THE ROLE OF SURGERY AND DISEASE LOAD IN REFRACTORY CHRONIC RHINOSINUSITIS

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Contents

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Ahmed Bassiouni

This work is dedicated to all the special people in my life.

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Thesis Abstract

Chronic rhinosinusitis (CRS) is chronic inflammation of the sinonasal mucosa. It is a disease of significant impact on public health, one that affects about 10-15% of the population. Functional Endoscopic Sinus Surgery (FESS) is the "gold standard" surgical treatment for CRS; its original philosophy or concepts are based upon the sinonasal mucociliary clearance studies by Messerklinger and Stammberger, which emphasize the role of the osteo-meatal complex (OMC). However, although the success rate of FESS is about 90%, there is a subgroup of patients who exhibit no improvement, and thus require repeated surgeries. This subgroup of patients suffers from refractory chronic rhinosinusitis (rCRS), which is the main focus of this thesis. In this thesis the current understanding of the pathogenesis and causes of surgical failure in CRS are reviewed. This thesis presents the hypothesis that our understanding of the pathogenesis of CRS has advanced since the original concepts of FESS were put forward, and that patients who develop rCRS have other pathogenic features that cannot be addressed by these concepts. We revisit middle turbinate lateralization (MTL) as a surgery-related factor of rCRS in Chapter 6, and we pose the question: Is MTL a complication associated with worse surgical outcomes, or just a harmless sequela, of the surgical destabilization of the middle turbinate during sinus surgery? Our findings show that MTL plays a role in surgical failure and requiring revision surgery, but suggest that the clinical significance of MTL may be related to frontal sinus obstruction and not necessarily to the OMC. We then present two novel hypotheses: the inflammatory load hypothesis in Chapter 7, and the irreversible disease hypothesis in Chapter 8. In Chapter 9, we investigate nasal polyp recurrence in CRS with Nasal Polyposis (CRSwNP) as an important cause of rCRS. We study the patterns of polyp recurrence and the clinical factors associated with more aggressive recurrence. The findings show that firstly, comorbid factors such as asthma and aspirin sensitivity contribute to the disease load and rCRS; and secondly, that more aggressive surgical removal of that disease load and maximal opening of the sinuses through a frontal drillout procedure improve the surgical outcome and disease control for these rCRS patients. We then proceed to investigate the relevance of our two novel hypotheses to refractory CRSwNP through a histopathological study in Chapter 10. We also describe the evolution of the inflammatory load in patients with rCRS from first to second surgery, a topic rarely addressed in the literature. We found that a higher inflammatory load is present in patients

who fail surgery and go on to develop refractory CRS, when compared to patients who respond to surgery, with a particular significance to the eosinophilic load. In summary, our findings suggest that the inflammatory load is associated with long-term surgical outcomes. The recommendation based upon findings in this thesis is that surgery offered for CRS should be viewed as a tool for addressing and controlling disease load, and not just for the conservative clearance of disease of the OMC.

Publications arising from this thesis

Bassiouni A, Wormald P-J. Airway remodeling in chronic rhinosinusitis. Global Atlas of allergic rhinitis and chronic rhinosinusitis. Cezmi A, Hellings P, Agache I, editors. European Academy of Allergy and Clinical Immunology; 2015 Jun.

Bassiouni A, Chen PG, Naidoo Y, Wormald P-J. Clinical significance of middle turbinate lateralization after endoscopic sinus surgery. Laryngoscope. 2015 Jan;125(1):36–41.

Bassiouni A, Naidoo Y, Wormald P-J. Does mucosal remodeling in chronic rhinosinusitis result in irreversible mucosal disease? Laryngoscope. 2012 Jan;122(1):225–9.

Bassiouni A, Chen PG, Wormald P-J. Mucosal remodeling and reversibility in chronic rhinosinusitis. Curr Opin Allergy Clin Immunol. 2013 Feb;13(1):4–12.

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Bassiouni A, Ou J, Rajiv R, Cantero D, Vreugde S, Wormald P-J. Subepithelial Inflammatory Load and Basement Membrane Thickening in Refractory Chronic Rhinosinusitis with Nasal Polyposis: A Histopathological Study. Int Forum Allergy Rhinol. Forthcoming 2016.

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Chapter 1 Chronic Rhinosinusitis

1.1 Overview of the chapter

This thesis ultimately revolves around chronic rhinosinusitis (CRS). This chapter introduces this disease with a definition, clinical picture, classifications as well as management.

1.2 CRS: Definition and Diagnosis

CRS is a chronic inflammatory condition of the mucosa lining the nose and sinonasal cavities. The diagnosis is based on identification of (a) symptoms, (b) clinical examination (c) investigations.

The definition of CRS according to The 2012 European Position Paper on Rhinosinusitis (EPOS)[1]

"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"

…with the duration of symptoms described as:

"more than (or equal)12 weeks symptoms without complete resolution of symptoms"

According to the American Rhinosinusitis Task Force (ARTF):[2]:

"Chronic rhinosinusitis is a group of disorders characterized by inflammation of the mucosa of the nose and paranasal sinuses of at least 12 consecutive weeks' duration."

By examining Table 1-1, we notice that the three guidelines offer both a "clinical definition"" and a "research definition". We can also note a slight difference between the EPOS and other definitions for the clinical definition: whereas EPOS requires either endoscopic proof or radiologic proof; the other two guidelines requires confirmation on (endoscopic) examination.

However, there is consensus that a pure symptom-based diagnosis will frequently yield false positives. As such, examination (through anterior rhinoscopy or nasendoscopy) or investigation (typically a CT scan) should be considered necessary for the diagnosis of CRS, with exceptions only given to special circumstances: such as for epidemiological studies; or for preliminary

diagnoses by non-ENT surgeons, especially in cases of insufficient resources for a CT or an endoscopy.

Table 1-1: Diagnosis of CRS

	American Rhinosinusitis Task Force[2]	EPOS 2012[1]	5 National societies (AAAAI/AAOA/AAO-HNS/ACAAI/ARS)[3]
Clinical diagnosis	2 major factor, 1 major factor 2 minor symptoms, or nasal purulence on exam concrete signs on physical examination are needed to make a diagnosis	presence of two or more symptoms one of which should be either nasal blockage or nasal discharge (anterior/posterior nasal drip): ± facial pain/pressure; ± reduction or loss of smell; for ≥12 weeks; and either endoscopic signs of or CT scan changes	Objective documentation is required by means of direct visualization of the middle meatus through anterior rhinoscopy (after decongestion) or nasal endoscopy to assert the accurate diagnosis of CRS.
Diagnosis for research	Should have a CT scan or quality photoendoscopy performed to confirm the diagnosis.	For research purposes chronic rhinosinusitis (CRS) is defined as per the clinical definition. For the purpose of a study, the differentiation between CRSsNP and CRSwNP must be based on endoscopy.	A positive sinus CT scan is required for the research definition of both CRSsNP and CRSwNP.

In the studies included in this thesis, we follow the "CRS diagnosis for research purposes", in which the recommendations of all three panels in Table 1-1 are very similar, since they require confirmation of the patients' subjective symptoms by nasendoscopy +/- imaging.

1.3 Epidemiology

1.3.1 Prevalence/incidence

In the USA, chronic sinusitis is estimated to affect about 12.5% of the population.[4] In Europe, a 2011 GA2LEN (Global Allergy and Asthma European Network project) multicenter study (19 centers, 12 countries in Europe) concluded that the overall prevalence of CRS according to EPOS criteria was 10.9%, and described CRS as an "underestimated" disease.[5] In Australia, according to the Australian National Health Survey, 9.2% of the Australian population (about 1.8 million people) had chronic sinusitis in 2004–2005.[6]

1.3.2 Impact on economy and public health

In the United States, CRS accounts for a conservative estimate of 18-22 million physician visits in the United States each year.[7]. It also accounts for 70 million restricted activity days annually.[8] CRS has been identified as the fifth most common diagnosis associated with antibiotic prescriptions[9] and around 500,000 surgical procedures are performed on the paranasal sinuses annually.[10] The overall health care expenditures attributable to sinusitis in 1996 (in the US) were estimated at $5.8 billion.[11]

1.4 Clinical picture

1.4.1 Symptoms

CRS is a disease with a huge impact on quality of life (QOL). Research showed that CRS had a higher impact on QOL measures of bodily pain and social functioning than diseases such as congestive heart failure, angina, chronic obstructive pulmonary disease, and back pain.[12]

Symptoms of the disease are listed in Table 1-2. These symptoms occur as a result of the inflammatory state of the nose and sinuses.

Table 1-2 Symptoms and signs of CRS

Symptoms	Examination of the nose findings (Endoscopic findings)
Major symptoms (of the nasal syndrome) • Nasal Blockage • Discharge • Post nasal drip • Facial pain/headache/discharge/ • Reduction/Alteration of the sense of smell	• Edema • Erythema • Discharge • Pus • Crusts, adhesions, fibrosis • Polypoid changes (cobblestoning) • Polyps
Other local symptoms • Cough • Ear pain/fullness	
Associated symptoms • Loss of energy and productivity • Lack of good quality sleep • Effect on the psyche (sadness, depression or embarrassment)	

1.4.2 Examination (Anterior rhinoscopy +/- Nasal endoscopy)

The sinonasal examination findings in CRS are listed in Table 1-2.

There have been efforts to objectively quantify the amount of disease as seen on nasal endoscopy, and one of the most well-known scoring systems in that regard is the Lund-Kennedy endoscopic score.[13] The Lund-Kennedy endoscopic scores five parameters (polyps, edema, scarring, discharge, crusting) on a three point scale (from 0 to 2).

Figure 1-1 Nasal Endoscopy: A view into the sinonasal cavity using the rigid endoscope, showing erythema, polypoid mucosa, and mucous discharge, all common endoscopic findings in CRS.
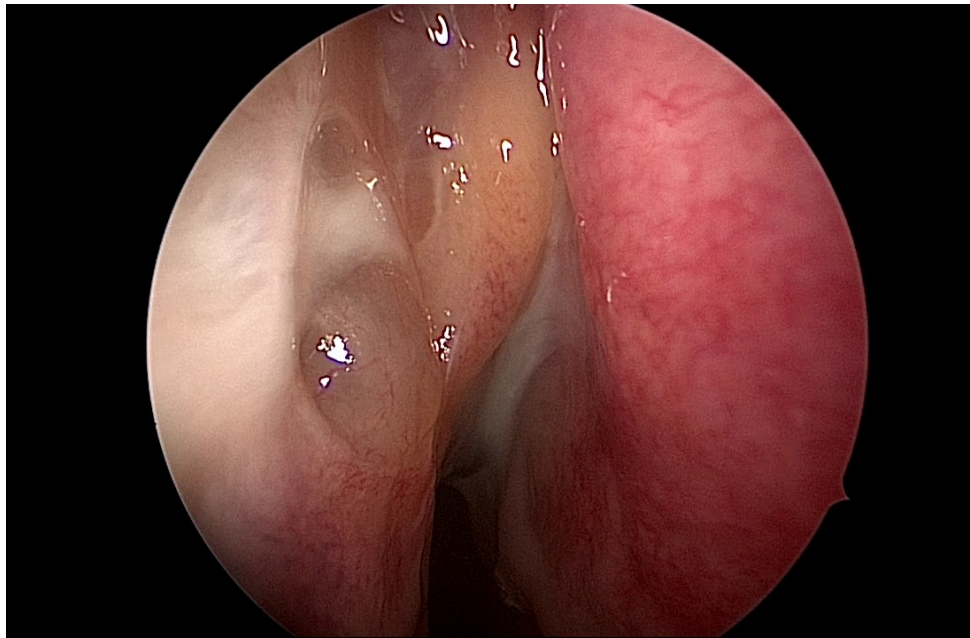
1.4.3 Computed Tomography (CT)

CT scan is now the gold standard radiological investigation. In addition to the confirmation of diagnosis, the CT scan allows pre-operative anatomical assessment and is the primary guide of the sinus surgeon to the patient's anatomy. The typical diagnostic findings are thickened mucosae often with opacified sinuses. Nasal polyps if present may be identified by their typical appearance. In various cases, some anatomical abnormalities may be found. These anatomical variations thought to predispose to CRS will be discussed in 1.6.1.3 below.

The CT scan also allows objective quantification of radiological disease, through standardized scoring systems such as the Lund-Mackay score,[14] which gives a score for each sinus (a score of 0 for no abnormality, 1 for partial opacification, 2 for complete opacification except in the OMC where the score is either 0 or 2).

Figure 1-2 A CT scan (coronal plane reconstruction) of the nasal sinuses in a CRS patient showing mucosal thickening and patchy opacification of sinus air cells.
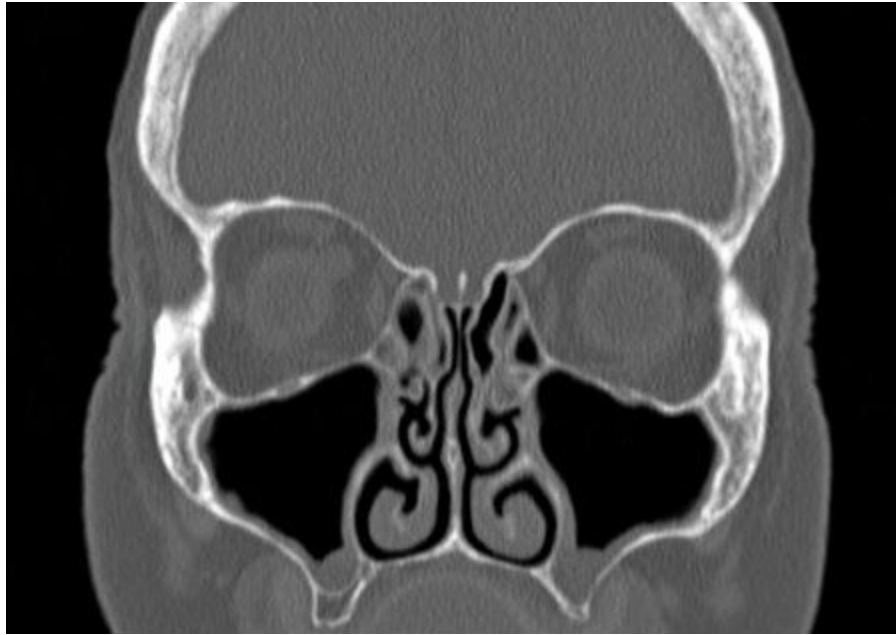
1.5 Classifications

CRS is a group of disorders characterized by sinonasal inflammation. CRS is an umbrella term that includes multiple subtypes. Owing to the complexity of these subtypes (and even wide disparity between some of them), multiple classification systems have been devised, in an attempt to describe the wide range of conditions covered by the umbrella term of CRS. This section will list the most common approaches to classification of CRS.

1.5.1 CRSsNP and CRSwNP: a clinical classification

This is the most common classification used in the literature as well as by clinicians.

The classification depends on the obvious presence (or absence) of the characteristic sinonasal polyps upon examination of the nose. Polyps are pale, edematous pedunculated lesions, commonly seen arising from the middle meatus (although can be found in all of the sinuses). Previous consensus is that detection of polyps bilaterally, only in the middle meatus (visualized lateral to the middle turbinate) is the ultimate criterion for making the final distinction.[1]

However, there exist cases where this distinction between CRSsNP and CRSwNP becomes problematic.

These cases include:

(a) CRSsNP but with polypoid changes/cobblestoned mucosa. These cases are interesting because their origin (and perhaps fate?) is unknown. Can CRSsNP switch phenotype to CRSwNP? Do (CRSsNP and CRSwNP) both represent two stages, albeit on one continuum (of severity)? Is polypoid disease a middle stage of progression between CRSsNP and CRSwNP? Or are they (CRSsNP and CRSwNP) completely distinct disease entities? And if they are completely distinct entities, how should we classify polypoid changes?

(b) CRSwNP having undergone surgery, will often make it harder to detect polyps, especially typical polyps "protruding" from the middle meatus. These cases challenge the arbitrary criterion set out to distinguish the two phenotypes, since a patient classified as CRSsNP could exhibit small-sized polyps contained within the sinuses and have not grown to protrude into the middle meatus yet. The EPOS 2012 guidelines[1] have addressed this topic, and they recommend that unless polyps are present and visible in the middle meatus, these cases should be classified as CRSsNP (despite the possibility that polyps may have been diagnosed before surgery).

These ambiguous, difficult-to-classify cases are a disadvantage of this, otherwise simple and effective, classification system, and emphasize the need for better classifications.

1.5.2 Histopathologic classification

Due to the existence of some difficult to classify cases as mentioned above, histological characterization and classification of the disease is often sought, based on evidence that inflammatory and tissue remodeling profiles in CRSsNP and CRSwNP are distinct. The most commonly used histological classification is based upon the presence or absence of eosinophils. This also happens to coincide with the results of previous studies reporting that CRSwNP cases tend to be more eosinophilic, while CRSsNP cases tend to be more non-eosinophilic (sometimes neutrophilic). Consequently, in this classification we would have eosinophilic CRS and non-eosinophilic CRS. Another commonly-mentioned type would be eosinophilic-mucus CRS (EMCRS).[15] This is a subtype of eosinophilic CRS which is characterized by a high level of eosinophils in the mucus with the characteristic appearance of Charcot-Leyden crystals.

1.5.3 Allergic fungal sinusitis

This is a special type of CRS in which Type I hypersensitivity to fungal antigens is a defining feature. The most widely used AFS diagnostic criteria are those developed by Bent and Kuhn in 1994.[16]

Bent and Kuhn Characteristics of this disease (major diagnostic criteria) include:

(a) CRS with nasal polyposis: unilateral affection of the sinuses common and sometimes affection is asymmetrical. AFS is different from other (opportunistic) diseases of fungal origin in that the patient is usually immunocompetent. Comorbidity with asthma is common.

(b) Characteristic viscid mucus, usually with a brownish tinge "peanut buttery" usually labelled "fungal mucus".. Upon microscopic examination, this mucus is usually eosinophil rich. Detection of fungal hyphae in the mucus is common and Charcot-Leyden crystals may be observed.

(c) CT shows characteristic tiny hyperdense areas in the affected opacified sinuses, leading to "double densities", which is a particularly characteristic sign of the presence of fungus. Another common CT feature is bone erosion, however, with no invasion of soft tissue. AFS is non-invasive and thus should not be confused with acute invasive fungal sinusitis. Other fungal sinus disorders must be excluded at diagnosis.[17]

(d) Elevated IgE with detection of specific IgE against fungal antigens (for example alternaria or aspergillus), indicating an active Type I hypersensitivity reaction.

(e) Positive fungal stain.

1.5.4 Other syndromes causing CRS ("Other CRS")

Aside from the typical CRSsNP and CRSwNP cases, there are less common conditions causing CRS symptoms or a "CRS-like syndrome". These cases are still considered under the umbrella term of CRS, however, these cases are usually excluded from general CRS research studies (EPOS 2012 recommendation).[1] The most important of these relatively uncommon conditions will be listed and touched upon briefly in this section. However, they are not the subject of interest of this thesis and as such, were excluded from all thesis studies in subsequent chapters.

1.5.4.1 Cystic fibrosis

Cystic fibrosis is a multi-organ disease brought about by abnormal mucosal ion transport, namely of sodium and chloride. This abnormal transport mechanism is secondary to various mutations in the CFTR gene (Cystic fibrosis transmembrane conductance regulator). CFTR is a transmembrane ion channel. Mutations in the CFTR gene may lead to either absent or defective CFTR protein, ultimately result in depressed Chloride (Cl^-) secretion. Intracellular chloride retention moves cations (such as Sodium; Na^+) into the cells, leading to loss of secretory volume of mucosal secretions and increase in viscosity. In the airways, mucus becomes increasingly viscid, making the physiological beating action of the airway epithelium cilia ineffective in (mucociliary clearance failure). The resultant accumulation of uncleared secretions encourages a hypoxic environment which provides a great niche for anaerobic organisms to flourish. Moreover, mutations in CFTR affect the epithelial innate immune function resulting in exaggerated and ineffective airway inflammation that fails to eradicate pathogens, suggesting that CF is not only a disease of impaired mucociliary function, but also a disease of mucosal immune deficiency.[18] The disease is thus characterized by development of bacterial biofilms and persisting *Pseudomonas aeruginosa* infections.[19]

In the nose, CF syndrome causes rhinosinusitis in 63% of the patients and is accompanied by formation of nasal polyps in 25%.[20] CF polyps have a distinct pathology when compared to general CRSwNP cases, exhibiting mainly a neutrophilic profile and different perturbations in innate markers.[21,22]

1.5.4.2 - Primary ciliary dyskinesia

Primary ciliary dyskinesia is a term given to a group of rare disorders that involve an abnormality in any of the proteins necessary for the assembly, structure, or normal function of the cilia. The primary genetic defect is usually inherited in an autosomal recessive fashion and 90% of the cases involve a ciliary ultrastructural abnormality, mostly in a dynein arm.[23,24] The disease typically presents in childhood or adolescence.[25,26] The ciliary abnormality leads to a global dysfunction of ciliary motility and mucociliary clearance. This manifests as a systemic disease which, besides nasal affection (chronic sinusitis), exhibits chronic otitis media, repeated lung infections, chronic bronchitis, bronchiectasis and sometimes situs inversus (the triad of sinusitis, bronchitis and situs inversus is known as Kartagener's syndrome).

1.5.4.3 - Granulomatous disease

These include infective causes (such as Rhinoscleroma or Tuberculosis), inflammatory (such as Sarcoidosis, Wegener's disease or Churg-Strauss syndrome), or neoplastic (T-cell lymphoma).[27]

1.5.4.4 - Gross immunodeficiency disorders (congenital or acquired)

Sinusitis can be a complication of immunodeficiency. A series of studies have identified serum immunoglobulin (Ig) deficiency in patients with recurrent rhinosinusitis and/or recalcitrant CRS.[28–32] The prevalence of these deficiencies has been estimated around 5-15%. These deficiencies were found mainly in the major Ig classes (Ig-G, Ig-A, or Ig-M), whilst a lower proportion of patients have low titres in more than one class of Igs that could be diagnosed as common variable immunodeficiency (CVID). [28–32] Although these rates are higher in CRS patients than in the general population, true prevalence remains unclear.[33] Specific Antibody Deficiency (SAD) is the failure to mount an immunoglobulin response against pneumococcal antigens (such as post-anti-pneumococcal vaccination) and has been reported in CRS.[32] These results suggest the measurement of serum immunoglobulins a part of the workup of patients with CRS who are showing severe disease and/or repeated infections not responsive to therapy. In three different studies, the prevalence of rhinosinusitis in HIV-positive patients ranges was reported as 54%, 35% and 12%.[34–36] One study showed higher risk of rhinosinusitis related to the total T-cell count, while another risk was related to diagnosis with AIDS, suggesting an association of disease with severity of the acquired immunodeficiency.

1.5.5 CRS endotyping

CRS endotyping is as an attempt to subclassify CRS according to insights gained mainly from histopathology, the nature of the inflammatory profile as well the pathophysiologic mechanisms involved. The heterogeneity of CRS has promoted the concept that CRS consists of groups of biological subtypes, or "endotypes".[37] CRS endotypes therefore contrast with "clinical" phenotypes (which classify CRS according to clinically-evident, gross manifestations), the most famous being the absence/presence of polyps. (See 1.5.1 above1.5.1) This follows the similar proposal to endotype asthma.[38] Each endotype would be predicted to follow a certain clinical behaviour or course.

Endotyping CRS could be described as an active area of research.[37] Despite this fact, there has been well-characterized endotypes that have already been described in the literature.[39] Within

CRSwNP, the endotype exhibiting high IL-5 expression is mostly associated with asthma co-morbidity, high total IgE as well as presence of IgE to Staphylococcal superantigens.[40] The typical neutrophilic profile of cystic fibrosis polyps[21] has also been described as an endotype.[39]

1.5.6 Conclusion

In summary, there exist many classification systems for CRS. This is due to the heterogeneous nature of the disease, which illustrates the difficulty of doing CRS research. The simplicity and practicality of one classification, CRSsNP versus CRSwNP, gained it widespread usage and recognition. Despite this, this system is not without its limitations. The most valuable classification is that which is able to provide more insightful prognostication and guide medical and surgical treatment options and this supports efforts to unravel CRS endotypes

1.6 CRS Etiology

To date, the exact cause of the disease is unknown. The pathogenesis of the disease is also complex and only partially understood, especially with regard to the initiating factor which triggers the inflammatory cascade. However, current consensus is that CRS is a multifactorial disease. This involves interaction(s) between host factors and environmental factors. Moreover, the relative contributions of each factor to disease etiology vary from one patient to the other. In this section, these factors will be briefly discussed.

1.6.1 First: Host factors

1.6.1.1 Genetics

The presence of a genetic predisposition to CRS has been suspected based on reports of positive family history of the disease.[41] DNA studies enable researchers to investigate genetic variants and whether they are associated with a particular trait or disease.

The genetic variants or polymorphisms that have been associated with CRS in the literature have been summarized in the review by Hsu et al.[42] These variations include: mutations in Cystic fibrosis Transport Receptor (CFTR) involved in chloride ion transport; genes encoding human leukocyte antigens (HLAs); genes involved in innate immunity; genes involved in Th2 inflammation; and other genes involved in inflammatory response and remodeling. The literature thus provides preliminary evidence for involvement of genetics in predisposition of CRS. There

are some challenges facing the generalization of the findings of these genetic studies.[42] Firstly, these studies provide associations, which do not imply causation. Secondly, studies need to be adequately powered, which requires sizable cohorts and, consequently, more expensive studies. Thirdly, genome variant findings do not account for gene-environment interactions, which are thought to occur due to epigenetic modifications to the genome.[43] Lastly, many of the positive findings have not been replicated in other studies and thus many findings still require to be confirmed. However, the most consistant finding is the association of CFTR mutations with CRS. [42] The most important study is the case-control study by Wang et al.,[44] in which the DNA of 147 CRS patients and 123 non-CRS controls was investigated for 16 mutations that account for 85% of cystic fibrosis in the general population. They found that the proportion of CRS patients who were found to have a CF mutation was 7%, versus 2% in the control group, and the difference was reported as significantly significant.[44]

1.6.1.2 Aspirin sensitivity

Hypersensitivity to aspirin is associated with chronic rhinosinusitis and nasal polyposis. This triad is known as "aspirin triad" or "Samter's triad", and is more recently termed "Aspirin-Exacerbated Respiratory Disease" (AERD). Administration of aspirin (and NSAIDs) in these patients can produce asthma, nasal symptoms, urticaria, angioedema, or in some cases even anaphylaxis. A systematic review estimated that AERD occurs in 7% of typical adult asthmatic patients, and twice that number in patients with severe asthma.[45] This hypersensitivity to aspirin (and often, to other NSAIDs) is not a Type 1 hypersensitivity reaction, but is associated with a disturbance in eicosanoid metabolism, such that there is an upregulation of the pro-inflammatory cysteinyl leukotrienes (cysLTs), with reduction in the anti-inflammatory prostaglandin E2 (PGE2). This imbalance lies at the heart of the pathogenesis of AERD. NSAIDs cause Cyclo-oxygenase 1 (COX-1) inhibition, which leads to worsen the imbalance between the increased cysteinyl leukotrienes (down the 5-lipo-oxygenase (5-LO) pathway) and the reduced PGE2 (downstream of COX enzymes).

1.6.1.3 Anatomical factors

It has been thought that unfavorable anatomic variants of the bony sinuses can lead to impaired mucociliary drainage, which could predispose to the development of CRS. This is to some extent

supported by the theory that ostio-meatal complex (OMC) obstruction put forward by Messerklinger as important in the development of CRS.

In one study,[46] scans of 200 patients with symptoms of CRS were reviewed and the authors concluded that there is a significant association between the presence of some anatomic variations (septal deviation, bilateral concha bullosa, medial deviation of uncinate process, Haller cell, ethmoidal bulla hypertrophic, agger nasi cell) – and the presence of mucosal disease.[46] This study however lacked comparison with a non-CRS control group and thus could not establish a specific association.[46]

In another study,[47] the authors reviewed 328 consecutive CT scans to investigate the association between the presence of frontal sinus cells in the frontal recess (thought to cause narrowing of the frontal recess) and mucosal thickening in the frontal sinus. They found a significant association of frontal sinus mucosal thickening with the presence of frontal sinus cells.[47] In contrast, another study of 70 scans showed no association between frontal sinus mucosal affection and frontal sinus or agger nasi cells.[48]

In the study by Bolger et al, [49] the authors concluded that the frequency of sinus anatomical variations in patients with CRS symptoms were not significantly different that their frequency in a control group undergoing CT scans for non-sinus complaints.[49] The only variation reported as more common in the sinus group was concha bullosa.[49] Zinreich et al. however showed in a cohort of 320, that OMC obstruction was not different between those with and without concha bullosa.[50] These results agree with similar findings by other studies that reported no increased rate of mucosal disease in patients with concha bullosa.[51,52]

In a study by Nouraei et al.,[53] the authors deployed multivariate regression to determine the association between anatomic variants and mucosal disease in 278 consecutive scans and concluded that bony anatomic variants are not associated with increased risk of mucosal disease.[53]Traditionally, OMC obstruction has been considered to be a significant factor in causing CRS. OMC obstruction however is not present in all patients,[54] suggesting that it could not be the main pathogenetic event in the development of CRS. These opposing findings of all the above studies indicate that the pathogenicity of sinus bony anatomy (as a precipitator of sinus ostial or OMC obstruction) probably plays a minor causative role in CRS, but they also indicate that it may play a role on a patient-to-patient basis. This patient-to-patient assessment has also

been previously suggested by Bolger et al.[49] Based on this suggestion, we could further hypothesize the presence of a group of patients who have a predominant obstructive disease, as opposed to patients who have a more widespread inflammatory component. These patients showing mainly obstructive disease are not well characterized in the literature as a distinct group of CRS, and further research is needed to delineate them through clear criteria.

1.6.2 Second: Environmental factors

1.6.2.1 Bacteria

CRS has always been a disease closely associated with bacterial infection. Despite this fact, CRS is not simply an infectious disease, but a complex inflammatory disease. Studies looking into the bacteriology of CRS have reported a positive bacterial culture rates of about 83%.[55,56] The most commonly occurring organism detected by culture is *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*. A recent survey of 513 cases of CRS sinus cultures[56] showed highest rates of cultures were for both *Staphyloccylococcus aureus* (35%) and *Pseudomonas* (9%). A similar culture rate of *Staphyloccylococcus aureus* (35%) was reported in another study by Gittelman et al.[57] Another study reported a culture rate of 17% for *Pseudomonas aeruginosa*.[58] Bacteria are an important clinical modifier as secondary bacterial infections cause exacerbations of disease, leading to the symptomatic suffering of patients and often necessitating antibiotics for treatment.

