# Novel therapeutics development for complex chronic rhinosinusitis: evaluating a low-dose, long-term macrolide in a double-blind, randomized, placebo-controlled trial

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## DEDICATION

To my children Eva, Theodore, and Alexander

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## I. Abstract English

**Background**: Chronic rhinosinusitis (CRS) is one of the most common chronic diseases in the developed world, with a significant proportion of patients remaining refractory to the standard of care treatment. This difficult-to-treat population is often left with few therapeutic options that provide low-morbidity, low-cost, and long-term meaningful symptomatologic improvement.

**Objectives**: Evaluate the efficacy of a low-dose, long-term macrolide in a high-risk and carefully selected CRS population, refractory to appropriate medical (budesonide irrigations [BNI]) and surgical therapy (endoscopic sinus surgery [ESS]). Evaluate factors associated with treatment response following low-dose, long-term macrolide. Evaluate the effect of low-dose, long-term macrolide in modulating the sinonasal microbiome.

**Methodology**: Patients at high-risk of disease recurrence following ESS+BNI were enrolled in a prospective clinical trial (Phase 1) at a tertiary care center. Serologic samples, microbial swabs, and patient-reported outcomes measures (PROM) were completed on the day of surgery (Visit-1) and four months postoperatively (Visit-2). Outcomes were evaluated using the Lund-Kennedy endoscopic score. If disease persisted on Visit-2, they were randomized (Phase 2) to receive 250gm azithromycin or a placebo three times per week for 16 weeks (Visit-3).

**Results**: A total of 128 patients were enrolled in the Phase 1 prospective trial. At the first four-month follow-up, 48 patients showed disease persistence and were randomized to azithromycin or placebo. Overall, azithromycin did not show a statistically significant difference in disease clearance at Visit-3 compared to placebo (54% vs. 33%, respectively; p=0.146). When patients with aspirin-exacerbated respiratory disease (AERD) were excluded from the analysis, azithromycin showed a significant improvement in disease clearance compared to placebo (71% vs. 35%, respectively; p=0.031), with a number needed to treat of 3 (2.8). In a subgroup analysis

looking at all patients with disease clearance, those on azithromycin reported significantly better PROM improvements than patients on placebo ($p=0.046$). Twenty patients received azithromycin in an open-label setting, either at Visit-2 or following failure of placebo, with 14 of 20 (70%) showing disease clearance. When analyzing the sinonasal microbiome, patients on azithromycin demonstrated a significant log-fold decrease in 29 different operational taxonomic units (OTUs) of *Staphylococcus aureus* compared to patients on placebo, while also showing a significant difference in beta diversity ($p<0.001$). There were no patient-reported side effects associated with the use of azithromycin.

**Conclusion**: Oral 250mg azithromycin given three times a week is a low-cost, low-morbidity, easy to administer treatment regimen which shows both clinical and statistically significantly better disease clearance rates for non-AERD patients with CRS failing standard medical and surgical therapy. Furthermore, we demonstrate that the use of azithromycin offers avenues to modulate the sinonasal microbiome, demonstrating the lasting positive effect it may have on re-establishing sinonasal microbial equilibrium.

**Clinicaltrials.gov:** NCT02307825

**Key Words:** chronic rhinosinusitis; endoscopic sinus surgery; azithromycin; Staphylococcus aureus; aspirin-exacerbated respiratory disease; double-blind, randomized, placebo-controlled trial; sinonasal microbiome; alpha and beta diversity

## II. Abstract French

**Contexte**: La rhinosinusite chronique (RSC) est une des plus communes maladies chroniques du monde développé, avec une proportion significative de patients qui demeurent réfractaires au traitement conventionnel. Cette population difficile à soigner reste souvent avec peu d'options thérapeutiques qui offrent peu de morbidité, un coût abordable, et avec une amélioration symptomatologique importante et durable.

**Objectifs** : Évaluer l'efficacité d'un macrolide à petite dose et pour une période prolongée dans une population avec RSC à haut-risque d'être réfractaire au traitement approprié médical (irrigations nasales avec budesonide - INB) et au traitement chirurgical (chirurgie endoscopique des sinus-CES). Évaluer les facteurs associés avec une réponse au traitement de macrolide à basse dose, long-terme. Évaluer l'effet du macrolide à basse dose, long-terme, sur la modulation du microbiome sinonasal.

**Méthodes** : L'étude a été séparée en deux parties principales. Phase 1 : étude clinique prospective dans un centre tertiaire dans lequel des patients à haut risque d'échec au traitement post CES+INB ont été recrutés. Des bilans sanguins, des cultures microbiologiques, des questionnaires cliniques ont été complétés le jour de la chirurgie (Visite-1) et quatre mois postopératoire (Visite-2). L'issue clinique a été mesurée avec le score endoscopique Lund-Kennedy. Phase 2 : s'il y avait persistance de maladie à la Visite-3, les patients étaient randomisés à recevoir soit 250mg d'azithromycine ou un placébo trois fois par semaine pour 16 semaines (Visite-3).

**Résultats:** Un total de 128 patients ont été recrutés dans la Phase 1. À la Visite-2, 48 patients ont démontrés une persistance de maladie et ont été randomisés à l'azithromycine ou le placébo. Globalement, l'azithromycine n'a pas démontré une différence statistiquement significative au niveau de la clairance de la maladie à la Visite-3, comparé au placébo (54% vs. 33%,

respectivement; p=0.146). Lorsque les patients avec la maladie respiratoire exacerbée par aspirine (MREA) étaient exclus de l'analyse, l'azithromycine démontrait un bénéfice statistiquement significatif comparé au placébo au niveau de la clairance de la maladie (71% vs. 35%, respectivement; p=0.031), avec un nombre à traiter de 3 (2.8). Dans une analyse de sous-groupe évaluant tous les patients ayant démontrés une clairance de maladie, les patients ayant reçu l'azithromycine ont reporté une amélioration de symptômes significativement plus importante que ceux sur placébo (p=0.046). Vingt patients ont reçu l'azithromycine hors randomisation, soit à la Visite-2, ou bien après un échec au placébo, avec 14/20 (70%) ayant une clairance de maladie. Lors des analyses du microbiome sinonasal, les patients sur azithromycine ont démontrés une diminution logarithmique significative dans 29 unités taxonomiques opérationnelles différentes de *Staphylococcus aureus* comparés aux patients sur placébo, tout en démontrant aussi une différence significative dans la diversité beta (p<0.001). Il n'y a eu aucun rapport d'effet secondaire associé à la prise d'azithromycine.

**Conclusions** : L'azithromycine prise oralement à 250mg, trois fois par semaine, pour 16 semaines est un traitement peu coûteux, avec peu de morbidité, et facile à administrer qui démontre un taux de clairance de maladie cliniquement et statistiquement plus important que le placébo chez les patients non-MREA qui ont un échec au traitement standard médical et chirurgical. De plus, nous avons démontrer que l'azithromycine peut offrir des avenues importantes à la modulation du microbiome sinonasal, démontrant l'effet positif durable qu'il pourrait avoir sur le rétablissement de l'équilibre microbiologique sinonasal.

**Clinicaltrials.gov:** NCT02307825

**Mots clés:** rhinosinusite chronique; chirurgie endoscopique des sinus; azithromycine; Staphylococcus aureus; maladie respiratoire exacerbée-par-l'aspirine; étude clinique randomisée à double-insu; microbiome sinonasal; diversité alpha et beta

## III. Acknowledgements

My sincere appreciation goes first and foremost to my supervisor Dr. John Sampalis who believed in the importance, potential, and clinical significance of my work. Dr. Sampalis was instrumental in motivating me to pursue the PhD program of Experimental Surgery. He has demonstrated continuous support throughout this journey and will remain as a mentor throughout my academic career. The independence he has given me through this process has been a wonderful learning experience and has provided me with significant academic growth.

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To all the patients who underwent rigorous examinations filled with questionnaires, samplings, phone calls, and repeat visits, thank you for voluntarily participating in our research

and helping us strive towards defining the pathophysiology of this heavily burdening disease of complex chronic rhinosinusitis, while investigating novel therapeutic avenues that we believe will help lessen the morbidity of this disease.

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## IV. List of commonly used abbreviations

| | |
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| CRS | Chronic rhinosinusitis |
| CRSwNP | Chronic rhinosinusitis with nasal polyposis |
| CRSsNP | Chronic rhinosinusitis without nasal polyposis |
| ESS | Endoscopic sinus surgery |
| BID | Twice daily |
| BUD | Budesonide |
| BNI | Budesonide nasal irrigations |
| AZI | Azithromycin |
| AERD | Aspirin-exacerbated respiratory disease |
| hsCRP | High sensitivity C-Reactive Protein |
| CBC | Complete blood count |
| IgE | Immunoglobulin E |
| MCID | Minimal clinically important difference |
| PROM | Patient reported outcome measures |
| SNSS | Sino-nasal symptom score |
| SNOT-22 | Sino-nasal outcome test – 22 questions |
| kIU | Kilo international units |
| NNT | Number needed to treat |

## V. Contribution to original knowledge

This thesis follows the manuscript-based thesis outline. This work has contributed significant findings in the effort to better understand the pathophysiology of refractory chronic rhinosinusitis. We provide the scientific community with findings from a project scaffolded on a robust methodology that involves extensive and multifaceted data collection points, including serum biomarkers, sinonasal microbiome, patient reported outcome measures, and endoscopic clinical findings at every key stage of the trial. As such, readers can appreciate detailed findings according to the stage of the disease, beginning from a pre-operative state (active disease) to a post-operative state (persistent disease), followed by a post-experimental drug state (disease clearance vs. disease persistence).

Through our work, we have identified a specific CRS phenotype which shows a clear response to a low-cost, low-morbidity, and easy to use regimen for this difficult-to-treat disease. Furthermore, we demonstrate the potential effect of the experimental drug on the sinonasal microbiome and its ability to modulate it. All work conducted and findings presented are entirely original, while the methodology developed is reproducible and can be used as a template for future work in the field of therapeutics development and microbiome analyses for CRS.

*Contribution to the literature*

Throughout the completion of the Phase 1 prospective trial, followed by the Phase 2 randomized control trial, we have been fortunate to collect a breadth of knowledge and data on refractory CRS and the experimental drug in question (azithromycin). A principal manuscript was published on the clinical outcomes of the randomized control trial which reports the clinical outcomes of patients on the experimental drug versus a placebo:

1. Azithromycin in refractory CRS following ESS and corticosteroid irrigations: double-blind, randomized, placebo-controlled trial *International Forum of Allergy & Rhinology.* [Epub September 2020]

Additional manuscripts reporting the findings from our subgroup analyses or associated research questions can be found below:

2. Increased *Staphylococcus aureus* prevalence characterizes the elderly CRS population. *International Forum of Allergy & Rhinology.* [Under review]
3. The Effect of Radiation and Chemoradiation Therapy on the Head and Neck Mucosal Microbiome: A Review. *Frontiers Oncology*. [Epub December 2021]
4. Azithromycin downregulates gene expression of pro-inflammatory cytokine IL-1β and serine protease activation pathway required by SARS-CoV-2 cell infection. *American Journal of Respiratory Cell and Molecular Biology.* 63(5):707-709. November 2020 [Epub August 2020]
5. Low-dose and long-term azithromycin significantly decreases Staphylococcus aureus in the microbiome of refractory CRS patients. *International Forum of Allergy & Rhinology.* 11(2):93-105. February 2021 [Epub July 2020]
6. Can a panel of serum inflammatory biomarkers predict endoscopic sinus surgery outcomes for chronic rhinosinusitis? *International Forum of Allergy & Rhinology*. [In process of submission]
7. Eustachian tube symptoms are frequent in chronic rhinosinusitis and respond well to endoscopic sinus surgery. *Rhinology*. 56(2):118-121. June 2018.
8. *Staphylococcus aureus* on sinus culture is associated with recurrence of CRS after ESS. *Frontiers in Cellular and Infection Microbiology-Clinical Microbiology*. 15(8):150. May 2018

Work from this PhD thesis has been extensively presented at the regional, national, and international level. Furthermore, the scientific community has recognized and awarded this work with various prizes, in addition to the aforementioned funding agencies, starting with the Daniel Tassé award (2018) for the top research project from the Department of Surgery at the University of Montreal, and most recently as the best oral presentation at the Congress of the International Rhinologic Society/Congress of European Rhinologic Society/Congress of the International Society of Inflammation and Allergy of the Nose (2021).

## VI. Contribution of authors

Anastasios Maniakas: study design, recruited patients, tabulated and analyzed the data, performed culture sampling and endoscopic evaluations, and was principal writer for all associated thesis manuscripts

Axel Eluid Renteria Flores: recruited patients and tabulated data, sinonasal microbiome data analyses, microbiome manuscript preparation and critical review

Marc-Henri Asmar: recruited patients, tabulated data, and serum inflammatory biomarkers manuscript preparation

Smriti Nayan: recruited patients, performed sampling and endoscopic evaluations

Saud Alromaih: recruited patients, performed sampling and endoscopic evaluations

Leandra Mfuna Endam: coordinated the research laboratory, and managed all equipment supplies and purchases

Martin Desrosiers: study design, performed culture sampling, mentorship, critical review of lead manuscript

John Sampalis: study design, statistical methodology, mentorship, manuscript critical review

## VII. Introduction

Chronic rhinosinusitis (CRS) is characterized as an inflammatory disease of the paranasal sinuses affecting one in ten adults in the western countries[1]. Almost one in three patients will not respond to standard medical and surgical therapy[2], classifying them as refractory CRS patients that will require additional treatment which often involves high-dose corticosteroids, multiple antibiotic regimens, and repeat surgeries. Unfortunately, these efforts often fail, causing long-term morbidity, absenteeism, and depression, all at a significant socio-economic cost.

In the past decade, there has been a push towards the development and trialing of novel therapeutics for CRS, primarily in the field of biologics. Although certain studies remain favorable, the general consensus is that it is not clear which biologic should be favored, and it is still very difficult to adequately predict which patient will respond to biologics, as response rates vary between 50% and 70%, as reported in the most recent Cochrane review[3]. Therefore, considering the exuberantly elevated cost of biologics compared to conventional treatment, such treatment options cannot be used or maintained in a general healthcare setting.

Identifying novel therapeutics can also occur in the setting of drug repurposing, also known as drug repositioning, reprofiling, or re-tasking. This involves the use of de-risked compounds previously approved by healthcare government bodies such as Health Canada to be used for other diseases that may or may not be clinically or physiopathologically associated with the new targeted disease. In the current setting, we identified the potential of a macrolide, azithromycin, commonly used in both acute lower respiratory diseases and in chronic respiratory diseases, such as Cystic Fibrosis. Based on its molecular profiling and mechanism of action, as well as favorable findings from a practice audit we conducted and demonstrated a good response in refractory CRS patients[4], we hypothesized that azithromycin may be a promising low-cost, low-morbidity drug that can

potentially control refractory CRS. Macrolides have been previously trialed and reported for rhinosinusitis patients[5-10] with significantly varied outcomes, rendering any conclusion on the true effect of macrolides unclear. This is mostly due to the high heterogeneity of the study populations, as well as the methodology of most of these studies which involved administering the drug to all patients with CRS. Therefore, there is uncertainty in the literature regarding the characterization and identification of the appropriate CRS patient population that may potentially respond to macrolides, the associated risk factors, clinical and serologic biomarkers, as well as the underlying effect of azithromycin on the sinonasal microbiome.

This thesis aims to fill this gap in the literature by presenting a robust randomized control trial on a prospectively followed population deemed at high-risk of disease recurrence/persistence following appropriate medical and surgical treatment for CRS.

## VIII. Literature review

In this section, I will present key information that will help the reader understand the basic anatomy, hypotheses around the development of CRS and treatment options, and the socio-economic burden of the disease. I will then present my approach to developing a birth cohort in CRS, followed by presenting the various characteristics of interest of macrolides, focusing specifically on azithromycin.

### 1. The surgical anatomy of the paranasal sinuses[11]

The paranasal sinuses are anatomically divided into the maxillary, sphenoid, frontal, and ethmoidal sinuses, the latter being further subdivided into anterior and posterior cells. The maxillary sinuses are found within the maxillary bone, beneath the eye and above the superior teeth, and are the largest of them all. The sphenoid sinuses are the deepest in the skull and are closely associated with important neurovascular structures, such as the internal carotid artery, the optic nerve, and the sella turcica. The frontal sinuses vary in size and pneumatisation and are found above the eyes in the most anterior portion of the skull. The ethmoid sinuses are the central structures of the nose and are closely associated to the medial wall of the orbit, the base of skull, and the olfactory system. Surgery to address diseases of the paranasal sinuses can be performed in a minimally invasive endoscopic approach or using an open approach which is associated to significantly higher morbidity and complications. Patients presenting with benign sinus disease are typically managed using a direct endoscopic transnasal approach, also known as endoscopic sinus surgery (ESS). Surgery for benign sinus disease is usually managed under general anesthesia and requires the adequate knowledge of the anatomy and experience of using the minimally invasive tools and avoiding severe complications.

