in reducing the size of nasal polyps (74). This study was in a small patient group and consisted of 2 single intravenous injections of mepolizumab or placebo with a follow-up of 8 weeks. Therefore it is interesting to find out if mepolizumab in a larger population with more injections could also reduce the need for surgery in patient with CRSwNP.

In chapter 2 we report on a large randomised, double-blind, placebo-controlled, multicenter, multinational (Belgium, the Netherlands, and United Kingdom) trial to determine whether mepolizumab could reduce the need for surgery in patients with severe, recurrent bilateral nasal polyps on topical corticoid therapy. This Phase II study based on a composite endpoint of reductions in endoscopic nasal polyposis score and nasal polyposis severity VAS score, demonstrated a statistically significant reduction in the proportion of patients eligible for surgery 4 weeks after the last dose at week 25 in the mepolizumab group compared with placebo. These results were supported by clinically significant improvement of symptoms and QoL SNOT-22 scores in the mepolizumab group compared with placebo. These results suggest that mepolizumab can improve the QoL and may reduce the need for surgery for patients with refractory CRSwNP.

Mepolizumab inhibits eosinophilic inflammation, which is present in many nasal polyps (75). In the mepolizumab group was a significant reduction in eosinophil counts at week 25 together with improvement of nasal symptoms and a reduced need for surgery. We did not find any improvement in long function on the other hand in patients with concomitant asthma, even though mepolizumab is an approved treatment for severe eosinophilic asthma. The reason for this could be that in this study only patients with mild or moderate asthmatic disease were included, not with severe eosinophilic asthma.

Not all patients with CRSwNP in this study showed a significant improvement after treatment with mepolizumab. The reason why some patients do not respond to the medicine, remains unclear. There was no difference found between responders and non-responders is VAS scores, QoL, clinical pharmacodynamics, blood eosinophil counts, anti IL-5 levels or comorbidities.

With the improved understanding of the different pathophysiologic pathways of CRSwNP, the treatment options are enhanced. With the arrival of new biologicals, we are challenged to select patients eligible for each specific treatment and predicting their therapeutic response. By the development of different biologicals there is a shift from general medical treatment of CRSwNP to a more target-specific treatment and personalised therapy for patients with certain endotypes in the future.

Concluding remarks

This thesis focused on analyzing and thereby optimizing different treatments and obtaining further insight in the pathophysiologic mechanisms of CRSwNP. We showed

for the first time that the active disease duration of CRSwNP in patients seems to be relatively constant at about eleven years, regardless of the age of onset, and that the epithelium could play a substantial role in the formation of nasal polyps, especially in neoplasm formation and cell cycle control. Furthermore we showed advantages of using the microdebrider in FESS and that FESS is a safe and effective treatment in children with CRSwNP, even if they have CF. By performing a systematic review we can advise the use of preoperative corticosteroids to reduce operative time and blood loss, and postoperative use of corticosteroids to improve endoscopic scores and reduce recurrence rates. And finally we showed that treatment with mepolizumab added daily to nasal corticosteroids might offer a viable alternative to surgery in a selected group of patients with severe, recurrent nasal polyposis requiring surgery.

Even though this thesis has led to an improvement of our understanding of the pathophysiology of CRSwNP and impact of the current treatments, still many gaps remain and treating CRSwNP can be very difficult. To further improve our care for patients with CRSwNP in the future we should focus more on precision medicine (PM). PM is already of major interest in other medical domains such as oncology, allergy and chronic airways disease. PM is a medical model aiming at the customization of healthcare tailored to the individual patients. PM consists of four principles, namely personalised care, prediction of treatment success, prevention of disease and patient participation in the elaboration of the treatment plan (76). The combination of these four pillars is expected to improve treatment outcomes. Most principles of PM can be implemented easily without major costs, such as providing patients with information about the effectiveness of different treatment modalities, informing patients about the impact of CRSwNP on asthma, and preventing progression of the disease and secondary prevention of the onset of asthma (77). However, the implementation of personalised care in patients with CRSwNP, is still in progress. The current knowledge that most nasal polyps are characterized by Th2 inflammatory patterns could be a possible therapeutic target for intervention. However, we are not sure whether Th2 cytokines are the cause or an effect of the disease. With the arrival of new biologicals, we are challenged to select patients eligible for each specific treatment and predicting their therapeutic response. Given the high costs of these new biologicals, improving patient selection by developing specific predictors will be very important to further implement this as a treatment in the future. It will be a great challenge to further investigate the etiology, endotyping, biomarkers and treatment (medical and surgical) of CRSwNP, for us to better understand this disease and develop new and better treatments for our patients.