1.6.2.2 Biofilms

Bacterial biofilms present in the sinuses have been associated with a worse clinical picture and disease outcomes.[59–61]

An investigation into which species were associated with worse disease demonstrated that Staph aureus was constantly associated with worse outcomes and disease severity.[62,63] On the other hand, species like *Haemophilus influenzae* was associated with milder disease forms.[62]

Biofilms were also demonstrated to be able to generate an innate immune response on sinonasal mucosa.[64,65] This response has been described as mostly promoting Th1/Th17-like response.[66,67]

All these studies provide powerful evidence that bacteria (in the form of biofilms) are a very important disease-modifying factor in CRS. However, few studies demonstrated a link between biofilms and the archetypical Th2 profile picture of CRS. The closest study to investigate this is

the study by Foreman et al.[68] (elaborated on in 1.8.5 below) which correlated biofilm presence with Th-2 skewed cytokine profile, independent of superantigen status. However, stronger evidence, using in vitro and in vivo models, is needed to confirm a causative association.

1.6.2.3 Superantigens

Staphylococcus aureus enterotoxins capable of acting as superantigens have been demonstrated to be associated with the Th2 response. The studies detailing the role of superantigens in CRS are discussed in 1.8.4 below.

1.6.2.4 The Microbiome

Traditional culture techniques are limited in identifying all bacteria associated with both healthy sinuses and diseased sinuses. DNA-based detection methods (such as 16S-rRNA based methods) are both ultra-sensitive and less biased, and therefore able to provide a more complete description of the whole microbial population occupying a certain environment, termed the microbiome. Microbiome studies thus have the potential to cause a paradigm shift in how the role of bacteria is perceived in CRS. In contrast to the particular-species-as-pathogen paradigm, microbiome studies allow the evaluation of the balance between the various components (species) of the microbiome, and how it relates to health versus disease. A disturbed evenness of a microbiome, with reduced species diversity thus has the potential to cause disease. Although microbiome studies are few and relatively novel in the CRS literature, multiple studies already reported that a reduced diversity of the microbiota is characteristic of CRS patients, when compared to healthy non-CRS controls.[69,70]

1.6.2.5 Fungi

Fungi have always been thought to play a role in CRS since the study by Ponikau et al.,[71] which employed a sensitive method of detection. Current evidence mostly rejects a true causative role for fungi in CRS.[72] Nevertheless, in a small percentage of CRS patients, fungal elements continue to be detected in CRS pathology specimens and/or grow in culture. The role of these fungi (whether as innocent bystanders or as disease modifiers), is still not fully understood. A recent study of the fungal microbiome showed a more diverse sinonasal fungal population never demonstrated using conventional culture methods.[73]

A less studied role is the interaction between fungus and bacteria in the sinuses. In a sheep model study of sinusitis, sheep were inoculated with *Staphylococcus aureus* as well as *Aspergillus fumigatus* and *Alternaria alternata* and observed for detection of development of fungal biofilm.[74] Significant fungal biofilm only occurred when *Staphylococcus aureus* was co-inoculated with the fungi and was not related to the presence or absence of immune sensitization to fungi. To assess if this phenomena was due only to the *Staphylococcus aureus*, fungi were co-innoculated with other bacteria and a ciliary toxin. Fungal biofilm developed in the presence of all bacteria as well as with a ciliary injury (induced by the ciliotoxin) indicating that fungal growth needed the presence of a mucosal injury.[74] These findings are of clinical interest and point to a more opportunistic (rather than causative) fungal colonization of the sinuses.A more prominent disease-modifying role of fungus could be observed in AFS, which has a characteristic clinical picture. This role involves a Type-1 IgE-mediated allergy against fungal antigens. This particular subgroup (AFS) has been described in 1.5.3 above.

1.6.2.6 Viruses

Viruses in CRS are an under-studied topic. A virus acting as an initial triggering factor for the production of CRS could be a plausible hypothesis. However, there remains no tangible evidence to support this hypothesis. A viral hypothesis has been proposed for the origination of aspirin-exacerbated respiratory disease,[75] but as yet there is no solid evidence to support it.

Respiratory Viruses were detected in nasal washes and mucosae of CRS patients, in rates higher than controls.[76] Several in vitro experiments showed that Rhinovirus impairs the mucociliary and barrier function of nasal epithelial cells.[77,78] Other studies suggest an immunomodulatory role,[79] and increased attachment of bacteria to epithelial cells following viral infection in vitro.[80] The results of these studies suggest there may be a role for viruses in the early CRS or in CRS exacerbations.

1.7 CRS Histopathological findings

CRS is a chronic inflammatory condition. The pathologic picture is thus that of inflammatory infiltration with varying grades of severity. The type of inflammatory infiltrate determines the inflammatory profile, which may vary in various CRS endotypes (see Histopathologic Classification)

The histopathologic mucosal findings in CRS include the following:

1.7.1 Inflammatory cell infiltrate

Eosinophils are the hallmark of the inflammatory infiltrate in CRS. Despite this fact, some cases have non-eosinophilic inflammation. It has been described that the eosinophilic inflammation is more characteristic of CRSwNP than CRSsNP.[81] CRSwNP in Asian populations tend to be more non-eosinophilic, when compared to Caucasian populations.[82]

Figure 1-3 Eosinophilic mucosal inflammation in CRS
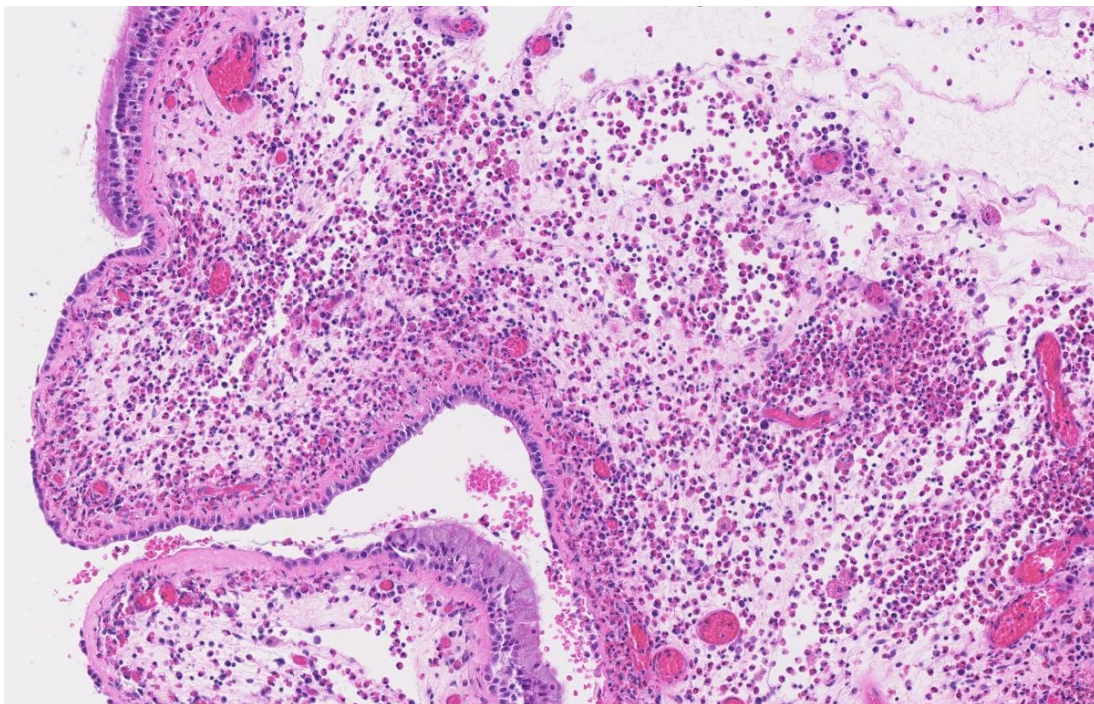
1.7.2 Mucosal edema

Edema is usually one of the initial macroscopic signs of an unhealthy mucosa. Edema is also found in patients after surgery, as a local reaction to surgical manipulation. However this post-surgical edema usually resolves with time, is considered part of the mucosal healing process and do not indicate a chronic unhealthy state.

1.7.3 Epithelial injury and shedding

Epithelial dysfunction is one of the consequences of CRS. The features of this dysfunction include:

(a) Epithelial shedding, loss of epithelial cells

(b) Loss of epithelial cell differentiation and squamous metaplasia[83]

(c) Ciliary ultrastructural defects.[83] These include presence of compound cilia, cilia showing abnormal microtubular patterns or numbers, and absence of dynein arms.

(d) Impairment of barrier function

These abnormalities can be explained through several factors:

(a) Production of eosinophil products, which cause tissue injury. (See 4.3.1 below)

(b) Interaction of underlying inflammatory cells with epthelial cells.[37]

(c) Injurious micro-organisms in the sinus cavity. For example, a recent study[84] points towards a potential role for strain-specific *Staphylococcus aureus*-secreted products in compromising epithelial barrier function. These has been shown in vitro to cause a concentration-dependent decline in electrical impedance across primary human nasal epithelial cell cultures grown at air-liquid interface, with the disruption of the tight junctions between the cells.[84]

1.7.4 Goblet cell hyperplasia (Goblet cell metaplasia)

Goblet cell hyperplasia is a characteristic feature of the chronic inflammatory state. This effect is mainly brought about through the action of IL-13. IL-13 is considered a Th2 cytokine, and plays an important role in the mucociliary differentiation of human nasal epithelial cells. IL-4, another Th2 cytokine, is also involved in this process. Both IL-13 and IL-4 have been shown to have a common receptor (IL-13Rα1/IL-4Rα) and thus activate a common signaling pathway involving STAT-6. This pathway ultimately leads to an increase in the proportion of secretory cells.[85,86]

1.7.5 Fibrosis/Mucosal Remodeling

Remodeling is a series of gradual structural modifications in the mucosa that could ultimately lead to irreversible changes. It has been found that mucosa in the sinuses show signs of remodeling in the context of CRS. These changes include increased myofibroblast activation and increased collagen deposition, and a thickened subepithelial basement membrane. In CRSwNP, there is an added dimension, which may help explain the growth of hyperplastic polyps, which includes fibronectin deposition (cite) and pseudocyst formation.[87]

Remodeling in CRS can be considered a recently described phenomenon (when compared to remodeling research in asthma) and its clinical implications in CRS have not been explored. One potential implication for remodeling is irreversible mucosal disease, which has already been demonstrated in asthmatic lower airways. The potential for irreversibility comes from the fact that once collagen is deposited, it is difficult to reverse with topical steroids.[88] More elaboration on mucosal remodeling and its potential role in CRS will be found in Chapter 8.

1.7.6 Osteitis/Bone remodeling

Features of bony erosion or, conversely, bony deposition (neo-osteogenesis) is a feature, albeit not universal, of CRS. Concurrent osteitis occurs in 36–53% of CRS patients.[89] The bone can act as a site of inflammatory activity, which can play a role in propagating sinus inflammation. Osteitis has been demonstrated to be association with: a worse clinical picture (as per endoscopic and CT scores);[89,90] revision cases compared to primary cases; increased mucosal inflammation; tissue and serum eosinophilia; and worse treatment outcomes. (cite for each) Bony erosion is also a common feature in AFS.

1.7.7 Mucus

The increase in goblet cells leads to a corresponding increase in mucus secretion. With a defective mucociliary clearance, mucus stagnates in the lumen. It then plays a role in blocking sinus passages and air cells. Mucus in CRS usually shows infiltration with eosinophils. These cases in which mucus shows widespread infiltration with eosinophils are usually termed eosinophilic mucus chronic rhinosinusitis.[15] Eosinophils also degranulate in the mucus releasing the contents of the granules, which include ECP and MBP, which contribute to epithelial injury. Charcot-Leyden crystals are usually detected in eosinophilic mucus. These crystals are eosinophil breakdown products, since eosinophils produce Charcot-Leyden crystal protein. Macroscopically, eosinophilic mucus is typically thick and viscous.

1.8 CRS Pathophysiology

The mechanism, by which the ultimate histopathological and clinical picture of CRS is reached, remains at best partially understood. All "broad" theories that had been proposed will be discussed in this section; these include recent as well as historical theories.

1.8.1 Inadequate resolution of acute sinusitis

Chronic sinusitis was primarily thought of as a natural progression of inadequately treated acute rhinosinusitis (ARS). This theory is considered historical as the explanation was found inadequate as evidence mounted that ARS and CRS are different clinical entities.

1.8.2 Osteomeatal complex obstruction

The belief that CRS is primarily a disease of sinonasal obstruction rests on the mucociliary clearance studies of Messerklinger and Stammberger. Messerklinger and Stammberger studied the pathways of mucociliary clearance (MCC) in the sinuses. They found that all cilia in the sinuses beat in an organized deliberate fashion towards the natural ostium of the sinus. This establishes a natural drainage pathway for each sinus. The drainage pathways of the sinuses would intersect at a common anatomical region at the middle meatus, which is called the osteomeatal complex (OMC). (Figure 1-4) The OMC is central to drainage from all the anterior sinuses.

Figure 1-4 CT scan illustrating the osteomeatal complex (OMC).
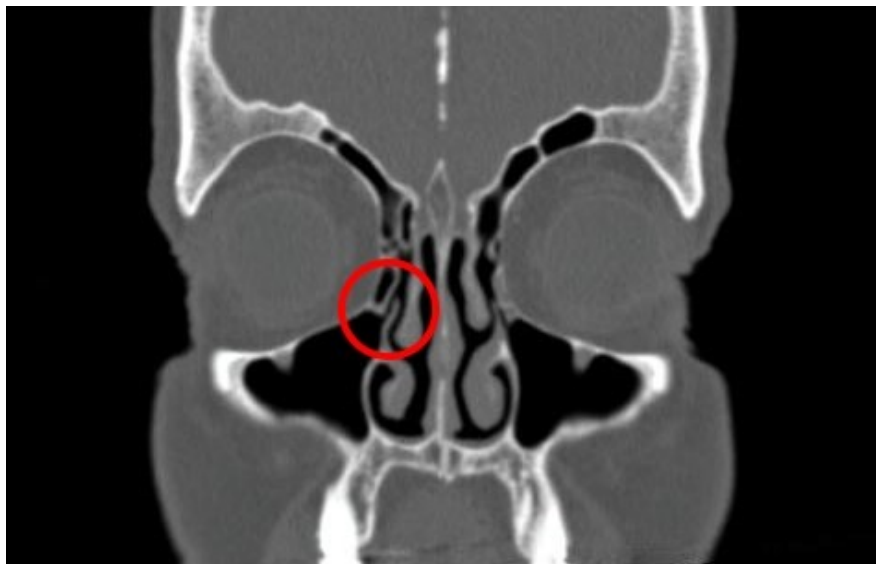
Messerklinger and Stammberger later hypothesized that obstruction at the OMC is the cardinal pathogenetic event in the development of CRS, since it leads to obstruction of drainage from the frontal, maxillary and anterior ethmoidal air cells. Obstruction subsequently results in the build-up of secretions, leading to secondary (persistent) infection which could not be cleared, mucosal thickening, and ultimately, CRS.

This theory has been instrumental in the development of the original concepts of Functional Endoscopic Sinus Surgery (FESS), which eventually became the gold standard in the surgical treatment of CRS. FESS depends on a large extent to clearance of obstruction at the OMC.

However, some recent studies report about a subset of CRS patients who do not exhibit OMC obstruction,[53,54] which rules it out as the central pathognomonic feature of the disease. Moreover, adequate clearance of OMC disease during FESS does not preclude against disease recurrence. In Chapter 5, we formulate our thesis against an exclusive rule of OMC in surgical management of CRS.

1.8.3 Fungal hypothesis

The fungal hypothesis was the one of the first to propose a specific aetiologic organism for CRS. The first strong proposition of a fungal hypothesis comes from the study by Ponikau in 1999.[71] Ponikau et al. found that fungal cultures of nasal secretions were positive in almost all patients in their study cohort (202 of 210 consecutive CRS patients, 96%).[71] This ubiquitous finding was later confirmed in another study (by the Graz group).[91] However, both studies reported fungal detection as well in (almost) all non-CRS controls.[71,91] Despite this fact, the findings were met with active enthusiasm, and trials were designed to test the efficacy of antifungal medication in CRS. Various clinical trials have reported no beneficial outcomes gained from antifungal therapy (Amphotericin B administered topically as irrigations), on the contrary, side effects of Amphotericin B were reported.[92–94] One of these trials failed to demonstrate any significant changes in inflammatory markers with Amphotericin B irrigations.[95] A recent meta-analysis confirms there is no evidence to support the use of antifungal therapy.[96] It was the failure of antifungal medication that led to the current consensus that the fungal hypothesis is not an adequate explanation for CRS.[72]

However, fungal spores and hyphae could play a role as a disease modifier, especially in a subgroup of CRS termed allergic fungal rhinosinusitis, or allergical fungal sinusitis (AFS). This role constitutes of provocation of IgE-mediated inflammation against fungal allergens. (See 1.5.3 above and also 1.6.2.5 above)

1.8.4 Superantigen theory

The superantigen theory assumes a major role for *Staphylococcus aureus* in the disease process. *Staphylococcus aureus* enterotoxins (in particular enterotoxin B and enterotoxin A) can act as superantigens. This means that they are able to cause widespread, non-specific, polyclonal activation of T cells. This occurs through their binding of the variable β (Vβ) chain of the T cell receptor-major histocompatibility complex class II (MHC-2), independent from the antigen-specific binding site. And since there are only a relatively limited number of Vβ recombinations, the proportion of lymphocytes activated is much higher through the classical antigen presentation mechanism Downstream effects of these activated T cells include production of Th2 cytokines (such as IL-4 and IL-5), recruitment of eosinophils, and polyclonal activation of B cells with consequent production of IgEs.

The initial finding was the detection of IgE against Staphylococcus aureus enterotoxins in nasal polyp tissue by Bachert et al.[97] Moreover, exposing nasal polyp tissue to Staphylococcus aureus Enterotoxin B led to increased release of IL-5 and IL-13, when compared to normal mucosa.[98] Tripathi et al. investigated the TCR Vβ chain repertoire using flow cytometry in nasal polyps for evidence of a superantigen effect (TCR VB subset expansion), and reported its detection in 58.3% of the cases.[99]

But since the evidence for superantigen effect has not been found in all patients, it is difficult to implicate them as the causative agent that triggers CRSwNP. Some authors opined that they should be regarded only as disease modifiers.[100] Indeed, there is evidence that a specific CRS endotype associated with specific IgE to Staphylococcal enterotoxins may be related to some disease characteristics, such as prediction of asthma comorbidity.[40,101]

1.8.5 Biofilm theory

It has been suggested that bacterial biofilms can play the role as an initiator (or at least of an early pathogenic role) in the disease process.[102] Biofilms are aggregates "communities" of bacterial cells, enclosed together in a polysaccharide matrix and attached to a surface. In the sinuses, these thus aggregates are clumped in the mucus and attach to the mucosal surface. This is in contrast to free-floating "planktonic" bacteria. This arrangement allows the bacteria to acquire various properties, including: (a) resistance to antibiotics; (b) evasion of immune responses; and (c) acting as a nidus for repeated infections.

These properties aid bacteria in their pathogenicity and are consistent with the findings of multiple studies associating biofilms with various features of worse disease. (See 1.6.2.2 above)

Virulent organisms such as Staphylococcus aureus or Pseudomonas aeruginosa were traditionally associated with worse outcomes. Staphylococcus aureus in particular is a classical CRS organism that is capable of: (a) intracellular residence, evading treatments and the immune system; (b) acting as a source of exotoxin superantigens.(see 1.8.4 above) A study by Foreman et al.[68] investigated the association of biofilm status and superantigen production (simultaneously) with the inflammatory cytokines in the mucosa. Using linear discriminant analysis, their findings suggested that biofilm status was associated with indicators of Th2 bias (IL5 and ECP) while positive superantigen detection was associated with an IgE, MPO and IFN-γ.[68] However, recent research shows that biofilms on the sinonasal mucosa are more likely to promote a Th1/Th17 response,[66,67] leaving how the pathognomonic Th2 response evolves unexplained. Thus stronger evidence linking biofilms with the Th2-skewed CRS pathology has yet to become available, and most probably need the development of a long-term animal model for CRS.

1.8.6 Eicosanoid metabolism disturbance

In the study by Rocca-Ferrer et al., IL1-β stimulated nasal polyp fibroblasts in vitro showed evidence of eicosanoid metabolism disturbances, regardless of the state aspirin intolerance of the donor.[103] Increased synthesis of pro-inflammatory leukotrienes and decreased synthesis of anti-inflammatory prostaglandins (PGE2) have thus been proposed as a mechanism not just for aspirin-sensitive nasal polyps but also aspirin-tolerant CRSwNP.[1,103]. This theory needs further investigation to accurately delineate the specific eicosanoid pathway disturbances and their variations between the aspirin tolerant and aspirin intolerant populations.

1.8.7 Immune barrier hypothesis

This hypothesis was based on reviewing the literature for all previously-mentioned organisms that have been reported to play a pathogenic role in the disease. The authors[104] concluded that, whichever the eliciting organism, there has to exist a mechanical/functional compromise in the sinonasal mucosa resulting in a loss of its immune barrier function. This would lead to unopposed interaction of the organisms (or allergens) in the lumen with the underlying mucosal elements, initiating the cascade of chronic inflammation. The barrier defects could be host-

related (genetic or epigenetic) or acquired (environmental). According to this hypothesis, proposed triggers (microbes or allergens) cease to be causative agents and become disease modifiers, in light of the immune barrier deficiency.[104]

1.8.8 Conclusion

Despite the fact that the exact pathogenetic mechanism of CRS is not completely understood, some conclusions can be made. CRS is a complex multi-factorial disease that could not be ascribed to one etiologic agent. The pathogenesis most probably involves interaction between host and environmental factors. A combination of host factors lead to a certain predisposition which, in the presence of the enabling environmental factors, ultimately result in disease development. The immune barrier hypothesis[104] is possibly the closest "broad" hypothesis to describe this process. Even in the presence of all disease "prerequisites", a "chicken-or-egg" triggering question still remains - whether the pathogenetic environmental factor(s) compromises the host's immune barrier first; or whether the immune barrier is already compromised due to an intrinsic host factor. The incomplete understanding is moreover compounded by the presence of multiple disease phenotypes (which includes for example CRSsNP and CRSwNP). Between the clinical phenotypes, the percentage of participation of each pathogenetic factor will differ. The problem is even more compounded by the fact that each CRS phenotype exhibits several endotypes. Each CRS endotype represents a different histopathological picture or inflammatory profile. More probable than not, different factors would also play different roles in each endotype. A possible conclusion could be that different pathogenetic courses ultimately lead to the final clinical picture of the CRS syndrome.

1.9 Medical Management of CRS

Upon initial presentation with confirmed CRS, patients are given a trial of medical treatment. This initial treatment is considered standard clinical practice. In this section, we discuss medical treatment options available.

The mainstay of medical treatment of CRS is a combination of (a) anti-inflammatory medication (b) anti-microbial medication (c) saline washes.

1.9.1 First: Anti-inflammatory medication

1.9.1.1 Steroids

Steroids are the mainstay of medical treatment of CRS for their anti-inflammatory action. These actions could be summarized in:

1) Effect on disease:

    The effectiveness of steroids in relieving patients' symptoms and improving quality of life has been documented in several clinical trials.[105–107] Use of steroids also results in improvement in endoscopic and healing scores.[108,109]

2) Effect on inflammation/inflammatory markers:

    a. inhibiting recruitment of inflammatory cells, mainly eosinophils into the nasal mucosa. This occurs through inhibition of leukocyte adhesion and chemotaxis.[110,111]

    b. enhancing apoptosis of as well as reducing the viability of eosinophils in the nasal mucosa[112–114]

    c. reduction in local levels of eosinophil products such as eotaxin[108,115]

    d. reduction in levels of pro-inflammatory cytokines, including the Th2 cytokines[108,110,116]

3) Effect on microbes:

    Recently, there have been studies that suggest a potential antibacterial role for steroids, due to a demonstrated direct inhibitory effect on growth of *Staphylococcus aureus* biofilms in vitro, albeit at higher dosages.[117] One can also hypothesize that such an effect can also occur indirectly, through modulation of the mucosal inflammatory profile. However, the clinical significance of this effect and whether it contributes substantially to the benefits of steroid therapy in CRS is yet to be determined.

1.9.1.2 Anti-Leukotrienes

Antileukotrienes (Leukotriene Receptor antagonists) act as competitive antagonists at the CysLT1 receptor. This group of drugs includes montelukast and zafirlukast. A recent review suggests that montelukast is an effective adjunctive therapy in CRSwNP, when used in

conjunction with topical and/or oral steroids (based on three randomized trials).[118] Despite the fact that the mode of action of these drugs would be particularly suited to address the pathophysiology of these CRS patients suffering from AERD, there is no current evidence in the literature to suggest that they have increased effectiveness in aspirin-sensitive versus aspirin-tolerant patients.[118] Their use in CRSsNP has also not been evaluated.[118]

1.9.1.3 Biologicals (Mono-clonal antibodies)

Monoclonal antibodies offer a more targeted approach to suppression of inflammation. They aim at blocking specific cytokines or pathways that play an important role in disease progresssion. This represents an alternative to the widespread less-specific inhibition caused by corticosteroids.

Anti-IL5 (reslizumab and mepolizumab) has been used in CRS in two trials.[119,120] It has been shown to reduce nasal polyp size, serum and nasal eosinophil cationic protein (ECP) levels in CRSwNP patients, with responders being the patients with high IL-5 levels.[119,120]

Anti-IgE (omalizumab) mechanism of action is through neutralizing IgE in nasal tissue (as well as systemically). To date, there are two RCTs for omalizumab in CRS. One trial[121] showed rather limited benefit, since it failed to show additional benefit on top of the standard maximal medical therapy. The other trial,[122] done in CRSwNP patients with comorbid asthma, demonstrated a reduction in polyp size as well as reduction in upper and lower airway symptoms.[122]

1.9.2 Second: Anti-microbial medication

1.9.2.1 Antibiotics:

The current recommendation is to treat secondary infection flare-ups on top of CRS, as evidenced by presence of purulent secretions.[1,123] This recommendation appears reasonable in the light of CRS being understood as a complex inflammatory (and not an infectious) disease entity. There is no data to support the routine long-term use (> 3 weeks) of antibiotics in the treatment of CRS.[123] All routine antibiotic use in CRS is oral.

1.9.2.1.1 Choice of antibiotic

Oral antibiotics are commonly used in CRS. Broad-spectrum with adequate gram-positive cover such as amoxicillin/clavulanic acid is a typical choice. However, there are few published studies that compare the efficacies of different antibiotic regimens. If mucopurulence is visible and

accessible, a swab can be taken for culture and sensitivity, and choice of medication can be decided based on bacterial susceptibility.

Conversely, topical antibiotics are currently not recommended for routine use in CRS.[123] The use of topical mupirocin washes post-surgery for treatment of multi-drug resistant Staphylococcus aureus showed promise in a recent RCT. However, upon cessation of treatment, a significant number of patients had microbiological and symptomatic relapse.[124,125]

1.9.2.1.2 Major drawbacks of antibiotic therapy

1.9.2.1.2.1 *Insufficient effectiveness against biofilms or intracellular pathogens*
It has been hypothesized that most of the bacteria in the sinuses exist in a biofilm state; biofilms are naturally resistant to antibiotics at standard doses.[126] Consequently, antibiotics can act as temporizing measures in case of a biofilm infection, but do not offer long-term microbial eradication. This has been demonstrated in a recent RCT investigating mupirocin and systemic amoxicillin/clavulanic acid in CRS, where microbiological and symptomatic relapse occurred following cessation of systemic antibiotic therapy.[124,125] The biofilm therefore acts as a nidus for repeated infection, a common occurrence in the course of CRS (as shown through typing studies).[127,128] Moreover, recent evidence showed that Staphylococcus aureus is capable of invading epithelial cells.[129] The intracellular resistance is thought to be a virulence mechanism, allowing the bacteria to escape the host immune response, to play a role in persistence of infection. Intracellular bacteria are naturally more resistant to antibiotic treatment, since antibiotics have to have adequate intracellular penetration and efficacy inside the cells.[130] Indeed, CRS patients who had intracellular *Staphylococcus aureus* positive had a significantly higher risk of clinical and microbiological relapse of their disease compared to patients who did not.[131]

1.9.2.1.2.2 *Disruption of the microbiome*
Conceptualizing the role of bacteria in CRS in terms of a microbial population that, not only includes pathogens, but also includes harmless commensals (which can be actually play a beneficial role or "probiotic") challenges conventional "antibiotic versus pathogen" prescription pattern. Broad-spectrum antibiotics inadvertently kill harmless probiotic bacteria[132] and disturb the balance between probiotic and harmful organisms in the microbiome. This disturbs the delicate homeostasis of host-microbiota mutualism.[133]

In a preliminary study upon the effect of antibiotic prescriptions on the microbiome in CRS, antibiotics were found to reduce microbial diversity.[134] A highly diverse microbiome is thought to associate with health and reduced diversity is thought to be associated with diseased states. The particular effects have not been studied yet and more studies are needed to investigate antibiotic administration effects on the CRS microbiome in various contexts (pre-operatively, peri-operatively and post-operatively).

1.9.2.1.2.3 Antibiotic resistance

CRS is commonly cited as the fifth most common diagnosis associated with antibiotic prescriptions in the United States.[9] Another US five-year national study found that a primary diagnosis of unspecified CRS accounted for 7.1% of antibiotic prescriptions, more than any other primary diagnosis documented in ambulatory care visits.[135] Antibiotic use as part of the "maximal medical treatment" is an almost universal practice by Otolaryngologists and Rhinologists surveyed in the US and UK.[136–138] These patterns call for rationalization of use, based on solid evidence-based grounds, where the indication of antibiotics will be most effective. The overuse of antibiotics leads to a possible looming global crisis, owing to the rise of multi-drug resistant bacteria "super-bugs".[139,140]

1.9.2.1.3 The need for antibiotic-alternative approaches

The limitations of antibiotic therapy listed in 1.9.2.1.2 above call for new approaches. These approaches should include:

(a) Use of natural antimicrobial peptides produced by naturally-ocurring bacteria:

These molecules are called Bacteriocins and they offer an alternative to traditional antibiotics.[141] They can have narrow or broad spectrum activity.[141]

(b) Use of alternative anti-biofilm treatments:

Some of these agents have proved effective against *Staphylococcus aureus* biofilms in vitro or in vivo. Examples include manuka honey[142,143], and colloidal silver[117].