The paranasal sinuses are confined within the skull and vary in size and shape. Their borders are bony and can also vary in thickness, making the surgical approach quite variable from one patient to the next. To better appreciate the individual variances of the paranasal sinuses, a computed tomography of the sinuses is regularly performed prior to ESS to help guide the surgeon and further characterize the severity and extent of the disease at hand. This can be seen by characteristic signs such as thickening or sclerosing of the bony sinus walls, or the presence of high-density opacifications within the sinuses themselves.

When performing ESS for inflammatory sinus diseases such as chronic rhinosinusitis, the altered mucosa can render the surgery challenging. This challenge is further increased when patients have undergone previous surgeries, increasing complications such as intra-orbital damage, cerebrospinal fluid leak with damage of the skull base, amongst others. As such, the more revision surgeries performed, the higher the likelihood of intra and postoperative complications.

## 2. Chronic rhinosinusitis physiopathology

Chronic rhinosinusitis (CRS) is characterized as a chronic inflammation of the paranasal sinuses. Although significant advances have been made in the recent years to further characterize this disease, it remains a complex disease with a highly heterogeneous population. For simplicity, the scientific community had initially established two main sub-groups or general phenotypes: patients with nasal polyposis (CRSwNP), and patients without nasal polyposis (CRSsNP). In the most recent European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS2020)[12] an additional classification was introduced, that of the endotype of dominance: “type 2” and “non-type 2”. The majority of CRSwNP patients are considered to be type 2, and conversely the majority of CRSsNP patients are non-type 2. However, these phenotypes and endotypes are not perfectly

aligned; 15% of CRSwNP patients are considered non-type 2 in western populations[13]. Patients harboring CRSsNP may often have idiopathic or odontogenic causes (as maxillary/upper teeth often protrude into the maxillary sinus and can cause chronic irritation), or more rarely can be due to immunodeficiencies, vasculitis, or other immune disorders. Patients presenting with CRSwNP often have a more 'inflammatory' aspect to their disease and comorbid conditions; the main cause remains idiopathic, although there is a much higher association with genetic, metabolic, or immunologic diseases[14]. Other than systemic diseases, such as cystic fibrosis or ciliary dyskinesia, associated with CRS, factors such as high eosinophilia, chronic allergies, recurrent bacterial infections, family history, and asthma, amongst others have been associated with CRS. With regard to family history, there is an increased prevalence of CRSwNP in patients having a first-degree relative with nasal polyps, compared to controls[15]. Phenotyping and genotyping studies have become one of the main topics of research in the field, as the race to identify therapeutics that offer adequate disease control and/or clearance is being overpopulated by expensive biologics.

Cardinal symptomatology associated with CRS include the presence of chronic purulent discharge, facial pain, nasal obstruction, nasal congestion, and loss of sense of smell. These symptoms can be quite debilitating not only on a clinical level, but on a functional or social level, with patients complaining of chronic fatigue, absenteeism, loss of concentration, isolation, and depression, amongst others. In patients with mild disease, a short course of intranasal corticosteroids with saline irrigation offers disease control and/or clearance, as long as the patient remains diligent and adherent to therapy. In patients with more severe symptoms, a short course of oral (systemic) corticosteroids will be given to rapidly control the inflammatory state, with or without the necessity for an oral antibiotic as there is often an associated episode of acute rhinosinusitis which may exacerbate symptoms. When maximal standard medical therapy is

attained and/or the patient begins to show intolerance to medical treatments, ESS is recommended in an effort to remove the polyposis and obstruction, aerate the sinuses, and provide the paranasal cavities with adequate drainage options.

### 3. Refractory chronic rhinosinusitis: epidemiology and socioeconomic costs

Chronic rhinosinusitis is considered to be one of the most frequent chronic inflammatory diseases with approximately 10% of the population currently affected[1,16]. Surgical therapy is common with more than 400,000 sinus surgeries performed per year in the United States alone[17]. Unfortunately, up to one in three[2] patients with CRS will fail ESS and post-operative medical therapy, rendering them refractory to maximal medico-surgical treatment. Patients with refractory disease present with reduced quality of life, poor sleep, fatigue, and acute exacerbations of their CRS, resulting in significant increases in the economic burden of the disease[18], presently estimated at $10,000 annually per refractory patient[19], significantly surpassing other chronic disease such as severe asthma ($7,300), chronic migraine ($5,800), and diabetes ($3,900). Furthermore, the overall direct and indirect costs related to CRS-related losses in work productivity are 13 billion and >20 billion per year in the USA alone, respectively[20].

### 4. Refractory chronic rhinosinusitis: risk factors

Refractory CRS not only poses an important economic burden, but its pathogenesis remains elusive, which ultimately stalls the development of novel therapeutic strategies. Authors have reported various factors associated with ESS failure, whether it is specific anatomical anomalies or associated medical comorbidities, amongst several other reported etiologies[21]. Our group has described certain parameters which may render a patient at a higher risk of ESS with maximal

medical therapy failure, such as having had prior ESS for CRS, elevated serum IgE (≥150 kIU/L) and/or eosinophil levels (≥500 cells/mm), a young age (≤38), Gram-negative bacteria in the sinonasal flora, or intraoperative findings of eosinophilic mucin[2]. Findings from our group have demonstrated that prior ESS was actually one of the highest risk factors for treatment failure, with more than 80% of patients who failed ESS+BNI had had at least one ESS prior to enrollment[11]. The burden of revision ESS is also depicted in the frequency of procedures one patient may undergo in an attempt to control the disease, with our group reporting that out of 80 patients having had prior ESS, close to 45% had undergone more than 1 and as many as 5 procedures[11]. By having patients undergo revision surgery, they are placed at an increased risk of developing intra and postoperative complications, such as cerebrospinal fluid fistulas, major intra and post-operative hemorrhage, among others, which are far from negligible and are known to significantly increase in frequency in previously operated sinonasal cavities. Furthermore, it has been reported that less than 2/3 of patients undergoing ESS found that their post-operative improvement in symptoms matched their expectations[22]. Therefore, identifying therapeutic options that offer long-term disease control and/or clearance are paramount to avoid additional surgeries that not only increase complication rates, but also poorly influence patient quality of life.

In addition to the strong correlation between a history of prior ESS and treatment failure, virulent microbial pathogens such as *Staphylococcus aureus* have also been established as major factors in determining if a patient is at risk of being refractory to standard treatment for CRS. Work from our group has demonstrated that nearly half of all patients failing standard medical and surgical treatment are actively colonized with *S. aureus* at their first follow-up visit, compared to 1 in 8 patients that do well[11]. What is even more compelling is that in patients that harbored *S. aureus* in the pre-operative setting, the rate of pathogen clearance was directly correlated with

disease clearance at their first follow-up following ESS and BNI; i.e. even though pre-operative *S. aureus* is a negative predictive factor for disease clearance, if cleared followed appropriate ESS+BNI, disease clearance was highly significantly more probable. The latter effect is likely multifactorial, with one reason being the ability for this pathogen to form biofilms[23] in the sinonasal cavity, as well its ability to penetrate the mucosal epithelium[24] and form intracellular small-colony variants and evade immunity[25], rendering local and/or systemic therapies futile. Overall, the role for *S. aureus* in disease development and/or persistence is no longer suspected, but rather is an evident entity. More in depth microbiome studies are however lacking in the literature and as such have become a major focus of this thesis.

### 5. Identifying a 'birth cohort' in chronic rhinosinusitis

One of the principal issues the scientific community has faced with this disease is that little is known about the early events leading to the development of CRS. In other respiratory diseases, such as asthma and allergic disorders, birth cohorts can track a population from an early age and have helped identify causal relationships between specific risk factors and disease development[26]. Unfortunately, there is no such cohort for CRS. Therefore, identifying a CRS birth cohort was the first challenge to overcome in order to ensure that we carefully selected and defined a study population that is as homogeneous as possible and phenotypically similar as possible, while remaining in a real-world trial setting. Using the day of ESS as the starting point for patient monitoring does offer such an opportunity, as all patients included in the proposed prospective trial will have undergone the same ESS, followed by administration of the same intranasal corticosteroid with nasal saline irrigations. Unlike asthma and other atopic disorders, CRS disease can be cleared with surgery, albeit often temporary. Thus, monitoring patients after ESS can allow

us to profile changes in molecular mechanisms and the microbiome as the healing cavity regenerates epithelium and repopulates its microbial flora. These early post-ESS changes appear important to disease recurrence, as outcome appears relatively stable following the six month post-ESS time point[19]. This setting therefore offers a privileged environment to identify factors associated with disease clearance or persistence.

## 6. Macrolides: antimicrobial pharmacokinetics

Macrolides are macrocyclical lactones consisting of 14, 15, and 16-member-ringed compounds. They represent a distinct group of antimicrobials characterized by similar chemical structures, mechanisms of action and resistance, but vary in the different pharmacokinetic parameters, and spectrum of activity[27]. They represent a very large class (>2000 compounds) of both natural substances isolated from various organisms such as fungi, as well as synthetic molecules. Erythromycin is the prototype of this class of antimicrobials. Azithromycin, the drug of interest in this thesis, is a 15-membered compound derived directly from erythromycin A through 'ring expansion'. This renders azithromycin more stable, while allowing for improved activity against gram-negative bacteria[27]. Generally, macrolides are inhibitors of RNA-dependent protein synthesis, reversibly binding to the 23S ribosomal RNA in the 50S-subunit of prokaryotic ribosomes. It is postulated that macrolides inhibit protein elongation, by inhibiting the peptidyl transfer reactions causing detachment of incomplete peptide chains, overall leading to increased concentration of immature proteins[28]. Azithromycin is considered to be bacteriostatic, i.e. inhibiting bacterial proliferation, exhibiting a broad spectrum of antimicrobial activity against many gram-positive and gram-negative bacteria. Furthermore, it presents an increased activity against gram-negative bacteria, compared to erythromycin and clarithromycin. Several of these

gram-negative pathogens include *Haemophilus influenzae*, *Moraxella catarrhalis*, *Haemophilus parainfluenzae*, amongst others, which are also associated with higher disease recurrence in CRS[4].

Azithromycin is rapidly absorbed with a bioavailability estimated at 37%, producing a peak of 0.62mg/L within 2.3 hours of oral administration[27]. The drug is 50% protein bound at clinically achievable serum concentrations, although protein binding rates rapidly decrease as concentrations increase. Absorbed azithromycin is primarily found in the bile in an unchanged state, while fecal and urinary excretion account for only a minimal route of elimination[27]. The terminal half-life of azithromycin after a standard dose of 500mg once daily is 35-40 hours, making it more than suitable for once-daily dosing[29]. Additionally, azithromycin, unlike erythromycin and clarithromycin, displays extensive tissue distribution and persistence, with its half-life estimated to be at 3.2 days in nasal and pharyngeal tissue such as the tonsils[29]. Lastly, azithromycin has also been shown to have high intracellular penetration, allowing for eradication of difficult-to-treat intracellular organisms[27].

In addition to the direct effect on bacteria, antibacterial agents may also demonstrate a prolonged effect on bacterial growth, also known as Post Antibiotic Effect (PAE). PAE specifically refers to the time delay in bacterial regrowth after the antimicrobial agent is no longer present in the tissue or serum[27]. Azithromycin, in contrast to other macrolides, has a strong PAE[30], making it an excellent candidate when trying to identify novel therapeutics for a disease such as CRS; having a strong PAE allows for reduced dosage and cost, lower toxicity, and a better compliance among patients[31].

## 7. Macrolides: anti-inflammatory properties

There has been a growing interest in the anti-inflammatory and immune-modulating properties of macrolides, with low-dose, long-term regimens being prescribed to trigger an immune response, and not necessarily for their antibiotic effect. Macrolides have been studied in other chronic respiratory diseases, such as cystic fibrosis, with low-dose administration having shown to be beneficial in the long-term[32-34].

Azithromycin specifically has been shown to contain anti-inflammatory and immunomodulatory properties primarily based on the inhibition of cytokine secretion, mucus synthesis and secretion, and neutrophil migration and adhesion[35]. These effects in turn alter bacterial biofilm formation, promote inflammatory cell apoptosis, while inhibiting key inflammatory transcription factors such as nuclear factor-κB (NF-κB). Collectively, there is decreased local tissue damage by inflammatory cells such as neutrophils, which is paramount in the wound healing equilibrium required in a post-operative and highly inflammatory CRS setting. Interestingly, these immunomodulatory effects are not seen by 16-member ringed macrolides, but mainly shared by 14- and 15-member ringed agents such as clarithromycin and azithromycin, respectively[36]. Lastly, and most importantly, the immunomodulatory effects seen with these macrolides are attainable following low-dose administration, significantly decreasing the risk of toxicity or major adverse effects associated with their use.

## 8. Macrolides: adverse effects

Macrolides are generally well-tolerated antimicrobial agents. Most commonly, mild gastrointestinal tract complaints may be reported, such as stomach pain, nausea, diarrhea, and/or vomiting. However, these side effects are principally noted with erythromycin, while compounds

such as azithromycin tend to have such effects to a lesser extent. Long-term use of azithromycin has been associated with temporary hearing loss, although this remains reversible[37].

Of more important concern is the association between macrolides and their potential exacerbation of cardiac disease. Certain macrolides are known to be proarrhythmic and may be associated with an increased risk in sudden cardiac death[38]. Specifically, erythromycin and clarithromycin can increase the risk of serious ventricular arrhythmias[39,40]. Although, azithromycin was previously reported to be relatively free of cardiotoxic effects, it has been associated with a small absolute increase in sudden cardiovascular deaths, principally in patients with a high baseline risk of cardiovascular disease[41]. Furthermore, these events occurred in a patient population receiving significantly higher doses than those used in low-dose, long-term regimens. Overall, careful selection of candidates and a thorough review of medical histories, primarily cardiovascular disease, are warranted when considering the administration of azithromycin.

## IX. Research question, hypothesis, and objectives

Chronic rhinosinusitis has a major impact on quality of life, and, although most patients respond to standard medical and surgical therapy, there is an ever-increasing proportion of patients that remain refractory to treatment. Treatment options for this refractory population currently offer inadequate outcomes and may be associated with significant complications and costs. Identifying solutions with low-morbidity, low-risk, and low-cost is key to controlling this disease which affects one in ten adults. The use of macrolides for CRS has proven controversial in terms of its applicability and intended population. Administering macrolides such as azithromycin in a "one-size fits all" approach is evidently ineffective, and although low-risk, can potentially be harmful.

This thesis aims to answer the following research questions:

1) What are the characteristics of a high-risk, refractory CRS population and are there biomarkers and/or risk factors that can help predict standard treatment failure?
2) Does low-dose, long-term azithromycin offer better disease clearance than a placebo?
3) How does azithromycin modulate CRS biomarkers and the sinonasal microbiome in this high-risk, refractory population?

We hypothesized that patients with prior sinus interventions and highly virulent pathogens such as Gram-negative bacteria and *Staphylococcus aureus* would be associated with a poorer outcome post-ESS and BNI, making them candidates for a low-dose, long-term course of azithromycin. We further hypothesized that azithromycin would show clinically and statistically significantly better outcomes to placebo. Finally, we hypothesized that low-dose, long-term azithromycin would alter the sinonasal microbiome significantly through its underlying immunomodulatory properties.

Our objectives follow the overall outline of this project:

1) Prospectively follow a cohort of CRS patients at high-risk of treatment failure and assess their disease evolution from the pre-operative state, to post-ESS+BNI and evaluate epidemiological, clinical, biochemical and microbial factors associated with disease persistence.
2) Assess the efficacy of low-dose, long-term azithromycin in disease clearance in patients having failed ESS and postoperative BNI.
3) Analyze the change in symptomatological, serological, and microbial biomarkers following treatment with low-dose, long-term azithromycin in patients having failed ESS and postoperative BNI.

## X. Phase 1 – Identifying and characterizing the population at risk of treatment failure through a prospective clinical trial

The following manuscript reports the first of a two-phased clinical trial. In this work, we have recruited consecutive patients with a diagnosis of CRS undergoing ESS after failed medical therapy. Following surgery all patients received daily budesonide nasal irrigations (BNI) for 16 weeks and were re-evaluated for disease persistence (poor outcome) or clearance (good outcome).