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Appendices &

SUMMARY
SAMENVATTING
AUTHORS AND AFFILIATIONS
PORTFOLIO
ABOUT THE AUTHOR
DANKWOORD

SUMMARY

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a chronic disease which has a significant negative impact on the quality of life (QoL) and brings along high costs because of high medical resource usage and high societal costs. Despite decades of research on the pathophysiology and treatment of CRSwNP, still a group of patients remain symptomatic under current state-of-the-art medical and surgical treatment. In order to keep improving our treatment for patients with CRSwNP, we need to critically asses our current therapeutic options and gain more insight in the pathophysiology of CRSwNP. This thesis focused on analyzing and thereby optimizing different treatments and obtaining further insight in the pathophysiologic mechanisms of CRSwNP.

In **Chapter 2** we describe a large randomised, double-blind, placebo-controlled, multicenter, multinational (Belgium, the Netherlands, and United Kingdom) trial to determine whether mepolizumab could reduce the need for surgery in patients with severe, recurrent bilateral nasal polyps on topical corticoid therapy. This Phase II study based on a composite endpoint of reductions in endoscopic nasal polyposis score and nasal polyposis severity VAS score, demonstrated a statistically significant reduction in the proportion of patients eligible for surgery 4 weeks after the last dose at week 25 in the mepolizumab group compared with placebo. These results were supported by clinically significant improvement of symptoms and QoL SNOT-22 scores in the mepolizumab group compared with placebo. These results suggest that mepolizumab added to daily nasal corticosteroids might offer a viable alternative to surgery in a selected group of patients with severe, recurrent nasal polyposis requiring surgery.

In **Chapter 3** the use of the microdebrider is compared to conventional instruments in FESS for patients with CRSwNP. We demonstrated that operating patients with CRSwNP with the microdebrider on top of traditional instruments is safe and very time efficient. Operating with the microdebrider was quicker mainly because it improves visualization due to the continuous suctioning and thereby no need to repeatedly exchange instruments in the nose each time. Furthermore we found that the user-friendliness of the microdebrider is very high, which means surgeons find it easier to operate patients with CRSwNP with the microdebrider than without.

In **Chapter 4** we performed a systematic review and meta-analysis of randomised controlled trials for operative outcomes on the perioperative role of corticosteroids. The aim of this study was to systematically review all existing evidence on the role of corticosteroids in patients undergoing FESS. We demonstrated that operation time and estimated blood loss were significantly lower, and surgical field quality was significantly better in the local and/or systemic steroid group compared to the non-steroid group. There was no difference in postoperative pain scores when intraoperative corticosteroids were used. The postoperative use of corticosteroids was shown to significantly improve endoscopic scores, but there was no significant difference in

symptom scores when corticosteroids were used. The use of corticosteroids was not associated with an increased risk of sinusitis. There was a reduced recurrence rate when postoperative corticosteroids were used in patients with CRSwNP, however this role is unclear in patients with CRSsNP.

In **Chapter 5** we assessed the long-term results of FESS in children with CRSwNP with CF and without CF and determined outcome, symptoms, quality of life and complications. We showed that FESS is a very effective and safe treatment in children with CRSwNP even in children with CF. Overall QoL has significantly improved in 78% of the patients at long-term follow-up, especially in the domain of nasal symptoms. In total 14% of the children needed a revision and there were no complications.

Chapter 6 explores the potential contribution of nasal epithelial cells to the pathophysiology of CRSwNP. We performed micro-array expression profiling on epithelial cells from CRSwNP patients and healthy controls to analyze the role of polyp epithelium in the pathogenesis of CRSwNP. We showed that 27 genes were significantly different between healthy individuals and patients with CRSwNP. Many of these genes could be linked to pathogenic mechanisms in neoplasm formation and cell cycle control. Hereby we have contributed to the notion that epithelium could play a substantial role in the formation of nasal polyps.

In **Chapter 7** we adopted FESS as an objective sign of active/uncontrolled disease and measured the time between first and final surgical intervention with a follow-up of 10 years. Active disease duration of CRSwNP was determined by looking at the relation between age, total number of times of sinus surgery and age at the time of the first operation ever. We showed for the first time that the active disease duration of CRSwNP in patients is relatively constant at about eleven years, regardless of the age of onset. This could be a first indication that CRSwNP is a self-limiting disease.