(c) Exploiting microbial antagonism:

These include the use of probiotic bacteria that competes against the most common CRS bacterial pathogens, such as Staphylococcus aureus or Pseudomonas aeruginosa. For example, *Staphylococcus epidermidis* has been shown to compete against *Staphylococcus aureus*. In a

mouse model of sinus inflammation, *Staphylococcus epidermidis* added to *Staphylococcus aureus* was able to reduce the amount of goblet cells in inflamed sinonasal epithelium.[144]

Bacteriophages are viruses that kill bacteria and are specific to a single bacterial species or strain. Proof of concept of a specific phage against Staphylococcus aureus has been demonstrated both in vitro and an in vivo sheep model of sinusitis.[145,145] As such, use of bacteriophage cocktails to control the microbial population offers an exciting potential avenue of future antimicrobial therapy.

1.9.2.2 Antifungals:

Antifungals were suggested to be a component of medical therapy based on the fungal theory put forward initially by Ponikau et al.[71] However, topical antifungals have been extensively investigated in RCTs and the evidence recommends against their usage,[1,96,123] since their side effects override their negligible benefit in general CRS patients.(discussed briefly in 1.8.3 above)

A special exception to the above rule may be AFS, where treatment with oral antifungals showed symptomatic benefit in at least three uncontrolled retrospective series.[146–148] and thus may remain an option in this specific subgroup of patients. More recently, a randomized controlled study of various post-operative antifungal regimens was done by Khalil et al. showed reduced recurrence rates of AFS in patients using intranasal topical fluconazole, compared to no-antifungal-treatment controls.[149] However, with the high risk of side effects of antifungal medication (such as elevation in liver enzymes), further research is needed for the proper indication of antifungals in CRS and AFS.

1.9.3 Third: Saline Irrigations

Saline irrigations are an important component of the management of CRS. Recent systemic reviews and consensus guidelines report high-level evidence of symptomatic benefit in patients performing regular saline washes for their sinuses.[1,150] The benefit seems to arise from the mechanical effect, which prevents buildup of secretions, improves mucociliary clearance, thus relieving obstruction and improving nasal airway patency.[151]

There is no evidence for the superiority of hypertonic saline versus physiological (normotonic) saline, in fact, the use of hypertonic saline was associated with more feelings of nasal burning and irritation at least in one trial.[151]

1.10   Surgical Management of CRS

Patients who fail maximal medical treatment are usually offered surgery. Today, the current gold standard for surgical treatment of CRS is functional endoscopic sinus surgery (FESS). In this section, we briefly discuss the philosophy of FESS and a brief history of how it grew to acquire its position as the standard management.

1.10.1   Historical external approaches to the sinuses

Before the introduction of the endoscope, sinus surgery was done through the "traditional" external (open) approaches. For each sinus, a certain procedure evolved to become the standard approach to that sinus at the beginnings of the twentieth century. These "traditional" procedures/approaches[152] include:

1) The external ethmoidectomy for the ethmoid sinus

2) The intra-nasal antrostomy and Caldwell-Luc procedure for the maxillary sinus

3) The osteoplastic flap procedure for the frontal sinus

4) The transantral or trans ethmoid approach for the sphenoid sinus

5) Simple polypectomy for nasal polyps (this is a traditional, but not an open procedure, performed with a headlight and forceps)

At that time, intranasal access was also attempted, but was abandoned at that time mostly due to poor visualisation and a high rate of intracranial complications.[152]

Although the open procedures provided adequate field of vision, the cosmetic results were a major disadvantages. Moreover, the main surgical objective at that time was the removal of all sinonasal mucosa (being considered as 'irreversibly diseased'); which led to increased risks of scarring, neo-osteoneogenesis, and mucocele-formation.[153] The wide surgical field of the open approaches is still sometimes required, for example in the case of huge intranasal malignancies. However for the treatment of CRS, and after the introduction of the endoscope, the use of the open techniques can now be considered historical (except for occasional exceptions).

Another traditional procedure (albeit not an open procedure) for the treatment nasal polyps, is (simple) polypectomy. This procedure involves using a Tilley-Henkel or snare forceps to extirpate nasal polyps. It can also been done in the outpatient setting. Polypectomy suffers from

high rates of recurrence, up to 75% with long-term follow-up.[154] It has been regarded as providing only temporary relief.[155] Nowadays, endoscopic sinus surgical techniques have mostly replaced polypectomy, since they showed better outcomes with lower rates of recurrence.[155–157]

1.10.2 Introduction of the endoscope

Early attempts for endoscopic visualisation of the sinuses came at the beginning of the 1900s with Hirschman, Reichert, Spielberg and Maltz (the latter coined the term "sinuscope").[153,158] However, due to the limitations of their instrument(s), these efforts remained solitary and were not further expanded into standard practice. It was not until Hopkins developed a more advanced instrument (the Hopkins rod-lens endoscope system) in the 1960s with more powerful resolution and lighting, that a revolution in studying the using endoscopes became possible. Messerklinger was able to make use of the technology, which allowed him to study the endoscopic anatomy of the sinuses and the patterns of their mucociliary clearance endoscopically. He published his work in the reference book "Endoscopy of the nose".[159] During the next decade, these studies enabled endoscopic sinus surgery pioneers Messerklinger, Stammberger, Wigand, Draf, Kennedy and others to further develop surgical techniques to ultimately constitute a surgical practice known as "functional endoscopic sinus surgery", a term that was coined by Kennedy in 1985.[160]

1.10.3 Functional Endoscopic Sinus Surgery (FESS)

1.10.3.1 Original concept

The original concept of FESS can be summarized as follows:

1) A pathologic process (mucosal thickening, build-up of secretions, anatomic bony variation or abnormality) causes blockage at the osteomeatal complex (OMC). (Figure 1-5)

2) OMC obstruction hinders muco-ciliary clearance from the maxillary, frontal and anterior ethmoid sinuses, with consequent buildup of secretions in the sinuses and promotion of sinus mucosal thickening.

3) The surgeons target the blockage at the OMC.

4) Consequences of this approach: Relief of sinus ostial blockage, Restoration of sinus ventilation, Restoration of normal mucociliary clearance. Symptomatic relief occurs as a result of these (beneficial) consequences.

This concept was heavily inspired by the sinonasal mucociliary clearance studies of Messerklinger. It is also important to mention how the concept of FESS can be considered a true paradigm-shift in the surgical treatment of CRS. This can be summarized in two points:

1) Shift of sinus surgery from external approaches to a minimally-invasive transnasal approach. This shift was enabled by the technological advancement in the development of endoscopes. This was not only important for cosmetic results, but the improved visibility resulted in lower morbidity and shortened hospital stays.

2) Shift to a more targeted and conservative surgical philosophy. (hence "functional" sinus surgery). This is compared to the preceding surgical practice which viewed surgery for CRS as an extensive excision of all diseased mucosal surfaces. As a result, the traditional radical approaches (see 1.10.1 above) that aim at excising the mucosa (such as the Caldwell-Luc procedure for example) started falling out of favor, and its use now became reserved for special situations.

Figure 1-5 CT scan showing obstruction of the OMC.
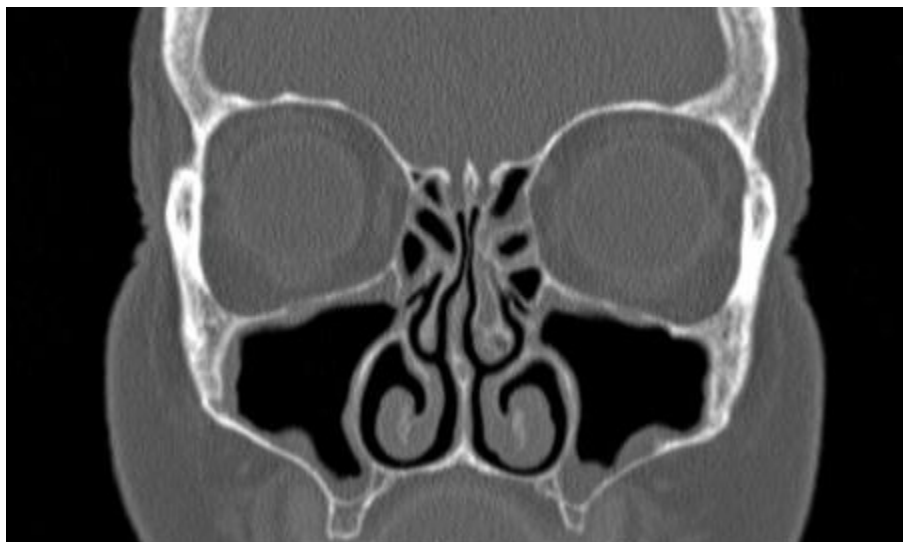
1.10.3.2   Success rates of Endoscopic Sinus Surgery

The estimated success rate of FESS is about 90%.[161,162]. Terris and Davidson reviewed 10 large series undergoing sinus surgery for CRS, with a total of 1,713 patients and reported a similar improvement rate (89%).[163] Dalziel et al. reviewed the efficacy of sinus surgery for CRSwNP and reported an improvement up to 88%.[164] Naidoo et al. reported a complete resolution of symptoms in 85 out of 109 (78%) patients undergoing primary sinus surgery and a revision surgery rate of 9%.[165] This is similar to the results of Smith et al. who reported a 75% clinically significant improvement in QOL scores in 302 patients.[166] Five-year outcomes reported from the English national comparative audit (1,459 patients) found a 15-20% revision surgery rate.[157]

Compared to primary cases, success rates for revision cases drop significantly (down to 70%-80%),[167–169] making previous sinus surgery a negative prognostic factor.[166,170,171]

There is substantial level 4 (and some level 2) evidence for the efficacy of ESS.[172] But despite the success of endoscopic sinus surgery to address the majority of CRS cases, there exists a subgroup of patients who fail (one or multiple attempts of) surgical intervention. This group suffering from "surgical failures" will be reviewed in Chapter 3.

1.11   Conclusion of the chapter

CRS is a chronic inflammatory disorder of the sinuses that has a large impact on patients' QOL. CRS is thought of as a disease of multi-factorial etiology and complex pathogenesis, involving both host and environmental factors. An initial trial of maximal medical therapy is prescribed, which includes steroids (often alongside antibiotics) and saline douches. Patients who fail maximal medical therapy are offered surgery. The invention of the endoscope has revolutionized surgical treatment of sinusitis. Functional endoscopic sinus surgery (FESS) has become the gold standard of surgical treatment of CRS. The success rate of FESS is about 90% but reduces significantly with revision cases leaving a small subgroup of patients complaining of severe disease that is non-responsive to repeated medical and surgical treatments. (These refractory patients will be the focus of Chapter 3).

Chapter 2 Extensive surgical techniques for CRS

2.1 Full-House FESS and Extent of Surgery

The Full-House FESS (FHFESS) is a term used to describe endoscopic surgery that includes a maxillary antrostomy, complete (anterior and posterior) ethmoidectomy, wide sphenoidotomy, and a Draf-2A frontal sinusotomy.[173] The philosophy of FHFESS is to address the affected sinuses, irrespective of the presence of specific sinus-related symptoms. As such, a CRS patient would undergo a FHFESS if there was radiological evidence of pansinusitis with opacities in all sinuses. This is of particular relevance for the surgical decision to address the frontal sinus. For example, a patient may not complain of frontal headaches but may have frontal sinus mucosal thickening on CT scan.[165] In the FHFESS concept this patient's frontal sinus should be addressed surgically, through a frontal recess clearance and Draf-2A frontal sinusotomy, irrespective frontal sinus specific symptoms.[165] In this way, the philosophy of FHFESS can be considered a maximization of the techniques of FESS, to include all the diseased sinuses. A recent retrospective review of FHFESS patients noted a success rate of 92%, with only 8 out of 109 (8%) required revision surgery.[165]

This concept of "full-house surgery" highlights the need for the debate as to how the extent of surgery and relates to outcomes of patients after sinus surgery. Should all diseased sinuses be addressed during surgery? How much diseased tissue should be removed?

Review of the literature produced multiple studies that supported better outcomes for more extensive surgery as well as the complete removal of diseased tissue. A study by Materson et al showed a positive role for more extensive surgery comparing a total ethmoidectomy versus anterior ethmoidectomy [174] while other studies showed that more complete removal of polyps or polypoid mucosa led to lower rates of recurrence and consequently better outcomes.[174–176]

2.2 Radical maxillary sinus procedures

The philosophy of conventional FESS for treating inflammation in the maxillary sinus was to address OMC disease, and thus restore aeration and mucociliary drainage to the sinus. This

usually is done by performing an uncinectomy; or an uncinectomy with a middle meatal antrostomy (MMA). Special consideration is given to avoid the phenomena of recirculating mucus in the presence of an accessory ostium by joining the accessory ostium with the natural ostium. Nevertheless, despite this surgery ongoing maxillary sinusitis may persist [177] and various (more radical) procedures have thus been described to address chronic maxillary sinusitis resistant to the conventional treatment.

Upon review of the literature, we found that the surgical procedures for refractory maxillary sinusitis depended on two concepts.

The first concept involves complete removal of mucosa perceived to be dysfunctional or "irreversibly-diseased" in the sinus. An example of this type of procedures is the traditional Caldwell-Luc procedure. Cutler et al. performed the Caldwell-Luc in patients who failed conventional treatment in the form of middle meatal antrostomy with a success rate of 92%.[178] Another positive result has been reported by Abd el-Fattah et al., who performed a Caldwell-Luc like procedure, described as "radical antrectomy", and reported better outcomes when compared to conventional MMA.[179] Other authors reported superior outcomes for the Caldwell-Luc compared to conventional FESS in patients with comorbid asthma and aspirin sensitivity.[180,181] A more recent and more conservative technique for addressing severely-diseased maxillary sinus is the canine fossa trephine (CFT) approach. This technique differs from the Caldwell-Luc procedure in that the mucosa is not stripped to expose the underlying bone and thus there is less risk of scarring and sinus cavity contraction, and of the side effects that have been reported with the Caldwell-Luc. The CFT approach has been reported to provide better outcomes with reduced disease recurrence rate.[176]

The second concept is allowance of gravity-dependent mucociliary clearance of the dysfunctional mucosa through maximal ostium-widening procedures. In addition to the creation of a maximally enlarged maxillary ostium, gravity-dependent drainage may also be assisted through an extra inferior extension obtained through partial excision of the inferior turbinate. These procedures have become to be known as maxillary mega-antrostomy and/or modified medial maxillectomy, with reported good outcomes for treatment of refractory maxillary sinuses.[182,183]

2.3 Draf-III/EMLP Frontal drillout procedure: the successor of the external osteoplastic flap

In the 1920s, the external fronto-ethmoidectomy described by Lynch was indicated for restoration of frontal sinus drainage. However, this procedure has been shown to have a 30% failure rate. The Lynch procedure for frontal sinusitis has been largely replaced by the osteoplastic flap procedure with fat obliteration described by Goodale and Montgomery in the 1960s, which had a lower rate of recurrence and complications. Since the 1960s to the introduction of endoscopic surgery, frontal sinus obliteration became the gold standard for treatment of chronic frontal sinusitis. With the development of endoscopic techniques, more transnasal approaches for tackling the frontal recess and restoration of frontal sinus drainage came to largely replace the external osteoplastic flap approach. Now the osteoplastic flap is reserved as a salvage procedure for refractory cases of frontal disease and for access for tumors in the frontal sinus. As new powered instruments such as drills developed, Draf-III/endoscopic modified Lothrop procedure (EMLP)/Frontal drillout surgery has allowed the creation of a wide neo-ostium for drainage of the intractably diseased frontal sinus. This procedure aims to create the largest possible opening between the frontal sinus and the nose.[184] The first step in this procedure is the creation of a septal window. Through this window, the instruments and burrs can gain access to the frontal recess of the contralateral side. Drilling of the entire frontal sinus floor, frontal beak and frontal intersinus septum, leads to conjoint drainage of right and left frontal sinuses and creates one large neo-ostium. Wormald reported a 93% primary success rate after an average follow-up of 21.9 months.[185] A recent systematic review and meta-analysis of the EMLP/Draf 3 demonstrated that frontal sinus patency occurs in about 95% of cases, with 82% of cases reporting improvement of symptoms and a low complication rate of 1-4%.[186]

In a recent study[187] of the long-term outcomes of the EMLP, Naidoo et al. reviewed the outcome of EMLP over 10 years on a single-surgeon cohort of 229 patients. They reported a 97% patency rate and only about 5% need for revision EMLP for persistence of symptoms.[187]

2.4 Nasalization

Nasalization is a procedure that has been described and performed by Jankowski's group in France for nasal polyposis. The concept of the surgery is radical, but is done through an

endoscopic transnasal approach. The surgery includes extensive polypectomy, radical ethmoidectomy, antrostomy, sphenoidotomy, exposure of the frontal ostium, and middle turbinate resection. The procedure involves dissecting of the mucosa from the underlying periosteum on the medial orbital wall, ethmoidal medial (turbinate) wall and ethmoid roof.[188]

Jankowski et al. compared five-year follow-up results of nasalization (in 39 patients), to functional ethmoidectomy (37 patients) and reported better symptom improvement, better long-term olfaction outcomes[189] as well as lower total polyp recurrence rate (22.7% in the nasalisation group, versus 58.3% in the ethmoidectomy group).[190]

2.5 Denker's procedure

Denker's procedure is a radical procedure that was in the past performed through an external approach in which the ethmoid, the lateral wall of the nasal cavity, and the middle and lower turbinate were being removed. (i.e. all walls separating the sinonasal cavities and all diseased mucosa was removed). This results in creation of a large single cavity. This cavity extends vertically from the roof of the ethmoid to the floor of the nose and horizontally from nasal septum to the lateral wall of the maxillary sinus.

The procedure was first described by Denker for sinonasal cavity malignancy,[191] but multiple authors described its use for CRS patients refractory to standard conservative procedures. The authors who performed this procedure for CRS cited the need to lower inflammatory burden and allow maximal drainage and aeration in patients with rCRS deemed incapable of restoring their normal mucociliary clearance.

Kerrebijn et al.[192] described improvement in symptom outcomes in 56 patients who have received this procedure during the period from 1979 to 1990. Eighty-two of patients reported improvement of nasal discharge, 89% reported a return of the sense of smell, 87% of patients reported improvement of headaches. Wreesman et al.[193] retrospectively investigated 82 patients who underwent Denker's procedure in the period of 1986 to 1997. Eighty-four percent of the patients reported significant reduction in various nasal symptoms, including headache, obstruction, anterior and post-nasal discharge. Patients who had concomitant asthma also reported improvement in their lower airway symptoms.[193] Videler at al. have investigated prospectively the results of Denker's procedure on 24 patients with rCRS who had undergone a

median of 6 previous failed sinonasal operations (range, 3-11).[194] After the procedure, 74% of patients reported improvement of feelings of congestion, 70% reported improvement in rhinorrhea, while 60% reported improvement in nasal obstruction. The authors also reported significant improvement of pain in 23 patients upon QOL testing.[195]

2.6 Conclusion of the Chapter

Extensive/Radical surgical procedures are possible treatment options for patients with rCRS who fail standard endoscopic procedures. The reasons for improvement with the radical approach are not completely understood. Various authors have opined that a proportion of rCRS patients should not be expected to have recovery of their normal mucociliary clearance function after surgery and thus the goals of surgery should be different. They cited multiple reasons for improvement seen with radical surgery, including gain of maximal drainage and aeration of the sinuses, improvement of topical medication access, reduction of inflammation, and removal of osteitic bone.

Apart from the radical procedures discussed in this chapter, the extent of surgery is an under-researched topic in sinus surgery outcomes. Are an uncinectomy and anterior ethmoidectomy sufficient? Or should all the diseased sinuses be opened and cleared? What is the optimum size of a particular sinus ostium? When should we address the frontal sinus? These are a few examples of unanswered questions. The few studies published in the literature tend to support more complete surgery with better outcomes for severely diseased patients with more extensive surgeries.

More research is needed on the extent of surgery and the role of radical ancillary procedures in the surgical treatment of CRS.

Chapter 3 Refractory CRS

3.1 Definition

A formal of definition of a clinical entity of "CRS resistant to treatment" has not been clearly standardized in the literature.

The first attempt at providing a definition for these difficult cases comes from the EPOS 2012 document: patients considered to have "Difficult-to-treat Rhinosinusitis" are those who complain of persistent symptoms despite appropriate treatment (recommended medication and surgery).[1]

 "Difficult-to-treat Rhinosinusitis" is defined as:[1]

"Patients who do not reach an acceptable level of control despite adequate surgery, intranasal corticosteroid treatment and up to 2 short courses of antibiotics or systemic corticosteroids in the last year."

However this definition to date has not being frequently used by authors in the literature.

Difficult to treat CRS patients include to a group of "severe chronic upper airway diseases" (SCUADs). SCUADs have been defined as:

"those patients whose symptoms are inadequately controlled despite adequate (ie, effective, safe, and acceptable) pharmacologic treatment based on guidelines."

SCUADs include: uncontrolled allergic rhinitis, nonallergic rhinitis, chronic rhinosinusitis, and aspirin-exacerbated respiratory diseases, or occupational airway diseases. These patients have impaired quality of life, social functioning, sleep, and school/work performance.[196]

3.2 Causes of disease recalcitrance and surgical failure

3.2.1 Surgery-related factors (or anatomic factors)

These factors are iatrogenic factors resulting from surgical manipulation during previous surgery and are thought to contribute to failure and subsequent need of revision. We reviewed these factors as presented in Table 3-1.

Table 3-1 Anatomical findings in revision sinus surgery patients; these findings are thought to have contributed to surgical failure.

	Ramadan et al.[197]	Musy and Kountakis[198]	Chiu and Vaughan[199]	Khalil et al.[200]	Gore et al.[201]
MTL +/- adhesions between the MT and lateral nasal wall	25%–56%	78%	35.8%	11.1%	11%
Maxillary ostium stenosis	27%	39%	Not explicitly reported	Not explicitly reported	
Residual air cells in the ethmoids	30.7%	64% anterior ethmoids; 41% posterior ethmoids; 49% agger nasi	79.1%	92.1% anterior ethmoids; 96% posterior ethmoids	75% anterior and posterior ethmoids; 64% agger nasi
frontal recess or frontal sinus ostium scarring/stenosis	25%	50%	49.3%	96% frontal recess	
Accessory maxillary sinus ostium	15%	4%	Not explicitly reported	Not explicitly reported	
Retained uncinate	Not explicitly reported	37%	38.8%	57.1%	64%

We conclude from Table 3-1 that residual cells, middle turbinate lateralization and frontal recess/sinus scarring are important findings in revision surgery patients. However, our ability to implicate these factors as the true cause of surgical failure is limited. One reason is that most of these studies are descriptions of findings at the time of revision surgery, and we could only retrospectively try to infer the relative contribution of each factor to failure.

3.2.2 Patient-related or disease-related factors

3.2.2.1 Asthma

A large number of studies show that asthma is a significant negative prognostic factor in CRS.
The conclusions of these studies are summarized in Table 3-2.

Table 3-2 Studies showing a negative effect for asthma on post-operative outcomes

Study	Study Population	Outcome(s) for asthmatic patients
Dejima et al.[202]	88 CRS patients	Worse symptomatic and endoscopic picture improvement after surgery for asthma-positive patients
Zhang et al.[203]	510 CRS patients	Asthma and biofilm-forming bacteria were associated with more than 2-fold increased odds of revision ESS after adjustment for CRS risk factors.
Matsuwaki et al.[204]	65 CRS patients	Patients with asthma were likely to experience recurrence of CRS within 5 years after surgery.
Kim et al.[205]	98 CRS patients	Asthmatics exhibited significantly worse postoperative endoscopic outcomes compared with non-asthmatics. Asthma an independent predictor of success upon multivariate analysis.
Smith et al.[206]	119 CRS patients	Asthmatic patients had worse absolute post-operative endoscopy scores.
Wynn and Har-El[171]	118 CRSwNP patients, with a minimum Lund-McKay score of 16 and a Kennedy computed tomography stage 3 or 4	History of previous sinus surgery or asthma predicted higher polyp recurrence and revision surgery rates.

3.2.2.2 Aspirin-exacerbated respiratory disease (AERD)

Amar et al.[207] followed-up 18 patients with Samter's triad and compared their long-term outcomes (a 6-year retrospective review) to a control group of 43 CRS with no triad. When compared to the non-triad patients, they found that Samter's triad patients had a higher prevalence in three of five symptoms (nasal discharge, congestion and anosmia) as well as a higher need to use nasal steroids (67% versus 36%) at their last follow-up. Moreover, triad patients will require over seven times as many revision operations during follow-up as non-triad patients.[207] In another long-follow-up study, Schaitkin et al. found that Samter's triad patients required more revision surgery than non-triad patients (36% versus 23%).[208] Batra et al.[209] retrospectively reviewed 25 asthmatic CRSwNP patients (18 aspirin-sensitive i.e. Samter's triad; and 9 aspirin-tolerant) and found that the aspirin-sensitive patients did not have a statistical improvement in sinonasal symptoms at 1-year post-operative follow-up, compared with the ASA tolerant group.[209] Smith et al.[206] prospectively reviewed 119 adult CRS patients with a mean follow-up of 1.4 years after ESS. They found that patients with aspirin intolerance had worse symptoms and that aspirin-intolerant asthmatic patients had less improvement after surgery when compared to aspirin-tolerant asthmatics.[206] In another retrospective study, Kim and Kountakis found that patients with Samter's triad had undergone approximately 10 times more sinus surgery than patients without Samter's triad. Samter's triad patients also had higher rates of symptom recurrence at 6-months post-operative follow-up.[210]

3.2.2.3 High-grade eosinophilia

The eosinophil is the major effector cell in CRS and eosinophilia is a hallmark histopathological feature in CRS affected nasal tissue. Eosinophilia, either systemic (measured in the serum) or local (measured in the mucosal or polyp tissue) has been associated with various negative prognostic measures in CRS. The pathologic role of the eosinophil in CRS is discussed in Chapter 4, whilst the role of high-grade eosinophilia in refractory CRS is discussed more in Chapter 7.

3.2.2.4 Allergic fungal sinusitis

Allergic fungal sinusitis (AFS) represents a specific and characteristic subgroup of CRS patients. The clinical features of AFS have been discussed in 1.5.3 above. An underlying intense inflammatory response against fungal antigens is thought to underlie the condition. This intense

inflammation leads to a severe clinical picture, with frequent bony involvement with occasional extension beyond the sinuses. Their postoperative course is marked by a high rate of persistent symptoms and recurrence of disease,[17,211]. The rates of recurrence have been reported to be as high as 50%.[146] Kupferberg et al. showed that at least 80% of patients suffer from recurrence once they are weaned off corticosteroids.[212] Schubert and Goetz showed that administration of oral corticosteroids after surgery have been found to reduce rates of requiring revision surgery.[213] This suggests that anti-inflammatory treatment is essential to control this severe form of disease. The results of antifungal medication in AFS were discussed in 1.9.2.2 above.

3.2.2.5 Aggressive nasal polyp recurrence

Polyp recurrence is an important cause of surgical failure. The risk of polyp recurrence after surgery has been reported to reach a rate as high as 60%.[171,214] The presence of polyps has been previously described as the most common endoscopic abnormality in patients undergoing revision surgery.[208,215]

Some patients suffer from rapid and aggressive polyp recurrence, while others exhibit a slower rate of recurrence. The classical subgroups with aggressive recurrence are those with comorbid asthma, and even more so if they are aspirin-intolerant (Samter's triad).[171,210,216–218] The aggressive recurrence in Samter's triad patients may be linked to the disturbance in their eicosanoid metabolic pathways, which causes excessive production of cysteinyl leukotrienes.[219] The other link is the presence of higher eosinophil infiltration in the polyp tissue of these patients with unified airway disease, which was found to correlate with higher rates of recurrence.[220,221]

3.3 Conclusion of the chapter

In this chapter we discussed Refractory CRS. We find that the definition of refractory CRS in the literature is variable, although some authors have noted the importance of characterizing this group.[1,196] Surgery-related factors can be theoretically considered as avoidable factors as the skills of the surgeons improve. We find that residual cells, frontal sinus ostium stenosis and middle turbinate lateralization are the most common surgical factors associated with surgical failure. The ability to implicate them as true causes of surgical failure using current evidence is however limited.

On the other hand, disease or patient-related factors of recalcitrance lead to surgical failure independent of (the avoidable) surgical factors. This means that these factors can lead to surgical failure independent of OMC obstruction. These patients are thus more difficult to manage, since failure is prone to occur despite having an initial adequate and effective surgery.

We also note a particular group of refractory patients are those who require revision surgery. These patients may be different from patients with recalcitrant symptoms but who do not progress to needing further surgery. Repeated hospitalization and re-operation poses a burden on the patients which is both a health burden as well as a financial one. The costs to the healthcare system at large are also great: in the USA, an estimated 500,000 operations are done on the paranasal sinuses each year.[10] This thesis will explore the causes of surgical failure defined as the need for undergoing revision surgery. The formulation of our main thesis and the aims will be discussed in Chapter 5.

Chapter 4 The Eosinophil in CRS

4.1 Definition, histology and physiology

Eosinophils are bone-marrow derived granulocytes. They were first described in 1879 by Paul Ehrlich, who discovered their characteristic pink colour when stained with the acid aniline dye eosin. This acidophilic staining pattern owes to the eosinophil's contents, rich in cationic proteins. Another distinguishing histological feature is the bilobed nucleus.

In terms of function, eosinophils are considered effector cells, which play a role in immunoregulation, defense against pathogens (in particular, helminths) and tissue remodeling. The effector function of the eosinophil is mainly mediated through its wide array of products it is able to secrete. The secretions are summarized in Table 4-1 .

Table 4-1 Eosinophil products (non-exhaustive list)

Cationic proteins	Eosinophilic major basic protein (MBP)
	Eosinophil cationic protein (ECP)
	Eosinophil-derived neurotoxin (EDN)
	Eosinophil peroxidase (EPO)
Cytokines	IL2-6, IL10, IL12, IL13, IFN-g, TNF-alpha, GM-CSF
Chemokines	Eotaxin/CCL11
	RANTES/CCL5
Lipid mediators	Prostaglandins
	Leukotrienes
Eicosanoid metabolism enzymes	such as 5-Lipoxygenase and LTC4 synthase

The cytoplasm of eosinophils serve the secretory function as it contains secretory granules. These granules have a unique ultrastructural morphology. They are limited by a membrane and

compartmentalized through "intra-granular" membranes made up of tubular structures. They are capable of holding the vast majority of the eosinophils secretory products. Eosinophils also possess lipid bodies, which store inflammatory lipid mediators and thus play an important role in eicosanoid metabolism and production. They are characterized by their round immunodense appearance on electron microscopy.