This study was registered on Clinicatrials.gov, NCT02307825

The manuscript was published in the *Frontiers in Cellular and Infection Microbiology-Clinical Microbiology*

## XI. Manuscript 1

### TITLE PAGE

**Complete title:** *Staphylococcus aureus* on sinus culture is associated with recurrence of chronic rhinosinusitis after endoscopic sinus surgery

**Type of article:** Original contribution

**Running title:** *S. aureus* culture is associated with recurrence of CRS after ESS

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DISCLOSURES:

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This study was registered on Clinicatrials.gov, NCT02307825

**SUMMARY**

**Objectives:** Identify whether identification of S Aureus on conventional culture is a predictor of success or failure after ESS followed by budesonide nasal irrigations (BUD) in chronic rhinosinusitis(CRS) patients at high risk of recurrence.

**Methodology:** Prospective clinical trial including 116 patients from a tertiary care center at high-risk of disease recurrence following ESS+BUD. Blood samples, microbial swabs, and SNSS/SNOT-22 were taken on the day of surgery (Visit-1) and four months postoperatively (Visit-2). Outcomes were evaluated using symptoms and mucosal status as assessed by the Lund-Kennedy endoscopic score.

**Results:**

75 patients (69.4%) attained SNOT-22 MCID or higher. (Mean=33.4, range 9 to 75). Objective documentation of recurrence of disease, as defined by combined endoscopic/symptomatic criteria, was noted in 58/116 patients (50%). Revision surgery was associated with a significantly higher rate of disease recurrence (60.0% vs. 28.0%; $p<0.001$). Culture for *Staphylococcus aureus* was associated with disease recurrence, preoperatively and at 4 months post-surgery ($p=0.020$; $p<0.001$). This was restricted to post-operative cultures in the revision group (10.0 vs. 48.8%; $p<0.001$). Other factors associated with poor outcome included intolerance to non-steroidal anti-inflammatory drugs ($p=0.036$). Significantly higher Lund-Kennedy scores in the recurrence groups despite similar symptom intensity, emphasising the importance of considering objective outcome in addition to patient-reported ones.

**Conclusion:** Patients undergoing revision ESS are at high risk of disease recurrence, even when budesonide irrigations are used post operatively. Presence of *S. aureus* on culture pre-operatively or at 4 months post-ESS is associated with a negative outcome. This suggests that *S. aureus* negatively influences outcome, possibly via a number of mechanisms, including interactions with the i) immune system, ii) regeneration and repair of the sinus epithelium, or iii) via interference with the sinus microbiome. This suggests that *S. aureus* may be a simple and inexpensive biomarker for disease severity and indicates a clear need to better appreciate *S. aureus* on how it contributes mechanistically to disease development and persistence in order to develop targeted therapeutic strategies.

**Keywords:** Chronic Rhinosinusitis, Staphylococcus aureus, Revision Surgery, Endoscopic Sinus Surgery, Budesonide Nasal Irrigation

## INTRODUCTION

Chronic rhinosinusitis (CRS) is characterized as a chronic inflammation of the paranasal sinuses and is considered to be one of the most frequent chronic inflammatory diseases with approximately 10% of the population currently affected[1,16]. Surgical therapy is common with more than 400,000 sinus surgeries performed per year in the United States alone[17]. Unfortunately, up to one in three[2] patients with CRS will fail endoscopic sinus surgery (ESS) and post-operative medical therapy, rendering them refractory to maximal medico-surgical treatment. Patients with refractory disease present with reduced quality of life, poor sleep, fatigue, and acute exacerbations of their CRS, resulting in significant increases in the economic burden of the disease[18], presently estimated at $10,000 annually per refractory patient, or 12.8 billion overall in the United-States alone.

Unfortunately, despite the high costs of CRS, its pathogenesis remains elusive, which stalls the development of novel therapeutic strategies. Authors have reported various factors associated with ESS failure, whether it be anatomical anomalies or associated medical comorbidities, amongst several other reported etiologies[6]. Furthermore, our group and others have described disease parameters which may render a patient at a higher risk of revision ESS with maximal medical therapy failure, such as having had prior ESS for CRS, elevated serum IgE (≥150 kIU/L) and/or eosinophil levels (≥500 cells/mm), a young age (≤38)[7].

One of the principal issues is that little is known about the early events leading to the development of CRS. Monitoring patients prospectively following ESS may represent a means of improving our understanding. While in other respiratory diseases asthma and allergic disorders, birth cohorts which track a population from an early age have helped identify factors influencing development of disease[8] we unfortunately do not yet have 'birth cohorts' for CRS. However,

monitoring sinus cavities after ESS may represent an opportunity. CRS is different from asthma and other atopic disorders as surgical therapy is available which can clear disease. Despite the fact that this post-ESS control may only be temporary, monitoring patients after ESS can almost be considered as a birth cohort since following surgery, we can profile changes in molecular mechanisms and microbiome as the healing cavity regenerates epithelium and repopulates its microbiome. These early post-ESS changes appear important to disease recurrence, as outcome appears relatively stable following the six month post-ESS time point[5]. This setting may thus offer a privileged environment to identify factors associated with disease remission or recurrence.

We hypothesized that patients with prior sinus interventions and highly virulent pathogens such as Gram negative bacteria and *Staphylococcus aureus* would be associated with a poorer outcome post-ESS. In order to prospectively evaluate this hypothesis and further identify factors influencing disease development, we performed a clinical trial evaluating the post-ESS disease evolution of a group of CRS patients at high risk of recurrence following ESS, assessing epidemiological, clinical, biochemical and microbial outcomes following ESS for CRS.

## MATERIALS AND METHODS

### *Study design and patient population*

A prospective study was undertaken between November 2014 and December 2016 in our tertiary medical center to monitor post-ESS evolution in a cohort of CRS (with or without nasal polyposis) patients older than 18 years of age at a high-risk of disease recurrence. All surgeries were performed by the senior author (M.D.), who performed all of the post-operative management and assessments. This study was approved by the Ethics Review Board of the University of Montreal Health Center (Centre Hospitalier de l'Université de Montréal) CHUM-14.140.

All patients were recruited and informed written consent was obtained prior to the surgery. Recruitment was performed by a member of the research group not involved in the patient's care on the day of surgery on consecutive patients between the study dates who met inclusion criteria. Patients were included if they had at least one of the criteria used to qualify a patient at a "high-risk" of recurrence previously listed. Exclusion criteria included patients who had received topical or systemic antibiotic up to 4 weeks prior to the surgical intervention. In addition, patients with cystic fibrosis, inverted papilloma, osteoma, cystic masses, mucoceles, skull base lesions, or any other sinonasal tumors were excluded from the study.

All patients were managed with mucosal- and middle-turbinate sparing endoscopic sinus surgery as required for disease clearance, which usually involved complete sphenoethmoidectomy and frontal sinusotomy. Septoplasty was performed as required for access, usually using a targeted endoscopic technique. All patients received broad-spectrum antibiotics and an oral prednisone taper for 14 days following surgery. Patients were seen for cavity cleaning at 14 days (+/- 3 days), at which point post-operative once-daily nasal irrigations with 1.0 mg budesonide ampules (BUD) (Pulmicort Respules, AstraZeneca, Mississauga, ON, Canada) was initiated and continued throughout the rest of the four-month observation period.

Assessments were performed on the day of surgery (Visit 1) and repeated at four months postoperatively (Visit 2).

***Patient evaluation***

All patients had the following laboratory tests at recruitment and at four-month follow-up: total IgE, High Sensitivity C-Reactive Protein (hsCRP) and complete blood count (CBC). Serum was also retrieved and preserved for future analyses at every visit. Patient-centered outcomes were

assessed using the Sino-Nasal Symptom Score (SNSS) as well as the Sino-Nasal Outcome Test (SNOT-22)[9] questionnaires. Endoscopic evaluation of the sinonasal cavities was performed at Visit 2 and scored using the modified Lund-Kennedy scoring system[10].

***Definition of disease recurrence vs. remission***

In order to minimize variability of assessment due to varied patient perceptions of disease, we used a rigorous definition using endoscopic assessment of mucosal disease. We based this concept on the criteria used for diagnosis of CRS, where symptoms alone cannot be relied upon and objective evidence of disease must be present. We thus defined 'disease remission' as absence of mucosal disease on endoscopy. Absence of disease was defined endoscopically as oedema ≤1 on the modified Lund-Kennedy mucosal grading scale. Any evidence of recurrence of polyposis was deemed to have recurrent disease.

***Bacteriology***

Swab culture sampling was performed at the beginning of every ESS and at Visit 2 at the level of the ethmoid bulla using a thin aluminum wire swab with a mini-tip swab (BBL Cultureswab PLUS – BD Diagnostics Inc., Franklin Lakes, NJ) under direct rigid endoscopy. Care was taken to avoid contaminating the swab by touching the nasal vestibule or cavity wall. Samples were processed by the hospital laboratory where they were plated and streaked for aerobic bacteria isolation on a mannitol salt agar, a chocolate Haemophilus agar, and a MacConkey agar (Oxoid Inc, Nepean, ON). Anaerobic bacteria isolation was performed on a Brucella Agar with 5% Sheep blood and in a Schaedler Anaerobe Broth (Oxoid Inc, Nepean, ON).

***Statistical analyses***

All data were tabulated using Microsoft Excel and all statistical analyses were performed using STATA 13.1 (STATACorp LP, College Station, TX). Quantitative variables are presented to describe patient medical and surgical history as well as associated pathologies. A two-tailed Pearson Chi-square or Fisher's exact tests were used to analyze the prevalence and proportion of demographic variables and specific bacterial strains between patients. SNSS and SNOT-22 scores as well as laboratory values amongst patients before and after ESS were evaluated using a two-sample Student T-test with unequal variances. A Pearson correlation coefficient was used to evaluate any clinically significant correlation between continuous variables. The differential relative abundance of any bacterial species between culture types was measured using a Wilcoxon signed-rank test. For all statistical analyses, a $p<0.05$ was considered statistically significant.

**RESULTS**

Between November 2014 and December 2016, a total of 116 high-risk patients meeting our inclusion criteria, interested in participating and available for follow-up underwent ESS for CRSwNP or CRSsNP. Demographics are found in Table 1. Overall, patients responded well to ESS with a clinically and statistically significant mean decrease in twelve-point SNSS and SNOT-22 scores of 3.4 and 21.3, respectively. SNSS and SNOT-22 questionnaires demonstrated a strong correlation between each other, both at Visit 1 ($r=0.762$; $p<0.001$) and Visit 2 ($r=0.761$; $p<0.001$). Using the minimal clinically important difference (MCID) for the SNOT-22 of 9.5[11], 75 patients (69.4%) attained SNOT-22 MCID or higher at four months (Mean=33.4, range 9 to 75). Preoperative SNSS and SNOT-22 scores demonstrated a moderate correlation with postoperative Visit 2 SNSS and SNOT-22 scores ($r=0.385$; $p<0.001$ and $r=0.395$; $p<0.001$, respectively). Visit

2 SNOT-22 had a small correlation with Lund-Kennedy total scores (r=0.236; p=0.014), while Visit 1 SNSS and SNOT-22, and Visit 2 SNSS scores had no correlation with Lund-Kennedy Visit 2 scores.

***Disease Remission versus Recurrence***

Using our strict criteria for disease, recurrence post ESS+BUD was significantly associated with patients undergoing revision ESS (p=0.001), or with an allergy/intolerance to non-steroidal anti-inflammatory drugs (NSAID) (p=0.036) (Table 2). While there was a trend towards higher failure rates in patients with asthma (p=0.080) or CRS without polyposis (p=0.061), this did not attain significance. No laboratory values were significantly different between patients having disease remission or recurrence post-ESS. Furthermore, there was no statistically significant difference between the 'remission' and 'recurrence' groups with regard to the SNSS and SNOT-22 scores at Visit 2 (p=0.306 and p=0.098, respectively).

Bacterial swabs demonstrated a statistically significant association between the presence of *Staphylococcus aureus* preoperatively and disease recurrence following ESS+BUD at four months postoperatively. The presence of *S. aureus* at Visit 2 was also significantly associated with disease recurrence. In the group with disease recurrence, 14 patients had *S. aureus* preoperatively, of which 3 (21%) had a *S. aureus*-free microbial swab postoperatively, while there were 9 (64%) who were not able to clear it and an additional 15 neo-colonizations.

***Revision ESS patients: Disease Remission vs. Recurrence***

A subgroup analysis was performed looking at only patients undergoing revision ESS for CRS (n=80) (Table 3). Of these, 35 (43.8%) had had more than one ESS for CRS prior to their

recruitment to this study (range 2 to 5). Sixty percent (n=48) showed endoscopic signs of disease recurrence at Visit 2. Again, the presence of *Staphylococcus aureus* postoperatively was significantly associated with failure. Having seasonal allergies was a minor statistically significant protective factor in this subgroup of patients. There were no significantly differences in the various laboratory values evaluated.

## DISCUSSION

Refractory CRS unresponsive to maximal medical and surgical therapy remains an important and growing burden and may even be underestimated in certain subgroups. In this study, we evaluated the outcome of primary or revision endoscopic sinus surgery followed by four months of budesonide nasal irrigations in a subgroup of patients deemed at a high risk of disease recurrence after ESS. In previous retrospective reports from our group, we had reported a recurrence rate slightly over 30%[2,4]. In this prospective trial, using well-documented rigorous endoscopic basement of mucosal disease, failure rates are seen to be higher, attaining 50% in this high risk patient population.

Patients with previous ESS were at the highest risk of treatment failure. This is of particular interest as more than 80% of patients who failed ESS+BUD had had at least one ESS prior to enrollment. This remains in line with the current literature that revision ESS is a poor prognostic factor[42,43]. The burden of revision ESS is also depicted in the frequency of procedures one patient may undergo in an attempt to control the disease. In this study, of the 80 patients having had prior ESS, close to 45% had undergone more than 1 and as many as 5 procedures. This rate is comparable to some recent studies which demonstrate a similar problematic[43]. Patients are therefore put at risk to the various intra and postoperative complications associated with ESS,

which are far from negligible and are known to significantly increase in frequency in previously operated sinonasal cavities. What remains certain is the need for additional clinically relevant therapeutic options that can offer a long-term alternate solution to revision ESS.

The most interesting finding in our prospective study was the contribution of microbes to early disease recurrence. Colonization with *Staphylococcus aureus* was present in nearly half of all patients having failed ESS+BUD at 4 months, compared to only 13% of patients with disease remission. This finding is in line with the current literature where a positive culture for *S. aureus* is described as a poor prognostic factor post-ESS[44]. Interestingly, when present preoperatively, *S. aureus* was cleared in 75% of patients with disease remission, but only 21% cleared it in the group with disease recurrence.

A role for *S. aureus* in disease development or persistence has been long suspected. In an earlier publication, we showed that recovery of *S aureus* is higher in diseased post-ESS cavities than in healthy ones[16]. This may represent isolate-specific behaviour in *S. aureus*. Our group has previously demonstrated that biofilm-forming capacity by *S. aureus* was associated with worse outcome[17], and Tan et al. have demonstrated that the presence of intraepithelial forms of small-colony variant *S. aureus* was associated with a negative outcome[18], possibly via reduction of immunity[19].

While the mechanisms for this have been postulated to include i) modulation of the immune system via enterotoxin-mediated super antigen activation of the immune system and ii) bacterial biofilm formation by *S. aureus*, recent evidence suggest that *S. aureus* may also employ other strategies, rendering it a realistic marker of disease development and a target for therapy. In addition to already described virulence strategies for *S. aureus*, our group has recently suggested two novel ones: i) An immunomodulatory effect, modulated via induction of excessively high

levels of IL-10, an anti-inflammatory cytokine[20,21], and ii) a deleterious effect on in-vitro models of epithelial regeneration and wound repair, particularly in cell cultures raised form CRS patients.[22]

What remains to be determined, and where research groups are focusing significant time, is the origin of the mucosal appearance of this pathogen, either through neo-colonization from the external environment or the nasal vestibule introduced into the ethmoid cavities at the time of surgery or via planktonic dissemination following surgical disruption of an existing *S. aureus* biofilm[44]. This does not appear to be influenced by the use of post–operative antibiotics: in a prospective trial comparing post-operative use of Chinese herbal medicine, amoxicillin, and placebo following ESS, colonization with S Aureus was present to an equal level in all three groups, with approximately one-half of all post-ESS patients showing evidence of neo-colonization with S Aureus[23]. Additionally, the question of whether some individuals are genetically predisposed to be susceptible to *S. aureus* colonization. A previous pooling-based genome wide association scan (pGWAS)[24] has suggested that variations in candidate genes implicated in bacterial engulfment and destruction as well those impacting epithelial barrier function are associated with *S. aureus* carriage in CRS patients, suggesting a potential genetically-determined basis to susceptibility.