Chapter 8 comprises the general discussion, overall conclusions and future perspectives of this research.

SAMENVATTING

Chronische rhinosinusitis met neuspoliepen (CRSwNP) is een chronische ziekte die een negatieve impact heeft op de kwaliteit van leven en daarnaast hoge kosten met zich meebrengt vanwege frequent gebruik van medische voorzieningen en hoge sociale kosten. Ondanks tientallen jaren van onderzoek naar de pathofysiologie en behandelingen van CRSwNP, bestaat er nog steeds een groep patiënten welke klachten blijven houden ondanks optimale medicamenteuze en chirurgische behandeling. Om onze behandelmogelijkheden te kunnen blijven verbeteren voor patiënten met CRSwNP, moeten we kritisch onze huidige therapeutische mogelijkheden onderzoeken en daarnaast meer inzicht krijgen in de pathofysiologie van CRSwNP. Dit proefschrift is erop gericht de huidige behandelmogelijkheden te analyseren en daardoor te optimaliseren, en daarnaast meer inzicht te verkrijgen in de onderliggende pathofysiologische mechanismen van CRSwNP.

In **Hoofdstuk 2** beschrijven we een grote gerandomiseerde, dubbelblinde, placebo-gecontroleerde, multicenter, multinationale studie om te kijken of mepolizumab de noodzaak tot een operatie kan afwenden, bij patiënten met ernstige bilaterale CRSwNP die corticosteroïd neusspray gebruiken. Deze fase 2 studie, welke als primaire eindpunten de afname van endoscopische poliepscore en de nasale symptoom score (VAS) hanteert, laat een statistisch significante reductie zien in het aantal patiënten dat een operatie nodig had 4 weken na de laatste dosis in de mepolizumab groep. Deze resultaten worden onderbouwd door een klinisch significante afname van symptomen en verbetering in kwaliteit van leven (SNOT-22) in de mepolizumab groep vergeleken met de placebo groep. Deze resultaten suggereren dat mepolizumab toegevoegd aan het dagelijks gebruik van corticosteroïden, een alternatief zou kunnen bieden voor chirurgie in een geselecteerde groep patiënten met ernstige terugkerende neuspoliepen die in aanmerking zouden komen voor chirurgie.

In **Hoofdstuk 3** wordt het gebruik van de microdebrider (shaver) naast conventionele instrumenten vergeleken met het gebruik van conventionele instrumenten alleen in functionele endoscopische bijholtechirurgie (FESS) bij patiënten met CRSwNP. Hier demonstreerden wij dat het opereren van patiënten met CRSwNP met de microdebrider naast conventionele instrumenten veilig is en erg efficiënt. Opereren met de microdebrider was sneller met name vanwege het goede zicht tijdens het opereren door de continue zuigfunctie, waardoor de chirurg de instrumenten niet telkens hoefde te wisselen in de neus. Verder lieten we hier zien dat de microdebrider zeer gebruiksvriendelijk is, wat betekent dat de chirurg het makkelijker vindt om patiënten met CRSwNP te opereren met de microdebrider dan zonder.

In **Hoofdstuk 4** hebben we een systematisch review en meta-analyse uitgevoerd van gerandomiseerde studies naar de operatieve uitkomsten van de perioperatieve rol van corticosteroïden. Het doel van deze studie was om systematisch alle studies te beoordelen over de rol van corticosteroïden rondom bijholtechirurgie.

We demonstreerden hier dat operatieduur en bloedverlies significant lager waren wanneer patiënten preoperatief lokale en/of systemische corticosteroïden hadden gebruikt in vergelijking met patiënten die dit niet hadden gedaan. Er was geen verschil in postoperatieve pijnscores bij intra-operatief gebruik van corticosteroïden. Postoperatief gebruik van corticosteroïden liet een significante verbetering in endoscopische scores zien, echter geen significante verbetering van symptoomscores. Het gebruik van corticosteroïden is niet geassocieerd met een verhoogd risico op sinusitis. Er was een afgenomen recidiefkans als er postoperatief corticosteroïden werden gebruik bij patiënten met CRSwNP, alleen bij patiënten met CRS zonder neuspoliepen bleef dit nog onduidelijk.