Piece-meal degranulation is the main process through which the contents of the secretory granules are released by activated eosinophils. Through this process, vesicular transport of material from the secretory granules occurs from the cytoplasm to the cell surface. The release of products into the extra-cellular space occurs in the absence of granule-plasma membrane fusion. Degranulation increases in response to specific eosinophil-activating or degranulating stimuli, which includes RANTES, Eotaxin or PAF. This results in an increase in the number of emptying granules in the cytoplasm. Granule contents also get secreted through classical exocytosis (which would involve granule-cell membrane fusion, in contrast to piecemeal degranulation).

4.2 Life cycle of eosinophils

4.2.1 Eosinophilopoiesis

Differentiation of haematopoietic stem cells into eosinophil occurs in the bone marrow. Haematopoietic stem cells differentiate into multipotent cells and finally into eosinophil progenitor cells (EoPs) marked by CD34+IL5RA+. These EoPs are lineage-committed (i.e. eventually give rise only to eosinophils). The process of maturation from now on is driven by a number of important cytokines, namely IL-5, GM-CSF and IL-3. Under homeostatic conditions, and for a "basal" production of eosinophils, lineage commitment and terminal differentiation of lineage-committed EoPs to produce mature eosinophils occurs through a delicate balance between various transcription factors. The basal eosinophil line commitment is thus "transcriptionally regulated". The fine interplay between the transcription factors involves an essential instructive role for GATA-1, a requirement for PU-1, as well as a coordinated temporal (up- and down-) expression of various C/EBP isoforms. The end-result of these coordinated efforts is the production of a mature terminally-differentiated eosinophil, able to transcribe the eosinophil-specific genes/products.[222]IL-5 (through its interaction with IL5-RA) is the most important cytokine in eosinophil expansion, differentiation, survival as well as

activation/degranulation, recruitment into sites of inflammation. Whilst IL-3 and GM-CSF possess actions as well on other myeloid precursors, IL-5 is a specific pro-eosinophil actor.[222]

4.2.2 Migration from the blood stream to tissues

The migration of eosinophils from the blood to peripheral tissues is termed "eosinophil trafficking".[223] Successful trafficking is dependent on a coordinated series of chemotaxis and adhesion steps, requiring chemotactic agents (eosinophilotactic chemokines) and adhesion molecules respectively. The first stage is successful attachment of eosinophils to the endothelium under physiological flow conditions. This involves successful tethering and rolling. This stage is highly dependent on Selectins. Endothelial P-Selectin appears to be particularly important for the initial step of tethering, since eosinophils express PSGL-1 (the P-Selectin ligand).[224–226] Th2 cytokines such as IL-4 appear to play an important role in promoting this P-Selectin adhesion step.[225,226]

Eosinophils in the blood stream also express L-Selectin (CD62L), which then bind to L-Selectin ligands on the surface of vascular endothelial cells (such as the carbohydrate, Sialyl-Lewis X). Anti-L-Selectin antibodies inhibited eosinophil attachment in vitro under flow conditions.[227] The L-Selectin based mechanism was also important for a eosinophil to adhere to an attached eosinophil (already-attached to the endothelium) i.e. leukocyte-leukocyte adhesion, forming a string of adhered cells to the endothelium.[228] Another study suggests that P-Selectin was important for primary endothelium-eosinophil tethering, while L-Selectin is more important for the following inter-eosinophil adhesions.[225,229] The significance of L-Selectin adhesion were confirmed in CRS studies.[230–232]

In addition to (and perhaps, directly following) the selectin-mediated attachment, VCAM-1-mediated attachment was essential for successful rolling and adhesion.[225,229,233] This makes integrins, in addition to selectins, crucial for eosinophil recruitment. There are at least seven types of integrins expressed on eosinophils.[223] The most important of the integrins in mediating eosinophil adhesion appear to be α4β1 (VLA-4, a β1 integrin) and αMβ2 (CD11b/CD18 or MAC-1, a β2 integrin). The first binds to endothelial VCAM-1 while the latter binds to endothelial ICAM-1. VCAM-1 is able to also bind another beta-2 integrin, αDβ2.

Interestingly, integrins can be said to undergo "activation". Their conformational state can determine whether an integrin is functional for cell adhesion and migration.[234] Activation of

integrins is accomplished by "inside-out" signaling, i.e. chemokines or cytokines interact with eosinophils, which initiates intracellular signaling pathways that upregulate expression of integrins and modulate their cytoplasmic domains, leading to a change in the affinity of the binding achieved.[234–236]

Integrin signaling stimulates eosinophils to form podosomes, [237,238] regulates the activation and degranulation states of the eosinophil,[239–241] and enhances its survival.[242–244] These modulations prepare the eosinophil for its eventual migration through inflammatory tissue in the following stages. On the other hand, adhesion through VCAM-1 activates intracellular signaling in endothelial cells to enable transendothelial migration (see 4.2.3 below).

4.2.3 Transendothelial migration

Once firm attachment has been achieved, migration of eosinophils through the vascular endothelium takes place (transendothelial migration). Transendothelial migration is an intricate process that requires a combination or 'cross-talk' of adhesion molecules plus the presence of a chemotactic gradient.[245] The chemotactic gradient is achieved by eosinophilotactic CCR3-ligand chemokines such as eotaxins and RANTES; while the adhesion mechanism involved in this stage is mainly beta-1 integrin (VCAM-1) mediated. However, there is also a role for beta-2 integrin (ICAM-1 mediated binding),[223,246–248] for a shift occurs from a predominant beta-1-integrin dependent mechanism towards a predominant beta-2-integrin mediated mechanism. This shift occurs through the action of chemokines.[248–250]Integrin-mediated attachment of eosinophils to endothelial cells (mainly through VCAM-1) activates intracellular signaling in endothelial cells,[223] (Eosinophil trafficking book) which ultimately leads to the opening of inter-endothelial cell gaps for facilitation of leukocyte 'extravasation'.[223,251] Eosinophils transmigrate between endothelial cells (paracellularly)- a process involving junctional disorganization/rearrangement of the endothelium,[252,253] through a proteolytic and ROS-dependent mechanism.

This process is augmented by various cytokines which, through activating endothelial cells (by way of upregulating the expression of various adhesion molecules on their surface), support increased eosinophil trafficking in areas of inflammation.

4.2.4 Role of IL-5

Not surprisingly, IL-5 plays an important role in the above described attachment stages. Priming of eosinophils with IL-5 promotes increased rate of transendothelial migration in conjunction with eosinophilotactic chemokines.[254] IL-5 also increases podosome formation in eosinophils.[237,238] Podosomes allow eosinophils to become more motile and thus increase their migratory abilities and has been associated with beta1-integrin-VCAM-1 adhesion.[237,238] IL-5 has been consistently reported to enhance αMβ2 integrin-mediated eosinophil adhesion, through increased activation and expression.[237,255–257] This effect occurs as an example of the "inside-out" signaling mechanism described above.[235,236] Eosinophils present in IL-5-rich BAL fluid after allergen challenge had a hyperadhesive phenotype associated with increased surface expression of αMβ2 and activation of β2 integrins.[258] Anti-IL-5 attenuated activation and surface density of β2 integrins on circulating eosinophils in ten subjects with asthma who administered anti-IL-5 intravenously.[259]

4.2.5 Transepithelial migration

By this stage, the eosinophil has migrated to the site of inflammation and settled in tissue. In the airway, this site is the mucosa, usually subepithelial, in the lamina propria. However, in airway inflammatory diseases such as CRS or asthma, this is not the final destination for some eosinophils, for eosinophils continue to migrate through to end up in the (sinus or bronchial) lumen. Consequently, after transepithelial migration, eosinophils participate in the formation of eosinophilic mucus. Transepithelial migration as a topic has received less study than transendothelial migration. However, several studies showed that, similar to transendothelial migration, this step is also dependent on adhesion molecules (integrins)[260] and chemokines (eotaxin).[261]

4.2.6 End of life

In the absence of inflammatory signaling, eosinophils (either circulating or tissue-based) have an average life span of 3-4 days, and thus are programmed to die if no exogenous stimulus exist.[262,223] Apoptosis is the predominant way of eosinophil death,[223] and involves caspase activation. On the other hand, pro-survival molecules act to increase the life span and these include GM-CSF and IL-5.

Eosinophil cytolysis (necrosis) is another mode of eosinophil death, which involves rupture of the cell membrane after loss of its integrity. With cytolysis, the contents of the eosinophil are released into the extra-cellular space. Cytolysis has been demonstrated to occur in an ultrastructural study of CRSwNP, in at least one fourth of the eosinophils.[263] Interestingly, secretory granules have cytokine and chemokine receptors on their granule surface. They are thus able to function as independent secretory organelles upon chemical stimulation, even after the death of their mother cell.[264]

4.3 Pathologic roles of the eosinophil

In this section, we will elaborate on the various injurious effects of eosinophils in the nasal mucosa, illustrating how they are the major effector cells in CRS.

4.3.1 Tissue Injury

Tissue injury caused by eosinophils is mainly mediated by contents of their proteinaceous products, the eosinophil granule proteins. These products have possibly evolved for eosinophils to ward off parasitic infections, as they have been shown to be toxic to helminths.[265] Tissue injury caused by these products is an unfortunate byproduct of this evolution. Eosinophil products are stored in granules in their cytoplasms (these granules can be easily seen on light microscopy). When eosinophils release the contents into the extra-cellular space, they are said to degranulate. This releases their products into the surrounding environment, causing tissue injury.

In CRS, epithelial cell injury and shedding has been observed in situ.[266,267] This injury contributes to dysfunction of the epithelial barrier, and could thus form an important component of the disease pathophysiology, according to the Immune Barrier theory. (See 1.8.71.8.7 above). This injury is mainly brought about by the cytotoxic eosinophil granule proteins. In various studies, cytotoxicity to epithelial cells has been demonstrated for: eosinophilic major basic protein (MBP);[268,269] Eosinophil cationic protein (ECP);[270] and Eosinophil peroxidase (EPO), either alone or through the glucose-oxidase halide system.[268,270] Eosinophil derived neurotoxin (EDN), although biochemically similar to ECP, did not cause toxicity.[270]

4.3.2 Pro-inflammatory and immunomodulatory cytokines and products

The eosinophil is able to influence inflammation through its products. A list of eosinophil secretory products is found in Table 4-1. The most important to note in the context of CRS, is the

ability of the eosinophil to influence Th2 inflammation (part of the adaptive immunity) through ability to secrete Th2-polarizing cytokines. These cytokines include IL-4, IL-5 and IL-13. IL-4 has the ability to drive the differentiation of naive T helper type 0 (Th0) lymphocytes into Th2 cells. IL-13 is another Th2 cytokine that has an important role in goblet cell differentiation and mucus production. Eosinophil secreted chemokines such as CCL17 and CCL22 were also shown to play a role in the recruitment of effector Th2 cells into sites of inflammation.[271]

4.3.3 Antigen presentation

Antigen presentation to naïve T lymphocytes constitutes an important step in the inflammatory cascade. This job (carried out by what is termed as antigen-presenting cells or APCs) is mainly undertaken by activated dendritic cells (therefore they are called "professional" APCs).

In Brief, the mechanism of antigen presentation occurs through the interaction of the TCR on naïve T lymphocytes with the antigen-MHC-II complex on the surface of the APC. In addition, it a costimulatory signal in the form of CD28 interaction (on the lymphocyte) with CD80 and CD86 (on the APC) for eventual survival and proliferation of the lymphocyte.[272]

Eosinophils, independent of dendritic cells and other inflammatory cells, have also been found to be able to function as APCs in the airways (i.e. act as "non-professional" APCs). This has been demonstrated both in vitro and in murine models in vivo.[272] First, eosinophils have been known to be able to express MHC-II molecules on their surface (once primed by GM-CSF).[273] Another study showed that transendothelial migration of eosinophils led to an increase in MHC-II expression on eosinophils.[274] This means that eosinophils gain the ability to act as APCs once they migrate to the peripheral tissue (including the airway). Airway eosinophils also express the costimulatory molecules of antigen presentation (CD80 and CD86), such that their mechanism of antigen presentation is typical CD86- and CD80-dependent.[275] An interesting finding is that antigen presentation through eosinophils was found to bias towards a Th2-type response, as it preferentially stimulated $CD4^+$ lymphocytes to produce IL-4, IL-5, IL-13, but not interferon (IFN)-γ, both in vitro and in vivo.[276,277] This biased mode of antigen presentation constitutes a sinister role in allergic diseases, as it further propagates the local Th2 milieu.

4.3.4 Steroid resistance

The glucocorticoid receptor-β isoform (GR-β) is a steroid action inhibitor that is associated with steroid insensitivity. At least two different groups reported an increase in GR-β expression in nasal polyps compared to nasal mucosa. Hamilos et al. showed a higher number of inflammatory cells staining for GRβ in nasal polyps compared with nasal mucosa (40% versus 16%).[278] Pujols et al. reported higher expression of GR- β in nasal polyps, when compared to nasal mucosa.[279] Moreover, nasal polyps with more than 3% of inflammatory cells had higher GR- β levels than both nasal mucosa (P<0.01) and polyps with lowe than 3% of inflammatory cells.[279] In another study, eosinophilic CRS is associated with an increased expression of GR-β.[280] Another study by Pujols et al. showed a negative correlation between eosinophil counts and glucocorticoid receptor-β expression.[281] These results propose that a higher inflammatory load increases resistance to steroid therapy (through upregulating GR-β and downregulating GR-α), and that eosinophilic CRS is inherently more resistant to steroid therapy than non-eosinophilic CRS.

4.3.5 Role in remodeling

Evidence suggests that eosinophils play an important part in the remodeling process. Remodeling is the term used to describe the set of structural changes that happens in the sinuses and is a feature of CRS. Firstly, eosinophils (along with other inflammatory cells) are an important source of transforming growth factor β (TGF-β). TGF-β is considered an important protein driving collagen deposition[282] and TGF-β signaling is considered a master regulator controlling the pattern of remodeling in CRS.[283] TGF-β induces fibroblast proliferation and the differentiation of fibroblasts into (activated) myofibroblasts. Myofibroblasts are responsible for deposition of collagen and extracellular matrix. Subepithelial Basement Membrane (BM) thickening, occurring secondary to increased collagen deposition, is a hallmark of mucosal remodeling and has been investigated in various studies as a surrogate for remodeling.[284] Secondly, BM thickness has been reported to correlate with the density of underlying eosinophils both in sinusitis and asthma.[285,286] Thirdly, eosinophils are capable of producing profibrotic cytokines.[88]. Fourthly (and perhaps most importantly), is the strong evidence for eosinophil involvement obtained from allergen-induced remodeling experiments in gene-knockout mice. Eosinophil-deficient mice were found not to suffer from increased peribronchiolar collagen

deposition and airway smooth muscle, when compared with sham control mice, indicating a critical role for eosinophils in the remodeling process.[287]

4.3.6 Eosinophil chemotaxis and autocrine persistence

The eosinophil is able to attract more eosinophils to the site of inflammation, through the effect of cytokines and chemokines that possess an autocrine effect. Eosinophils are a major producer of IL-5 (a major upregulator of eosinophilopoiesis and the major upregulator of eosinophilia in tissue sites). They are also capable of producing the other eosinophilopoietins IL-3 and GM-CSF. Eosinophil attractant chemokines secreted by eosinophils include eotaxins[288–290] and RANTES.[291]

4.4 Conclusion of the chapter

Eosinophils have multiple pathogenic factors in CRS. This makes them (the) major effector cells in CRS. This would also make them a good candidate for a predictor of worse disease. The associated Th2 cytokine milieu intensifies these pathogenic effects and works to recruit more eosinophils to the site of inflammation. This leads us to ask the questions: What is the clinical significance of eosinophilia in CRS? Could they play a role in determining disease prognosis? These questions are more elaborated in Chapter 7, where we formulate and discuss a hypothesis for the prognostic value for inflammation (and eosinophilia) in CRS.

Chapter 5 Thesis

5.1 Refractory CRS and questioning an exclusive role for the OMC

The research focus of this thesis is the subgroup of patients who fail surgery (and whom we term "Refractory CRS (rCRS)"). For these rCRS patients, a number of studies have described the various factors that contribute to surgical failure. The relative contribution of each factor (versus the rest) to surgical failure is poorly understood. In the past a major role was ascribed to OMC obstruction (by the early concepts of FESS) in disease pathogenesis. However, the importance of this role becomes doubtful because: *(a)* there is a subgroup of patients who continue to suffer from refractory disease despite adequate surgery which includes clearance of OMC; *(b)* clearance of the OMC does not result in clearance of disease of the anterior ethmoids and frontal in a significant number of patients; and *(c)* improved outcomes have been reported with more extensive surgery in severe patients, when compared to a more conservative functional approach.

5.2 Our Research

Our research first looks at one of the important surgery-related anatomical causes of rCRS, middle turbinate lateralization (MTL), and its role in surgical failure. The rationale previously cited in the literature is the close proximity of the middle turbinate to the OMC, such that lateralization of the middle turbinate would cause OMC obstruction. Review of the literature shows little evidence for this proposed mechanism, leading us to the first study in defining a role for MTL in surgical failure.

With regard to the rCRS group we propose two novel hypotheses. First, the role of the eosinophil in CRS is examined. Here we propose the hypothesis (the inflammatory load hypothesis) that the eosinophil plays a major role in the grade of inflammation in CRS (rather than the OMC obstruction) in determining long-term prognosis in patients, and is thus central to the development of refractory CRS. The role of removal of the eosinophil with more extensive surgery is explored as a possible explanation for improved outcomes with these surgeries. Next we examine the relatively recently-described pathophysiological concept of mucosal remodeling

in CRS as a possible contributing factor to rCRS through the development of irreversible mucosal disease.

Nasal polyp recurrence is a common finding in revision surgery patients. A higher rate of recurrence has been correlated to the inflammatory load in multiple studies. We thus describe the patterns of nasal polyp recurrence after surgery as well as the clinical factors associated with more aggressive recurrence. We also investigate, in light of the newly proposed inflammatory load hypothesis, a potential role for the Draf-3 frontal drillout procedure in influencing outcome (nasal polyp recurrence and long-term need for revision surgery).

Finally these hypotheses are tested by investigating histopathological slides of sinonasal mucosa collected from a group of refractory CRS patients.

5.3 Thesis Aims

The aims of this work can thus be summarized in the following points:

1) Investigate the clinical significance of middle turbinate lateralization through studying the association of post-operative middle turbinate lateralization in CRS with sinus symptoms and the subsequent need of revision sinus surgery.

2) Describe the clinical relevance of the following 2 hypotheses:

   a. The inflammatory load hypothesis

   b. The irreversible disease hypothesis

3) Characterize the patterns of nasal polyp recurrence after endoscopic sinus surgery and investigate the role of a radical/extensive surgical option (namely the Draf-3 frontal drillout procedure) in influencing outcome

4) Investigate inflammatory load and mucosal remodeling in rCRS patients through a histopathological study

Chapter 6 Clinical significance of middle turbinate lateralization post-endoscopic sinus surgery

6.1 Statement of Authorship

Title of Paper	Clinical significance of middle turbinate lateralization after endoscopic sinus surgery.
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Publication Style
Publication Details	Bassiouni A, Chen PG, Naidoo Y, Wormald P-J. Clinical significance of middle turbinate lateralization after endoscopic sinus surgery. Laryngoscope. 2015 Jan;125(1):36–41.

Principal Author

Name of Principal Author (Candidate)	Ahmed Bassiouni		
Contribution to the Paper	Project design, literature review, data collection, data analysis, manuscript preparation		
Overall percentage (%)	70%		
Signature		Date	18/06/2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:
  i.   the candidate's stated contribution to the publication is accurate (as detailed above);
  ii.   permission is granted for the candidate in include the publication in the thesis; and
  iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Philip G Chen		
Contribution to the Paper	Project design, literature review, data collection, manuscript editing		
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Signature		Date	18/06/2015

Name of Co-Author	Peter-John Wormald		
Contribution to the Paper	Project supervision, manuscript editing		
Signature		Date	18/06/2015

6.2 Citation

Bassiouni A, Chen PG, Naidoo Y, Wormald P-J. Clinical significance of middle turbinate lateralization after endoscopic sinus surgery. Laryngoscope. 2015 Jan;125(1):36–41.

6.3 Abstract

Objectives/Hypothesis: To investigate the clinical significance of middle turbinate lateralization (MTL) occurrence post-endoscopic sinus surgery (ESS) for chronic rhinosinusitis, namely, association with post-operative symptoms and eventual need for undergoing revision surgery.

Study Design: Retrospective chart review of consecutive post-operative follow-up appointments (November 2009–May 2011), for patients who had had full-house ESS. (Post-hoc analysis)

Methods: Endoscopic video recordings were reviewed by a blinded reviewer to determine occurrence of MTL (any portion of the MT touching the lateral nasal wall). Post-operative symptom questionnaires using the Adelaide scoring system were collected. Records were reviewed to determine need for revision surgeries during follow-up.

Results: 151 patients had follow-up with video-endoscopy in the duration 2009-2011. No statistically significant association between MTL and symptoms was found ($p > 0.05$). 21% of patients with MTL required revision, versus 9% in those who had no MTL ($p = 0.07$). Log-rank test showed that there was a statistically significant difference between the revision surgery survival curves for the MTL and no MTL groups ($p = 0.03$). Controlling for the inability to examine the frontal sinus, the difference between the two survival functions increased ($p = 0.005$).

Conclusions: MTL was not associated with patient-reported symptoms, but may be associated with a more rapid need for future revision surgery. We hypothesize that this effect is related to interference with the frontal sinus.

Level of evidence: 4

6.4 Introduction

The middle turbinate (MT) is viewed as having important functions in nasal air flow and olfaction. It is also an important anatomical landmark that is also in close proximity to the osteomeatal complex (OMC). Middle turbinate lateralization (MTL) has thus been linked to OMC obstruction, an event which adversely impacts the mucociliary drainage and ventilation of the sinuses. This explanation provided the theoretical foundation to view MTL as an undesirable side effect after sinus surgery, in line with the original concept of FESS.[292] Indeed, the reputation of MTL as a significant complication of sinus surgery rests on multiple reports that demonstrated a high percentage of MTL prevalence in patients undergoing revision surgeries.[168,197–199,208] That led surgeons to develop numerous techniques devised specifically to reduce MTL. Nevertheless, these previous reports lacked a control group, and reported MTL only during the (second) revision surgery, as a pre- or intra-operative finding.

On the other hand, higher level evidence comes from studies that examined outcomes of middle turbinate (MT) resection, rather than investigating MTL per se. These studies reported improvements in olfaction and endoscopic picture;[293] delayed nasal polyp recurrence;[294] and delayed need for revision surgery.[295] Although these studies have not examined MTL, we hypothesized that some of these reported benefits of MT resection may be due to eliminating the risk of MTL post-operatively.

We have recently examined the factors that are potentially associated with an increased risk of MTL (identifying none that were statistically significant).[296] The aim of this follow-up study is to investigate the clinical significance of MTL, namely: the association between the occurrence of MTL after surgery, and patient-reported post-operative symptoms as well as long-term need for revision surgery.

6.5 Methods

6.5.1 Study design

Post-hoc subgroup analysis of a retrospective study[296] (data collected prospectively). The study was approved by the Institution Ethics Board (reference HREC/12/TQEHLMH/121).

6.5.2 Inclusion criteria

Consecutive sampling of all CRS patients (including CRSsNP and CRSwNP) attending the tertiary rhinology practice of the senior author (P.J.W.) for a follow-up appointment (November 2009 - May 2011) following "full house" endoscopic sinus surgery (FH ESS) in the period of 2003–2009. The following groups were excluded:

- Patients who did not receive any post-operative follow-up with the senior author nor had post-operative video-endoscopic recordings available during this period.

- Patients who did not have frontal sinusotomies as part of their surgeries

- Patients who had a Draf-III frontal drillout /modified Lothrop procedure

- Absence of a middle turbinate

6.5.3 Surgical technique

All surgeries were complete spheno-ethmoidectomies with frontal ostial clearance (so-called full house ESS (FH ESS)) indicated for CRS after failed medical treatment. Surgery included uni- or bi-lateral middle meatal antrostomies, ethmoidectomies and frontal sinusotomies. All frontal sinusotomies were Draf-2A dissections and the dissection technique has been previously published,[165] and included the axillary flap approach[297] to access the frontal recess in all patients.

At the discretion of the surgeon, unstable MTs (floppy or lacking structure) were sutured to the septum using 4-0 Vicryl Rapide (Ethicon, Somerville, NJ) tied to a PS-2 curved needle, as previously described.[296]

6.5.4 Assessment of MTL after surgery

Video-endoscopic recordings from the included patient cohort were reviewed as previously described.[296] In brief, the post-operative status/anatomical location of the middle turbinate was recorded to assess lateralization and the reviewer was blinded to all details of the patients' history and surgery. MTL was defined as any portion of the body or head of the MT contacting the lateral nasal wall, as previously described.[296] The reviewer also recorded whether the frontal sinus could be visualized, independent of MTL. In this paper, patients were classified into two groups (an MTL-positive group and an MTL-negative group) for statistical comparisons. Patients

belonged to the MTL-positive group if they had evidence of MTL on either side at their last follow-up visit.

6.5.5 Outcome variables

The first group of outcome variables was subjective post-operative patient-reported symptoms. Post-operative Adelaide CRS symptom scoring[298] questionnaires were sent to all operated patients in June 2011, after their last video-recorded follow-up visit. This questionnaire includes questions on a 5-point scale about the severity of 5 major CRS symptoms (nasal obstruction, rhinorrhea, post-nasal drip, headache, anosmia) plus an overall 8-point disease severity effect on quality of life (QOL). Presence/Absence of a symptom was determined according to the Adelaide symptom scoring system, with a score of 1 meaning absence of the symptom. The second outcome variable was whether patients had required revision surgery during their follow-up and the dates of these revision procedures were recorded. Revision surgery was offered to patients who had ongoing persistent CRS symptoms with radiographic evidence of disease after failure of at least 6 months of maximal medical therapy.

6.5.6 Statistical analysis

All statistics were done using the R statistical software (R Foundation for Statistical Computing, Vienna, Austria)[299] and the IPython[300] notebook. Statistical significance was taken at the traditional 0.05 level. Fisher's exact tests were used to test for an unequal confounding effect of clinical variables between the MTL-positive group and the MTL-negative group. Kruskal-Wallis rank sum tests were employed for assessing the association of MTL with the collected post-operative symptom scores. Univariate logistic regression models were used to assess the association of MTL with the presence/absence of the 5 major post-operative symptoms on the questionnaire. Fisher's exact tests were employed for the association of MTL with the need for revision surgery. Survival analysis was also used and Kaplan-Meier survival curves for were plotted. Any revision procedure done for the patient to date was taken as the outcome of the survival analysis, with survival defined as duration between their initial surgery and their revision surgery. For patients who did not require revision surgery, the date of their last-follow-up visit was recorded and they were considered as right-censored observations. Log-rank test was used to test significant difference between survival curves of the MTL-positive and MTL-

negative groups. The stratified log-rank test was also used to control for the additional variable "inability to examine the frontal sinus during endoscopy".

6.6 Results

6.6.1 Baseline characteristics

A total number of 151 patients had follow-up with video-endoscopy in the duration 2009-2011. These had undergone FH ESS in the period March 2003 - February 2011. Table 6-1 shows the baseline characteristics of the study cohort. MTL occurred in 38 cases (25%). No significant unequal confounding effect of sex, primary/revision status, polyp status, asthma status, or smoking status was found between the MTL-positive group and the MTL-negative group (Fisher's exact tests, $p > 0.05$).

Table 6-1 Baseline characteristics of the study cohort.

N	151	
Age at time of operation	Mean 50.5 years (SD 14.3)	
Follow-up duration	Mean 40.9 months (SD 26.1)	
Sex†	Male: 85	Female: 66
Type of surgery†	Primary surgery: 71	Revision surgery: 80
Nasal polyp status†	CRSsNP: 81	CRSwNP: 70
Asthma status†	Non-asthmatic: 97	Asthmatic: 54
Smoking status†	Non-smoker: 145	Smoker: 6
Middle Turbinate	Not lateralized: 113	Lateralized: 38 (with inability to examine the frontal sinus in 28)

CRSsNP = chronic rhinosinusitis sans nasal polyposis

CRSwNP = chronic rhinosinusitis with nasal polyposis

† Fisher's exact tests excluded unequal confounding of these variables upon middle turbinate status (p > 0.05)

6.6.2 MTL and patient-reported post-operative symptoms

The association between MTL and post-operative symptoms (as recorded on the Adelaide symptom severity questionnaire) was studied using Kruskal-Wallis tests. Sixty-nine patients (39% female; 51% primary surgeries; 54% CRSwNP; 40% asthmatic;) had replied to the post-operative questionnaires sent by first-class mail and were included in this analysis. This found no significant association between MTL and any of the symptom scores recorded. (Table 6-2) We then tested for an association between MTL and mere presence/absence of symptoms using univariate logistic regression models and this also was not significant. (Table 6-2)

Table 6-2 Assessing the association between MTL and post-operative symptoms: Kruskal-Wallis tests for symptom scores and univariate logistic regression models for absence/presence of symptoms.

	Kruskal-Wallis	Logistic
Independent variable	p-value	p-value
Nasal obstruction	0.492	0.387
Rhinorrhoea	0.8223	0.959
Post-nasal drip	0.9092	0.316485
Headache/Facial pain	0.2305	0.2244
Anosmia	0.6146	0.681
Effect of overall symptoms on your QOL	0.9195	_
Total of the 5 symptoms	0.5877	_
Total of 5 symptoms + Overall effect on QOL	0.7665	_

QOL = Quality of Life

6.6.3 MTL and revision surgery

The association between MTL and revision surgery was examined, employing Fisher's exact test and results are shown in Table 6-3. First, this was studied on the cohort of patients who have replied to the questionnaire. Owing to variable durations of post-operative follow-up, we have done these analyses on various subgroups, according to the duration of follow-up they received (more than 6, 12, and 24 months). (Table 6-3) The results showed lower proportion of patients undergoing revision surgery in the non-lateralized group, however this was not statistically significant. ($p > 0.05$; Table 6-3)

Table 6-3 Assessing the association between MTL and undergoing revision surgery

Cohort	Middle turbinate		Fisher's exact p-value
	Not lateralized	Lateralized	
Cohort with follow-up ≥ 6 months (N=143)	9/105 (8.6%) required revision	8/38 (21%) required revision	0.0746
Cohort with follow-up ≥ 12 months (N=134)	9/97 (9.3%) required revision	8/37 (21.6%) required revision	0.07916
Cohort with follow-up ≥ 24 months (N=110)	9/80 (11.25%) required revision	8/30 (26.7%) required revision	0.07244

6.6.4 Survival analysis of the need for revision

To overcome the variable follow-up durations for each patient, we also employed survival analysis to analyse the survival curves for the revision surgery outcome variable. Kaplan-Meier survival curves are shown in Figure 6-1. Log-rank test showed a statistically significant difference between those with MTL and those with no MTL (Chi-square= 4.6, on 1 degrees of freedom, $p = 0.0316$). We then repeated the same comparison, but controlled for the "inability to examine the frontal sinus" variable using a stratified log-rank test. This gave a $p=0.0049$ (Chi-square= 7.9, on 1 degrees of freedom), i.e. the p-value became more significant after controlling

for the inability to examine the frontal sinus, implying that the difference between the two survival curves is moreover increased after controlling for this variable.