Asthma with nasal polyposis and intolerance to non-steroidal anti-inflammatories, also known as aspirin exacerbated respiratory disease (AERD), was also a significant association to ESS+BUD failure. Of 17 patients with this diagnosis, 13 (76.5%) had disease recurrence. These findings are concordant with the current literature on this disease and complement the findings of Mendelsohn et al.[21] that demonstrated increased rates of disease recurrence and revision ESS.

Interestingly, although more elevated in the group with disease recurrence, SNSS and SNOT-22 scores were not statistically significantly different between the two groups at either time-point. Our findings did demonstrate a strong correlation between the two scores and further strengthened the comparability of the SNSS to the SNOT-22 score in evaluating active disease. However, SNOT-22 scores showed only a small correlation, although significant, to a poor Lund-Kennedy score. This discrepancy is possibly due to the self-reporting bias on subjective measures. The significantly higher Lund-Kennedy scores observed in the disease-recurrence groups despite similar symptom intensity, emphasising the importance of considering objective outcome in addition to patient-reported ones.

A potential limitation of our study can be argued to that 4 months may be considered as a short follow up period after ESS. However, several authors have previously shown that endoscopic signs of recurrence are seen as early as four months[25,26] and that these appear stable over an additional twelve month follow up period[4]. Furthermore, data on SNSS and SNOT-22 are self-reported and patients may either not accurately recollect the severity or prevalence of their symptoms leading to a recollection bias, it can also lead to a response bias due to personal perceptive influences on one's symptomatology. It is for these exact reasons that studies using subjective questionnaires require an objective measurable value, such as the Lund-Kennedy endoscopic score.

## CONCLUSION

Patients undergoing revision ESS are at high risk of disease recurrence, even when budesonide irrigations are used postoperatively. Presence of *S. aureus* on culture pre-operatively or at 4 months post-ESS is associated with a negative outcome. This suggests that *S. aureus*

negatively influences outcome, possibly via a number of potential interactions with the i) immune system, ii) regeneration and repair of the sinus epithelium, or iii) via interference with the sinus microbiome. This suggests that *S. aureus* may be a simple and inexpensive biomarker for disease severity and indicates a clear need to better appreciate *S. aureus'* mechanistic contribution to disease development and persistence in order to develop targeted therapeutic strategies. Additional studies on microbiome and sinus mucosal gene expression will help in the understanding of several persisting pathophysiologic queries.

## AUTHORSHIP CONTRIBUTION

Anastasios Maniakas: Study design; Data collection and analysis; Manuscript drafting, reviewing, final approval, accountability for all aspects of the work

Marc-Henri Asmar: Data collection and analysis; Manuscript reviewing, final approval, accountability for all aspects of the work

Axel Eluid Renteria Flores: Data collection; Manuscript reviewing, final approval, accountability for all aspects of the work

Smriti Nayan: Data collection; Manuscript reviewing; final approval, accountability for all aspects of the work

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Leandra Mfuna Endam: Data collection and analysis; Manuscript drafting; final approval, accountability for all aspects of the work

Martin Desrosiers: Study design; Data analysis; Manuscript writing, reviewing, final approval, accountability for all aspects of the work

## CONFLICT OF INTEREST

No conflict of interest

**REFERENCES**


1. Hastan D, Fokkens WJ, Bachert Cet al. Chronic rhinosinusitis in Europe--an underestimated disease. A GA(2)LEN study. Allergy 2011; 66:1216-1223.
2. Chen Y, Dales R, Lin M. The epidemiology of chronic rhinosinusitis in Canadians. The Laryngoscope 2003; 113:1199-1205.
3. Anand VK. Epidemiology and economic impact of rhinosinusitis. The Annals of otology, rhinology & laryngology Supplement 2004; 193:3-5.
4. DeConde AS, Soler ZM. Chronic rhinosinusitis: Epidemiology and burden of disease. American Journal of Rhinology & Allergy 2016; 30:134-139.
5. Rudmik L, Smith TL, Schlosser RJ, Hwang PH, Mace JC, Soler ZM. Productivity costs in patients with refractory chronic rhinosinusitis. The Laryngoscope 2014.
6. Mendelsohn D, Jeremic G, Wright ED, Rotenberg BW. Revision rates after endoscopic sinus surgery: a recurrence analysis. The Annals of otology, rhinology, and laryngology 2011; 120:162-166.
7. Nader M-E, Abou-Jaoude P, Cabaluna M, Desrosiers M. Using response to a standardized treatment to identify phenotypes for genetic studies of chronic rhinosinusitis. J Otolaryngol Head Neck Surg 2010; 39:69-75. (
8. Jackson DJ, Gern JE, Lemanske RF Jr. Lessons learned from birth cohort studies conducted in diverse environments. J Allergy Clin Immunol. 2017 Feb;139(2):379-386. doi: 10.1016/j.jaci.2016.12.941.)
9. Piccirillo JF, Merritt MG, Jr., Richards ML. Psychometric and clinimetric validity of the 20-Item Sino-Nasal Outcome Test (SNOT-20). Otolaryngol Head Neck Surg 2002; 126:41-47.

10. Psaltis AJ1, Li G, Vaezeafshar R, Cho KS, Hwang PH. Modification of the Lund-Kennedy endoscopic scoring system improves its reliability and correlation with patient-reported outcome measures. Laryngoscope. 2014 Oct;124(10):2216-23. doi: 10.1002/lary.24654. Epub 2014 Apr 2.

11. Browne JP, Hopkins C, Slack R, Cano SJ. The Sino-Nasal Outcome Test (SNOT): can we make it more clinically meaningful? Otolaryngol Head Neck Surg 2007; 136:736-741.

12. Maniakas A, Desrosiers M. Azithromycin add-on therapy in high-risk postendoscopic sinus surgery patients failing corticosteroid irrigations: A clinical practice audit. American journal of rhinology & allergy 2014; 28:151-155.

13. Lee JY, Lee SW, Lee JD. Comparison of the surgical outcome between primary and revision endoscopic sinus surgery for chronic rhinosinusitis with nasal polyposis. American journal of otolaryngology 2008; 29:379-384.

14. Philpott C, Hopkins C, Erskine Set al. The burden of revision sinonasal surgery in the UK-data from the Chronic Rhinosinusitis Epidemiology Study (CRES): a cross-sectional study. BMJ open 2015; 5:e006680.

15. Jervis-Bardy J, Foreman A, Boase S, Valentine R, Wormald P-J. What is the origin of Staphylococcus aureus in the early postoperative sinonasal cavity? International forum of allergy & rhinology 2011; 1:308-312.

16. Al-Shemari H, Abou-Hamad W, Libman M, Desrosiers M. Bacteriology of the sinus cavities of asymptomatic individuals after endoscopic sinus surgery. J Otolaryngol. 2007 Feb;36(1):43-8.

17. Bendouah Z, Barbeau J, Hamad W, Desrosiers M. Biofilm formation by Staphylococcus aureus and Pseudomonas aeruginosa is associated with an unfavourable evolution after

surgery for chronic sinusitis and nasal polyposis. Otolaryngology - Head and Neck Surgery Vol 134, Issue 6, Pages 991-996. 2006.

18. Tan NC, Cooksley CM, Roscioli E, Drilling AJ, Douglas R, Wormald PJ, Vreugde S. Small-colony variants and phenotype switching of intracellular Staphylococcus aureus in chronic rhinosinusitis. Allergy. 2014 Oct;69(10):1364-71. doi: 10.1111/all.12457. Epub 2014 Jul 29.

19. Ou JJJ, Drilling AJ, Cooksley C, et al. Reduced Innate Immune Response to a Staphylococcus aureus Small Colony Variant Compared to Its Wild-Type Parent Strain. Frontiers in Cellular and Infection Microbiology. 2016;6:187. doi:10.3389/fcimb.2016.00187.

20. Chau TA, McCully ML, Brintnell W, et al. Toll-like receptor 2 ligands on the staphylococcal cell wall downregulate superantigen-induced T cell activation and prevent toxic shock syndrome. Nature Med.2009;15(6):641-8.

21 Schwartz JS, Al-Mot S, Endam MF, Alromaih S, Madrenas J, Desrosiers M.Bacterial immune evasion via an IL-10 mediated host response, a novel pathophysiologic mechanism for chronic rhinosinusitis. Rhinology. 2017 Sep 1;55(3):227-233. doi: 10.4193/Rhin16.199.PMID: 28315920

22. Valera F, Ruffin M, Adam D, Maillé E, Ibrahim B, Berubé J, Rousseau S, Brochiero E, Desrosiers MY. Staphylococcus aureus impairs sinonasal epithelial repair: effects in CRSwNP and control subjects. Journal of Allergy and Clinical Immunology, *in press.*

23. Liang KL, Su YC, Tsai CC, Lin JS, Jiang RS, Su MC. Postoperative care with Chinese herbal medicine or amoxicillin after functional endoscopic sinus surgery: a randomized,

double-blind, placebo-controlled study. Am J Rhinol Allergy. 2011 May-Jun;25(3):170-5. doi: 10.2500/ajra.2011.25.3610.

24. Cormier C, Mfuna Endam L, Filali-Mouhim A, Boisvert P, Boulet LP, Boulay ME, Vallée-Smedja S, Bossé Y, Desrosiers M. A pooling-based genomewide association study identifies genetic variants associated with Staphylococcus aureus colonization in chronic rhinosinusitis patients. Int Forum Allergy Rhinol. 2014 Mar;4(3):207-15. doi: 10.1002/alr.21276. Epub 2014 Jan 15. PMID: 24431132

25. Amali A, Saedi B, Rahavi-Ezabadi S, Ghazavi H, Hassanpoor N. Long-term postoperative azithromycin in patients with chronic rhinosinusitis: A randomized clinical trial. American Journal of Rhinology & Allergy 2015; 29:421-424.

26. Stjarne P, Olsson P, Alenius M. Use of mometasone furoate to prevent polyp relapse after endoscopic sinus surgery. Arch Otolaryngol Head Neck Surg 2009; 135:296-302.

**Table 1. Patient demographics**

| | |
|---|---|
| **Number of patients recruited** | **116** |
| **Age (range)** | 49 (20-78) |
| **Sex (Male:Female)** | 57 M:59 F |
| **Asthma** | 75 (64.7%) |
| **Tobacco use** | |
| **Never** | 52 (44.8%) |
| **Active** | 19 (16.4%) |
| **Former** | 45 (38.8%) |
| **Polyposis** | 108 (93%) |
| **Race** | |
| **Caucasian** | 104 (89.7%) |
| **Hispanic** | 6 (5.2%) |
| **Asian** | 2 (1.7%) |
| **Arabic** | 4 (3.4%) |
| **Previous ESS** | 80 (69.0%) |
| **Number of previous ESS (if applicable)** | 1.8 (0-5) |
| **Allergies (all types)** | 90 (77.6%) |
| **Seasonal** | 44 (37.9%) |
| **NSAID** | 23 (19.8%) |
| **Total IgE kIU/L** | 239.6 (0-1600) |
| **Eosinophils cells/mm** | 431.6 (0-1390) |

**Table 2. Disease remission versus recurrence in all patients**

| **Total:** ***116 patients*** | **Remission** | **Recurrence** | **p value** |
|---|---|---|---|
| **Patients** | 58 | 58 | |
| **Age** | 49.7 | 48.0 | p=0.490 |
| **Male** | 27 (46.6%) | 30 (51.7%) | p=0.577 |
| **Female** | 31 (53.4%) | 28 (48.3%) | |
| **Asthma** | 33 (56.9%) | 42 (72.4%) | p=0.080 |
| **Previous ESS** | 32 (55.2%) | 48 (82.8%) | ***p=0.001*** |
| **Smoking** | | | |
| **Never** | 24 (41.4%) | 28 (48.3%) | p=0.591 |
| **Active** | 11 (19.0%) | 8 (13.8%) | |
| **Former** | 23 (39.6%) | 22 (37.9%) | |
| **Polyposis** | 57 (98.3%) | 51 (87.9%) | p=0.061 |
| **Race** | | | |
| **Caucasian** | 52 (89.7%) | 52 (89.7%) | p=1.000 |
| **Hispanic** | 3 (5.2%) | 3 (5.2%) | |
| **Asian** | 1 (1.7%) | 1 (1.7%) | |
| **Arabic** | 2 (3.4%) | 2 (3.4%) | |
| **Allergies (all types)** | 48 (82.8%) | 42 (72.4%) | p=0.182 |
| **Seasonal** | 27 (46.6%) | 17 (29.3%) | p=0.056 |
| **NSAID** | 7 (12.1%) | 16 (27.6%) | ***p=0.036*** |
| **S. aureus (pre-ESS)** | 4 (7.3%) | 14 (24%) | ***p=0.020*** |
| **S. aureus (post-ESS)** | 7 (13.0%) | 24 (47.1%) | ***p<0.001*** |
| **P. aeruginosa (pre-ESS)** | 1 (1.8%) | 5 (8.6%) | p=0.207 |
| **P. aeruginosa (post-ESS)** | 1 (1.9%) | 6 (11.8%) | p=0.056 |
| **Gram negative (pre-ESS)** | 13 (23.6%) | 12 (20.7%) | p=0.706 |
| **Gram negative (post-ESS)** | 6 (11.1%) | 13 (25.5%) | p=0.056 |
| **SNSS (post-ESS)** | 4.4 | 5.2 | p=0.306 |
| **SNOT22 (post-ESS)** | 25.5 | 32.6 | p=0.098 |
| **Lund-Kennedy (post-ESS)** | 0.98 | 6.3 | ***p<0.001*** |

**Table 3. Disease remission versus recurrence in revision ESS patients**

| **Total:** ***80 patients*** | **Remission** | **Recurrence** | **p value** |
|---|---|---|---|
| **Patients** | 32 (40%) | 48 (60%) | |
| **Age** | 52.9 | 50.0 | p=0.295 |
| **Male** | 16 (50%) | 26 (54.2%) | p=0.715 |
| **Female** | 16 (50%) | 22 (45.8%) | |
| **Asthma** | 22 (68.8%) | 37 (77.1%) | p=0.407 |
| **Smoking** | | | |
| **Never** | 12 (37.5%) | 22 (45.8%) | p=0.485 |
| **Active** | 8 (25.0%) | 7 (14.6%) | |
| **Former** | 12 (37.5%) | 19 (39.6%) | |
| **Polyposis** | 31 (96.9%) | 42 (87.5%) | p=0.233 |
| **Race** | | | |
| **Caucasian** | 28 (87.5%) | 44 (91.7%) | p=0.321 |
| **Hispanic** | 1 (3.1%) | 3 (6.3%) | |
| **Asian** | 1 (3.1%) | 1 (2.1%) | |
| **Arabic** | 2 (6.3%) | 0 (0.0%) | |
| **Allergies (all types)** | 28 (87.5%) | 35 (72.9%) | p=0.118 |
| **Seasonal** | 14 (43.8%) | 11 (22.9%) | ***p=0.049*** |
| **NSAID** | 4 (12.5%) | 16 (33.3%) | ***p=0.039*** |
| **S. aureus (pre-ESS)** | 3 (9.7%) | 12 (25%) | p=0.141 |
| **S. aureus (post-ESS)** | 3 (10.0%) | 20 (48.8%) | ***p<0.001*** |
| **P. aeruginosa (pre-ESS)** | 1 (3.2%) | 5 (10.4%) | p=0.395 |
| **P. aeruginosa (post-ESS)** | 0 (0.0%) | 5 (12.2%) | p=0.069 |
| **Gram negative (pre-ESS)** | 7 (22.6%) | 11 (22.9%) | p=1.000 |
| **Gram negative (post-ESS)** | 5 (16.7%) | 10 (24.4%) | p=0.560 |
| **SNSS (post-ESS)** | 4.8 | 5.3 | p=0.660 |
| **SNOT22 (post-ESS)** | 29.2 | 31.9 | p=0.619 |
| **Lund-Kennedy (post-ESS)** | 1.0 | 6.1 | ***p<0.001*** |

## XII. Serum biomarker sub-group analysis

The overall complexity around the pathophysiology of CRS, and even more so refractory CRS, is one of the most important barriers for clinicians and researchers in the field. In Section XI, we reported our findings on a large cohort of patients prospectively followed post-ESS and daily BNI. The most significant demographic risk factor identified was a history of prior ESS, while the most relevant biomarker associated with treatment failure was the presence of *S. aureus* both in a pre-operative and/or post-operative setting.

Serum biomarkers that can be easily retrieved through routine blood samples would be ideal, offering high reproducibility, generalisability, and cost-efficiency. We therefore attempted to identify additional biomarkers that may help predict disease outcome in this prospective group by evaluating commonly studied biomarkers such as high sensitivity CRP (hsCRP), total serum IgE, lymphocyte subtyping CD3, CD4, and CD8, and overall white blood cell counts, as well three biomarkers of recent interest in the field of CRS: Monocyte Chemoattractant Protein 1 (MCP1), Keratin 6A (KRT6A), and Small Proline-Rich Protein 3 (SPRR3), using enzyme-linked immunoassay (ELISA). To confidently characterize the latter three biomarkers, tissue samples were also retrieved intra-operatively to assess and compare their presence in serum versus tissue samples.