In **Hoofdstuk 5** onderzochten we de langetermijnresultaten van FESS bij kinderen met CRSwNP met en zonder Cystic Fibrosis (CF) om specifiek te kijken naar uitkomst, symptomen, kwaliteit van leven en complicaties. Deze studie toonde dat FESS effectief en veilig is bij kinderen met CRSwNP, zelfs bij kinderen met CF. De algemene kwaliteit van leven was significant verbeterd bij 78% van de patiënten bij lange termijn follow-up, met name in het domein van nasale symptomen. In totaal had 14% van de kinderen revisiechirurgie nodig en waren er geen complicaties.

Hoofstuk 6 exploreert de potentiële contributie van neusepitheel in de pathofysiologie van CRSwNP. Hier hebben we micro-array expressieprofilering uitgevoerd op epitheelcellen van patiënten met CRSwNP en gezonde controles om de rol van epitheelcellen in de pathogenese van CRSwNP te onderzoeken. Er werden 27 significant verschillende genen gevonden tussen gezonde controles en patiënten met CRSwNP. Veel van deze genen kunnen gelinkt worden aan pathologische mechanismes in neoplasmaformatie en celcycluscontrole. Hierdoor hebben we bijgedragen aan het idee dat epitheelcellen mogelijk een substantiële rol spelen in de formatie van neuspoliepen.

In **Hoofdstuk 7** hebben we FESS gebruikt als objectief teken van activiteit van ziekte/ongecontroleerde ziekte en hebben we de tijd tussen de eerste en laatste chirurgische interventie bepaald met een follow-up van 10 jaar. De duur van de ziekteactiviteit werd geanalyseerd door te kijken naar de relatie tussen de leeftijd van patiënt, totaal aantal operaties, en leeftijd bij de eerste operatie ooit. Hier wordt voor de eerste keer ooit gedemonstreerd dat de duur van ziekteactiviteit van CRSwNP redelijk constant lijkt, namelijk ongeveer 11 jaar, ongeacht leeftijd ten tijde van start van de ziekte. Dit zou een eerste aanwijzing kunnen zijn dat CRSwNP een self-limiting ziekte is.

In **Hoofdstuk 8** worden de conclusies van het onderzoek genoemd gevolgd door een algemene discussie en perspectieven voor de toekomst.

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Courses

2011	Practical Biostatistics (ECTS 1.1)
2011	Clinical Data Management (ECTS 0.3)
2011	Reference Manager Basic (ECTS
2011	Pubmed (ECTS 0.1 ECTS)
2011	Oral Presentation in English (ECTS 0.8)
2011	Scientific writing in English (ECTS 1.5)
2010	Good Clinical Practice (GlaxoSmithKline) (ECTS 1.0)

Presentations

2017	Expression profiling of nasal polyp epithelial cells. *Oral Presentation, Rhinology World Congress.* August 2017, Hong Kong (ECTS 0.5)
2016	Expression profiling of nasal polyp epithelial cells. *Oral Presentation, EUFOREA.* November 2016, Brussels. (ECTS 0.5)
2016	Chronic rhinosinusitis with nasal polyps appears to be a 'self-limiting disease' lasting approximately 11 years. *Oral presentation, ERS Congress.* July 2016, Stockholm. (ECTS 0.5)
2016	Nasal polyps in children. *Oral presentation in Symposium, ERS Congress.* July 2016, Stockholm. (ECTS 0.5)
2015	Expression profiling of nasal polyp epithelial cells identifies two distinct phenotypes and suggests a role for neurogenic inflammation. *Oral presentation, ORL-HNS.* June 2015 Prague (ECTS 0.5)
2015	Kinderrhinologie: diagnostiek en behandeling van CRS bij kinderen. *Regionale refereermiddag AMC.* June 2015, Amsterdam (ECTS 0.5)
2015	Expressie profielen van epitheelcellen van neuspoliepen laten twee verschillende fenotypen zien en rol voor neurogene inflammatie. *Oral presentation, KNO Vergadering.* April 2015, Nieuwegein (ECTS 0.5)
2015	Expression profiling of nasal polyp epithelial cells identifies two distinct phenotypes and suggests a role for neurogenic inflammation. *Oral presentation, SERIN congress.* March 2015, Stockholm (ECTS 0.5)

2014 Nieuwe inzichten in polyposis nasi. *Oral presentation, Wetenschapsdag AMC.* November 2014, Amsterdam (ECTS 0.5)

2014 When do we operate on children with chronic rhinosinusitis. *Symposium, ERS Congress.* June 2014, Amsterdam (ECTS 0.5)