Figure 6-1 Kaplan-Meier survival curves for the middle turbinate lateralization (MTL) and no-MTL groups. There was a statistically significant difference between the two curves (log-rank test, P = .0316).
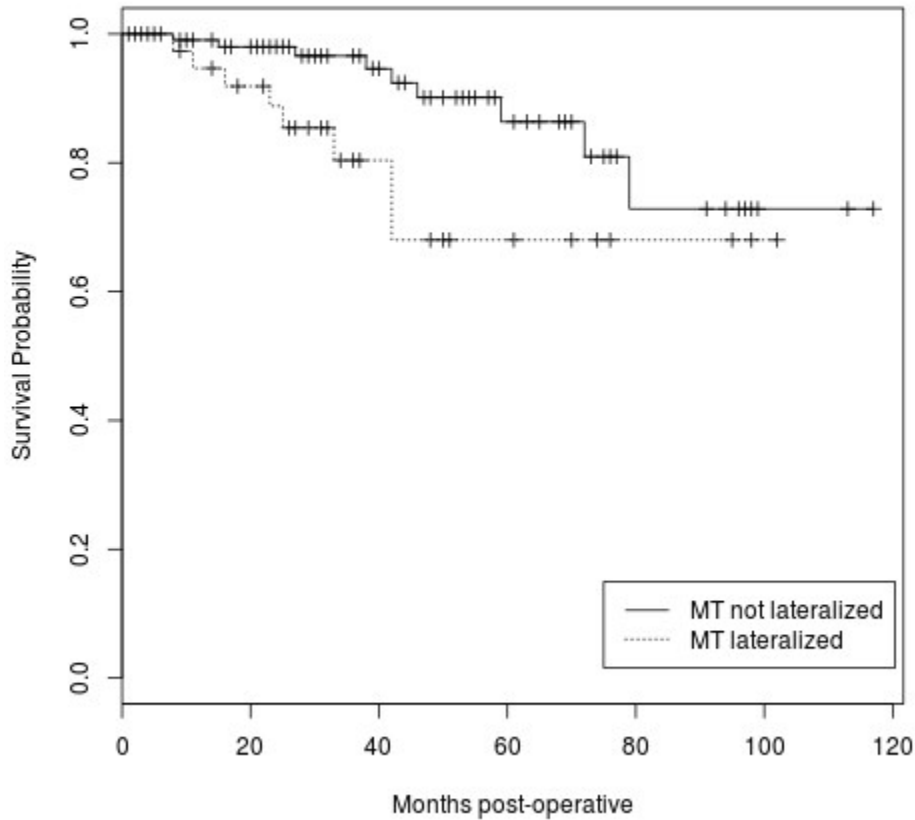
6.7 Discussion

Our aim was to ascertain whether occurrence of MTL following surgery was associated with a poorer post-operative outcome. In this paper, we investigated its relationship to post-operative symptoms and the need for revision surgery. Our results found that although MTL is not associated with post-operative symptoms, it may be associated with an accelerated risk of requiring revision surgery.

MTL is a relatively common phenomenon following endoscopic sinus surgery. We have recently reported an incidence of approximately 15-22% in a cohort of 124 patients[296] Reasons for the occurrence of MTL post-operatively is not yet clear, since we have previously shown that there was no association between MTL and pre-operative clinical variables (including sex, asthma, polyp status, primary versus revision surgery), or specific operative interventions (including septoplasty and concha bullosa reduction).[296] MTL may therefore occur due to unknown surgical intra-operative events that lead to destabilization of the middle turbinate or to an anatomical predisposition.

The clinical question remains: is MTL a complication of sinus surgery, or is it just a harmless sequela? Multiple previous studies[168,197–199,208] showed MTL to be a significant factor (ranging from 22 to 75%), in patients requiring revision surgeries. (Table 6-4) The authors of these studies concluded that MTL was a significant complication of sinus surgery that increased the risk of revision surgery. One of the explanations offered was that MTL, and resultant adhesions, lead to OMC obstruction and recurrent disease. However, due to: (a) the retrospective nature of these studies, (b) the description of MTL only at the time of the second revision procedures, and (c) the explicit absence of an internal control group; we suggest further research is necessary before this can be supported. Our study, while limited by being a retrospective review, attempts to improve the evidence, using prospectively collected data, and maintaining a timeline between events (timeline shown in Figure 6-2).

Figure 6-2 Timeline of various events for our study cohort.
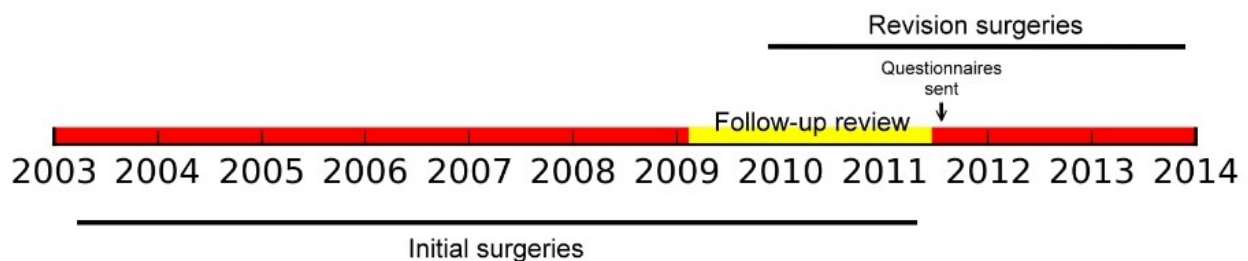
Our results hold several implications for current clinical practice and should be therefore discussed in that context. First, MTL alone was not found to correlate with patient symptoms. (Table 6-2) However, a limitation to this finding is that the questionnaire was not done at the time of the endoscopic proof of lateralization, but rather at a fixed later time-point; the limitation

being imposed by the post-hoc nature of this analysis. Unfortunately, there exists no higher level evidence in the literature pertaining to this association to the best of our knowledge. Secondly, although MTL was associated with an increase in the proportion of those who require later revision surgery (from 11.25% to 26.7% with ≥ 24 months follow-up; Table 6-3), this did not reach statistical significance (albeit may be described as a trend, p = 0.07; Table 6-3). Survival analysis results nevertheless showed patients with MTL had worse survival curves, when compared to those who had no MTL. (Figure 6-1) This suggests that MTL alone is not a central component of the disease process, but rather plays a role as a contributing factor to a worse outcome.

Table 6-4 Studies reporting MTL as a finding of high occurrence, in patients requiring revision surgeries.

Study	N	Finding
Lazar et al.[168] (1992)	673	**43%** of 63 patients who required revision had significant adhesions between the MT and the lateral nasal wall
Schaitkin et al.[208] (1993)	91	**22%** of 23 patients who required revision had adhesions between the MT and lateral nasal wall
Ramadan et al.[197] (1999)	398	In 52 requiring revision, most (**25-56%**) had adhesions, often involving a lateralized MT.
Musy and Kountakis[198] (2004)	80	Lateralization of the MT found in **78%** of 80 patients undergoing revision surgery.
Chiu and Vaughan[199] (2004)	67	Lateralized remnants of the MT with scarring of the frontal recess were seen in **35.8%** of patients undergoing revision surgery.

There appears to be no published higher level evidence explicitly examining MTL and its effect on CRS outcomes. However, strong evidence exists in studies reporting benefits to MT resection. Soler et al.[293] prospectively followed up 47 patients who had bilateral MT resection and 195 patients who had no bilateral MT resection. They found no significant difference between the two groups in various QOL questionnaires, however they reported improved olfaction and endoscopic pictures for patients undergoing resection.[293] Wu et al.[295] retrospectively investigated factors affecting duration to revision surgery and reported a longer duration to revision in patients who underwent middle turbinate resection rather than preservation (4.56 vs. 3.93 years).[295] In another prospective study, Marchioni et al. reported reduction in rates of nasal recurrence with MT resection,[294] this result being perhaps consistent with a hypothesized reduction in disease load with more extensive surgery. [301,302] Our study did not include a group which underwent MT resections and thus could not provide comparability to the aforementioned studies. However, our results suggest that the reported benefits of MT resection can be, at least partially, attributed to abolishing the risk of MTL post-operatively.

Another interesting observation, albeit statistical, can be found in the result of the stratified log-rank test, which showed increased difference between the survival functions when controlling for the ability to endoscopically examine the frontal sinus by the surgeon. This suggests that MTL may only be significant clinically (accelerating the need for revision) when it interferes with the frontal sinus pathway, and thus its relation to ethmoidal and maxillary drainage at the OMC (the OMC regarded as the convergence point of all these sinuses) may be of lower importance clinically. The concept of OMC obstruction alone, as a central and universal pathogenetic process in all CRS subgroups has recently been questioned.[54,301,303] Hosemann et al.[304] and Naidoo et al.[165] have previously demonstrated the importance of frontal ostial stenosis for frontal sinus outcomes. A valid explanation to link our present results with the previous results by Hosemann[304] and Naidoo[165] is offered, whilst attempting to avoid a more simplistic OMC obstruction view. (Figure 6-3) Significant MTL would prevent penetration of topical steroids, as well as harbor infections. This could lead to a persistent inflammatory load[301], which could accelerate remodelling/fibrosis at the frontal recess, eventually ending in frontal ostium scarring and stenosis. (Figure 6-3) Severe scarring at the frontal ostium may ultimately lead to a dysfunctional[284,305] frontal sinus, necessitating extensive surgery such as in the form of a Draf-3 frontal drillout[306,307]. Prospective controlled studies are required to prove any of these

hypothesized associations, and thus it is important to note the limitation of our retrospective study.

Figure 6-3 Hypothesized adverse mechanism of middle turbinate lateralization (MTL). (a) Postoperative middle turbinate lateralization occurs as a side effect of sinus surgery. (b) MTL harbors repeated infections that do not clear easily as well as reduces penetration of topical medication, leading to a persistent inflammatory load (c). (d) Persistent inflammatory load leads to accelerated fibrosis/remodeling in the region, which eventually involves the frontal recess and frontal sinus ostium, ending in frontal scarring and stenosis.

Color Legend:

☐ Mucosa   ☐ Infection   ☐ Inflammatory load   ☐ Fibrosis/Remodeling
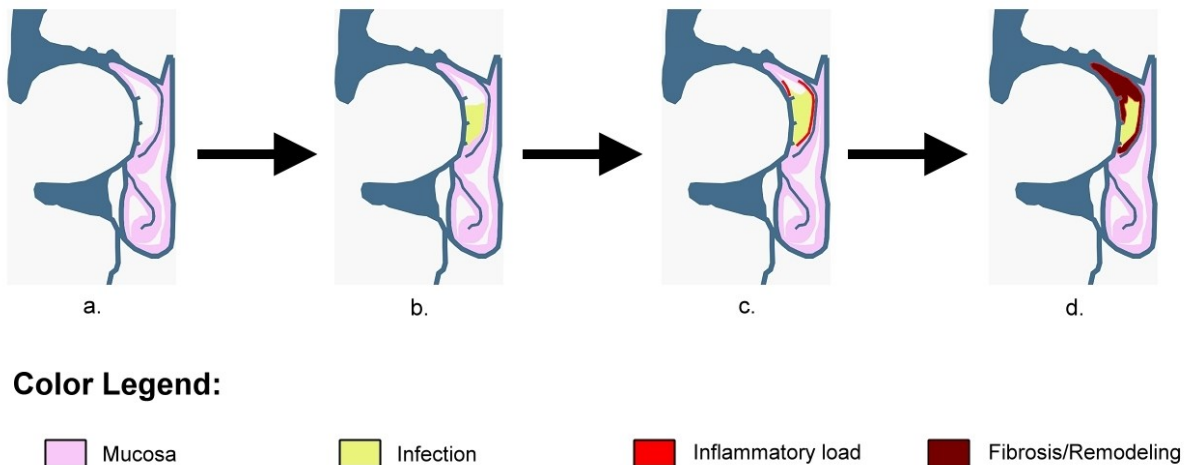
Our results support previous conclusions that MTL is of clinical significance and should be considered a complication of surgery. (Table 6-4) Further research should identify the best method of preventing MTL. We recently reported that middle turbinate suture conchopexy did not lead to a significant reduction in rate of MTL; however that study was limited by non-randomization.[296] Other techniques that have been described include controlled synechiae[308–310], metal clips[311], middle meatal spacers[312,313] and bioresorbable implants[314]. The effectiveness of these techniques in long-term outcomes is still unknown. MT resection is also a viable surgical option with published benefits, if done properly in a carefully selected subgroup of patients, when the MT is involved in the disease process.[293] Surgeons should record MTL, and in particular, inability to examine the frontal sinus in their post-operative follow-up notes, and regard it as a potential negative prognostic event. These variables are not included in the Lund-Kennedy endoscopic scoring system.[13] We therefore suggest that future research investigate development of an improved systematic method of recording and scoring follow-up endoscopic

surveillance. This method would record such important post-operative events that help better prognosticate long-term outcomes.

6.8 Conclusion

Our results show that post-operative MTL was not associated with short-term patient-reported symptoms, but may be associated with an accelerated need for revision surgery. We hypothesize that this effect is related to interference with the frontal sinus. Future prospective studies need to confirm the findings of this paper, better characterize the mechanism by which MTL contributes to the disease process, and improve techniques that prevent MTL from occurring.

Chapter 7 The inflammatory load hypothesis in refractory CRS

7.1 Statement of Authorship

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Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Publication Style
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Name of Principal Author (Candidate)	Ahmed Bassiouni		
Contribution to the Paper	Project design, literature review and reference collection, manuscript preparation		
Overall percentage (%)	70%		
Signature		Date	18/06/2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

  i. the candidate's stated contribution to the publication is accurate (as detailed above);

  ii. permission is granted for the candidate in include the publication in the thesis; and

  iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Yuresh Naidoo		
Contribution to the Paper	Literature review, manuscript editing		
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Contribution to the Paper	Project supervision, manuscript editing		
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7.3 Abstract

Through recent advances in research, our understanding of chronic rhinosinusitis (CRS) has evolved to consider it as an inflammatory condition of the mucosa brought about by multiple factors. However, surgical management is still ruled by the classical concepts of functional endoscopic sinus surgery (FESS), which emphasizes the importance of ostial obstruction and sinus ventilation. These concepts still fail to provide sufficient explanation for the presence of a subset of patients with refractory CRS, who fail to respond to conventional FESS. Recent outcome studies have shown that high-grade mucosal inflammation often results in a poor outcome and that this patient group may show improved results with more radical surgery. This review examines the "inflammatory load hypothesis" as a possible explanation. We hypothesize that the grade of the inflammation is the most important predictor of long-term outcomes. Surgery, therefore, has a significant role in not only reestablishing ventilation, but also with removing the inflammatory load in the affected sinuses. We suspect that in these severely diseased patients, a more radical removal of local pro-inflammatory factors during surgery may improve patient outcomes.

7.4 Introduction

Functional Endoscopic Sinus Surgery (FESS) is widely considered to be the gold standard in the surgical management of chronic rhinosinusitis (CRS) which has failed maximal medical therapy.[162] FESS emphasizes clearance of pathology at the osteo-meatal complex (OMC).[160,315] This concept suggests that clearing the obstruction of the common drainage pathway restores function by improving ventilation and allowing mucociliary clearance to normalize. FESS has

been shown to be successful, with reported success rates of 90% for primary FESS. [161,162] However, success in revision cases falls to 69.8%.[167]

Patients who fail FESS, and require multiple surgeries, are suffering from refractory chronic rhinosinusitis (rCRS).[316] Recent insights into the aetiopathogenesis of CRS suggest an increasing number of reasons for the existence of this small, but significant, subset of patients with rCRS. The original theories of FESS do not hold sufficient explanation as to why these patients fare badly with functional surgery. On the other hand, there is increasing evidence that many patients with rCRS benefit from more extensive or radical surgical options.[174,175,178,180,185,192,214,317]

The aim of this article is to review the literature in an attempt to understand why patients with rCRS fail standard functional surgery and to discuss potential alternative surgical options for this group of patients.

7.5 Revisiting historical concepts: Is a restoration of sinus ventilation and mucociliary function sufficient during surgery?

The theories behind FESS were based mainly upon the sinonasal mucociliary physiology studies of Messerklinger and Stammberger. Consequently, FESS placed great emphasis on sinus aeration and restoration of mucociliary function through clearance of blocked sinus ostia , with a particular focus on OMC disease. Although these concepts play a role in the disease process, they do not provide sufficient explanation as to why some patients (rCRS) do not benefit from functional surgery.

Does OMC disease, with the subsequent blockage of sinus ventilation and mucociliary drainage, truly represent the major pathogenic factor in CRS? A recent study showed that more than 35% of patients with CRS did not manifest OMC obstruction on CT scans.[54] Even though the efficacy of FESS in improving the mucociliary function is shown in multiple studies (Table 7-1) that looked at mucociliary function before and after FESS, the exact role of mucociliary drainage is not clear. Two studies[318,319] showed only a slight or non-significant improvement in mucociliary clearance, and while Inanli et al[320] reported a significant improvement, it still did not reach the level of normal healthy controls at 12 weeks. Hafner et al[321] showed that although the measured Saccharin Test (ST) transit times improved significantly in 17 of 22 patients post-FESS, it

remained prolonged in 5 patients and Asai et al[322] discovered that postoperative mucociliary function (as indicated by the ST) did not always correlate with the postoperative endoscopy. Some studies noted that, although OMC blockage cleared, MCC tended to be significantly prolonged in sinuses containing polyps when compared to sinuses without polyps.[322–324] Moreover, many patients reported absence of symptoms, while mucociliary function has still not fully recovered.

Table 7-1 Mucociliary function studies post-surgery

Study	Method of Measurement	Duration of Post-ESS Follow-up	Summary
Hafner et al[321] (1997)	Saccharin Test	4-10 months	Improvement of Mucociliary Transport
Huang et al[324] (2006)	Saccharin Test	8 weeks to 4 months	Improvement of Mucociliary Transport
Kaluskar et al[325] (1997)	Saccharin Test	6 months	Improvement of Mucociliary Transport
Inanli et al[320] (2000)	Saccharin Test	12 weeks	Improvement of Mucociliary Transport
Elwany et al[326] (1998)	Saccharin Test	3 months	Improvement of Mucociliary Transport
Min et al[327] (1995)	Saccharin Test	1, 6 and 12 months	Improvement of Mucociliary Transport
Myller et al[318] (2006)	Isotope method	9 months	*No significant Improvement*
Toskala and Rautiainen[319] (2004)	Isotope method	6 months	*No significant Improvement*
Behrbohm and Sydow[328]	Isotope method	between 6 and	Improvement of

(1991)		18 months	Mucociliary Transport
Dal et al[329] (1996)	Isotope method	3 weeks	Improvement of Mucociliary Transport
Ikeda et al[330] (1996)	Isotope method	Between 6 to 14 months	Improvement of Mucociliary Transport

7.6 The inflammatory load and its effect on disease severity and surgical outcome

CRS outcome studies have looked at the relationship between the inflammatory process and disease severity and prognosis, utilizing various parameters in an attempt to describe or quantify the degree of inflammation in the mucosa. These parameters include peripheral blood eosinophil count[331,332] and local mucosal eosinophilic infiltration, expressed as a percentage of inflammatory cells,[333,334] or as an absolute number of eosinophils per high-power field.[335–338]

Through these parameters, it was found that the grade of inflammation positively correlated with disease severity, using various subjective and objective criteria. Mucosal eosinophilia correlated with disease severity as measured by CT or endoscopy scores,[334–336,338] while other studies showed a similar correlation with systemic eosinophil counts.[332,337,339,340] Patients with Eosinophilic Mucus chronic rhinosinusitis (EMCRS) had higher symptom scores and higher rates of bone erosion on CT scans, than normal CRS patients without EM.[15] It was also found that with increasing eosinophilia in patients' sputum, the higher their CT scores and the greater the bone erosion exhibited on CT.[341,342] Patients with persistent nasal discharge after surgery had a significant predomination of eosinophils in their secretions, when compared with controls with no discharge.[343] This eosinophilia was still prominent even in the presence of a positive bacterial culture, a feature that normally gives a neutrophilic skew.[343]

Other studies analyzed how the inflammatory load correlated with postoperative outcomes. It was found that a higher grade of mucosal eosinophilia consistently predicted a worse prognosis with more likely recurrence of disease[204,344], a higher level of post-operative symptoms[232], and less improvement in post-operative QOL scores[166,345]. Similar conclusions were also drawn when serum eosinophilia was studied.[204,346] Another study found no correlation between the number of

mucosal eosinophils and surgical outcome but found an association between the number of IL-5 mRNA-producing cells and a worse prognosis.[347] From the literature it is apparent that the patients with the highest inflammatory load (those who represent the end of the 'inflammatory spectrum') are those with nasal polyposis (CRSwNP), concomitant asthma and/or aspirin intolerance.[97,331,333,348–350] These same patients experience worse post-operative subjective and objective outcomes, as well as higher recurrence rates and a higher need for revision surgery.[205–210,351,352]

This data illustrates that relieving an obstruction that hinders ventilation and mucociliary clearance is not the only determinate of surgical success, but that the inflammatory process, in which the eosinophil acts as the pivotal cell, is of equal significance in determining long-term prognosis. This is especially true in the more severe spectrum of CRS (the NP/asthma/aspirin-sensitive subgroups).

7.7 Understanding the pathophysiologic role of the eosinophil

To understand the previously-mentioned negative outcomes associated with a high eosinophilic inflammatory load, a quick review of the various pathophysiologic roles of the eosinophil is presented. (Figure 7-1) Eosinophil recruitment to the sinonasal mucosa occurs mainly through an IL-5 mediated process.[353] Eosinophils contribute to mucosal injury by secretion of toxic granule proteins, such as Major Basic Protein (MBP) and eosinophil cationic protein (ECP).[354,355]

Figure 7-1 The pathophysiologic roles of the eosinophil in the sinuses.
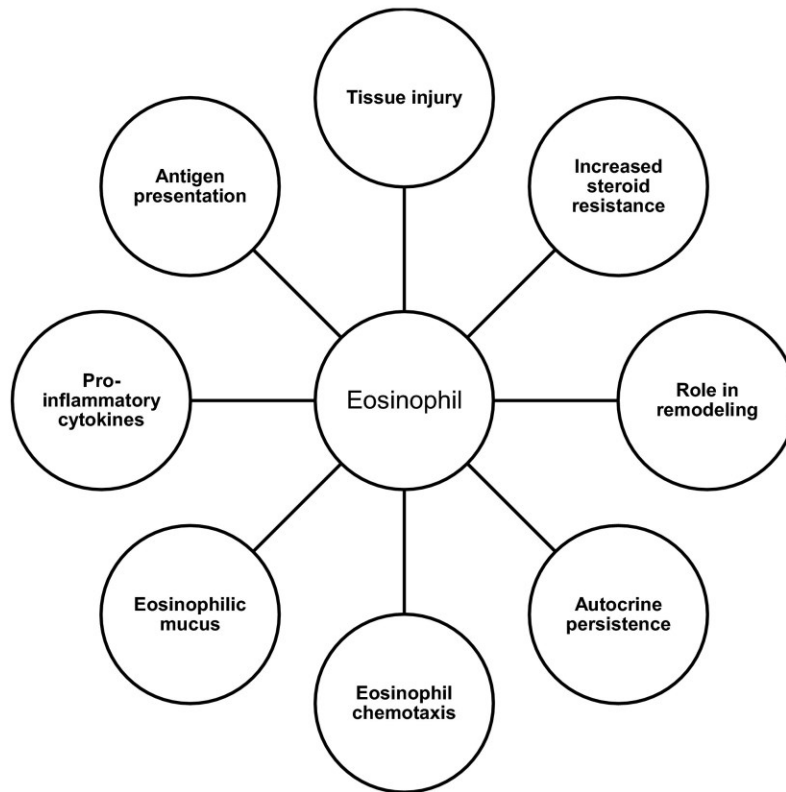
Eosinophils are also main producer of eotaxins[288–290] and their granule proteins, when released, stimulate eosinophils themselves to degranulate.[356] In this way, eosinophils contribute to the persistence of their own species in an autocrine fashion. Moreover, Eosinophilic CRS (ECRS) is associated with an increased expression of the Glucocorticoid Receptor beta (GR-β) isoform.[280] The GR-β is a steroid action inhibitor that is associated with steroid insensitivity. This means that ECRS, when compared to non-ECRS, is inherently more resistant to medical therapy.[280]

Apart from their direct effector functions, eosinophils also play an important immunomodulatory role. This occurs through their ability to secrete pro-inflammatory cytokines[357,358] and through functioning as antigen-processing and presenting cells[272] promoting TH-2 cell responses. Saitoh et al[285] showed that epithelial damage and basement membrane thickness in CRS correlated with the number of infiltrating eosinophils, suggesting that they also play a role in the process mucosal remodeling.[285] Epithelial cells in turn compound this ongoing inflammation by

promoting eosinophil survival and activation through the production of eotaxin-2,[359] GM-CSF[360,361] and neurotrophins[362].

In addition to infiltration within the mucosa, eosinophils also escape to the mucus produced within the sinus cavity itself,[363] with the epithelial production of eosinophilic mucous (EM). Antigenic material (for example, staphylococcal superantigens or fungal remnants) becomes entrapped in the thick viscous EM and persists locally, leading to continuous immune stimulation. EM contains toxic products from degranulated cells and leads to increased migration of eosinophils from the circulation to the nose, suggesting the presence of chemoattractants in the mucus that encourage eosinophil migration.[363] In this way EM is not just an end-product of disease, but plays an active role in disease perpetuation.

The end result is a self-sustaining self-perpetuating inflammation, independent of further allergenic stimulation, and resistant to standard therapy.

7.8 Radical Surgery: the surgical concept of "reducing the inflammatory load"

FESS currently produces excellent long-term results in patients without high-grade eosinophilic inflammation. However, a large proportion of patients in whom standard FESS fails have eosinophilic infiltration of the sinus mucosa. To some extent this can be seen as a consequence of conservative surgery addressing only the sinus ostia and not the significant load of eosinophils in the mucosa and the thick tenacious eosinophilic mucus in the sinuses. Many studies suggest that better outcomes can be achieved through a radical surgical approach.

In the early days of FESS surgery, FESS was compared to the more traditional but radical surgery of Caldwell-Luc (CL) and ethmoidectomies. In 1990, Mcfadden et al[180] reviewed 25 patients with Samter's triad. Sixteen patients underwent initial conservative ethmoidectomies using FESS philosophy. Of these 16 patients, 6 required subsequent surgery for recurrent disease. The remaining 9 of the 25 patients had initial radical procedures such as CL with intranasal and transantral sphenoethmoidectomies. None of these 9 patients required further surgery.[180] Another study comparing CL to FESS showed that the CL group needed reoperation in 4.8-7.3% of cases whereas the FESS group needed reoperation 18-27% of cases.[364] Ragheb and Duncavage also compared CL to MMAs in 153 patients and suggested that the subset of

patients with bronchial asthma may indeed benefit from the more radical approach offered by the CL as opposed to a FESS.[181] In a more recent study,[178] the traditional CL (with a radical removal of the mucosa) was performed on patients who failed, on average, two prior middle meatal antrostomies (MMAs). The response rate was 92%.[178] Although the CL retains some indications in the endoscopic era,[365] its use nowadays for CRS has almost been abandoned.

A more recent and slightly more conservative approach for severe maxillary sinusitis has been the Canine Fossa Trephine (CFT).[317,366] In CFT it is important to note that, contrary to CL, the mucosa is not stripped to the underlying bone. The sinus is cleared of all polypoid mucosa with the underlying basement membrane retained. The authors cite better access gained by this external approach with better clearance of polyps, pus and tenacious eosinophilic mucus from the sinus. This in turn led to less disease recurrence when compared to a matched historical cohort.[176] The CFT also gave better symptom control and better postoperative mucosal appearance on MRI than did the traditional FESS approach to the maxillary sinus.[317] Friedman and Kantsantonis[175] performed revision surgery for 100 patients with recurrent disease in the maxillary sinus that occurred despite functional surgery with a conventional MMA. But in the revision surgery, all recurrent or residual diseased mucosa was removed, including polyps, occasional mucoceles, and hyperplastic changes that occurred inside the sinus, followed by wide marsupialization into the posterior nasal vault. The overall polyp recurrence rate at 18 to 48 months after this revision surgery less than 5%, compared to 19.2% after the functional sphenoethmoidectomy with MMAs.[175]

The endoscopic modified Lothrop procedure (EMLP or Draf 3/frontal drillout) is a radical but successful procedure for persistent frontal sinusitis. Wormald[185] performed the EMLP for 83 patients with a dysfunctional frontal sinus. On an average follow-up of 21.9 months, the cure rate from the EMLP was 75%, in a cohort who had had a mean of six previous failed functional sinus operations.[185] We suggest that this radical ostium-widening procedure breaks the cycle of persistent frontal sinusitis by enabling the surgeon to gain better access and achieve better clearance of the inflammatory load in the normally difficult-to-access frontal sinuses (which, in many cases, are blocked by osteitic new bone formation in the frontal recess).