C-Reactive protein (CRP), a non-specific systemic biomarker of inflammation, is an independent prognostic factor for cardiovascular risk. High sensitivity CRP (hsCRP) is believed to represent early or low intensity chronic inflammatory processes, however neither have been used to document response to therapy in CRS patients[45]. Monocyte Chemotactic Protein 1 (MCP-1 or CCL2) is a Th2-associated chemokine, independent from eosinophils, that regulates the migration and infiltration of monocytes, memory cells, and natural killer cells. Monocytes

and macrophages produce MCP-1, stimulate IL-4 secretion, and mediate Th2 polarization of Th0 helper cells[46]. MCP-1 has been detected in CRS nasal polyps[47], with documented elevated serum levels in untreated CRS patients[48]. It has been suggested that MCP-1 may be involved in polyp development[47,49], while overexpression of MCP-1 has been reported in polyps retrieved from CRSwNP patients with AERD[50].

KRT6A (cytokeratin 6A) is a type II intermediate filament expressed in skin, corneal and several other mucosa[51], and is a marker of basal cells in the respiratory epithelium. KRT6A activity depends on extracellular matrix composition and may regulate cell migration of keratinocytes in response to epithelial damage, as seen with CRS and post-ESS mucosa[52]. Since KRT6A was previously identified as a candidate gene for CRS in a pooled genome-wide sequencing study[53], we postulated that its activity may reflect the 'barrier defect' that has been reported in the literature[54].

Small proline-rich proteins (SPRR) are a group of proteins participating in the epithelial differentiation complex (EDC) and are involved in epithelial regeneration and repair[55,56]. In atopic dermatitis, SPRR3 expression is increased in both lesional and non-lesional skin, with levels increasing proportionally with disease severity[57]. In a CRS cohort, SPRR3 gene expression was increased 8-fold when compared to a control group and was independent of atopy[56].

Patients recruited in this subgroup analysis had serum obtained for ELISA on the day of surgery and 16 weeks post-ESS and BNI by centrifuging a 4 mL sample at 2500 rpm for 8 min, with supernatant collected and stored at -80°C. Thawed plasma samples were assayed in a single batch for ELISA for human MCP-1 (BD OptEIA™, BD Biosciences, CA, USA), human KRT6A (Abcam #ab238013, Cambridge, MA, USA) and human SPRR3 (Abcam #ab218131, Cambridge, MA, USA). All procedures were done in duplicate according to the manufacturer's

instructions. Plasma MCP-1 concentrations (pg/mL), KRT6A (ng/mL) and SPRR3 (ng/mL) were extrapolated for all samples and the average value was calculated from duplicates.

Immunohistochemistry (IHC) staining for CCL2 (MCP-1) was performed on frozen sections using anti-human CCL2 antibody (Abcam, Cambridge, MA, USA). Immunofluorescence staining against KRT6A (Abcam #ab238013, Cambridge, MA, USA) and SPRR3 (Abcam #ab218131, Cambridge, MA, USA) was performed on paraffin-embedded formalin-fixed samples. This step was performed to ensure that the studied biomarkers are present in the sinonasal tissue as well.

Patients were paired according to disease outcome. A total of 26 patients from our prospectively collected trial (Phase 1) accepted to undergo the additional analyses included in this biomarker study. Thirteen had disease persistence, while the remainder had disease clearance.

**Table 2. Biomarker levels pre versus post-ESS+BNI**

*Statistically significant; CI: Confidence Interval; hsCRP: high sensitivity C-Reactive Protein; IgE: Immunoglobulin E; MCP1: Monocyte Chemoattractant Protein 1; KRT6A: Keratin 6A; SPRR3: Small Proline-Rich Protein 3

| | **Pre ESS (N = 26)** | | | **Post ESS (N = 26)** | | | |
|---|---|---|---|---|---|---|---|
| **Serum Biomarker** | n | Mean | 95% CI | n | Mean | 95% CI | P Value |
| **hsCRP (mg/L)** | 20 | 2.23 | 1.12 – 3.35 | 21 | 2.56 | 1.26 – 3.85 | 0.185 |
| **IgE (kIU/L)** | 26 | 330.81 | 63 – 598.6 | 26 | 257.65 | 55.5 – 459.8 | 0.051 |
| **CD3 (x10$^3$/μL)** | 21 | 1.49 | 1.25 – 1.74 | 20 | 1.40 | 1.2 – 1.6 | 0.413 |
| **CD4 (x10$^3$/μL)** | 21 | 0.96 | 0.77 – 1.16 | 20 | 0.87 | 0.76 – 0.97 | 0.443 |
| **CD8 (x10$^3$/μL)** | 21 | 0.48 | 0.36 – 0.6 | 20 | 0.48 | 0.33 – 0.63 | 0.191 |
| **Lymphocytes (x10$^3$/μL)** | 26 | 2.06 | 1.77 – 2.34 | 26 | 1.87 | 1.69 – 2.05 | 0.067 |
| **Eosinophils (x10$^3$/μL)** | 26 | 0.39 | 0.26 – 0.52 | 25 | 0.32 | 0.2 – 0.43 | 0.398 |
| ***Novel biomarkers (ELISA)*** | | | | | | | |
| **MCP1 (pg/mL)** | 26 | 141.83 | 110.7 – 173 | 26 | 55.41 | 40.3 – 70.5 | < 0.001 * |
| **KRT6A (ng/mL)** | 24 | 2.07 | 1.01 – 3.14 | 24 | 2.24 | 1.05 – 3.42 | 0.493 |
| **SPRR3 (ng/mL)** | 26 | 6.71 | 5.38 – 8.04 | 26 | 6.46 | 4.8 – 8.1 | 0.77 |

**Table 3. Biomarker levels according to disease outcome**

*Statistically significant; P Val [1]: Intragroup statistic (pre vs. post ESS); P Val [2]: Intergroup statistic (favorable vs. unfavorable); CI: Confidence Interval; ESS: Endoscopic Sinus Surgery; hs CRP: high sensitivity C-Reactive Protein; IgE: Immunoglobulin E; MCP1: Monocyte Chemoattractant Protein 1; KRT6A: Keratin 6A; SPRR3: Small Proline-Rich Protein 3

| | Disease Clearance | | | Disease persistence | | | |
|---|---|---|---|---|---|---|---|
| Serum Biomarker | n | Mean | 95% CI | n | Mean | 95% CI | P Val [2] |
| Pre ESS hs CRP (mg/L) | 12 | 2.26 | 0.55 – 3.96 | 8 | 2.20 | 0.5 – 3.89 | 0.97 |
| Post ESS hs CRP (mg/L) | 13 | 2.86 | 0.9 – 4.82 | 8 | 2.07 | 0.27 – 3.86 | 0.595 |
| P val [1] | | | 0.117 | | | 0.887 | |
| Pre ESS IgE (kIU/L) | 13 | 134.31 | 50.1 – 218.52 | 13 | 527.31 | -17.5 – 1072.1 | 0.545 |
| Post ESS IgE (kIU/L) | 13 | 127.62 | 56.35 – 198.9 | 13 | 387.69 | -27.2 – 802.56 | 0.724 |
| P val [1] | | | 0.929 | | | 0.013 * | |
| Pre ESS CD3 ($x10^3/\mu L$) | 13 | 1.25 | 1.04 – 1.46 | 8 | 1.9 | 1.42 – 2.37 | 0.004 * |
| Post ESS CD3 ($x10^3/\mu L$) | 12 | 1.25 | 1.04 – 1.46 | 8 | 1.62 | 1.22 – 2.01 | 0.055 |
| P value[1] | | | 0.904 | | | 0.406 | |
| Pre ESS CD4 ($x10^3/\mu L$) | 13 | 0.86 | 0.7 – 1.01 | 8 | 1.14 | 0.64 – 1.64 | 0.547 |
| Post ESS CD4 ($x10^3/\mu L$) | 12 | 0.87 | 0.72 – 1.02 | 8 | 0.86 | 0.66 – 1.06 | 0.945 |
| P val [1] | | | 0.954 | | | 0.233 | |
| Pre ESS CD8 ($x10^3/\mu L$) | 13 | 0.35 | 0.27 – 0.43 | 8 | 0.7 | 0.47 – 0.94 | 0.009 * |
| Post ESS CD8 ($x10^3/\mu L$) | 12 | 0.34 | 0.25 – 0.43 | 8 | 0.7 | 0.36 – 1.04 | 0.043 * |
| P val [1] | | | 0.542 | | | 0.982 | |
| Pre ESS Lymphocytes ($x10^3/\mu L$) | 13 | 1.8 | 1.48 – 2.12 | 13 | 2.31 | 1.84 – 2.78 | 0.062 |
| Post ESS Lymphocytes ($x10^3/\mu L$) | 13 | 1.64 | 1.45 – 1.83 | 13 | 2.09 | 1.81 – 2.37 | 0.008 * |
| P val [1] | | | 0.135 | | | 0.324 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Pre ESS Eosinophils ($x10^3/\mu L$) | 13 | 0.38 | 0.17 – 0.58 | 13 | 0.4 | 0.2 – 0.59 | 0.614 |
| Post ESS Eosinophils ($x10^3/\mu L$) | 13 | 0.33 | 0.13 – 0.53 | 12 | 0.3 | 0.16 – 0.45 | 0.852 |
| P val [1] | | | 0.247 | | | 0.661 | |
| *Novel biomarkers (ELISA)* | | | | | | | |
| Pre ESS MCP1 (pg/mL) | 13 | 144.77 | 115.2 – 174.4 | 13 | 138.89 | 78.6 – 199.2 | 0.851 |
| Post ESS MCP1 (pg/mL) | 13 | 49.61 | 34.18 – 65.04 | 13 | 61.22 | 32.98 – 89.46 | 0.762 |
| P val [1] | | | 0.001 * | | | 0.001 * | |
| Pre ESS KRT6A (ng/mL) | 12 | 1.84 | -0.04 – 3.73 | 12 | 2.3 | 0.97 – 3.63 | 0.089 |
| Post ESS KRT6A (ng/mL) | 12 | 2 | -0.24 – 4.23 | 12 | 2.47 | 1.21 – 3.74 | 0.101 |
| P val [1] | | | 0.308 | | | 0.937 | |
| Pre ESS SPRR3 (ng/mL) | 13 | 7.07 | 4.93 – 9.22 | 13 | 6.35 | 4.47 – 8.24 | 0.801 |
| Post ESS SPRR3 (ng/mL) | 13 | 5.56 | 3.73 – 7.4 | 13 | 7.36 | 4.43 – 10.29 | 0.264 |
| P val [1] | | | 0.227 | | | 0.463 | |

When comparing pre-ESS to post-ESS+BNI biomarker values, only MCP-1 showed a significant decrease in levels post-ESS. This decrease may be explained by the overall reduction in Type-2 inflammation associated with the pre-ESS heavy polyp burden. However, MCP-1 levels lacked sufficient discriminatory power to predict disease outcome and provided no insight as to why certain patients cleared their disease while their MCP-1 levels remained elevated.

The CD8+ T lymphocyte sub-population was the only studied serum biomarker that differed in concentrations depending on the outcome of the disease; there was a positive correlation between higher levels and disease clearance. Interestingly, despite its discriminative power for post-ESS outcomes, CD8+ levels did not vary between the pre and post-ESS setting,

suggesting that surgical intervention does not have a downstream effect on its CD8+ concentrations.

Overall, even though this biomarker analysis was performed on a small cohort, the attempt to identify novel biomarkers that may help predict disease outcome was deemed unsuccessful. The absence of significant changes in circulating inflammatory biomarkers suggests that mucosal changes may translate poorly to circulating serum biomarkers. Therefore, our strongest biomarker remains the presence or not of *S. aureus* in the sinonasal cavity as described in Section XI.

A comprehensive report and manuscript on this subgroup analysis has been prepared and is in the process of being submitted.

## XIII. Phase 2 – double-blind, placebo-controlled, randomized clinical trial

A total of 128 patients were ultimately enrolled and recruited in this two-phased trial, in an effort to meet the requirements of our power calculation for the Phase 2 RCT. 48 were randomized to placebo or azithromycin, three times a week, for 16 weeks. The results of this study are presented in two manuscripts – the first reports the clinical, symptomatologic, and endoscopic outcomes of the RCT, while the second (section XV) will provide an in depth analysis of the effect of ESS+BNI, with or with azithromycin, on the sinonasal microbiome.

This study was registered on Clinicatrials.gov, NCT02307825

The manuscript was published in the *International Journal of Allergy and Rhinology*

## XIV. Manuscript 2

**Title:** Azithromycin in high-risk, refractory CRS following ESS and corticosteroid irrigations: double-blind, randomized, placebo-controlled trial

**Type of article:** Original article

**Running title:** Low-dose azithromycin for refractory CRS

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DISCLOSURES:

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**ABSTRACT**

**Background**: Refractory chronic rhinosinusitis (CRS) remains a significant burden for patients, often leaving them with few therapeutic options that provide low-morbidity, long-term, and meaningful symptomatologic and endoscopic disease improvement. Macrolides have long-thought to offer both an immunomodulatory and anti-microbial effect. Our objective was to evaluate the efficacy of low-dose, long-term azithromycin in a carefully selected high-risk population failing appropriate medical therapy of budesonide nasal irrigations (BNI) and endoscopic sinus surgery (ESS).

**Methods**: A double-blind, randomized, placebo-controlled trial was completed in a single tertiary care center assessing the addition of 250mg of azithromycin, three times a week for 16 weeks in adults failing ESS followed by high-volume BNI. Associated comorbidities, as well as symptomatologic, microbiologic and serologic values were systematically collected.

**Results**: 128 patients were enrolled and underwent ESS followed by BNI. At the 4-month post-ESS visit, 48 patients showed disease persistence and were randomized to azithromycin or placebo. Overall, azithromycin compared to placebo did not show a statistically significant difference in disease clearance (54% vs. 33%, respectively)(p=0.146), although patients with disease clearance who were on azithromycin showed significantly better SNOT-22 score improvements than patients on placebo (18 vs. -0.9, respectively)(p=0.046). In a subgroup analysis excluding AERD patients, azithromycin statistically significantly improved disease clearance as compared to placebo (71% vs. 35%, respectively) (p=0.031), with a number needed to treat of 3 (2.8).

**Conclusion**: Low-dose azithromycin is a therapeutic option with few side-effects that may show favorable clinical outcomes in this difficult-to-treat population, especially if patients are AERD-negative.

This study was registered on Clinicatrials.gov, NCT02307825

**Keywords**

Chronic rhinosinusitis, Endoscopic sinus surgery, Azithromycin, Staphylococcus aureus, Aspirin-exacerbated respiratory disease, double-blind randomized placebo-controlled trial

## Introduction

Chronic rhinosinusitis (CRS) symptoms remain debilitating on a clinical level, as well as on a functional and social level, leading to loss of concentration, absenteeism[58], isolation, and depression[59], amongst others. Medical management often includes the conservative approach of daily saline irrigations and topical nasal corticosteroids, although, more often than not, patients will be subjected to the repetitive use of short-course high dose corticosteroids, and oral antibiotics. When appropriate medical therapy fails, patients generally undergo endoscopic sinus surgery (ESS), estimated to be performed approximately 400,000 times a year in the United States alone[17] and will incur a cost in excess of $20 billion per year[20] due to loss of productivity. Unfortunately, as many as one in three[2] patients with CRS may present with disease persistence following ESS and post-operative medical therapy, rendering them refractory to appropriate medico-surgical treatment.

CRS management is associated to high failure rates due to the multifactorial pathogenesis of the disease, which can influence disease progression. Identified contributing factors include high levels of eosinophilia, perennial and seasonal allergies, recurrent bacterial infections, family history of CRS, and asthma, as well as systemic diseases such as cystic fibrosis or ciliary dyskinesia. This mosaicism within the CRS population renders therapeutic development extremely challenging, as targetable phenotypes are difficult to define. Characterizing the CRS population that will be at a high risk of disease recurrence following appropriate medical and/or ESS remains an ongoing challenge. Using our group's criteria for high-risk patients[2], we demonstrated through a prospective clinical trial that presence of pre-ESS and post-ESS *Staphylococcus aureus* was a negative microbiological biomarker for disease recurrence in patients having undergone ESS followed by saline irrigations with budesonide respules (BNI)[60]. Our group further demonstrated

*S. aureus*'s deleterious effect on epithelial repair[61], which concorded to the clinical findings of the prospective trial.