2014 Long term results of FESS in children with nasal polyps. *Oral presentation, ERS Congress.* June 2014, Amsterdam (ECTS 0.5)

2014 Micro array gene analysis of nasal epithelial cells in patients with chronic rhinosinusitis and nasal polyps. *Oral presentation, ERS Congress.* June 2014, Amsterdam (ECTS 0.5)

2014 Micro array gene analysis of nasal epithelial cells in patients with chronic rhinosinusitis and nasal polyps. *Oral presentation, EAACI Congress.* June 2014, Copenhagen (ECTS 0.5)

2014 Micro array gene analysis of nasal epithelial cells in patients with chronic rhinosinusitis and nasal polyps. *Poster presentation, EAACI Congress.* June 2014, Copenhagen (ECTS 0.5)

2013 Neuspoliepen van 0-80 jaar: what's the difference? *Oral presentation, Allergiemiddag AMC.* May 2013, Amsterdam (ECTS 0.5)

2013 Long term results of FESS in children with nasal polyps. *Oral presentation, ORL-HNS Congress.* April 2013, Nice (ECTS 0.5)

2013 The microdebrider, a step forward or an expensive gadget? *Oral presentation, German ENT congress.* May 2013, Nürnberg (ECTS 0.5)

2013 Long-term results of FESS in children with nasal polyps. *Poster presentation, SERIN congress.* March 2013, Leuven (ECTS 0.5)

2012 Nasal polyps in the elderly. *Poster presentation, SERIN Congress.* March 2013, Leuven (ECTS 0.5)

2012 Long-term results of FESS in children with nasal polyps. *Oral presentation, 11th International Congress of the European Society of Pediatric Otorhinolaryngology.* May 2012, Amsterdam (ECTS 0.5)

2012 The microdebrider, a step forward or an expensive gadget? *Oral presentation, ERS Congress.* June 2012, Toulouse (ECTS 0.5)

2012 Polyposis nasi. *Oral presentation, Wetenschapsdag AMC.* September 2012, Amsterdam.

2011 De Shaver: een stap vooruit of een dure grap? *Oral presentation, 219e KNO Vergadering.* November 2011, Groningen (ECTS 0.5)

(Inter)national conferences

2017	Rhinology World Congress, Hong Kong (ECTS 0.75)
2016	European Rhinology Research Forum (EUFOREA), Brussels, Belgium (ECTS 0.5)
2016	Congress of the European Rhinologic Society, Stockholm, Sweden (ECTS 0.75)
2015	Congress of European Otorhinolaryngology and Head and Neck Surgery, Prague, Czech republic (ECTS 0.75)
2015	Symposium on Experimental Rhinology and Immunology of the Nose, Stockholm, Sweden (ECTS 0.5)
2014	Congress of the European Rhinologic Society, Amsterdam, the Netherlands (ECTS 0.75)
2014	European Academy of Allergy and Clinical Immunology, Copenhagen, Denmark (ECTS 0.75)
2013	Congress of European Otorhinolaryngology and Head and Neck Surgery, Nice, France (ECTS 0.75)
2013	German annual ENT meeting, Nürnberg, Germany (ECTS 0.25)
2013	Symposium on Experimental Rhinology and Immunology of the Nose, Leuven, Belgium (ECTS 0.5)
2012	International Congress of the European Society of Pediatric Otorhinolaryngology, Amsterdam, the Netherlands (ECTS 0.5)
2012	Congress of the European Rhinologic Society, Toulouse, France (ECTS 0.75)

Others

2014-2017	Board Member of Junior European Rhinologic Society

Awards

2014	EAACI Oral Presentation Prize
2014	Junior Member Grant for the ERS Congress
2014	EAACI Congress Scholarship
2013	Abstract Award winner, SERIN Congress
2012	European Rhinologic Society Junior Prize winner, ERS Congress