Other radical procedures have been described for complete clearance of severe disease in the sinuses. Masterson et al[174] reviewed CRSwNP patients who had a complete removal of all polyps

along with a radical ethmoidectomy and compared them to patients who underwent only anterior ethmoidectomies and found that extensive surgery led to a significant decrease in revision rate three years post-operatively.[174] Denker's procedure is another radical procedure in which all walls between the nasal fossa and the paranasal sinuses are removed, creating one large cavity reaching from the ethmoid roof to the floor of the nose and maxillary sinus and from the lateral wall of the maxillary sinus to the nasal septum. Denker's procedure was originally described by Denker for sinonasal malignancies, but has been performed as a last resort for rCRS. Kerrebijn et al performed it for 56 patients and reported relief of sinusitis with significant improvement in symptoms.[192] Videler et al[194,195] reported significant improvements in symptoms and quality of life measures from Denker's procedure and Wreesmann et al[193] in addition reported improvement of lower airway symptoms in asthmatic patients. Both suggested that radical surgery should be an option for patients who fail repetitive conservative functional surgery. [193,194]

A recent study supports the removal of all diseased mucosa with a significant reduction in inflammatory load through the nasalization procedure.[214] Nasalization is essentially similar to Denker's procedure in that it consists of a radical ethmoidectomy with removal of all bony lamellae (including the middle turbinate), plus wide opening of all sinus ostia. This creates one large cavity with all the sinuses marsupialized (or nasalized) into the nasal cavity. Jankowski et al[190,214] compared nasalization with functional ethmoidectomy in nasal polyposis (NP) patients and reported better long term results with better overall symptom improvement in the nasalization group.[190,214] The total recurrence rate was 22.7% in the nasalization group versus 58.3% in the ethmoidectomy group.[214]

The better outcomes of radical surgery (versus conservative) reported from the previous literature is not well explained. However, we assume that in some patients, benefit is obtained from a more radical removal of the inflammatory load. We hypothesize that, as the inflammatory momentum reaches a certain threshold, it becomes resistant to the conventional functional surgical intervention (i.e. clinically irreversible with FESS) and this explains the high rates of failure in these patients. Consequently, mere relief of ostial obstruction in high-grade disease is inadequate, and it becomes mandatory to clear the highest number of activated eosinophils possible. This is particularly relevant in the worse subset of NP with asthma and aspirin sensitivity. The objective is an attempt at "resetting the clock" – elimination of all inflammatory triggers that help perpetuate a vicious cycle of local inflammation. (Figure 7-2)

Figure 2. Factors contributing to the overall local inflammatory load.

We believe that this can only be achieved by a clearance of all polyps inside the sinuses, using appropriate powered and cutting angled instruments. The polyps inside the sinuses should be cleared to the basement membrane, since the highest number of activated eosinophils is present in the pedicle region of the polyp.[367] In addition to eosinophils, polyps that are retained in the sinuses after surgery still contain CD8+ memory T-cells in an activated state.[368–370] Leaving a large number of residual memory cells behind may lead to rapid recurrence of inflammation in the event of re-recognition of antigen especially if the eosninophilic mucous has not been adequately removed. All mucus and debris that can contain eosinophils (with their toxic granule proteins and pro-inflammatory cytokines) or antigenic material (fungal/staphylococcal) should

be meticulously removed and washed out from sinus cavities. This can be achieved relatively easily in the ethmoids and sphenoids but may need ancillary procedures in the maxillary sinus (CFT) and frontal sinus (EMLP).

It is important to note that the radical procedures cited (with the exception of the CL procedure) do not involve deliberate mucosal stripping. Mucosal stripping was reported to cause increased fibrosis and osteogenesis,[371–373] which may eventually result in a dysfunctional sinus[374]. However, other studies reported normal mucosal regeneration.[375,376] It was suggested that it is the preservation of an intact periosteum intact on the surface of the bone that allows normal regeneration without formation of scar tissue and without reducing the sinus cavity.[377]

This concept of complete eradication of inflammatory mediators should be employed in that subset of rCRS patients who fail to respond to conventional FESS. We believe this breaks the local vicious cycle of inflammation and consequently leads to better outcomes and, most importantly, reduce the long-term need for revision through induction of a longer period of disease remission. More research efforts should be spent in further delineation and definition of rCRS, in both CRSwNP and CRSsNP, based on clear objective criteria. We predict that the eosinophil will be the most important arbiter in such a definition.

7.9 Conclusion

Although FESS is the current gold standard, the extent of surgery employed remains highly variable and is not evidence-based. Many reports suggest that the inflammatory load is the most important predictor of long-term outcome. Patients with a high inflammatory load have a higher probability of being refractory to standard FESS. Although the definitive management of these rCRS patients remains uncertain in the literature, many reports point out a role for a more radical or extended surgeries in this group. We hypothesize that the benefit obtained comes through the eradication of pro-inflammatory factors (eosinophils in mucosa, eosinophilic mucus, fungal and staphylococcal antigens, bacterial load, osteitic bony lamellae) that contribute to the local inflammatory load. (Figure 7-2) Further research is needed to identify and select those patients who would benefit most from the extended approach over conventional FESS. As a concept, surgical candidates could be classified pre-operatively, according to definite criteria, into: (a) patients with mild disease (low-grade eosinophilic inflammatory load), reversible with functional surgery, in which OMC obstruction might explain the primary cause and (b) patients with severe

disease (high-grade eosinophilic inflammatory load), in which the inflammatory load plays a far more important role than OMC disease, for whom a more radical approach should be considered, in an attempt to reduce the inflammatory load.

Chapter 8 Clinical significance of remodeling in refractory CRS

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Name of Principal Author (Candidate)	Ahmed Bassiouni		
Contribution to the Paper	Project design, literature review and reference collection, manuscript preparation		
Overall percentage (%)	70%		
Signature		Date	18/06/2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

    i.   the candidate's stated contribution to the publication is accurate (as detailed above);

    ii.   permission is granted for the candidate in include the publication in the thesis; and

    iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Yuresh Naidoo		
Contribution to the Paper	Manuscript editing		
Signature		Date	18/06/2015
Name of Co-Author	Peter-John Wormald		
Contribution to the Paper	Project supervision, manuscript editing		
Signature		Date	18/06/2015

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8.3 Abstract

Mucosal remodeling in the sinuses is a recently-described phenomenon in which the mucosa undergoes potentially irreversible changes as a result of ongoing underlying inflammatory processes. Research into remodeling that occurs in the bronchial airways in asthmatic patients has led to modification of asthma treatment guidelines. However, remodeling in the sinuses has still not led to changes in current medical or surgical management of chronic rhinosinusitis. Upper airway remodeling constitutes a new area of research that poses many unanswered clinical questions and may potentially alter the management of patients with severe chronic rhinosinusitis.

8.4 Introduction

The phenomenon of mucosal remodeling of the respiratory passages was first described in the lower airways in asthmatic patients.[378] Although asthma was originally defined as a reversible disease of the lower airways, it was found that airflow obstruction is not always fully reversible. These non-reversible patients experienced persistent obstruction and a progressive loss in respiratory function, secondary to structural modifications in the mucosa of the lower respiratory passage.[379,380] The upper airway exhibits a similar remodeling phenomenon in the context of chronic rhinosinusitis (CRS).[266,381] This upper airway remodeling mirrors the lower airway remodeling that occurs in asthmatic patients.

The discovery of remodeling in asthma has led to a serious rethinking of how to approach patients and prompted modification of treatment guidelines.[382] In contrast, nothing has been published on the implications of remodeling in CRS. Although CRS was historically considered as an irreversible disease, current treatment regimens, either medical or surgical, depend on a concept of disease reversibility. Recent descriptions of mucosal remodeling in the sinuses however, may imply a similar phenomenon to that seen in asthma -"irreversible mucosal disease". In this paper we review some of the pathophysiological mechanisms, their clinical relevance and pose questions that need further investigation.

8.5 Collagen deposition and fibrosis as an irreversible end-stage of ongoing inflammation

Myofibroblasts are the cells responsible for collagen deposition and extracellular matrix (ECM) molecules in the airways. Fibroblasts and myofibroblasts (activated fibroblast phenotype) have been demonstrated in sinus mucosa in chronic sinus disease.[383] Fibroblast proliferation and myofibroblast differentiation is brought about mainly through the action of TGF-beta.[383,384] It is established that in chronic rhinosinusitis with nasal polyps (CRSwNP), there is an increased number of myofibroblasts and increased deposition of collagen.[88,385] Wang et al. studied the differential distribution of myofibrolasts and TGF-beta concentrations across the structure of nasal polyps and demonstrated that TGF-beta and myofibroblasts are mainly concentrated in the pedicle.[385]

Similarly in CRS without polyps (CRSsNP), the amount of subepithelial collagen was found to be significantly greater when compared with controls.[283,386] The process of collagen deposition was found to be irreversible with topical fluticasone propionate,[88] despite the fact that topical steroids effectively suppress earlier precursor steps, including the profibrotic IL-11, [88] and the TGF-beta-induced fibroblast proliferation and differentiation.[383,387,388]

This research poses some interesting clinical questions. For example, despite the fact that corticosteroids are effective in opposing eosinophilic inflammation[113,389], the 2007 European position paper on CRS and Nasal Polyposis[390] concluded that the results of using topical corticosteroids in CRSsNP are mixed and thus there is no evidence to support the routine use of corticosteroids in CRSsNP. On the other hand, topical steroids were stated to be effective in CRSwNP where they reduce polyp size, decrease nasal obstruction and anosmia.[390] It is also known that collagen deposition is a more prominent feature in CRSsNP than in CRSwNP.[283,391]

This raises the question as to whether the recent research into remodeling creates evidence for a change in the current patterns of anti-inflammatory medication usage in CRS? In asthma it appears that upper airway remodeling is proportional to the grade of the underlying mucosal inflammation. Inflammatory cells such as granulocytes and eosinophils are the main producers of TGF-beta isoforms in the nasal mucosa.[392–396] The number of infiltrating eosinophils in CRS has been shown to correlate with the basement membrane (BM) thickness.[285] Eosinophils also produce the profibrotic cytokines IL-11 and IL-17A. A correlation was found between deposition of collagen type I and expression of IL-11[88] and in another study, IL-17A was found to correlate with the degree of epithelial damage and basement membrane thickness.[397] CRS patients with concomitant asthma and/or aspirin intolerance have the highest grades of eosinophilic inflammation.[97,333,348–350] A markedly thickened BM was also found to be associated with this group .[398] Haruna et al showed that asthmatics had significantly increased TGF-beta and myofibroblasts compared with non-asthma and control groups.[349] Fibroblasts in turn helped recruit more eosinophils by producing eosinophil chemo-attractants such as eotaxin[399,400] and RANTES[401]. In this way, a fibroblast-eosinophil symbiosis helps maintain inflammation and remodeling.

This nexus of inflammation and remodeling raises further questions. Does fibrosis and 'irreversibility' occur earlier in CRS patients with asthma and/or aspirin intolerance? Does this

provide an explanation for the observed worse surgical outcomes[205–210,351,352] frequently reported in this subset of patients?

8.6 The temporal component of remodeling: Does a "window of opportunity" exist?

There seems to be a significant temporal component to the remodeling process. Basement membrane (BM) thickening is a feature of remodeling in CRS, similar to that seen in asthma.[266,398,402] Rehl et al[398] found that the duration of CRS symptoms correlated positively with BM thickness and that patients with a markedly thickened BM had a significantly greater duration of CRS symptoms compared to patients with a thinner BM.[398] Other studies found that collagen deposition and BM thickness were significantly greater in adult patients than in children.[386,403] This may be explained by the longer duration of symptoms in adults, or by the higher number of infiltrating eosinophils in adults.[386,403]

As previously mentioned, once collagen is deposited, it is difficult to reverse with topical steroids.[88] But if topical steroids are started earlier, this may decrease and prevent remodeling through acting on various precursor steps, including fibroblast proliferation and myofibroblast differentiation.[383,387,388] Thus, early medical and surgical intervention may help to render the mucosa amenable to treatment with topical steroids.

This issue raises a question of significant clinical importance: is there a "window of opportunity" in which early and effective treatment will help prevent, or at least delay, through inducing a long period of remission, these irreversible mucosal changes? By early intervention can we alter the process of remodeling and prevent progression to a state that is more resistant to conventional medical and surgical intervention? This question was put forward in asthma management and is a subject of ongoing debate.[404] The hypothesis of a "window of opportunity" in asthma therapy was based on reports that early inhaled steroid therapy in newly-diagnosed patients led to better long-term outcomes in terms of respiratory function and that improvements gained from treatment correlated negatively with the duration of symptoms pretreatment. This research should be repeated in CRS.

8.7 Surgery and irreversible mucosal changes

One of the principles in functional endoscopic sinus surgery (FESS) is the potential for disease reversibility. Stammberger argues that there is a high potential for even the most severe form of disease to normalize with functional surgery.[315] The concept of "remodeling" adds a new dimension to current surgical concepts, and challenges surgeons to define and clinically identify "histologically-irreversible disease".

The definition of what can be considered as "irreversible mucosal changes" after surgery is inconclusive and controversial.[160] Only a few authors[405,406] have demonstrated, using electron microscopy, "irreversible changes" in the mucosa before or after surgery. Fang[323] studied the normalization of maxillary sinus mucosa and mucociliary function after FESS and concluded that a long history of disease (more than seven years history) was an ominous sign for recovery.[323] Richtsmeier[177] reviewed 85 patients with refractory maxillary sinusitis and reported that one of the top ten causes of maxillary sinus surgery failure is persistent mucociliary flow dysfunction after surgery. Richtsmeier suggests that this dysfunctional sinus state is irreversible and is secondary to prolonged inflammation, host factors or iatrogenic injury.[177]

Since remodeling was first described, no studies formally investigated its relevance or impact on outcomes of surgery. However, some authors investigated radical surgical options for dealing with what was considered as "irreversible mucosal disease". Most of these studies either were done before the phenomenon of remodeling was established in the literature or simply do not refer to the phenomenon directly in text. Cutler et al[178] performed the traditional Caldewell Luc (CL) (with a radical removal of the mucosa) in patients who failed, on average, two prior middle meatal antrostomies (MMAs) and thus were considered, by the authors, clinically irreversible. They reported a response rate of 92%.[178] Kikawada et al[377] investigated the use of a high-pressure water jet (HPWJ) in 45 patients with severe maxillary sinus disease that failed maximal medical therapy and persisted for many months after an initial FESS. The HPWJ removed the mucosa but left the periosteum intact. The authors assumed that, if the periosteum could be preserved on the surface of the bone, normal mucosa could regenerate over the periosteum without formation of granulation or scar tissue and without reducing the size of the cavity, unlike the CL procedure. They concluded that this helped "cure" these patients and they reported no reduction of the cavity by scar tissue except in one side in one patient where all mucosa

(including periosteum) was removed.[377] Abd el-Fattah et al[179] compared functional MMAs with an endoscopic radical antrectomy procedure in a prospective study. In the radical antrectomy group, the mucosa in the maxillary sinus was completely removed using straight and curved forceps introduced through either a canine fossa puncture or through the antrostomy, under direct endoscopic visualization. They reported better outcomes from the radical group versus the MMA group.[179]

All the previous studies referred to "irreversible disease" but did not provide histological evidence of remodeling in the mucosa or how the patients enrolled differed in their histological or mucociliary function from other surgical candidates. Although the results reported were positive, there is no evidence that this can be explained by removal of irreversibly diseased mucosa, despite the concept being theoretically sound. Historically, it should be noted that CRS was considered an irreversible disease which supported the rationale of radical mucosal stripping procedures before the advent of FESS.

Other surgeons took a different approach in dealing with the apparently dysfunctional mucosa. Rather than resorting to stripping of the sinus mucosa, this alternative approach assumed that in recalcitrant maxillary sinuses, mucociliary clearance remained impaired even after adequate re-ventilation with a traditional MMA. The hypothesis was that these "dysfunctional" sinuses had become gravity-dependent, which required the creation of a maximally enlarged ostium with an inferior extension.[183] Rodriguez et al described a procedure of "extended maxillary sinusotomy", created by extending the middle meatal sinusotomy inferiorly into the inferior meatus.[407] In a similar technique, Woodwarth et al[183] reviewed the results of a "modified endoscopic medial maxillectomy" (MEMM) for chronic maxillary sinusitis refractory to middle meatal antrostomy. They performed the procedure on nineteen patients who had failed prior surgery, including 14 Caldwell-Luc procedures. They showed that MEMM is both a safe and an effective treatment for chronic maxillary sinusitis refractory to standard medical and endoscopic surgical management.[183] Cho and Hwang[408] reported a postoperative clinical resolution of 100% with a "maxillary mega-antrostomy", a procedure very similar to the above mentioned techniques, except that the extension of the opening is done through the posterior half of the inferior turbinate, instead of anteriorly.[408] Another similar radical ostium-widening procedure for the frontal sinus is the endoscopic modified Lothrop procedure (EMLP). Wormald[185] performed the EMLP as a salvage procedure for a failed frontal sinus in 83 patients. The patients had a mean of

six previous failed operations and had been then considered as clinically irreversible. On an average follow-up of 21.9 months, the cure rate from the EMLP was 75% (only 21 patients reported recurrent symptoms) and the frontal ostium patency rate was 93%.[185]

Based on the previous positive reports, a question can be posed. Can disease that is "irreversible with normal FESS" be effectively reversed only through more extensive or radical procedures? Can a dysfunctional sinus be restored again to function? How do these alternative surgical techniques interact differently with the mucosa than the way normal FESS does? Can a radical procedure bring about a normalization of mucosa that cannot be obtained through traditional functional surgery (FESS)? Are the better results explained by the enhanced ventilation obtained by creating a maximally-widened ostium? Is it due to better post-operative penetration of the sinuses by topical medication? Or is it due to greater intraoperative removal of inflammatory cells with a consequent decreased activation of the fibroblasts through the TGF-beta pathway? These questions are yet to be investigated.

8.8 Summary

Upper airway remodeling has important implications for recalcitrant chronic rhinosinusitis. The literature provides little information on its definition and clinical implications. Studies reporting dysfunctional or irreversible mucosa have to date only provided clinical selection criteria such as "failed prior surgeries" or "refractory to standard surgery" and have not offered formal histological evidence of remodeling, collagen deposition or any other objective histological changes. Recent histological descriptions of remodeling may in the future provide clearer more precise criteria for this entity. This would enable more focused research, with potential implications for the medical and surgical management of CRS.

Several questions (Figure 8-1) are raised in this review:

(1) How will remodeling affect the use of steroids in CRS? Do topical steroids have a role in the prevention or delay of an "irreversible end-stage" collagen deposition in both CRSsNP and CRSwNP?

(2) Does failure to treat early lead to later deterioration? Is there an optimal "window of opportunity", in which early diagnosis and intervention with anti-inflammatory medication or surgery will help delay mucosal remodeling and lead to better long-term outcomes?

(3) How can we properly define the elusive terms "irreversible disease" or "dysfunctional

sinus"? How can we diagnose patients reaching this irreversible stage, using clear and standardized clinical and/or histologic criteria?

(4) Will patients with "irreversible disease" benefit from alternative or more radical surgical options? How can we explain some of the positive results reported from radical options such as a canine fossa trephination, Caldwell-Luc or a mega-antrostomy for a dysfunctional maxillary sinus, or a modified Lothrop procedure for a dysfunctional frontal sinus?

Figure 8-1 Flow Diagram: Inflammation and remodeling as a basis of potential irreversible disease.
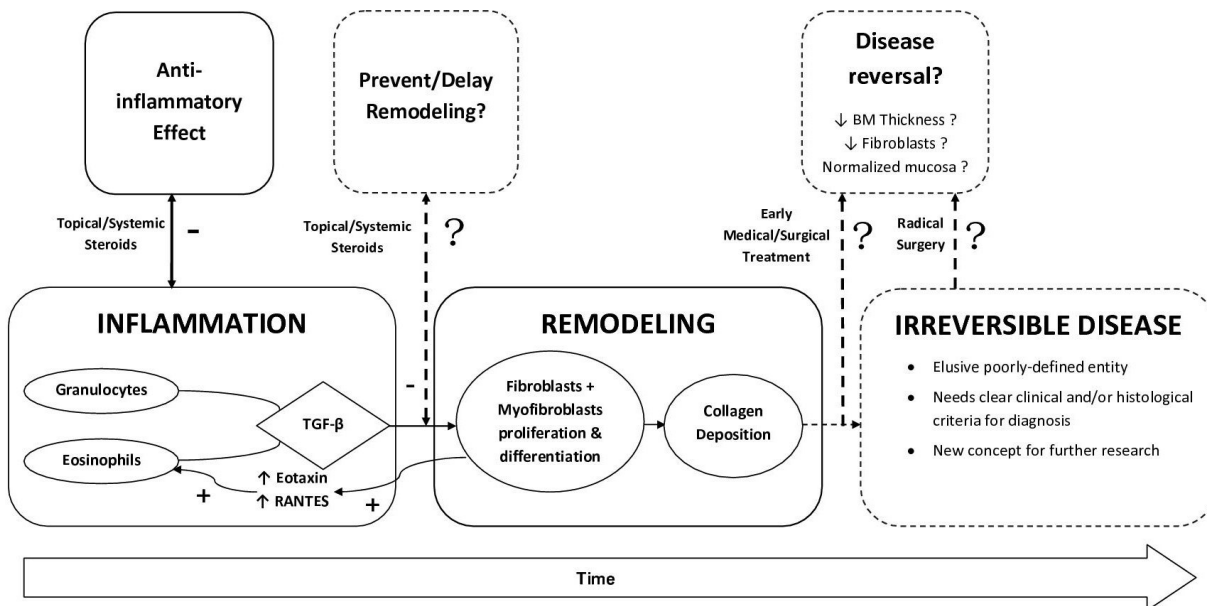
Although lower airway remodeling in asthma was extensively studied and later led to therapeutic implications, upper airway remodeling has not yet been translated into definite clinical recommendations. In fact, there is no proof to date that remodeling holds any significance in altering how we approach the management of patients with CRS. However, since current medical and surgical management is based on concepts of disease reversibility, the simple notion of the presence of "irreversible changes" warrants further research. The dilemma now for the

106

treating rhinologist is to identify patients with "irreversible" CRS. Ideally this would be a clinical diagnosis supported by defined histopathological changes in the sinus mucosa. This would allow treating surgeons to tailor the type and extent of surgery for their patients, with patients in the irreversible group perhaps undergoing more radical surgical procedures. The presence of an objective clear-cut definition of the elusive term "irreversible disease" may provide evidence in the future to modify current medical and surgical practice, especially in patients resistant to standard treatments.

Chapter 9 Role of surgery in nasal polyp recurrence

9.1 Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Ahmed Bassiouni		
Contribution to the Paper	Project design, data collection, data analysis, manuscript preparation		
Overall percentage (%)	80%		
Signature		Date	18/06/2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Peter-John Wormald		
Contribution to the Paper	Project supervision, manuscript editing		
Signature		Date	18/06/2015

9.2 Citation

Bassiouni A, Wormald P-J. Role of frontal sinus surgery in nasal polyp recurrence. Laryngoscope. 2013 Jan;123(1):36–41.

9.3 Abstract

Background: Patients with aggressive nasal polyp recurrence form an important subgroup of patients with refractory sinus disease.

Objectives: To establish patterns of polyp recurrence and evaluate the effect of frontal sinus surgery (Draf 2b vs Draf 3) on polyp recurrence.

Study design: retrospective cohort study reviewing 338 consecutive operations

Methods: Polyp recurrence was defined according to the Lund-Kennedy mucosal edema score. Survival analysis methods were used for statistics.

Results: After complete spheno-ethmoidectomies, Draf 2b frontal sinusotomies and middle meatal antrostomies, persistent polyp recurrence occurred in 19.8% after 6 months and increased to 22.7% of patients after 12 months. Polyps first recurred in the area of the frontal sinus/ostium (55%), followed by the ethmoids (38%). Asthma and aspirin sensitivity were the most important variables affecting recurrence (hazard ratios 1.71, 1.79 respectively, $p < 0.05$) The Draf 3 was a significant factor in reducing recurrence (especially in asthma and aspirin intolerant patients). Overall revision rate was 18% (follow-up duration > 12 months, median $= 29$ months), with 37% revision rate in the FESS group versus 7% in the Draf 3 group ($p < 0.001$). Survival analysis showed that the Draf 3 significantly reduced the risk of revision (Hazard ratio $= 0.258$, $p = 0.0026$).

Conclusion: Nasal Polyposis is characterized by a high rate of recurrence. The presence of asthma or aspirin intolerance leads to more aggressive recurrence, and in these patients, the Draf 3 drillout procedure becomes a good option for improved long-term outcomes and reducing the need for revision surgery.

Level of evidence: 2b (retrospective cohort)

Keywords: Chronic rhinosinusitis, Nasal polyposis, Nasal polyp recurrence, Endoscopic sinus surgery, Revision sinus surgery, Radical sinus surgery, Frontal sinus surgery, Frontal sinusotomy, Draf 3 procedure, Modified Lothrop procedure, Frontal drillout procedure

9.4 Introduction

One of the most important causes of surgical failure in chronic rhinosinusitis (CRS) with nasal polyposis (CRSwNP) patients is polyp recurrence. This may result in multiple surgeries and the perception that nasal polyps are difficult to cure. The rate of post-operative polyp recurrence has been reported to be as high as 60%.[171,214] Therefore, patients with diffuse polyposis and aggressive recurrence constitute an important subgroup of patients with refractory chronic rhinosinusitis (rCRS) who gain limited long-term benefit from standard surgical intervention.

Although CRSwNP has undergone extensive research, there are few studies examining post-operative polyp recurrence and the factors affecting it,[171,217,218,220,409] and many questions remain unclear or unanswered. In patients who have undergone surgery, the initial sites of polyp recurrence and the average time till recurrence are not reported. There is some contradictory evidence[409] for higher recurrence rates reported in asthmatic patients[217,218]. To date the role of bacteria and/or fungus is still to be clearly elucidated even though conditions with high recurrence rates such as allergic fungal sinusitis,[211,212] patients with S. aureus infections and high levels of staphylococcal superantigens[410] are associated with nasal polypoid disease. There are reports that more radical surgical approaches to polypoid disease lead to less polyp recurrence and better outcomes.[174,175,180,193,214] Although the Draf 3 procedure for the frontal sinus (also known as Endoscopic Modified Lothrop procedure) have achieved success in refractory patients resistant to standard ESS,[185,186,411,412] objective data on its effectiveness in controlling polyposis recurrence and its long-term outcomes are still lacking.

In this study, we retrospectively review CRSwNP cases to determine the patterns and the factors affecting polyp recurrence and their eventual prognosis. We also investigate whether the surgical approach to the frontal sinus plays a role in surgical outcome.

9.5 Methods

9.5.1 Study design

Retrospective cohort with a consecutive recruitment pattern

9.5.2 Study population

We included all patients consecutively attending to the tertiary rhinology practice of the senior author (PJW) who were diagnosed with CRSwNP and received endoscopic sinus surgery (ESS) during the period from 2003 to 2010. The diagnosis of nasal polyposis was made according the definition of CRSwNP for research purposes, in the European Position Paper on Rhinosinusitis and nasal polyps.[390] This clinical diagnosis requires the positive identification of polyps in or beyond the middle meatus upon anterior rhinoscopy.[390] All patients went through a standard trial of medical therapy including a 3 week course of systemic prednisolone, 2 month course of topical nasal steroid and saline washes and, if pus was present, culture-directed antibiotics. If medical treatment failed, patients underwent surgery.

9.5.3 Surgery

All operations were performed by a single surgeon (PJW). All patients had a standard complete spheno-ethmoidectomy, and frontal recess clearance with a Draf-2a frontal sinusotomy (so-called full-house ESS). Throughout this article, this group of patients will be referred to as having received a standard ESS. Patients who had undergone previous complete spheno-ethmoidectomies (either in another institution or by the senior author) and had medically resistant recurrence of polyps in the frontal sinus and frontal recess underwent a Draf 3/Modified Lothrop/ frontal drillout procedure in addition to a revision spheno-ethmoidectomy and clearance of the maxillary sinuses. The technique for the Draf 3 frontal drillout procedure was described in detail in previous publications.[185] Some patients (in both groups) also had canine fossa trephinations[366] for severe maxillary sinus disease. The middle turbinate was always partially removed in the Draf 3 procedure however it was never removed in the standard ESS group.

9.5.4 Post-operative care and management

Patients were managed post-operatively with a 3-week decreasing dose of systemic prednisolone, antibiotics and saline douches.

9.5.5 Recording recurrence and post-operative follow-up

During follow-up, the date of the first polyp recurrence was documented on endoscopic examination. Polyp recurrence was defined as the first documented postoperative re-appearance of a polyp-like structure (i.e. a Lund-Kennedy endoscopic score of three), regardless of size or

number. This excludes the common description "polypoid" mucosa or "cobblestoned" mucosa (a score of two on the Lund-Kennedy endoscopic grading). Sites of first appearance of polyps were recorded. Knowing that many early polyp recurrences resolve on regular medical treatment during follow-up we recorded the incidence of 'persisting polyp recurrence'. This we defined as polyps persisting (or increasing in size or number) despite ongoing topical medical treatment for three or more months. Revision surgery was offered to patients who had polyp recurrence with persistent symptoms greater than six months duration after failure of maximal medical therapy.

9.5.6 Statistical analysis

All statistical analyses were done using the R statistical software (R Foundation for Statistical Computing, Vienna, Austria). Survival analysis using the Cox model of proportional hazards (univariate and multivariate analysis) was used to analyze the relative effect of the different covariates on the duration of the post-operative polyp-free period and thus identify the most important variables. The variables studied included: (1) sex (2) age (3) smoking (4) asthma (5) aspirin sensitivity (6) primary versus revision surgery (7) number of previous operations (8) gastro-eosophageal reflux disease (9) CT scores (10) fungal detection (11) bacterial and staphylococcus aureus cultures (12) total IgE. Finally, we looked at the effect of the type of surgery (standard ESS versus Draf 3). Robust variance equivalents were calculated for final models to exclude any effect related to dependence of observations. Kaplan Meier survival curves were used for illustration. Statistical significance was taken at the 0.05 level.

9.6 Results

9.6.1 Study cohort characteristics

The study surveys 338 consecutive operations done for 299 CRSwNP patients. No patients were excluded from the consecutive sampling. The summary of operation characteristics are shown in 1. In the same period there were 419 patients who had CRSsNP who underwent surgery. These are not included in this study. The disease severity as measured by the average Lund and Mackay scores (LMS) in patients undergoing standard ESS with Draf 2a frontal sinusotomies and was 16.62 (SD=4.5). This is similar to the average disease severity LMS of 16.96 (SD=4.4) in the group receiving the Draf 3 procedure. The canine fossa trephine was used in 95/199 (47.7%) of the standard ESS group and in 69/139 (49.6%) of the Draf 3 group.