As the mechanistic evolution of refractory CRS is being defined, the development of novel therapeutics is following suit, with efforts being directed towards Type 2 immunomodulating targeted therapies used in severe eosinophilic asthma[62-65]. Although there is significant excitement in the medical community for these therapies, not all patients respond, and, as with asthma and skin disease, we will continue to struggle with patients whose disease is not controlled by Type 2 biologics[66]. Furthermore, the cost for such treatments, if used on a large scale in CRS patients, will lead to a substantial surge in the already heavy economic burden of CRS[18,20,58,19]. Immunomodulation is not restricted to Type 2 monoclonal antibodies or corticosteroids, as it can be an effect of other drugs classes, such as macrolides. Specifically, azithromycin has been reported to contain anti-inflammatory and immune-modulatory properties primarily based on the inhibition of cytokine secretion, mucus synthesis and secretion, and neutrophil migration and adhesion[35]. Additionally, azithromycin has been shown to reverse the mucosal healing defect seen in CRS, even in the presence of pathogenic organisms such as *S. aureus*[61]. A randomized clinical trial reported a reduction of CRS symptom recurrence following long-term low-dose azithromycin in patients having undergone ESS[5]. Our group also demonstrated retrospectively the benefit of giving low-dose, long-term azithromycin to a high-risk CRS population post-ESS[4]. Although the macrolide of choice[67-69], and the timing and/or duration of administration vary from one study to the next, most authors agree that the key to macrolide success in CRS lies in patient and phenotype[70] selection.

We aimed to assess the efficacy of low-dose azithromycin (AZI) in clearing disease in adults having failed ESS and post-op budesonide nasal irrigations (BNI).

## Methods

This study was approved by the Ethics Review Board of the University of Montreal Health Center (Centre Hospitalier de l'Université de Montréal) CHUM-14.140.

### *Phase 1: identification of patients with disease persistence*

Consecutive patients from a single institution were recruited and an informed consent was obtained prior to the surgery. On the day of surgery (Visit 1), all patients were managed with mucosal- and middle-turbinate sparing endoscopic sinus surgery as required for disease clearance, which involved complete sphenoethmoidectomy and frontal sinusotomy. Septoplasty was performed as needed for access, usually using a targeted endoscopic technique. At the end of the procedure a lavage was performed in each of the paranasal sinuses with antibiotic solution, followed by the placement of a bioresorbable packing. All patients received broad-spectrum antibiotics and oral prednisone taper for 14 days post-ESS. Patients were seen for cavity cleaning at 14 days (+/- 3 days), at which point post-operative once-daily nasal irrigations with 1.0mg budesonide ampules (Pulmicort Respules, AstraZeneca, Mississauga, ON, Canada) was initiated and continued throughout the 16-week observation period.

### *Phase 2: randomized control trial*

Patient assessment was performed 16 weeks post-ESS (Visit 2). A randomized, double-blind, placebo-controlled trial was undertaken in the patients demonstrating disease recurrence using 250mg azithromycin, three times a week, or matching placebo. Block randomization in groups of 4 was performed by the institution's research pharmacy. The research pharmacist provided the patients with 16 weeks' worth of capsules containing either azithromycin with lactose powder or lactose powder alone (Galenova Inc., Saint-Hyacinthe, QC, Canada); total=48 capsules.

Both patients and investigators were masked to the assigned drug. Patients were instructed to administer one capsule three times a week orally (Monday-Wednesday-Friday). Patients were seen 16-weeks later (Visit 3) for repeat assessment and study completion. Capsule administration compliance was monitored at Visit 3 by the research pharmacist. All randomized patients adequately completed their 48 capsules prior to Visit 3. Trial unmasking was completed following the last recorded patient visit.

***Patient evaluation and procedures***

Patient-centered outcomes were assessed at all three visits using the Sino-Nasal Symptom Score (SNSS) and the Sino-Nasal Outcome Test (SNOT-22)[71] questionnaires. Patients with nasal polyposis, asthma, and non-steroidal anti-inflammatory drug (NSAID) hypersensitivity were classified as patients with aspirin-exacerbated respiratory disease (AERD). Rigid endoscopic evaluation of the sinonasal cavities was performed and scored using the modified Lund-Kennedy scoring system[72] at Visits 2 and 3. Total IgE, high specificity C-Reactive Protein and a complete blood count was performed at each study visit, while an extra blood sample was retrieved for serum extraction and analysis. Swab culture sampling was performed at the beginning of every surgical procedure, and every study visit thereafter. Culture swabs were performed at the level of the ethmoid bulla using a thin aluminum wire swab with a mini-tip swab (BBL Cultureswab PLUS – BD Diagnostics Inc., Franklin Lakes, NJ) under direct rigid endoscopy. Care was taken to avoid contaminating the swab by touching the nasal vestibule or cavity walls. Samples were plated and streaked for aerobic bacteria isolation on a mannitol salt agar, a chocolate Haemophilus agar, and a MacConkey agar (Oxoid Inc, Nepean, ON). Anaerobic bacteria isolation was performed on a Brucella Agar with 5% Sheep blood and in a Schaedler Anaerobe Broth (Oxoid Inc, Nepean, ON).

A second culture swab was also performed and stored for 16S ribosomal RNA microbiome analyses[73].

***Inclusion and exclusion criteria***

Patients were included if they were ≥18 years of age, had a diagnosis of CRS[74], and had at least one of the criteria used to qualify a patient at high-risk of disease recurrence: history of previous sinus surgery, sinus surgery at ≤38 years of age, absolute eosinophilia ≥500cells/mm, total serum IgE levels ≥150kIU/L, sinus culture of a gram-negative organism at any point in time, and intraoperative finding of eosinophilic mucin. Exclusion criteria included patients who had received topical or systemic antibiotics up to 4 weeks prior to ESS. In addition, patients with immunodeficiencies, cystic fibrosis, inverted papilloma, osteoma, cystic masses, mucoceles, or any other sinonasal tumors were excluded from the study. Patients with any known level of cardiovascular disease were also excluded due to the literature on azithromycin and the small, but increased, risk in cardiovascular deaths, primarily in patients with high baseline cardiovascular disease[41].

***Outcomes: disease clearance versus disease persistence***

In order to minimize variability of assessment due to varied patient perceptions of disease, we used a rigorous definition using endoscopic assessment of mucosal disease. We based this concept on the criteria used for diagnosis of CRS, where symptoms alone cannot be relied upon and objective evidence of disease must be present. Our primary outcome was therefore based on endoscopic evaluation. We defined disease clearance as strict absence of mucosal disease on endoscopy (Figure 1) as defined by the modified Lund-Kennedy mucosal grading scale. Presence of disease was defined endoscopically as edema >1. Any evidence of polyposis (1 or 2 on modified Lund-Kennedy scale) was deemed to have recurrent (Visit 2) or persistent (Visit 3) disease.

Secondary outcomes included the evaluation of patient reported outcomes, sinonasal culture differences, and biochemical marker changes following randomization. SNOT-22 scores were assessed, and improvement was evaluated using the previously suggested minimal clinically important difference (MCID) of 8.9 points[75]. The SNSS MCID used is the previously reported value of 0.28[76].

***Statistical analysis***

Based on our previous work[4], we estimated that a sample size of 24 patients per treatment group would give 80% power (two tailed test at an α level of 0.05) to detect an effect size of 0.4 with the AZI group (disease clearance rate difference of 40%). Considering that response to ESS followed by BNI is approximately 60%[2], we estimated that we would require 150 patients enrolled in Visit 1.

Quantitative variables are presented to describe patient medical and surgical history as well as associated pathologies. A two-tailed Pearson Chi-square or Fisher's exact tests were used to analyze the prevalence and proportion of demographic variables and specific bacterial strains between patients. SNSS and SNOT-22 scores as well as laboratory values amongst patients before and after ESS were evaluated using a two-sample Student T-test, while the difference in score variability from one visit to the next was calculated using a one-way ANOVA. A Pearson correlation coefficient was used to evaluate any statistically significant correlation between continuous variables. The differential relative abundance of any bacterial species between culture types was measured using a Wilcoxon signed-rank test. Analyses were performed on the intention-to-treat population, defined as all patients who were randomly assigned. Data were analyzed according to assigned intervention, whether received or not.

This trial was registered at Clinicatrials.gov, NCT02307825. All statistical analyses were performed using STATA 13.1 (STATACorp LP, College Station, TX) with a $p<0.05$ considered statistically significant.

**Results**

Between November 2014 and January 2017, a total of 128 patients were enrolled and underwent ESS, followed by 16 weeks of BNI. Six patients moved away and 4 were lost to follow-up. Following the post-operative evaluation on Visit 2, 48 were randomly assigned to azithromycin three times a week (n=24) or placebo (n=24) (Figure 2). Final Visit 3 assessment was completed in August 2017, which was followed by trial unmasking. All patients completed their 16-week capsule regimen without any adverse events reported, other than one patient reporting soft stool during the period of capsule intake.

Patient demographics at Visit 2 were balanced across the treatment groups (Table 1), except for a slightly larger proportion of males in the placebo group, and higher serum IgE levels in the AZI group. Most patients were asthmatic (67%) or had undergone previous ESS (79%).

***Primary Outcome***

Using the endoscopic criteria for presence of disease, 13/24 (54%) of the AZI group patients had disease clearance , compared to 8/24 (33%) in the placebo group (p=0.146), with a relative risk reduction of 0.313 (95%CI, -0.066-0.692), and a number needed to treat of approximately 5 (4.8).There was no statistically significant difference in improvement of Lund-Kennedy scores.

***Primary Outcome Subgroup Analyses***

***Asthma as a comorbidity***

Asthma was associated with a poor outcome following randomization, regardless of group, with 70% of disease persistence occurring in asthmatics, compared to 23% in non-asthmatics (p=0.004). Specifically, AZI patients without asthma had an 88% disease clearance rate, compared to 38% in asthmatics (p=0.020), whereas there was no such statistically significant association in the placebo patients (p=0.112).

***AERD as a comorbidity***

When excluding patients with AERD (7 on AZI and 4 on placebo), AZI patients had a statistically significant higher rate of disease clearance compared to placebo patients (71% vs. 35%, p=0.031), with a relative risk reduction of 0.548 (95%CI, 0.356-0.740), and a number needed to treat of approximately 3 (2.8). For patients with AERD, 6 of 7 on AZI and 3 of 4 on placebo had disease persistence.

***Secondary Outcomes***

***SNOT-22 and SNSS MCID***

Patients with disease persistence versus clearance, regardless of randomization, did not differ in Visit 3 SNSS and SNOT-22 scores (p=0.661 and p=0.852, respectively). AZI patients achieved SNOT-22 MCID significantly more often than the placebo patients (41% vs. 17%; p=0.045), regardless of disease outcome. SNOT-22 Visit 2-to-Visit 3 improvement significantly correlated with a favorable outcome in AZI group (r=0.503; p=0.017), while there was no such correlation in the placebo group (r=-0.017; p=0.940). Furthermore, when only looking at patients with disease clearance, AZI patients had a significantly larger improvement in SNOT-22 scores

compared to the placebo patients (18.0 vs. -0.9; p=0.046) (Figure 3), while achieving MCID significantly more often (64% vs. 13%; p=0.026). Although SNSS scores showed statistically significant improvement from Visit 2-to-Visit 3 in both the placebo (1.25; 4.5 MCID) and AZI (1.5; 5.4 MCID) patients with disease clearance, the difference was not statistically significant (p=0.883).

***Microbiological outcome***

Visit 2 bacterial cultures were comparable between the groups (Table 1). Overall, at Visit 3, the AZI group had a lower prevalence of *S. aureus* than the placebo group (p=0.029) (Table 2). Furthermore, disease clearance was also associated with *S. aureus* clearance in the AZI group as only 1 of 13 (8%) had *S. aureus*, compared to 6/8 (75%) patients on placebo (p=0.002).

*Pseudomonas aeruginosa* (Gram-negative) was identified in 6 patients (3 placebo and 3 AZI) at Visit 2 (Table 1). In the AZI group, 2/3 were able to clear the pathogen, and simultaneously clear their disease at Visit 3, while all 3 placebo patients were unable to clear it at Visit 3 and also had disease persistence.

*Haemophilus influenzae* (Gram-negative) was present in 3 patients at Visit 2 (1 placebo and 2 AZI). Both AZI patients cleared their *H. influenzae* at Visit 3 and had disease clearance, while the placebo patient did not clear the pathogen and also had persistent disease.

Other Gram-negative species *Enterobacter cloacae*, *Enterobacter amnigenus*, and *Pantoea agglomerans*) were identified at Visit 2 (in 3 patients (1 placebo and 2 AZI). Both AZI patients cleared the pathogens, and one also had disease clearance, while the placebo patient cleared the pathogen but remained with persistent disease.

***Unblinded, open-label trial administration of azithromycin***

Ten patients initially included in Phase 1 of the study that were unable or unwilling to participate in the randomization received 16-week low-dose AZI in an unblinded manner at Visit 2, of which 9 had disease clearance. Furthermore, of the 16 patients with disease persistence following placebo (Visit 3), 4 abstained from it, 2 were lost to follow-up, and 10 received AZI. Of the latter 10, 5 had disease clearance, for a total open-label disease clearance rate of 70% (14/20).

***Side effects during trial***

One patient reported non-troublesome soft stool while on AZI that did not require cessation of therapy.

**Discussion**

Current literature suggests that poor prognosis and poor steroid responsiveness in patients with CRS is correlated with high NF-κB activation and neutrophilic nasal polyposis[77]. While this is a seldom-studied observation in CRS, a neutrophilic phenotype in asthma is associated with a more severe form of disease, refractory to corticosteroid therapy, a condition deemed steroid-insensitive asthma[78,79]. Through the initial phase of this real-world trial, we have been able to carefully select a subgroup of CRS patients unresponsive to ESS and post-operative budesonide nasal irrigations. Monitoring patients prospectively following ESS represents a unique opportunity to evaluate changes in symptomatology, molecular mechanisms, and the microbiome as the sinonasal cavity heals itself, regenerating its epithelium and microbiome.

Although the effect was not statistically significant, adding azithromycin led to more than half of the patients showing complete clearance of disease endoscopically, reducing the risk of disease persistence by 31%. When excluding the difficult-to-treat AERD patients[80], azithromycin

demonstrated significantly better disease clearance rates, reducing the risk of disease persistence by 55%, suggesting that azithromycin should be further considered for AERD-negative patients failing appropriate medical and surgical therapy for CRS. When analyzing our study's open-label response to azithromycin, 70% of patients had disease clearance, of which 36% were patients having initially failed placebo.

For the subgroup of patients showing disease clearance, azithromycin patients also had SNOT-22 improvement equivalent to >2 MCID, while patients on placebo had unchanged SNOT-22 scores. Therefore, in this trial, using a very rigorous marker (perfect endoscopy), although the effect of azithromycin may not be statistically significantly better compared to placebo, it may be clinically significant. These findings differ with the recent meta-analysis on macrolides in CRS patients which showed no difference in SNOT-22 improvement when compared to placebo[69].

On a microbiological level, our findings suggest that azithromycin patients that do well are able to not only clear their disease endoscopically but appear to be able to clear unfavorable pathogens such as *S. aureus, P. aeruginosa,* and *H. influenzae*. Interestingly, patients on placebo who had disease clearance were unable to clear their *S. aureus*, suggesting that they remain at an increased risk of disease recurrence[44] compared to the azithromycin group. The mechanism behind *S. aureus*'s CRS pathogenesis and tendency for poor prognosis has been described through various hypotheses, whether it be its biofilm-forming capacities[23], its intraepithelial presence[24], its potential for immunity reduction[25], or its impairment of epithelial repair[61], amongst others. What remains evident is that *S. aureus* is a strong marker for CRS outcome, both pre and post-ESS[60]. The benefit of adding azithromycin, and its capacity to influence *S. aureus* clearance, is demonstrated clinically in this study, while our group also previously showed that the mechanism behind this effect may be associated to the CRS epithelium healing properties azithromycin has

through its rho-kinase inhibitor-like properties, even in the presence of *S. aureus*[61]. Furthermore, we recently reported with an in-depth 16S rRNA microbiome analysis that patients on a low-dose azithromycin regimen showed a significant decrease in the abundance of sinonasal *S. aureus* variants, while patients on placebo instead showed a decrease in key species regulating a healthy sinonasal microbiome, such as *Bacteroides vulgatus* and *Lachnospiraceae spp*, resulting in a significant decrease in microbial diversity which may be in part responsible of disease persistence[73]. Overall, we can postulate that azithromycin's antibacterial, anti-inflammatory, and immunomodulating characteristics may play an important role in clearing key virulent sinonasal pathogens when given at a low-dose and long-term, providing an improved barrier and modified ecological niche.