List of publications

2017 **Activity of bacteriophages in removing biofilms of Pseudomonas aeruginosa isolates from chronic rhinosinusitis patients.**
Dr Stephanie Fong, Dr Amanda Drilling, Dr Sandra Morales, Dr Bradford A. Woodworth, Drs. **Marjolein. E. Cornet,** Prof Wytske J. Fokkens, A/Prof Alkis J. Psaltis, A/Prof Sarah Vreugde, Prof Peter-John Wormald
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2016 **Chronic rhinosinusitis: New understanding of specific and general Quality of life scores.**
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2016 **Chronic rhinosinusitis with nasal polyps appears to be a "self-limiting disease" lasting approximately eleven years.**
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2016 **Role of corticosteroids in functional endoscopic sinus surgery- a systematic review and meta-analysis**.
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Georgalas C, **Cornet M,** Adriaensen G, Reinartz S, Holland C, Prokopakis E, Fokkens W
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2013 **Long-term results of functional endoscopic sinus surgery in children with chronic rhinosinusitis with nasal polyps.**
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2012 **The microdebrider, a step forward or an expensive gadget?**
Cornet ME, Reinartz SM, Georgalas C, van Spronsen E, Fokkens WJ
Rhinology 2012, jun;50(2):191-8.

2012 **Role of innate immunity in the pathogenesis of chronic rhinosinusitis: progress and new avenues.**
Van Drunen CM, Mjosberg JM, Segboer CL, **Cornet ME**, Fokkens WJ
Curr Allergy Asthma Rep 2012 Apr; 12(2):120-6.

ABOUT THE AUTHOR

Marjolein Cornet was born on the 20th of June 1983 in Haarlem, the Netherlands. After graduation at the Stedelijk Gymnasium Haarlem (2001), she first enrolled in a Bachelor in Psychology in the University of Amsterdam. In 2002 she was decentrally selected and started her medical training in the University of Amsterdam. During her study in 2007 she followed a general internship in the RSU Mardi Lestari, Sragen, Indonesia (Java) and between 2002 and 2006 she worked as a parttime research assistent in Gynaecology/Radiology in the Academic Medical Centre, Amsterdam. Besides her work during this whole period she played field hockey on the highest level in the Netherlands at AH&BC and THC Hurley. In 2009 she graduated as a Medical Doctor from the University of Amsterdam and started working as a surgical resident at the Academic Medical Center in Amsterdam. In February 2010 she obtained her Advanced Trauma Life Support certification in the Harlem Hospital Center, New York, USA.

As of October 2010 Marjolein started her PhD project (Prof. dr. W.J. Fokkens, Dr. C. M. van Drunen) combined with clinical work as a resident at the department of Otorhinolaryngology. She presented her work in several international congresses and is as from 2014 on an active Board Member of the Junior European Rhinologic Society. After 1.5 year she continued her fulltime specialist training in Otorhinolaryngology and Head and Neck Surgery at the Academic Medical Center in Amsterdam (prof. dr. S. van der Baan, prof. dr. W.J. Fokkens, dr. S.M. Reinartz, dr. A.M. König and prof. dr. F.G. Dikkers). During her residency she also obtained her certification as a Medical Examiner of Divers (Scott Haldane Foundation). After finishing her specialist training in 2017 she obtained a position as an ENT surgeon at the Academic Medical Center, subspecialized in Rhinology.

She is married to Joost de Wit and they have one daughter, Cato (2016), and are expecting their second child in November.

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&

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Dirk, jij hebt onze onderzoeks-drie-eenheid voltooid. Ik zie je nog binnenkomen hier in het AMC inclusief mat en aardappel, maar je bent inmiddels wel goed veramsterdamd. We hebben samen van begin tot het einde alle onderzoeksperikelen besproken en lief en leed gedeeld. Ik bewonder je eeuwige geduld, beheersing en vriendelijkheid en kan nog heel veel van je leren.

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of Dom Benedictine in de hand. Ik bewonder enorm hoe jullie in het leven staan na alles wat jullie samen hebben meegemaakt. Ik hoop dat we nog heel veel jaren van het leven kunnen genieten. Lieve **Frederik, Yue, Johannes en Daphne**, ook al zien we elkaar niet vaak, als we elkaar zien is het altijd alsof het nooit anders is geweest. Ik ben enorm blij met jullie als schoonfamilie.

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Lieve **Cato**, ik weet niet wat ik zou kunnen schrijven wat recht zou doen aan hoe bijzonder je voor mij bent. Als je me lachend een kusje komt geven en je armpjes uitsteekt, kan ik wel huilen. Je bent mama's prinsesje en ik zal er altijd voor je zijn!

Lieve **Joost**, je bent het belangrijkste in mijn hele leven, wat zou ik moeten zonder jou. Jij bent precies wat ik nodig heb en houdt mij perfect in balans. Tijdens mijn hele promotie en met name de zware momenten heb je mij altijd bijgestaan zodat ik dit proefschrift kon afmaken. Je bent m'n 'allessie'.