Table 9-1 Characteristics of 338 operations

Total Number of operations	338 (representing 299 unique patients)		
Follow-up duration	Mean 20.5 months (zero follow-up in 58 patients)	Max: up to 86 months	
Revision status	Primary: 78	Revision: 260	
Number of previous operations	Mean: 2.338	Max: up to 25	
Asthma status	Positive 200	Negative 137	Unknown 1
Aspirin Sensitivity status	Positive 62	Negative 275	Unknown 1
Gastro-eosophageal Reflux Disase (GERD) status	Positive: 29	Negative 308	Unknown 1
Smoking status	Non-smoker 317	Smoker 20	Unknown 1
Lund-Mackay Scores	Mean: 16.79 (CT scores missing for 109 operations)	Range: 4 to 24	
Type of Surgery performed	Standard ESS: 199	Draf-3 Frontal Drillout: 139	

9.6.2 Incidence/frequency of recurrence

We defined the difference between a polyp that occurred and then resolved on medical treatment and a polyp that persisted despite medical treatment. The incidence of a polyp recurring in the total cohort of all patients who were followed up for greater than 6 months was 88/222 (39.65%). If the follow up was extended to 12 months or more (i.e. a smaller n), the polyp recurrence rate increased to 82/185 (44.3%). In the total cohort, the incidence of polyps that persisted despite medical treatment for at least 3 months or more was significantly less with 44/222 (19.8%) in those followed up 6 months or longer and 42/185 (22.7%) for those followed up 12 months or longer.

9.6.3 Sites of recurrence

On the first documentation of a polyp recurrence, the site of recurrence was recorded. (Table 9-2) Three cases were excluded from Table 9-2 due to non-recorded site of recurrence.

Table 9-2 Analysing 127 known sites of recurrences

Site of recurrence	Frequency (Percentage)
Frontal sinus area (including around the frontal ostium, frontal recess or inside the frontal sinus itself)	70 (55.1%)
Ethmoid cavities, including the spheno-ethmoidal recess	48 (37.8%)
Maxillary sinus and ostium	4 (3.1%)
Olfactory recess	3 (2.4%)
Middle meatus	2 (1.6%)
Total	127

9.6.4 Timing of recurrence

Figure 9-1 is a histogram which illustrates the timing of the initial polyp recurrence during the first four years after surgery.

Figure 9-1 Histogram illustrating the frequencies of timings of the first occurrence of a polyp recurrence throughout the first 4 years after surgery.

The chart shows recurrence frequencies plotted against post-operative months, with annotations: "95% of recurrences occur in the first three post-op years" and "79% of recurrences occur in the first two post-op years".
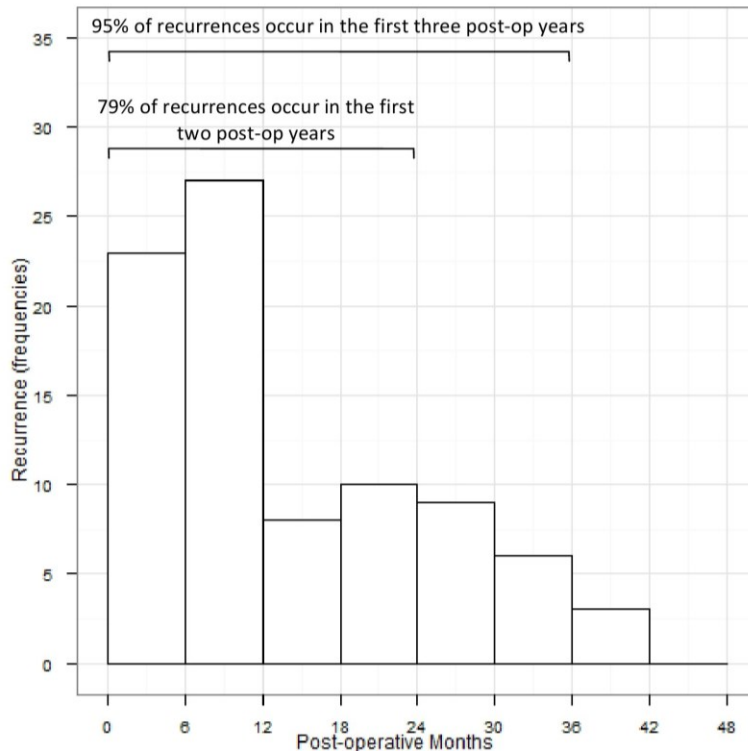
9.6.5 Significant factors in determining polyp recurrence

Using survival analysis, we studied the effect of the 12 different covariates (listed above in the methods section) on polyp recurrence in a Cox model of proportionate hazards. Survival times were considered as the duration beginning from the date of the operation, until the date of the first documented polyp recurrence seen post-operatively. At first, each variable was inserted singly into a Cox model to examine the univariate impact of each variable, followed by various multivariate analyses to identify the most important variables. The variables that had statistically significant impact on post-operative polyp recurrence with simultaneous sufficiently-high hazard ratios were asthma and aspirin intolerance. They were then inserted in a common multivariate model (Table 9-3) and their Kaplan Meier survival curves plotted. (Figure 9-2) Robust estimates of the standard error were calculated to exclude any bias that might have been caused by some patients having two surgeries (and thus appearing twice) in the cohort.

Figure 9-2 Kaplan-Meier survival curves (baseline, asthma, aspirin-sensitive asthma).
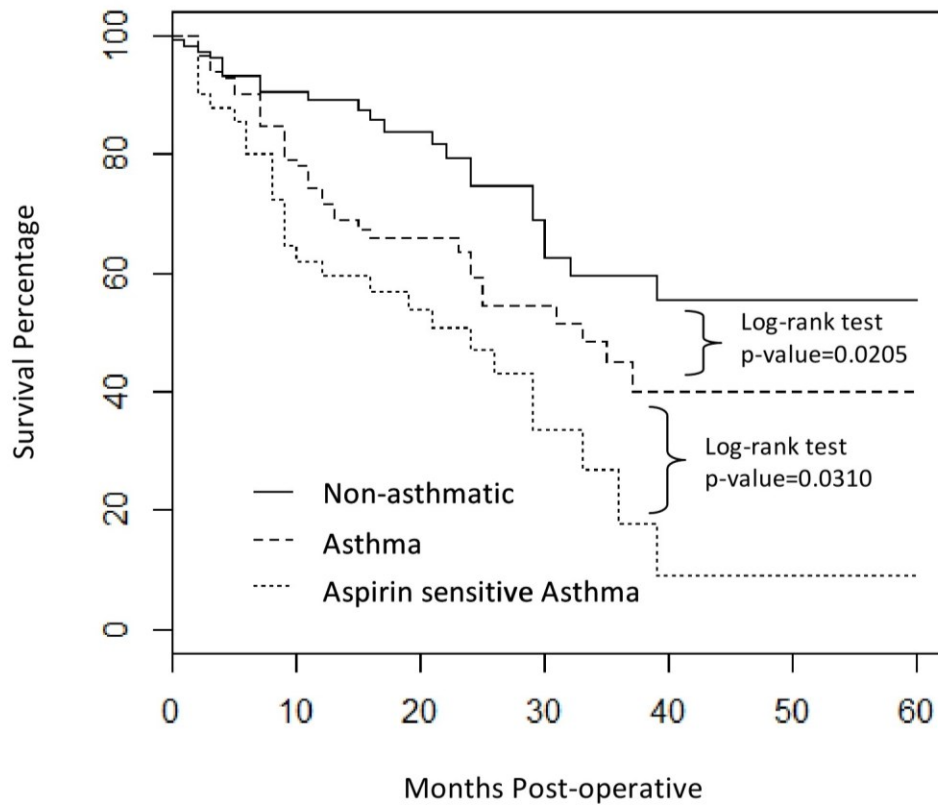
Table 9-3 Cox Model incorporating asthma, aspirin sensitivity as predictors of polyp recurrence.

	Hazard Ratio [95% CI]	p-value	Robust Standard Error	Robust p-value
Asthma	1.71 [1.02-2.86]	0.039	0.262	0.041
Aspirin sensitivity	1.79 [1.10-2.93]	0.019	0.251	0.020
Likelihood ratio test = 15.5 on 3 degrees of freedom, p=0.000429, n= 337				

Table 9-4 Polyp recurrence rate in standard ESS versus Draf-3 groups (follow-up duration 12 months or above). Fisher's exact test.

	Polyp recurrence rate			Persistent polyp (> 3 months) recurrence rate		
	ESS	Draf 3	p-value	ESS	Draf 3	p-value
All patients	49% (56/113)	36% (26/72)	0.095	26% (30/113)	16% (12/72)	0.150
Asthmatic patients	59% (40/67)	41% (20/48)	0.061	40% (27/67)	16% (8/48)	0.007*
Aspirin-sensitive patients	72% (13/18)	52% (9/17)	0.305	55% (10/18)	11% (2/17)	0.011*

*: statistically significant result at the 0.05 level.

9.6.6 The role of frontal sinus surgery on polyp recurrence

Patients in the standard ESS group (n = 199) had a recurrence rate of 59/139 (42%) when followed up for longer than 6 months. This increased to 56/113 (49%) when this cohort was followed up for longer than 12 months. Patients who had a complete spheno-ethmoidectomy, maxillary clearance and a Draf 3 opening of their frontal sinuses (n = 139) had a recurrence rate of 29/83 (35%) when followed for more than 6 months. This remained stable at 26/72 (36%) for those followed up for longer than 12 months. The rates of a persisting recurrence were less and are shown in Table 9-4. The proportion of recurrence in both the Draf 3 and the standard ESS groups were compared using Fisher's exact test. (Table 9-4)

We also tested the effect of Draf 3 on recurrence by inserting it into the Cox model containing the variables Asthma and Aspirin intolerance. The resulting model was statistically significant (p=0.000248), with patients who underwent a Draf 3 having a reduced hazard ratio of 0.65 (p = 0.059, robust p = 0.060, Table 9-5).

Table 9-5 The common Cox Model incorporating asthma, aspirin sensitivity and the frontal drillout state as predictors of polyp recurrence.

	Hazard Ratio [95% CI]	p-value	Robust Standard Error	Robust p-value
Asthma	1.77 [1.06-2.96]	0.028	0.259	0.030
Aspirin sensitivity	1.87 [1.13-3.09]	0.013	0.251	0.015
Draf-3 frontal drillout	0.65 [0.41-1.02]	0.059	0.228	0.060
Likelihood ratio test = 19.2 on 3 degrees of freedom, p=0.000245, n= 337				

9.6.7 Long-term fate (need for revision surgery)

The rates of undergoing revision surgery (follow-up duration 6 months or above) were as follows: 40/222 (18%) in all patients; 26/88 (29%) in patients who manifested post-operative polyp recurrence; 25/44 (57%) in patients who manifested persisting post-operative polyp recurrence.

Figure 9-3 Effect of the Draf 3 procedure on postoperative survival. Kaplan-Meier curves illustrating the effect of the Draf 3 procedure in reducing the rate of revision surgery. ESS = endoscopic sinus surgery.
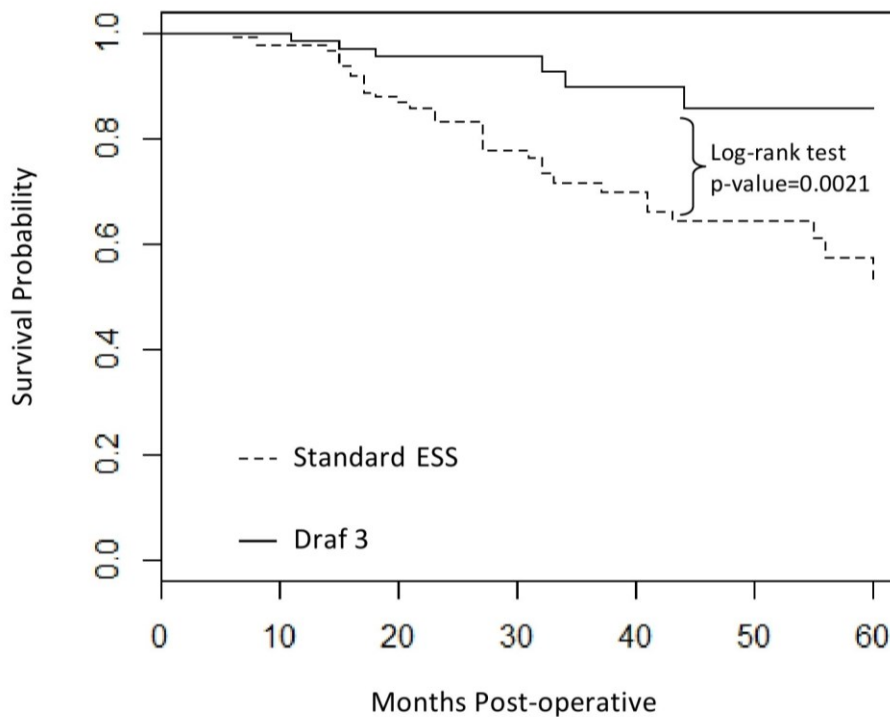
In order to test the effect of the Draf 3 procedure on long-term prognosis considering that all the other sinuses were treated exactly the same way in both groups, we compared the proportion of patients who required a revision surgery (persistent polyp recurrence plus symptoms for> 6 months) during their follow-up in both the Draf 3 and the non-Draf 3 groups using Pearson's chi square test. The Draf 3 group had statistically significant lower revision rates than the non-Draf 3 group (7% versus 32% in patients with follow-up > 6 months, median follow-up = 23.5 months, chi-square p-value = 0.001; and 7% versus 37% in patients with follow-up > 12 months, median follow-up = 29 months, chi-square p-value < 0.001). This was also confirmed by a significant

Log-rank test (p= 0.00206) comparing the survival curves between Draf 3 and non-Draf 3 groups, when revision surgery was taken as the event of survival. (Figure 9-3) Survival in Figure 9-3 was defined as the duration between initial surgery and revision surgery, with patients who have not undergone revision considered as right-censored observations. The difference between the two curves (Figure 9-3) remained statistically significant on multivariate Cox modeling (after adjusting for asthma and aspirin intolerance, Draf 3 hazard ratio = 0.258 [CI 0.1071 to 0.6236], p = 0.0026, median follow-up time = 14 months).

9.7 Discussion

Polyp recurrence rate in this study (in patients with follow-up longer than 6 months, median = 23.5 months) was 40% but only half of this group (20%) had a persisting recurrence lasting longer than 3 months. This recurrence rate is similar to the high rates (up to 60%) reported elsewhere in the literature.[171,214] However, it is important to differentiate between those who exhibit recurrent polyps which resolve on medical treatment and medically-resistant recurrent polyps, since the latter group has a higher risk of ultimately requiring further intervention. The most common site of recurrence of nasal polyps was the frontal sinus region (around the frontal ostium and frontal recess, followed by frontal sinus cavity). It is unclear from our data and from the literature if this is due to the narrowness of the frontal ostium with mucosal contact, residual disease left within the frontal sinuses or as a result of poor ventilation of the frontal sinuses.

In the literature there have been a number of studies that have compared traditional ESS with the more aggressive opening and removal of tissue in the sinuses. [174,175,214] Jankowski et al. previously reported that recurrence rate with the nasalization procedure was 22.7%, compared to 58.3% with a functional ethmoidectomy.[214] Masterson et al. compared radical ethmoidectomy with nasal polyp clearance to the results of anterior ethmoidectomy in the UK national audit and found that the radical approach significantly decreased the need for revision surgery from 12.3% to 4.0% at 36 months.[174] Friedman and Katsantonis[175] compared functional ethmoidectomy and antrostomy to a revision procedure that involved a wide anstrostomy plus a complete removal of all polyps and hyperplastic changes that remained in the maxillary sinuses despite a patent anstrostomy decreasing recurrence from 19.2% to 5%.[175] Our results show that the Draf 3 frontal drillout, offered as a more extensive surgical approach to refractory polyposis, may provide better recurrence control, further confirming the results reported by these papers.

The reason for this reduction in polyp recurrence remains unclear. We have previously hypothesized[301] that a greater reduction in the mucosal inflammatory load leads to better long-term outcomes and this explains the good outcomes reported from the more extensive procedures (such as the Draf 3 or the nasalization[214] procedure) and may in part be due to removal of the diseased inferior half of the middle turbinate in the Draf 3 procedure. The removal of the lower half of the turbinate may decrease the disease load as well as improve the ventilation in the posterior ethmoid and sphenoid complex. Tosun et al reported that polyp recurrence correlated with the eosinophilic content in the polyps.[220] More aggressive surgery should thus aim to clear all polyps in all of the sinuses, in order to reduce the eosinophilic inflammatory load.[301] This requires a meticulous removal of all polyps to their bases, since the highest concentration of eosinophils is present in the pedicle.[367] This becomes critical in the asthma and aspirin intolerant subgroups, since they exhibit higher degrees of mucosal eosinophilia.[333,348–350] The larger frontal sinus opening created during a Draf 3 procedure increases the ability of the surgeon to decrease the disease load by providing better access to the frontal sinuses and may thus result in lower polyp recurrence. The reduction may also be explained by increased ventilation of the frontal sinuses or simply be that it provides much improved post-operative penetration of topical medications to the frontal sinus ostium and ethmoid areas, which are the sites of highest recurrence. (Table 9-2)

It is important to note that the Draf 3 does not completely prevent polyp recurrence (Table 9-4) but is a significant factor in reducing persistence of polyps, indicating that the procedure allows better control of the disease. (Table 9-4) This beneficial effect of the Draf 3 in reducing polyp recurrence and persistence appears to become more evident in the asthma and aspirin sensitive subgroups. (Table 9-4) The most significant benefit however, is the long-term effect in reducing the need for revision surgery, (Figure 9-3) which has considerable implications on the patients' quality of life. It also has positive economic implications by decreasing the rate of hospitalization and re-operation. In our study, the risk of a Draf 3 operated patient requiring a revision at any time after the operation was approximately one quarter that of a standard ESS-operated patient. (Cox model hazard ratio = 0.258, median follow-up = 14 months). In patients followed up longer than 12 months (median = 29 months), the revision rate for the ESS group was 37%, compared to only 7% in the Draf 3 group. Reduced revision surgery may have an

element of bias as the surgeon who performs the primary surgery may be less inclined to revise the procedure.

One of the difficulties encountered when comparing these results with previous reports is the absence of a definition of 'polyp recurrence'. Two studies did not comment on how the diagnosis was formulated,[171,220] while others provided vague definitions. Mendelsohn et al.[218] defined polyp recurrence as a "first notation of recurrent NP after surgery", which suggests a re-diagnosis of NP according to the formal definition, thus implying only a recurrence of polyps within or beyond the middle meatus which might be difficult to assess accurately post-operatively when there is a loss of surgical landmarks. Garell et al.[409] suggested that diagnosis was based on endoscopic examination but did not give a formal definition of a recurrence although he placed recurrences into either a 'minor recurrence' or a 'major recurrence' group. In this study, we defined polyp recurrence as the first recorded appearance of a polyp structure (regardless size or number) during post-operative follow-up anterior endoscopy. This is equivalent to a grade 3 on the Lund-Kennedy mucosal scoring system (Figure 9-4; with 0 being normal mucosa; 1, edematous mucosa; 2, polypoid change with no frank polyps; 3, frank polyp). However, it is not the presence of a polyp that is clinically important rather the persistence of polyps and for such a persistent recurrence to ultimately require revision surgery.

Figure 9-4 The Lund-Kennedy mucosal edema score. In our study, a polyp recurrence was defined as a recurrence of a frank polyp (i.e., Lund-Kennedy score of 3) excluding polypoid/cobblestoned mucosa.
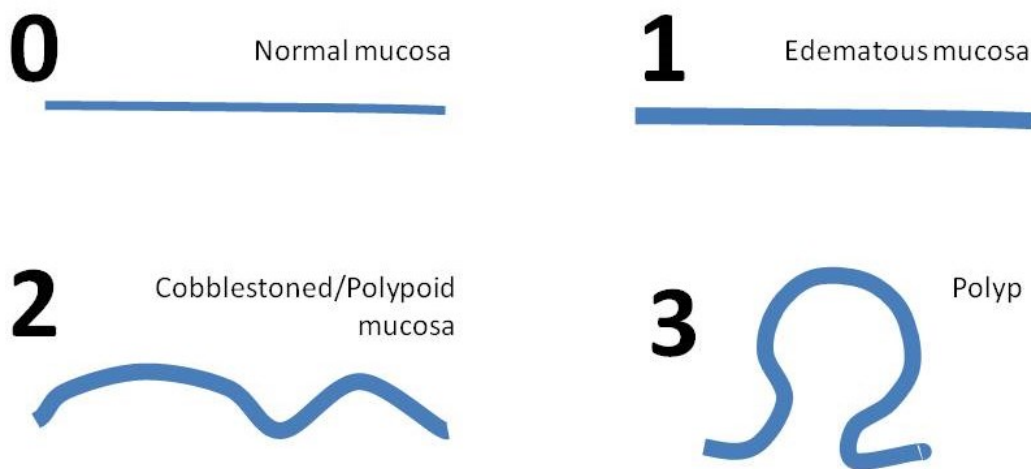
To overcome the problem of the wide varying times of follow-up periods and patients dropping out of follow-up, which complicates the added variable of the timing of polyp recurrence we employed survival analysis as a statistical method to investigate the impact of different covariates on recurrence as a single-event outcome. This method has been used by Albu et al[217] and Mendelsohn et al[218] to assess polyp recurrence. We found that the comorbidity with asthma and aspirin intolerance were the two most important clinical variables affecting polyp recurrence. (Figure 9-2) Table 9-3 shows that an aspirin-tolerant asthmatic patient has a 1.7 times higher risk of developing recurrence at any time after the operation than a normal patient, while an aspirin-intolerant asthmatic has a recurrence risk equaling 3.1 times that of a normal patient. (Table 9-3) These findings are similar to previously published papers.[217,218] Wynn and Har-el studied polyp recurrence in 118 patients and found that asthma was a significant factor in determining recurrence.[171] They also reported that the number of previous operations was an important variable,[171] however this was not corroborated in our cohort of patients. (Table 9-3)

To study the influence of fungal allergy, presence of fungus and staphylococcal superantigens in nasal polypoid disease, we assessed the value of clinical investigations such as intra-operative bacterial/staphylococcal cultures (endoscopically-guided swabs intentionally directed towards thick mucus or pus) in predicting recurrence. The multivariate analysis failed to show any significant effect on polyp recurrence. However, bacterial/fungal detection on microscopy and culture does not reflect a direct measure of the interaction of Staphylococcus aureus and fungus with the nasal mucosa, and therefore, these results need to be interpreted in this light. In addition total serum IgE was also not a significant predictor of recurrence.

9.8 Conclusion

Nasal Polyps are characterized by a high rate of recurrence. The presence of asthma or aspirin intolerance leads to more aggressive recurrence, and in these patients, the Draf 3 frontal drillout procedure, offered as a more aggressive surgical approach, may be a good option for improved long-term outcomes and reducing the need for revision surgery.

9.9 Acknowledgements

Chapter 10   Subepithelial inflammatory load and basement membrane thickening in refractory CRSwNP

10.1   Statement of Authorship

Title of Paper	Subepithelial inflammatory load and basement membrane thickening in refractory CRSwNP: a histopathological study
Publication Status	☐ Published ☑ Accepted for Publication ☐ Submitted for Publication ☐ Publication Style
Publication Details	This paper has been submitted to the peer-reviewed journal International Forum of Allergy and Rhinology in April 2015, and has been accepted for publication on 08/09/2015.

Principal Author

Name of Principal Author (Candidate)	Ahmed Bassiouni		
Contribution to the Paper	Project design, slide preparation and scanning, data collection, data analysis, manuscript preparation		
Overall percentage (%)	60%		
Signature		Date	18/06/2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

  i.   the candidate's stated contribution to the publication is accurate (as detailed above);

  ii.   permission is granted for the candidate in include the publication in the thesis; and

  iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Project supervision, manuscript editing		
Signature		Date	18/06/2015

10.2   Citation

Bassiouni A, Ou J, Rajiv R, Cantero D, Vreugde S, Wormald P-J. Subepithelial Inflammatory Load and Basement Membrane Thickening in Refractory Chronic Rhinosinusitis with Nasal Polyposis: A Histopathological Study. Int Forum Allergy Rhinol. Forthcoming 2016.

10.3   Abstract

Background:

A subgroup of chronic rhinosinusitis patients with nasal polyps (CRSwNP) patients is refractory to optimal surgical therapy and requires multiple revision sinus operations. Studies have shown that mucosal eosinophilia correlates with disease severity. We hypothesized that a high-grade tissue inflammatory load is associated with these refractory patients.

Methods:

A single-surgeon, retrospective case-control study comparing 20 CRSwNP patients requiring a second surgery during follow-up (refractory group) versus a matched cohort of 20 CRSwNP patients without needing further revision surgery (control group). H&E stained tissue harvested intra-operatively (×2 for the refractory group) were recalled for histopathological examination of subepithelial inflammation and basement membrane thickness.

Results:

The refractory group had a significantly higher average eosinophil count (49 versus 18), relative eosinophilia (55% versus 32%) as well as total inflammatory cell count (86 versus 49) than the control group ($p < 0.05$). However within the refractory group, the eosinophil-lymphocyte ratio was reduced from their first to their second (revision) surgeries while the total averaged

inflammatory cell count remained unchanged. No significant difference in BM thickness was found between the groups.

Conclusion:

These findings suggest that a higher inflammatory and eosinophilic load is associated with refractory disease and thus may be useful in predicting need for future revision surgery in CRSwNP.

Level of evidence: 4

Keywords: chronic rhinosinusitis, chronic rhinosinusitis with nasal polyposis, nasal polyposis, endoscopic sinus surgery, inflammatory load, mucosal eosinophilia, basement membrane thickness

10.4   Introduction

Endoscopic sinus surgery is currently considered the gold standard for chronic rhinosinusitis (CRS) that has failed medical treatment. Unfortunately there is still a group of patients who suffer repeated surgical failures with frequent re-operations and hospitalizations reducing quality of life and contributing to the economic burden of the healthcare system. These patients, who are mostly from the CRS with polyps subgroup, suffer from refractory chronic rhinosinusitis (rCRS).[301]

Traditionally, there have been a number of clinical and disease characteristics that have been deemed as predictors of a worse clinical picture and an overall negative prognosis. Comorbid asthma or aspirin intolerance are the traditional clinical variables that have been associated with a worse prognosis, since they are usually associated with more aggressive polyp recurrence.[171,217,218,302] They are also associated with a higher grade of inflammation,[331,333,348–350] serum and tissue (local) eosinophilia and have a higher disease load as measured by CT and endoscopy scores.[332,335,336,338–340]

Local tissue Eosinophilia has been previously associated with surgical adverse outcomes such as persistent post-operative symptoms[204,232,345,413] and increased polyp recurrence,[220,221] but to our knowledge, it has not been directly linked to the need for revision surgeries (i.e. surgical failure). We have previously hypothesized[301] that the inflammatory load (the amount of disease present in

all the sinuses) could be a predictor of long-term surgical prognosis and have raised the question[305] whether remodeling in the sinuses could play a role in rCRS through mucosal dysfunction[177,374]. However this remains a hypothesis with no scientific proof.[284] It is also interesting that, to date, inflammation in the mucosal tissue of CRS patients has rarely been examined at different time points. This may show the evolutionary patterns of inflammation and remodeling that occurs with the passage of time in this difficult-to-treat rCRS group.

To address these questions, this study aims to characterize the subepithelial inflammatory load and basement membrane thickening at the time of the initial surgery and at the revision surgery in a cohort of refractory CRSwNP patients, in order to investigate potential roles played by inflammation and remodeling in surgical failure.

10.5 Methods

10.5.1 Ethics Approval

This study has been approved by the Queen Elizabeth Hospital Human Research Ethics Committee under Application Number 2011072.

10.5.2 Study Design

This study is a retrospective review of histopathological mucosa and polyp specimens harvested prospectively from CRSwNP patients during endoscopic sinus surgery and sent for histopathological examination. The study could be divided into two main substudies.

Comparison of the matched rCRS and control groups: to test our previous hypothesis that a higher inflammatory load (and in particular eosinophilic load) would be associated with surgical failure and refractory sinusitis. This substudy is a retrospective matched case-control study design.

In the rCRS group: a study of the change in inflammatory load and subepithelial basement membrane thickness with time, through comparing tissue collected during the first surgery to those collected during the later revision surgery for the same patient, thus utilizing a paired (repeated-measures) design

10.5.3   Study Cohort inclusion criteria

Records of CRSwNP patients who attended to the tertiary rhinological practice of the senior author (P-J.W.) and who had undergone endoscopic sinus surgery for their nasal polyposis with the senior author during the period of 2004-2012 were reviewed. Diagnosis of CRSwNP was done according to the EPOS criteria.[1]

Patients with negative prognosis (i.e. those who eventually had disease persistence or recurrence during post-operative follow-up, and consequently attended for subsequent revision with the same surgeon, P-J.W.) were then selected. Revision surgery was offered to patients when they had persistent CRS symptoms with radiographic evidence of disease after failure of at least 6 months of maximal medical therapy. The routine maximal medical therapy prescribed for all CRSwNP patients consists of daily regular nasal saline washes, daily regular topical steroid nasal spray, and includes a 3 week tapering course of oral steroid (25mg of prednisolone for 7 days; then 12.5mg for 7 days; then 12.5mg on alternating days for 7 days).

Only patients who had tissue biopsy harvested during the initial and later revision surgeries that underwent histopathological investigation and had tissue blocks available in the archive were included. This resulted in a cohort of 34 patients, with 14 patients excluded due to missing tissue blocks, making a final study cohort of 20 patients, termed the "refractory group".

A matched cohort, consisting of 20 patients sampled from consecutive patients attending for surgery between 2008 and 2013. This cohort was matched on variables that could confound the grade of inflammation and clinical picture. The first group of variables matched were asthma, aspirin intolerance, and average number of previous operations (including an equal number of primary surgery cases in both refractory and control groups). After this step, matching was performed on the duration of follow-up, such that patients with follow-up less than 12 months were not included. Patients were included only when they had histology blocks available in the archive. We then assured through statistical testing that there is no significant difference in radiological disease severity score (Lund-Mackay CT scores) between the refractory group and this matched control group. This group will be termed the "control group".

10.5.4   Histology

For the study cohort, thin slides were ordered to be cut from all nasal tissue blocks available in the archive. Blocks containing only mucus with no tissue fragments were not used. When a single patient had more than one tissue block present in the archive, all of these were ordered and included in the study. Slides were then stained with routine Haematoxylin and Eosin (H&E) staining. All slides were then scanned using digital Whole-Slide Imaging technology (WSI), on the NanoZoomer Digital Pathology System (Hamamatsu Photonics, Hamamatsu City, Japan) under high resolution ($40\times$ objective magnification power). WSI allows us to survey the whole surface of the slide as the whole slide is saved in the form of a digital file.

10.5.5   Histopathological examination methodology

Digital slide files were then examined by the scorer using the Nanozoomer specialized software application "NDP.View". Histopathology scoring was done by one scorer, such that the scorer was blinded to the patient's clinical details and operation details. Scoring was done according to a systematized methodology as follows.

The whole slide surface was scanned at low magnification to determine appropriate areas for field selection/sampling. Fields are then marked at high magnification by utilizing the rectangular annotation tool in the NDP view software. This tool allows the scorer to draw a quadrangular area that could be set to a particular surface area. The scorer then proceeds to select 7 to 10 "representative" fields at high magnification.