Our study had certain limitations. Studying a high-risk population known to recur soon after ESS may lead to a strict selection bias, however it does allow us to adequately phenotype CRS patients, identifying the subgroups who will likely respond to azithromycin. Furthermore, using the SNSS and SNOT-22 questionnaires may lead to a recollection bias as they are self-reported, and patients may either not accurately recollect the severity or prevalence of their symptoms. They can also lead to a response bias due to personal perceptive influences on one's symptomatology. It is for these reasons that we emphasized our study outcomes primarily on objective and measurable values, such as the Lund-Kennedy endoscopic sore. Failure to reach statistical significance when comparing overall azithromycin to placebo group outcomes was likely due to the underpowered nature and the large effect size estimated for our study, as well as the higher population of AERD patients in the azithromycin group, leading to a potential Type 2 error. Once the AERD patients were removed from the analyses, azithromycin was shown to have a significant effect on disease outcome. Finally, the decision to administer azithromycin must be

made following careful evaluation of the patient's cardiovascular disease status due to prior reports of a small absolute increase in deaths in patients with high cardiovascular baseline risk[41], although these studies studied significantly higher individual doses than the regimen used in this trial. Also, minor side-effects such as loose stool and indigestion should be monitored and reported if they develop.

In summary, we report findings from a double-blind, randomized, placebo-controlled trial of low-dose, long-term azithromycin in patients failing appropriate medical and surgical therapy, where the primary outcome was to evaluate disease clearance. Overall, the effect of azithromycin was not statistically significantly better. However, when excluding patients with AERD, disease clearance rates were significantly higher in patients on azithromycin compared to placebo, suggesting that AERD patients with refractory CRS are likely a distinct phenotype that may not represent good candidates for azithromycin therapy. Finally, unlike patients on placebo, patients on azithromycin who cleared their disease also improved on a clinical level (>2 MCID on SNOT-22, and clearance of virulent pathogens).

**References**


1. Beswick DM, Mace JC, Rudmik L, Soler ZM, DeConde AS, Smith TL. Productivity changes following medical and surgical treatment of chronic rhinosinusitis by symptom domain. International forum of allergy & rhinology 2018; 8:1395-1405.
2. Schlosser RJ, Hyer JM, Smith TLet al. Depression-Specific Outcomes After Treatment of Chronic Rhinosinusitis. JAMA otolaryngology-- head & neck surgery 2016; 142:370-376.
3. Anand VK. Epidemiology and economic impact of rhinosinusitis. The Annals of otology, rhinology & laryngology Supplement 2004; 193:3-5.
4. Rudmik L. Economics of Chronic Rhinosinusitis. Curr Allergy Asthma Rep 2017; 17:20.
5. Nader M-E, Abou-Jaoude P, Cabaluna M, Desrosiers M. Using response to a standardized treatment to identify phenotypes for genetic studies of chronic rhinosinusitis. J Otolaryngol Head Neck Surg 2010; 39:69-75.
6. Maniakas A, Asmar MH, Renteria Flores AEet al. Staphylococcus aureus on Sinus Culture Is Associated With Recurrence of Chronic Rhinosinusitis After Endoscopic Sinus Surgery. Frontiers in cellular and infection microbiology 2018; 8:150.
7. Valera FCP, Ruffin M, Adam Det al. Staphylococcus aureus impairs sinonasal epithelial repair: Effects in patients with chronic rhinosinusitis with nasal polyps and control subjects. The Journal of allergy and clinical immunology 2019; 143:591-603.e593.
8. Bachert C, Han JK, Desrosiers Met al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. Lancet 2019; 394:1638-1650.

9. Pinto JM, Mehta N, DiTineo M, Wang J, Baroody FM, Naclerio RM. A randomized, double-blind, placebo-controlled trial of anti-IgE for chronic rhinosinusitis. Rhinology 2010; 48:318-324.

10. Gevaert P, Lang-Loidolt D, Lackner Aet al. Nasal IL-5 levels determine the response to anti-IL-5 treatment in patients with nasal polyps. The Journal of allergy and clinical immunology 2006; 118:1133-1141.

11. Bachert C, Sousa AR, Lund VJet al. Reduced need for surgery in severe nasal polyposis with mepolizumab: Randomized trial. The Journal of allergy and clinical immunology 2017; 140:1024-1031.e1014.

12. Edris A, De Feyter S, Maes T, Joos G, Lahousse L. Monoclonal antibodies in type 2 asthma: a systematic review and network meta-analysis. Respiratory research 2019; 20:179.

13. DeConde AS, Soler ZM. Chronic rhinosinusitis: Epidemiology and burden of disease. American journal of rhinology & allergy 2016; 30:134-139.

14. Rudmik L, Smith TL, Schlosser RJ, Hwang PH, Mace JC, Soler ZM. Productivity costs in patients with refractory chronic rhinosinusitis. The Laryngoscope 2014.

15. Tamaoki J. The effects of macrolides on inflammatory cells. Chest 2004; 125:41S-50S; quiz 51S.

16. Amali A, Saedi B, Rahavi-Ezabadi S, Ghazavi H, Hassanpoor N. Long-term postoperative azithromycin in patients with chronic rhinosinusitis: A randomized clinical trial. American journal of rhinology & allergy 2015; 29:421-424.

17. Maniakas A, Desrosiers M. Azithromycin add-on therapy in high-risk postendoscopic sinus surgery patients failing corticosteroid irrigations: A clinical practice audit. American journal of rhinology & allergy 2014; 28:151-155.

18. Suzuki H, Ikeda K, Honma Ret al. Prognostic factors of chronic rhinosinusitis under long-term low-dose macrolide therapy. ORL J Otorhinolaryngol Relat Spec 2000; 62:121-127.

19. Cervin A, Kalm O, Sandkull P, Lindberg S. One-year low-dose erythromycin treatment of persistent chronic sinusitis after sinus surgery: clinical outcome and effects on mucociliary parameters and nasal nitric oxide. Otolaryngology - Head & Neck Surgery 2002; 126:481-489.

20. Seresirikachorn K, Suwanparin N, Srisunthornphanich C, Chitsuthipakorn W, Kanjanawasee D, Snidvongs K. Factors of success of low-dose macrolides in chronic sinusitis: Systematic review and meta-analysis. The Laryngoscope 2019; 129:1510-1519.

21. Oakley GM, Christensen JM, Sacks R, Earls P, Harvey RJ. Characteristics of macrolide responders in persistent post-surgical rhinosinusitis. Rhinology 2018; 56:111-117.

22. Piccirillo JF, Merritt MG, Jr., Richards ML. Psychometric and clinimetric validity of the 20-Item Sino-Nasal Outcome Test (SNOT-20). Otolaryngol Head Neck Surg 2002; 126:41-47.

23. Psaltis AJ, Li G, Vaezeafshar R, Cho KS, Hwang PH. Modification of the Lund-Kennedy endoscopic scoring system improves its reliability and correlation with patient-reported outcome measures. The Laryngoscope 2014; 124:2216-2223.

24. Renteria AE, Maniakas A, Mfuna LE, Asmar MH, Gonzalez E, Desrosiers M. Low-dose and long-term azithromycin significantly decreases Staphylococcus aureus in the microbiome of refractory CRS patients. International forum of allergy & rhinology 2020.

25. Desrosiers M, Evans GA, Keith PKet al. Canadian clinical practice guidelines for acute and chronic rhinosinusitis. Allergy Asthma Clin Immunol 2011; 7:2.

26. Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the risk of cardiovascular death. New England Journal of Medicine 2012; 366:1881-1890.

27. Hopkins C, Gillett S, Slack R, Lund VJ, Browne JP. Psychometric validity of the 22-item Sinonasal Outcome Test. Clinical otolaryngology : official journal of ENT-UK ; official journal of Netherlands Society for Oto-Rhino-Laryngology & Cervico-Facial Surgery 2009; 34:447-454.

28. Barnes ML, Vaidyanathan S, Williamson PA, Lipworth BJ. The minimal clinically important difference in allergic rhinitis. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology 2010; 40:242-250.

29. Valera FCP, Scrideli C, Queinoz R, Gonzaiga Tone L, Anselmo-Lima WT. NF-kappaB expression predicts clinical outcome for nasal polyposis. Rhinology 2010; 48:408-441.

30. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma : evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest 2001; 119:1329-1336.

31. Moore WC, Meyers DA, Wenzel SEet al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010; 181:315-323.

32. Stevens WW, Peters AT, Hirsch AGet al. Clinical Characteristics of Patients with Chronic Rhinosinusitis with Nasal Polyps, Asthma, and Aspirin-Exacerbated Respiratory Disease. The journal of allergy and clinical immunology In practice 2017; 5:1061-1070.e1063.

33. Jervis-Bardy J, Foreman A, Boase S, Valentine R, Wormald P-J. What is the origin of Staphylococcus aureus in the early postoperative sinonasal cavity? International forum of allergy & rhinology 2011; 1:308-312.

34. Bendouah Z, Barbeau J, Hamad WA, Desrosiers M. Biofilm formation by Staphylococcus aureus and Pseudomonas aeruginosa is associated with an unfavorable evolution after surgery for chronic sinusitis and nasal polyposis. Otolaryngology - Head & Neck Surgery 2006; 134:991-996.

35. Tan NC, Cooksley CM, Roscioli Eet al. Small-colony variants and phenotype switching of intracellular Staphylococcus aureus in chronic rhinosinusitis. Allergy 2014; 69:1364-1371.

36. Ou JJ, Drilling AJ, Cooksley Cet al. Reduced Innate Immune Response to a Staphylococcus aureus Small Colony Variant Compared to Its Wild-Type Parent Strain. Frontiers in cellular and infection microbiology 2016; 6:187.

**Table 1.** Patient demographics and clinical characteristics of the 48 randomized patients at Visit 2 (intention-to-treat population). NSAID: non-steroidal anti-inflammatory drug; AERD: aspirin-exacerbated respiratory disease; ESS: endoscopic sinus surgery; SNSS: sino-nasal symptom score; SNOT-22: sino-nasal outcome test; hsCRP: high-specificity C-reactive protein. Bacterial findings using conventional bacteriology. Asterisk (*) denotes statistical significance

| | **All patients N = 48** | **Placebo N = 24** | **Azithromycin N = 24** | **P value** |
|---|---|---|---|---|
| **Age (range)** | 47 (20-75) | 44 (20-66) | 50 (25-75) | 0.120 |
| **Male** | 25 (52%) | 16 (67%) | 9 (38%) | 0.043* |
| **Tobacco use** | | | | |
| **Never** | 23 (48%) | 12 (50%) | 11 (46%) | 0.683 |
| **Active** | 6 (13%) | 2 (8%) | 4 (17%) | |
| **Former** | 19 (40%) | 10 (42%) | 9 (37%) | |
| **Race** | | | | |
| **Caucasian** | 42 (88%) | 23 (96%) | 19 (79%) | 0.223 |
| **Hispanic** | 3 (6%) | 0 (0%) | 3 (13%) | |
| **Asian** | 1 (2%) | 0 (0%) | 1 (4%) | |
| **Arabic** | 2 (4%) | 1 (4%) | 1 (4%) | |
| **Asthma** | 33 (69%) | 17 (71%) | 16 (67%) | 0.755 |
| **Allergies (all types)** | 35 (73%) | 16 (67%) | 19 (79%) | 0.330 |
| **Seasonal** | 15 (38%) | 5 (28%) | 10 (42%) | 0.204 |
| **NSAID** | 13 (33%) | 4 (17%) | 9 (37%) | 0.173 |
| **AERD** | 11 (23%) | 4 (17%) | 7 (29%) | 0.194 |
| **CRSwNP** | 43 (90%) | 22 (92%) | 21 (88%) | 0.637 |
| **Previous ESS** | 38 (79%) | 18 (75%) | 20 (83%) | 0.477 |
| **Number of previous ESS (range)** | 2 (1-4) | 2 (1-4) | 2 (1-4) | 1.000 |
| ***S. aureus* Visit 2** | 24 (50%) | 14 (58%) | 10 (42%) | 0.248 |
| ***P. aeruginosa* Visit 2** | 6 (13%) | 3 (13%) | 3 (13%) | 1.000 |
| **Gram negative bact Visit 2** | 12 (25%) | 5 (21%) | 7 (29%) | 0.505 |
| **SNSS Visit 2 (range)** | 5.1 (0-15) | 5.0 (0-15) | 5.1 (0-14) | 0.944 |
| **SNOT22 Visit 2 (range)** | 32.5 (1-87) | 30.9 (1-87) | 34.2 (2-83) | 0.626 |
| **Lund-Kennedy Visit 2 (range)** | 6.9 (2-12) | 6.9 (2-11) | 6.9 (2-12) | 0.959 |
| **IgE kIU/L Visit 2 (range)** | 285.2 (0-2110) | 154.6 (0-1380) | 421.4 (0-2110) | 0.047* |
| **hsCRP mg/L Visit 2 (range)** | 4.3 (0.2-46.1) | 3.6 (0.54-11.6) | 5.0 (0.23-46.1) | 0.497 |

**Table 2.** Visit 3 group characteristics from the 48 randomized patients. SNSS: sino-nasal symptom score; SNOT-22: sino-nasal outcome test; hsCRP: high-specificity C-reactive protein; Bacterial findings using conventional bacteriology. Asterisk (*) denotes statistical significance

| | **All patients N = 48** | **Placebo N = 24** | **Azithromycin N = 24** | **P value** |
|---|---|---|---|---|
| ***S. aureus* Visit 3** | 15 (31%) | 11 (46%) | 4 (17%) | 0.029* |
| ***P. aeruginosa* Visit 3** | 7 (15%) | 3 (13%) | 4 (17%) | 0.683 |
| **Gram negative bact Visit 3** | 12 (25%) | 7 ((29%) | 5 (21%) | 0.505 |
| **SNSS Visit 3 (range)** | 4.6 (0-13) | 4.9 (0-13) | 4.3 (0-11) | 0.491 |
| **SNOT22 Visit 3 (range)** | 27.9 (3-89) | 29.7 (6-89) | 26.1 (3-78) | 0.558 |
| **Lund-Kennedy Visit 3 (range)** | 5.0 (2-12) | 5.4 (2-11) | 4.7 (2-12) | 0.543 |
| **IgE kIU/L Visit 3 (range)** | 348.7 (0-2320) | 232.5 (0-2320) | 443.8 (0-1980) | 0.232 |
| **hsCRP mg/L Visit 3 (range)** | 2.7 (0.2-7.0) | 2.6 (0.2-7.0) | 2.7 (0.3-6.2) | 0.845 |

## Figures

**Figure 1.** Sinonasal cavity of a ‘disease clearance’ patient. There is no evidence of polyposis, edema, or purulent discharge.