The selection of "representative" fields followed a systematic protocol:

- Each field surface area was equal to 0.035 mm^2. This particular surface area was chosen as it was equal to the surface area of the High Power Field (HPF) captured using the light microscope available at our institution (Nikon Eclipse 90i Microscope, Nikon Corporation, Tokyo, Japan) using digital camera capture (Nikon Digital Sight, Nikon Corporation, Tokyo, Japan).

- Fields should be directly beneath the epithelium, to sample subepithelial inflammation.
- Seven to ten representative fields were chosen per slide. The high number of fields allows good sampling of the whole slide, in order to obtain representative and consistent average results. This sampling methodology is an advantage of using a WSI platform. (Figure 10-1)

- For each slide, the most severely affected field(s) of subepithelial inflammation was included in the chosen representative fields. This point is adapted from previous methodologies by Bhattacharya et al.[338] and Soler et al.[345]

- Large blood vessels were excluded as they appear to bias the surrounding inflammation in an exaggerated way compared to the rest of the tissue section

- Localized secondary lymphoid aggregates that are not representative of subepithelial inflammation were excluded.

- For each field, the following parameters were counted:

(a) Eosinophils

(b) Lymphoplasmacytic cells

(c) Neutrophils (polymorphonuclear)

(d) Subepithelial basement membrane thickness in micrometers, defined as the area of deposition of extracellular matrix immediately beneath of epithelial cells, separating the epithelium from the inflammation in the lamina propria, and almost totally clear of cells itself. This was done using the "linear measure" annotation tool in the NPD.View software.

(e) computed parameters for each field included: total cells (= a + b + c), relative eosinophilia (= a / total cells), relative lymphocytia (= b / total cells), relative neutrophilia (= c / total cells), and eosinophilic/lymphocytic ratio (= a / b).

Each of the above parameters was calculated for each field. An average value for each parameter per slide was then calculated (by addition then dividing on the number of fields). When more than one slide was available for a particular operation, average parameters were calculated, such that the final result was the average parameter values per operation.

Figure 10-1 Whole Slide Imaging (WSI) technology allows us to survey, adequately visualize and sample representative fields through the whole surface of the sectioned mucosa on the slide.
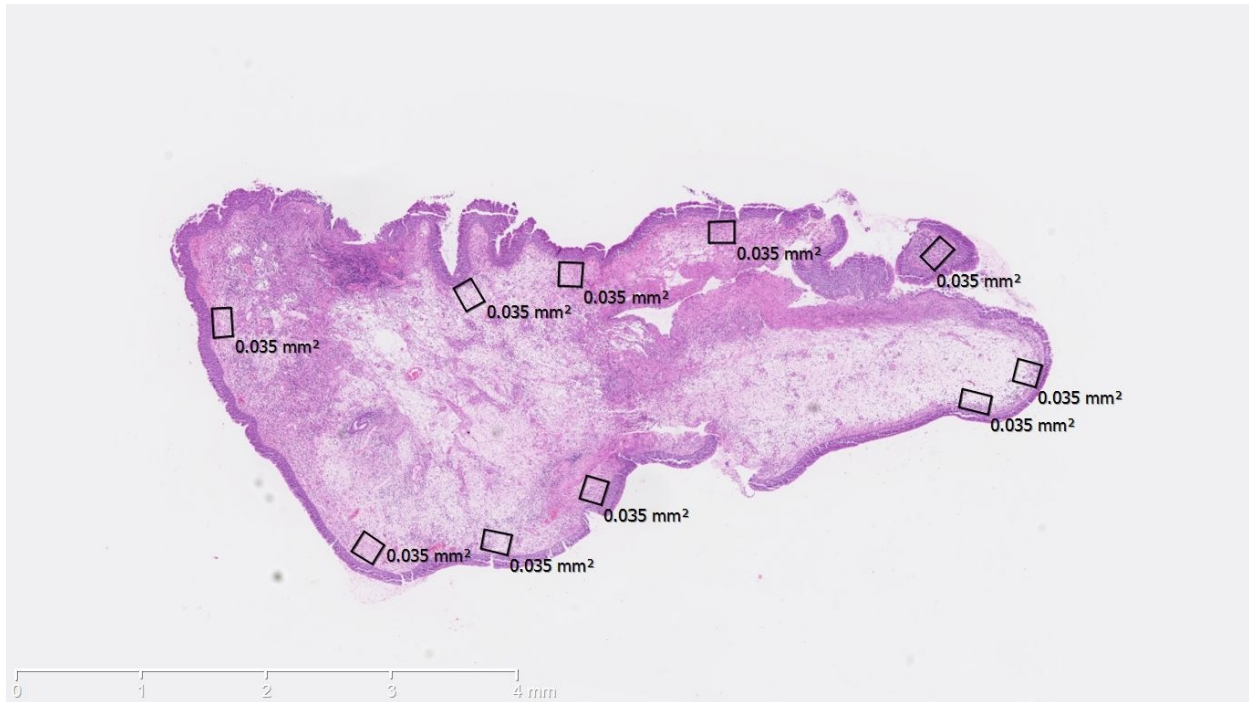
10.5.6   Statistics

For comparisons between the refractory and control groups, non-parametric Mann-Whitney-Wilcoxon tests (Wilcoxon rank sum tests) were used. For paired Comparisons between first and last (revision) operations in the rCRS group follow a paired (or a repetitive measure) design, so for these we utilized Wilcoxon signed rank tests. When exact p-values can't be computed in Wilcoxon tests due to ties or zero values, a continuity correction is applied. Statistical work was done using the *R statistical software* (R Foundation for Statistical Computing, Vienna, Austria)[299] through the *IPython*[300] notebook. Statistical significance was taken at the traditional 0.05 level.

10.6   Results

10.6.1   Basic characteristics of the study cohort

The clinical characteristics of the study cohort can be found in Table 10-1. Slides for 60 operations (40 patients) were reviewed. There was an average of 31 months of follow-up time/time between the initial to revision surgeries in the refractory group. The control group

were also followed up for 31 months. (Table 10-1)

Table 10-1 Characteristics of the study cohort

	Refractory group	Control group
N	20	20
Sex	M=12; F=8	M=11; F=9
Age (mean) †	43.6	51.35
Asthma	16 (80%)	16 (80%)
Aspirin sensitivity	5 (25%)	5 (25%)
Primary cases	5 (25%)	5 (25%)
Prev ops (mean) †	1.75	1.45
Follow-up duration or time to revision surgery (mean) †	31.3 months	30.6 months
Lund-Mackay score (mean) †	18.7	16.8

† = No significant difference between the two groups (t-test $p > 0.05$).

10.6.2 First substudy: Refractory group versus control group

Results of this substudy are found in Table 10-2 (n=20). Results can be summarized as follows: the rCRS group has a significantly (2.7×) higher absolute counts of eosinophils, as well as total inflammatory load (1.8×). Moreover, the rCRS group also shows a significantly higher relative eosinophilia per field. (55% versus 32%). The BM was of slightly less thickness in the refractory group but this was not statistically significant.

This comparison was then repeated for the subgroup that underwent primary surgeries (no previous sinus surgery) in both groups. This subgroup had only 5 patients in each group. The characteristics in both groups (matched) were as follows: 3 non-asthmatics, plus 2 asthmatics, plus 1 aspirin-sensitive asthmatic. The same observations for the control group used as a comparison were seen: the fold-change difference in eosinophilic load was even more pronounced in the refractory group (3.15× higher: 42.6 eosinophils/field in the refractory group,

versus 13.5 eosinophils/field in the control group; p = 0.016). This was also accompanied by a higher total inflammatory load (2.13× higher: 69.5 cells/field in the refractory group, versus 32.6 cells/field in the control group; p= 0.016) and a higher relative eosinophilia (58% versus 36%; p=0.095).

Table 10-2 Comparing the refractory group to the matched control group

Parameter	CRS Control	CRS Refractory	Wilcoxon rank sum test p-value
Eosinophils	**17.9** cells/field +/- 18.0	**48.8** cells/field +/- 26.6	**< 0.001***
Lymphocytes	**29.9** cells/field +/- 18.5	**36.3** cells/field +/- 23.9	0.40
Neutrophils	**0.9** cells/field +/- 1.6	**0.4** cells/field +/- 0.6	0.50
total cells	**48.6** cells/field +/- 29.6	**86.1** cells/field +/-31.5	**< 0.001***
relative eosinophilia	**32%** +/- 18%	**55%** +/- 18%	**< 0.001***
relative lymphocytia	**64%** +/- 19%	**44%** +/- 6%	**< 0.001***
Relative neutrophilia	**4%** +/- 6%	**1%** +/- 0.01%	0.23
Subepithelial BM thickness	**8.9** µms +/- 8.3	**6.8** µms +/- 2.2	0.46
eosinophilic/lymphocytic ratio	**0.65** +/- 0.56	**1.85** +/- 1.26	**< 0.001***

10.6.3 Second substudy: Following up the refractory group – comparing results of first versus revision operations

Results of this substudy (n=20) are found in Table 10-3. Within the rCRS group, the proportion of eosinophils reduced (53% to 45%, Wilcoxon signed rank $p < 0.05$) and the proportion of lymphocytes increased (46% to 54%, $p < 0.05$) from their first to their second (revision) operations, while the total averaged inflammatory cell count remained unchanged (~86 cells/field). This denoted a significant reduction in eosinophilic/lymphocytic ratio from 1.85 to 1.1.

Table 10-3 Comparing first to last (revision) operation in the refractory group

Parameter	First operation	Last (revision) operation	Wilcoxon signed rank test p-value
Eosinophils	**48.8** cells/field +/- 26.6	**40.7** cells/field +/- 17.8	0.34
Lymphocytes	**36.3** cells/field +/- 23.9	**44.9** cells/field +/- 20.3	**0.04***
Neutrophils	**0.4** cells/field +/- 0.6	**0.5** cells/field +/- 1.4	0.41
total cells	**86.1** cells/field +/-31.5	**86.1** cells/field +/- 25.4	0.75
relative eosinophilia	**55%** +/- 18%	**47%** +/- 15%	**0.02***
relative lymphocytia	**44%** +/- 6%	**52%** +/- 15%	**0.01***
Relative neutrophilia	**1%** +/- 0.01%	**1%** +/- 0.01%	0.32

Subepithelial BM thickness (μm)	**6.8** μms +/- 2.2	**6.5** μms +/- 2.5	0.43
eosinophilic/lymphocytic ratio	**1.85** +/- 1.26	**1.1** +/- 0.6	**0.02***

Subgroup analysis was done for the primary surgery group only (n=5, asthma=3, aspirin-sensitive=1). The subgroup analysis differed in that there was an increase in average absolute eosinophil count in the revision procedures, as well as an increase in the averaged total inflammatory load which was marginally significant (from to 69.5 cells/field to 94.5 cells/field, p = 0.062). There were no significant differences in BM thickness.

Despite the fall in relative eosinophilia from first to second surgery, the eosinophilic inflammation at the time of the second (revision) surgery (n=20) was still higher than the results of the control group, with: eosinophil counts of 40.7 eosinophils/field versus 17.9 (p < 0.001); total inflammatory load of 86.1 cells/field versus 48.6 (p < 0.001); and relative eosinophilia of 47% versus 32% (p < 0.001).

10.7   Discussion

In this retrospective study, we have demonstrated in a cohort of refractory CRSwNP patients a higher inflammatory load, suggesting an association between failure of surgery (long-term prognosis) and inflammatory load (in particularly eosinophilia). We also described the evolution of histopathology of CRSwNP in the same patients undergoing sinus surgery, by comparing tissue collected at first and revision surgeries.

Needing ultimately to undergo revision is perhaps a very important event in the course of the disease, since it affects the patients' QOL and exposes them to additional risks of hospitalization, surgery and anaesthesia. It also has important economic implications on both the patient and the healthcare system; the overall health care expenditures attributable to sinusitis in 1996 (in the US) were estimated at $5.8 billion,[11] with around 500,000 surgical procedures performed on the paranasal sinuses annually.[10] Therefore, this group of patients (termed "refractory CRS") should be intensively researched. Inflammatory and eosinophilic markers have been repeatedly

associated in previous studies with various negative prognostic events. (Summarized in

Table 10-4). However, no direct association has yet been shown between the local inflammatory

load and long-term need for revision surgery. Investigating the inflammatory load is even more

important in CRSwNP, since this group do not have classical osteomeatal complex (OMC)

disease[303] that potentially responds to limited, more functional surgery[303]

Table 10-4 Literature review – eosinophilic and inflammatory predictors of adverse surgical outcomes

Study	Outcome	Predictor
Zadeh et al.[346]	Polyp recurrence, Revision surgery	Serum eosinophilia
Matsuwaki et al.[204]	Post-operative symptoms	Serum eosinophilia, local eosinophilia
Myller et al.[232]	Post-operative symptoms	Local eosinophilia
Lavigne et al.[347]	Post-operative symptoms	IL-5 mRNA
Vlaminck et al.[413]	Post-operative symptoms, polyp recurrence	Local eosinophilia
Nakayama et al.[221]	Polyp recurrence	Local eosinophilia
Tosun et al.[220]	Polyp recurrence	Local eosinophilia
Soler et al.[345]	Post-operative QOL scores	Local eosinophilia
Smith et al.[166]	Post-operative QOL scores	Local eosinophilia
Van Zele et al.[414]	Polyp recurrence	Various local inflammatory markers including IL-5, ECP, IgE

In the first substudy, we compared the "refractory group" to a "matched control group",

according to various criteria that are supposed to affect inflammatory load and/or outcomes.

These criteria included asthma, aspirin intolerance, number of previous operations, follow-up duration, and radiological disease severity scores.

Our results have confirmed our initial hypothesis that refractory patients would exhibit a higher tissue inflammatory load. (Table 10-2) The refractory group showed higher eosinophils, lympho-plasmacytic cells and total cell counts per field. The absolute and relative eosinophil counts were particularly of statistical significance, suggesting that eosinophils play the most important role characteristic of disease refractoriness. The eosinophilic load was higher in the refractory group (for both their first and second surgeries), when compared to the control group. Another interesting finding is the tiny proportion of neutrophils, although this finding is similar to the finding of Soler et al.,[336] who reported the presence of neutrophils in only 0.7% of 147 subjects.[336] To avoid any confounding effect for previous sinus surgery in both groups, a subgroup analysis was done for patients who had zero previous operations i.e. primary surgeries (n=5); results of this showed similar tendencies to the parent comparison. The levels of eosinophilia in this study (average ~18-48/field) appear to be higher than have been reported in the literature. The size of the field we used (0.035mm^2) is based on a HPF capture through a digital camera and is smaller than a typical HPF obtained through the regular eyepiece. It is also smaller than the High Power Field (HPF) sizes that have been used in other studies.[338,345] Some studies also report HPF without reporting on the surface area of the field sampled.[415,416] This is a limitation when comparing this study with previously reported studies, since various microscopes would have different sizes for their lenses and thus different sized HPFs. Several studies have adopted 10 eosinophils/HPF as a cut-off value defining eosinophilia in CRS.[345,221,303] Our results suggest that this cut-off is conservative, especially if it is going to be used for predicting surgical prognosis, as all patients in both the refractory and control groups had significantly higher counts. However, we do recognize that our patients are attending a tertiary Rhinology clinic, and thus may not be representative of patients presenting at a primary healthcare facility. The differences may also be due to the different sampling and cell counting methodologies between the different studies.

Another recently raised question is whether remodeling in the sinuses plays a role in refractory disease.[305] A clinical phenomenon that had often been reported in the literature is the presence of irreversibly-diseased mucosa.[177,178,374] Ongoing collagen deposition in the context of mucosal remodeling could thus play a role in producing "dysfunctional mucosa", as it appears to respond

poorly to traditional surgical techniques and steroids.[284] Indeed, at least one study[416] have associated basement membrane thickening with a worse post-operative prognosis.[416] Subepithelial collagen deposition, leading to basement membrane thickening is a hallmark feature that has been particularly investigated in various human as well as animal model studies as a marker of remodeling.[284] In our study, we obtained measurements of the absolute thickness of subepithelial basemement membrane. Our results however did not show a significant difference between both groups (Table 10-2). This result could be interpreted as raising a question about the role played by remodeling in refractory patients. It could also be interpreted such that BM thickening is not the most appropriate feature to quantify mucosal remodeling, or that perhaps the sinuses sampled in this study were not irreversibly diseased. Another possible interpretation is that collagen deposition is not considered clinically "harmful" in CRSwNP, but plays a role in CRSsNP which shows higher tendency to produce fibrotic dysfunctional mucosa. A higher pro-fibrotic environment in CRSsNP (when compared to CRSwNP) has been shown in previous studies. This highlights the difficulty of defining and investigating irreversibly diseased mucosa in CRS.

The second substudy follows up the histopathology of the refractory group, to describe the evolution of inflammation with time, post-sinus surgery. This subject has been rarely addressed in the CRS literature. We are only aware of one similar study by Stoop et al [344] from 1993 which only examined eosinophils in recurrent polyps. In our results, we discover that there is persistence of total inflammatory load as per our original hypothesis.[301] This could be an indicator of persistent or ongoing disease in these patients. But contrary to our initial expectation, there was no increase in eosinophils; in fact there was a reduction. (Table 10-3) The statistically significant result was the reduction in the eosinophilia/lymphocyte ratio, caused by an increase in relative lymphocytes with reduction in relative eosinophilia. (Table 10-3) These results agree with the immunohistochemical findings of Stoop et al.[344] in which eosinophils in recurrent polyps after surgery were reduced in number at 6 and 12 months follow-up (23 and 15 patients, respectively).[344] This reduction could point towards the possibility that surgery decreases the inflammatory load and reduces the relative amount of eosinophils but not sufficiently to allow resolution and recovery of the mucosa. This picture could also be aided by the ongoing use of topical and intermittent oral steroid therapy. . It is important to note that although the medical therapy regimen was the same for all patients in our study and was prescribed by the same

surgeon, it is difficult to confirm patient compliance in the setting of a retrospective study. A clue to a possible immunomodulatory role played by surgery itself is that this trend did not appear in the "primary surgery only" subgroup analysis. This can suggest that in patients who had previous surgery, as the number of surgeries performed increases, surgery exerts changes on the mucosa not evident in patients undergoing their primary surgery. Another early hypothesis of ours[305] (that with passage of time, remodeling with collagen deposition would increase) was also not proven with this study, since there was no increase in BM thickness demonstrable. (Table 10-3) This indicates that remodeling is a more complex phenomenon and may not occur in a simple linear fashion with the passage of time and that other methods of quantification may be necessary in addition to measuring BM thickness.

10.8   Conclusion

Our findings suggest that a higher inflammatory and eosinophilic load is associated with refractory CRSwNP and thus may be useful in predicting the need for future revision surgery in CRSwNP patients. On the other hand, we could not demonstrate a role for basement membrane thickening in refractory CRSwNP. Our results support previous recommendations[415] for routine histopathological investigation for CRS. Our findings need to be confirmed in a future prospective study, to overcome the limitations of retrospective studies. Future studies should work on formulating standardized methods and specifying the exact histopathological parameter values of optimum predictive power.

10.9   Acknowledgements

Chapter 11   Thesis Synopsis

11.1   Synopsis of research findings

In Chapter 6, we investigated the clinical significance of middle turbinate lateralization (MTL), as being one of the surgery-related factors in rCRS. MTL had the reputation of being a complication of CRS that played a role in surgical failure, but with limited evidence in the literature. Our study in Chapter 6 improves upon previous evidence in the literature, in that it used a prospectively-collected data set, with a timeline between initial surgery, post-operative follow-up, and revision surgery. An interesting finding of our study suggests that MTL becomes significant when it occludes the frontal sinus. Traditionally, MTL was considered important due to its relationship to the OMC, but our study suggests that MTL relationship to frontal drainage pathway is more important. As it is difficult to imagine MTL totally obstructing drainage from the frontal sinus, we offered a hypothesis in which a significant partial obstruction leads to recurrent infection and inflammation, predisposing to persistent frontal sinusitis and ultimately frontal sinus ostial stenosis. Frontal sinus ostial stenosis has already been described as a negative prognostic factor in a number of previous studies.[165,304] Our newly generated hypothesis needs to be investigated in future studies of MTL.

In Chapter 7 and Chapter 8 we describe two novel hypotheses. These hypotheses were generated based upon a review of the advances of basic science research into the pathophysiology of chronic rhinosinusitis. CRS is a complex multi-factorial heterogeneous inflammatory disease that encompasses more than simple OMC obstructive process as described in the original theories of FESS. For example, the OMC obstruction theory does not explain why asthma, aspirin intolerance are related to poorer outcomes, and why are better outcomes reported with more extensive surgical approaches, as we have shown in Chapter 9 with the Draf-3 frontal drillout procedure. The two hypotheses (the inflammatory load hypothesis in Chapter 7; and the irreversible disease hypothesis in Chapter 8) thus attempt to reconcile the pathology of CRS with surgical prognosis, guided but what we already know from the CRS literature.

We then proceed in Chapter 9 to investigate nasal polyp recurrence, an important "disease-related" cause of refractory CRS. Our findings confirm previous reports in the literature that asthma and aspirin sensitivity are important factors in determining aggressive recurrence. In light

of the newly proposed inflammatory load hypothesis, and based on studies that reported better outcomes with more extensive surgery, we investigated a possible role of extensive Draf-3 frontal sinus surgery in determining post-operative polyp recurrence. We found that a Draf-3 frontal drillout procedure reduces the risk of polyp recurrence post-operatively. We also found that it reduces the need for undergoing revision surgery, such that the risk of undergoing revision was about 25% of those patients who had (functional) endoscopic sinus surgery without a Draf-3 frontal drillout procedure. These findings are novel.

Finally, in Chapter 10, we investigate the two hypotheses discussed in Chapter 7 and Chapter 8 through a histopathological study. We find that an inflammatory and eosinophilic load is higher in the cohort of patients who have failed surgery and required revision. Eosinophilic inflammation has been previously described as a negative prognostic marker, but has not been directly linked to the (long-term) need for revision surgery is the first study to suggest the existence of this association. On the other hand, we could not associate the thickness of the subepithelial basement membrane (which we used as a marker of remodeling/fibrosis) with the disease load or surgical prognosis.

11.2   Alternative views

In this section, we present alternative views from the literature.

Some authors support a concept of "minimal" intervention. This approach is exemplified in the practice of Minimally Invasive Sinus Technique (MIST). MIST is considered to be a targeted minimal standardized intranasal intervention, regardless of disease severity. Proponents of MIST cite for their surgical philosophy the following reasons: preservation of mucosa and turbinate tissue required for proper nasal and sinus physiology, leaving the primary (birth) ostia undisturbed (as most patients would not need a middle meatal antrostomy), which leads to a decrease in operative morbidity.[417] MIST is thus proclaimed as a true embodiment of the original conservative, functional principles of Messerklinger.[417] Some studies suggested that the outcomes of MIST are comparable, or even better than, the outcomes after FESS.[418,419] Despite this study, this technique has been criticized by some authors as incapable of addressing the underlying pathology in CRS.[420]

Another minimally invasive technique is the use of balloons to dilate sinus ostia (termed endoscopic balloon sinus ostial dilation, or sometimes balloon sinuplasty). It appears that the use of balloons for the sinuses is widespread at least in the USA, as one study showed that 8% of endoscopic sinus surgery cases in California, Florida, Maryland, and New York in 2011 utilized balloon technology.[421] The high-cost associated with balloon technology is another limitation to its use.[421] Some authors reported good outcomes with balloon sinuplasty in minimal diseased non-polyp patients,[422] while other authors did not.[423] A Cochrane systematic review found that there was not sufficient high-level evidence to support the use of endoscopic balloon dilation compared to conventional surgical.[424] Its status thus remains controversial in the literature.

11.3   Future directions

11.3.1   Refractory CRS and Long-term outcomes research

Refractory chronic rhinosinusitis (defined, as in this thesis, as surgical failure with requiring revision) is a difficult entity to study since the revision surgery may happen many years after the initial surgery. This sort of long-term outcomes research is often difficult to investigate in a prospective fashion. The answer lies in standardized (prospectively-collected) data. This would be preferably done in multi-centre based registries that are potentially capable of being representative of the whole population.

In this thesis, our hypotheses were concerned about the pathology of the disease and how it could affect prognosis, in particular the inflammatory load. Future studies could look into alternative and more accurate measures for characterising the inflammatory cell population in CRS, such as flow cytometry.

In addition studies could investigate other pathological features of CRS (other than inflammation) such as osteitis and neo-osteogenesis.

11.3.2   Origin of the Th2 response

We have shown that a higher inflammatory load was associated with the cohort of patients who require revision surgery. In particular, we showed the importance of the eosinophilic inflammation and its association with long-term outcome. Eosinophils are recruited and thrive in a Th2 cytokine environment. But how does the Th2-skew originate? Exciting research is currently under way investigating how Th2 responses are initiated and amplified.[425] The role of

epithelial-derived Th2 cytokines such as IL25 and IL33, TSLP and their interaction with ILC2 cells is an interesting avenue to explore. We have already investigated ILC2 cells in our department and we found them increased in CRSwNP patients, compared to CRSsNP and controls.[426] This finding is one of several in the literature that point to a role for these cells in CRS pathophysiology that requires further research.

11.3.3 Extent of surgery

What are the guidelines about the extent of surgery to be performed? There is little data about this topic in the literature, especially data that could be considered as high-level evidence. We show in this thesis through our literature review and study findings that more extensive surgery has been shown to provide better outcomes. For example, the Draf-3 procedure has been shown, by our department as well as by others, to be effective in surgical salvage of severely diseased patients. The next step is to organize a Randomized Controlled Trial to confirm these findings and better delineate the indications of the procedure.

11.3.4 CRS Endotypes

Defining CRS endotypes may be possible in the future once more accurate characterization of the inflammatory disease load is possible. Research efforts should look into grouping similar patients into subgroups (or "bins") that would share a particular disease course or clinical picture, or benefit from a particular course of management. These "bins" are known as CRS endotypes. Investigating CRS endotypes is an exciting venue of research for predicting the clinical course and prognosis of the disease. The Polyp versus Non-Polyp classification, albeit practical and effective, may not be of much use in predicting the outcome of medical and surgical treatment. (The shortcomings of this classification has been discussed in 1.5.1) More research is thus needed to elucidate the various CRS endotypes.

11.4 Conclusion

Based on insights gained from previous literature, in addition to insights derived from new research work done for the purpose of this thesis, we suggest that the OMC obstructive process is not sufficient to provide a universal explanation for surgical failure in rCRS patients as well as for the complex pathophysiology of CRS. Although there may be a subset of CRS patients presenting mainly with OMC obstructive pathology, these patients are not representative of the

whole heterogeneous population of CRS patients. Consequently, the OMC theory (which guided the original philosophy of FESS) should not dictate current surgical practice as a general rule. We therefore support that surgery should be viewed as a tool of reducing and controlling disease load, while allowing better penetration of topical anti-inflammatory medication. This view should be translated to surgical teaching as well as managing patients' expectations during pre-operative counseling. Surgical practice should be more guided by the characterization of the underlying disease's pathophysiology, and CRS is a heterogeneous entity comprising several endotypes.

In particular, we provide further evidence to an association between the inflammatory load and long-term outcome. We also provide novel evidence that the more extensive Draf-3 frontal sinus procedure is associated with better outcomes in CRSwNP patients, allowing it to be a viable option offered to patients with high risks of refractory disease.

In conclusion, the recommendation based upon this work is that characterization of the disease load (both its quality and its quantity) would better guide disease prognostication and surgical philosophy.

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Appendix i: List of Abbreviations

AERD	Aspirin-Exacerbated Respiratory Disease
AFS	Allergic fungal sinusitis
AIDS	Acquired immunodeficiency syndrome
APC	Antigen-presenting cell
ARS	Acute rhinosinusitis
BAL	Bronchoalveolar lavage
BM	Basement membrane
C/EBP	CCAAT-enhancer-binding proteins
CCL	Chemokine ligand
CD	Cluster of differentiation
CF	Cystic fibrosis
CFT	Canine Fossa Trephine
CFTR	Cystic fibrosis transmembrane conductance regulator
CL	Caldwell-Luc
COX	Cyclo-oxygenase
CRS	Chronic rhinosinusitis
CRSsNP	Chronic rhinosinusitis *sans* (without) nasal polyposis
CRSwNP	Chronic rhinosinusitis with nasal polyposis
CT	Computed Tomography
cysLTs	Cysteinyl leukotrienes
DNA	Deoxyribonucleic acid
ECP	Eosinophilic cationic protein
ECRS	Eosinophilic chronic rhinosinusitis
EMCRS	Eosinophilic mucus chronic rhinosinusitis
EMLP	Endoscopic modified Lothrop procedure
EoPs	Eosinophil progenitor cells
EPO	Eosinophil peroxidase
EPOS	European Position Paper on Rhinosinusitis
ESS	Endoscopic sinus surgery
FESS	Functional endoscopic sinus surgery
FHFESS	Full-House functional endoscopic sinus surgery
GATA	GATA transcription factors
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GR	Glucocorticoid receptor
H&E	Haematoxylin and Eosin
HIV	Human Immunodeficiency Virus

HLA	Human leukocyte antigen
HPF	High-power field
IFN-γ	Interferon gamma
Ig	Immunoglobulin
IgE	Immunoglobulin E
IL	Interleukin
ILC2	Innate Lymphoid Cell 2
LMS	Lund-Mackay score
MBP	Major Basic Protein
MCC	Mucociliary clearance
MEMM	Modified endoscopic medial maxillectomy
MHC	Major histocompatibility complex
MIST	Minimally Invasive Sinus Technique
MMA	Middle meatal antrostomy
MPO	Myeloperoxidase
mRNA	Messenger RNA
MT	Middle turbinate
MTL	Middle turbinate lateralization
NSAID	Non-steroidal anti-inflammatory drug
OMC	Osteo-meatal complex
PAF	Platelet-activated factor
PGE2	Prostaglandin E2
QOL	Quality of life
RANTES	Regulated on activation, normal T cell expressed and secreted
rCRS	Refractory chronic rhinosinusitis
RCT	Randomized controlled trial
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
rRNA	Ribosomal RNA
SCUADs	Severe chronic upper airway diseases
SD	Standard deviation
STAT	Signal Transducer and Activator of Transcription
TCR	T-cell receptor
TGF-β	Transforming growth factor β
Th2	T-helper 2
TSLP	Thymic stromal lymphopoietin
VCAM-1	Vascular cell adhesion protein 1
VLA-4	Very Late Antigen-4
WSI	Whole-Slide Imaging

Appendix ii: List of Figures

Appendix iii: List of Tables