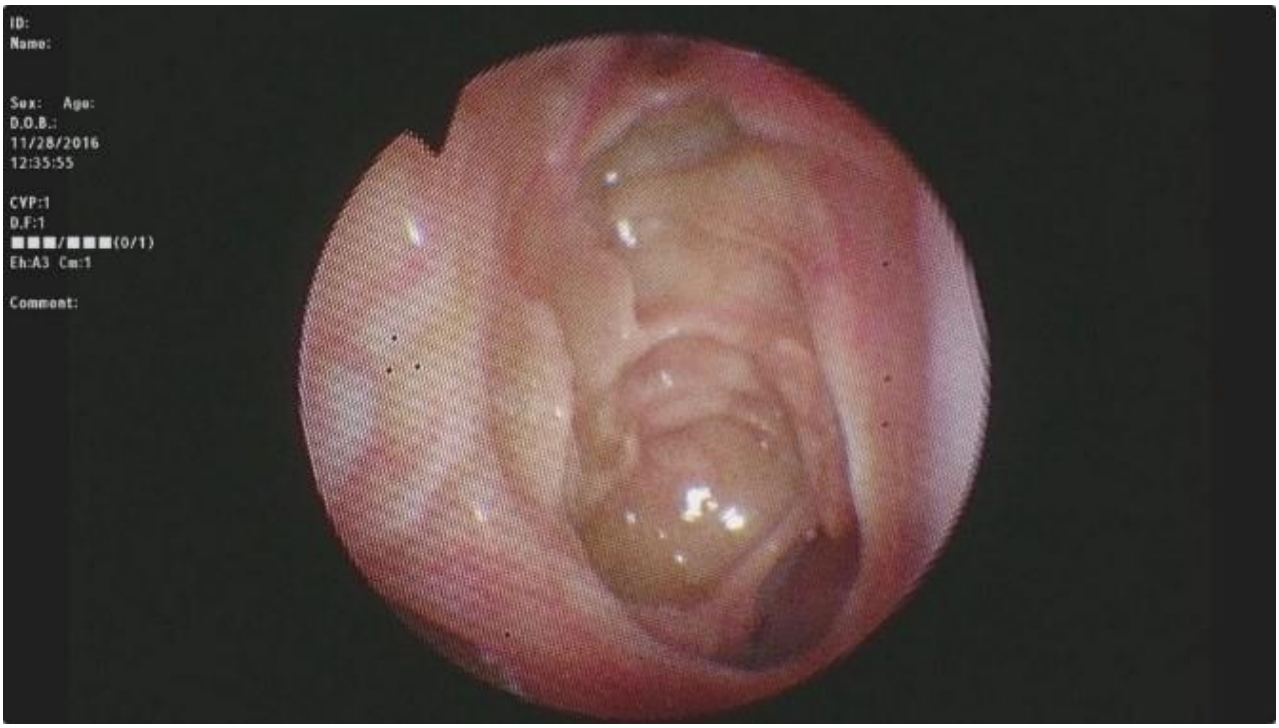


**Figure 2.** Patient trial profile. ESS=endoscopic sinus surgery; BNI=budesonide nasal irrigations

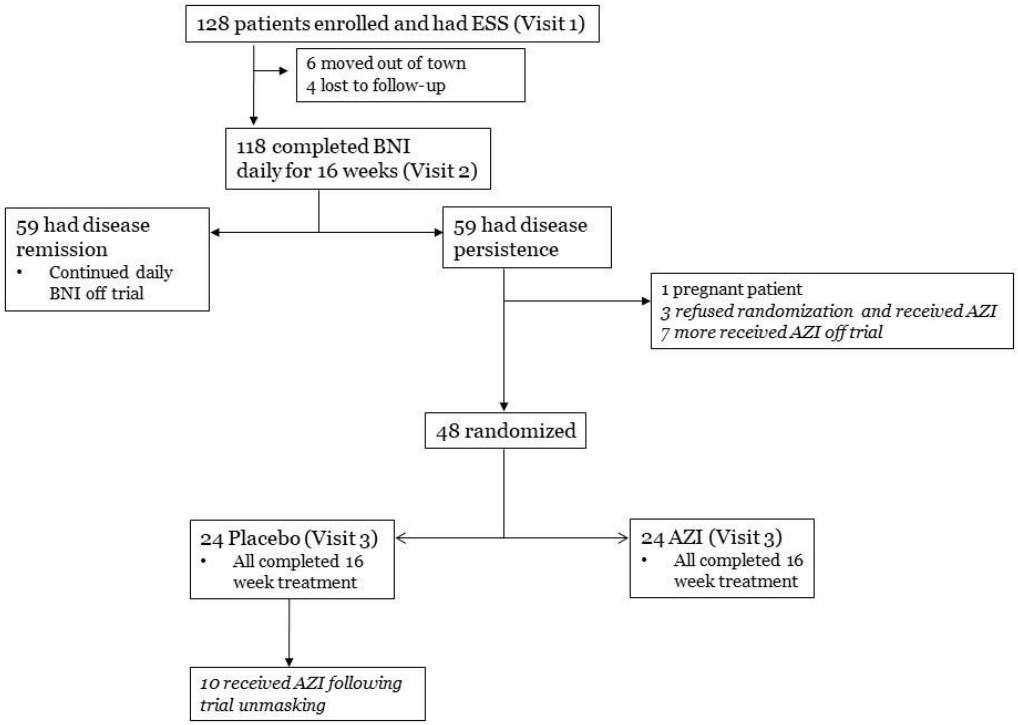
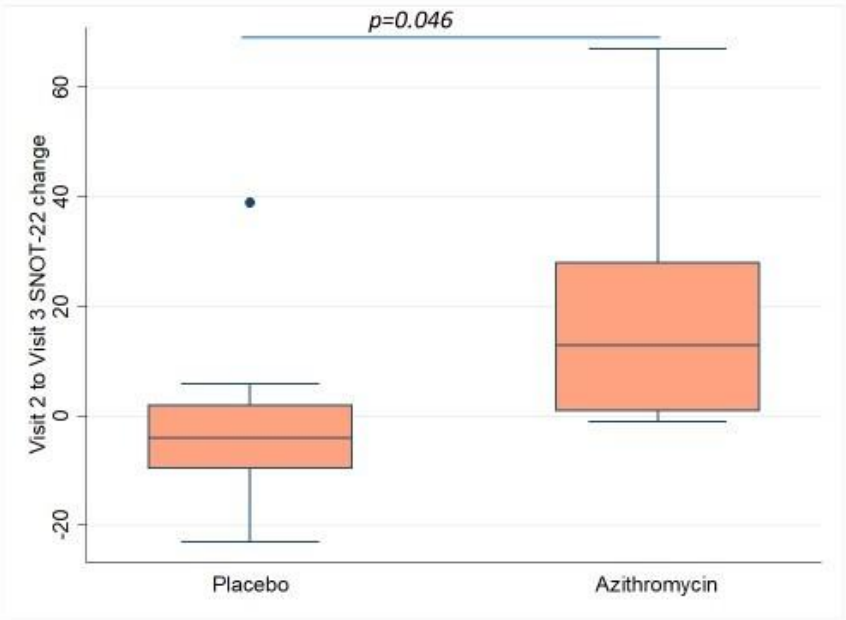


**Figure 3.** SNOT-22 score improvement between Visit 2 and Visit 3 in patients with a favorable outcome/disease clearance (Placebo=8 patients; AZI=13 patients).



## XV. Manuscript 3

**Title:** Low dose and long term azithromycin significantly decreases *Staphylococcus aureus* in the microbiome of refractory CRS patients

**Running Title:** Shift in refractory CRS microbiome following azithromycin

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**Abstract**

**Introduction**: The sinonasal microbiome is believed to play an important role in the pathophysiology of refractory chronic rhinosinusitis (CRS). We evaluated changes in the microbiome following a 4-month course of low-dose azithromycin. Assessing microbiome alterations following such a treatment may help identify underlying mechanisms of this drug.

**Method:** 48 adults with refractory CRS were enrolled in a double-blind, randomized, placebo controlled trial. Patients were randomized to 250mg of azithromycin or placebo three times weekly for 4 months. During this time, daily budesonide saline irrigations were continued. Sinonasal swabs were collected endoscopically-assisted prior to treatment initiation and at the end of it, and sent for 16S ribosomal RNA gene sequencing. High-resolution ANCHOR pipeline was used to infer and annotate putative species. The two patient groups were compared using DESeq2 differential abundance analysis.

**Results:** From initiation to the end of azithromycin treatment, patients showed a significant difference in beta diversity analysis ($P = 0.0004$) along with a significant decrease in 71 different OTUs of *Staphylococcus aureus* (FDR<0.05) obtained from the differential abundance analysis. This was not observed in placebo-treated patients. By the end of treatments, azithromycin-treated patients had a significant decrease in 29 different OTUs of *S. aureus* (FDR<0.05) when compared to placebo.

**Conclusion/Implications:** A 4-month course of 250 mg of azithromycin three times weekly in patients with refractory CRS significantly decreases *S. aureus* abundance in the sinonasal microbiome. Considering the pathogenic role of *S. aureus* in the refractory CRS population, azithromycin may constitute an additional therapeutic option to help control this disease.

## Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease with ever-growing economic burden in the Western World[81,82]. This burden reflects the chronicity of the disease which greatly affects quality of life (QOL) and productivity[81] to a greater degree than various other chronic illnesses, including cancer[83]. Among the many reasons this illness is so debilitating is its substantial proportion of patients with refractory disease[84]. In fact, it has been reported that 40% of patients will have nasal polyp recurrence within the first 6 months[85] and 20% of patients will require a revision ESS after five years[86].

The pathophysiology of this refractory CRS population remains unclear, which has stagnated the development of effective targeted therapies. Currently, ESS failures have been reported to be associated to several factors[21,87,88] that can be grouped into three major categories; epithelial defenses, immune status, and sinonasal microbiome[89]. Moreover, it has been documented that chronic inflammatory diseases, including CRS and diabetes, are associated with significant shifts in microbiomes[90,91]. In fact, the microbiome has been acknowledged to play an essential role in disease development, evolution, and persistence[90,92-94] readily interacting with its environment, notably the host's immune system[89].

Azithromycin, along with other macrolides, has been proposed as an adjunct anti-inflammatory agent in the management of CRS[4,95-97]. Given the contradictory results obtained in two clinical trials[98,99], the role of azithromycin in CRS patients is unclear[96,100]. Hence, its use in more targeted populations such as refractory CRS remain completely unexplored. Used for its anti-inflammatory, immunomodulatory, and antibacterial effects in cystic fibrosis[101] and chronic obstructive pulmonary disease[102], assessing its effect on the sinonasal microbiome may prove useful in better understanding CRS.

In this study, we analyzed the sinonasal microbiome of patients enrolled in a double-blind, randomized, placebo controlled trial, where patients were randomized to low-dose azithromycin or placebo following disease recurrence post-endoscopic sinus surgery (post-ESS). Based on the anti-inflammatory and immunomodulatory effect[103], notably its ability to activate macrophages[104], combined with its increased antibiotic concentrations in most peripheral tissues[105,106], we hypothesized azithromycin would significantly shift the sinonasal microbiome by restoring its diversity and decreasing the abundance of pathogenic bacterial species.

**Material and Methods**

This study was approved by the Ethics Review Board of the University of Montreal Health Center (Centre Hospitalier de l'Université de Montréal) CHUM-14.140.

**Study Design and Participants**

A double-blind randomized, placebo-controlled trial (ClinicalTrial.gov NCT0230782) was undertaken in our tertiary medical center to assess the effect of low-dose, long-term azithromycin in a cohort of refractory CRS patients post-ESS. All surgeries were performed by a single surgeon (M.D.), who performed all the postoperative management and assessments.

Patients were recruited and an informed written consent was obtained prior to the surgery. Recruitment was performed by a member of the research group not involved in the patient's care on the day of surgery. Patients were included if they had at least one of the following inclusion criteria that qualified them as "high-risk" for recurrence; prior ESS for CRS, elevated serum IgE (>150 kIU/L), eosinophil levels (>500 cells/mm) or a young age (>38 years) of disease presentation[85]. Exclusion criteria included patients < 18 years of age or patients who had received

topical or systemic antibiotics up to 4 weeks prior to the surgical intervention, patients with cystic fibrosis, inverted papilloma, osteoma, cystic masses, mucoceles, skull base lesions, or any other sinonasal tumors.

Patients were managed with mucosal and, when possible, middle-turbinate sparing ESS involving complete spheno-ethmoidectomy and frontal sinusotomy. No extended frontal sinus procedures (DRAF III or Lothrop) were included. Septoplasty was performed as required for access, usually using a targeted endoscopic technique. All patients received broad-spectrum antibiotics and an oral prednisone taper for 14 days following surgery. Patients were seen for cavity cleaning at 14 days (± 3 days), at which point they were all prescribed a once-daily nasal irrigation with 1.0 mg of budesonide ampules (Pulmicort Respules, AstraZeneca, Mississauga, ON, Canada) dissolved in 240ml of 0.9% saline for 4 months. Patient information and demographics was collected at the time of surgery. Assessments were performed 4 months post-operatively (pre-treatment visit) and 8 months post-operatively (post-treatment visit). On the pre-treatment visit if the patient was deemed to have disease recurrence, the patient was randomized to receive 250mg of azithromycin (Zythromax, Pfizer Canada, Montreal, QC, Canada) PO or placebo three times per week for an additional 4 months while continuing nasal irrigation with budesonide. Block randomization in groups of four was performed by the institution's research pharmacy. Patients were seen again 4 months later (post-treatment visit) for a repeat assessment and study completion. It is important to underline that patients with acute exacerbations between the pre- and post-treatment visits requiring oral or additional intranasal steroids or any antibiotics would be excluded from the analysis.

**Patient Evaluation**

The 48 randomized patients had a nasal swab taken at the level of the ethmoid bulla using a thin aluminum wire swab with a mini-tip swab (BBL Cultureswab PLUS – BD Diagnostics Inc., Franklin Lakes, NJ) under direct rigid endoscopy. This was done 4 month post-op (pre-treatment visit) prior to the initiation of the experimental treatment and at the end of the treatment, 8 months post-op (post-treatment visit) for 16S ribosomal RNA analysis. Endoscopic evaluation of the sinonasal cavities was performed at both visits and was scored using the modified Lund-Kennedy scoring system to determine disease recurrence or remission[107]. Symptoms were assessed using the Sino-nasal Outcome Test 22 item (SNOT-22)[75] also at both visits. Lund-McKay scores were obtained from pre-op sinus CT-scans.

**Study Definition of Recurrence**

To minimize variability of assessment due to the subjective perception of patient-reported disease symptoms, we used an objective standardized method in the modified Lund-Kennedy mucosal grading scale. We thus defined recurrence as total grading score of ≥4 on the modified Lund-Kennedy mucosal grading scale.

**Population demographic analysis**

Demographic characteristics among groups were analyzed using GraphPad Prism version 6.00 for Mac OS X (GraphPad Software, La Jolla, CA: www.graphpad.com) using Fischer's exact test and the student t-test according to the nature of the data (Table 1) with statistical significance set at $p < 0.05$.

**16S Ribosomal RNA Studies**

Swab cultures taken from all recruited patients at the pre- and post-treatment visits and sent to Surette Laboratory (Hamilton, ON, Canada) where DNA extraction, amplification and sequencing was performed.

**DNA Extraction, 16S rRNA gene amplification and sequencing**

Briefly, DNA was extracted from the whole swabs according to Surette Laboratory's standardized protocol. Total DNA concentration was measured with a Nanodrop 2000c Spectrophotometers (Fisher Scientific, Hampton, NH, USA). The quality of the extracted DNA was evaluated on a 1% agarose gel. DNA extracted blanks were also part of the controls.

Libraries were prepared by amplifying the V3 hypervariable region of the 16S rRNA gene based on a modified version of the libraries described by Bartram et al[108]. Primers used were GC-341F and 518R (5'-CCTACGGGAGGCAGCAG-3' (Forward), 5'-ATTACCGCGGCTGCTG-3' (Reverse)[109]. PCR amplification of the V3 region was done according to the standardized Surette Laboratory protocol. Amplicons were normalised according to the obtained concentrations prior to sequencing. Sequencing was performed on the MiSeq platform (Illumina Inc., San Diego, CA, USA) with the 250-base-pairs paired-end chemistry over 8 runs.

**Bioinformatics pipeline and amplicon processing**

Raw paired-end sequences were processed using ANCHOR bioinformatics pipeline[110]. Briefly, sequences were aligned and dereplicated before selection of operational taxonomic units (OTUs) using a count threshold of three across all samples. Annotation queried four sequence repositories with strict BLASTn criteria (>99% identity and coverage): NCBI curated bacterial and Archaea

RefSeq, NCBI nt, SILVA and Ribosomal Database Project (RDP). When the highest identity/coverage was shared amongst multiple different putative annotation, all were retained and reported; borrowed from the idea of secondary annotation in metatranscriptomics[111]. Amplicons with low-counts (<14) were binned to high-count sequences in a second BLASTn, using a lower threshold of >98% identity/coverage (secondary count capture).

**Diversity and differential abundance analysis**

To be included in the analysis, patients needed to have available samples in both pre- and post-treatment visits. A total of 36 samples were compared (an exhaustive patient sample flow chart is presented in Figure 1). Relative abundances were calculated from raw counts and samples were excluded when they had specific OTU abundances higher than three standard deviations calculated from the mean of all the samples. Alpha diversity was measured using Shannon and inverse Simpson indices within Phyloseq R package[112]. Beta diversity was estimated using Bray-Curtis dissimilarity and the Constrained Analysis of Principal Coordinates (CAP) ordination method in addition to Principal Coordinate Analysis (PCoA). Dispersion ellipses were drawn using veganCovEllipse function from Vegan package in R[113]. Significant distance was evaluated between the groups using non-parametric analysis of similarities (ANOSIM) and permutational MANOVA (PERMANOVA) on normalized counts based on Bray distances (R Vegan package). Differential abundance analysis on 16S rRNA gene amplicons was performed using DESeq2[114], which can perform well with uneven library sizes and sparsity common to 16S rRNA gene data[111,115,116]. A differential abundance selection parameter of false discovery rate (FDR; Benjamini-Hochberg procedure) <0.05 was applied. Raw counts were transformed using regularised log transformation across samples (rlog function, R phyloseq package).

## Results

*Patient demographics*

Thirty-six patients had both pre-operative and post-operative samples that were available for microbiome analysis studies. This population demographics is summarized in Table 1. The latter was mainly characterized by a high prevalence of asthma, allergies and CRS with nasal polyposis. There were no significant differences between azithromycin and placebo groups in any of the collected demographical data.

*Microbiome pre-treatment comparison*

Prior to the initiation of the experimental treatment, patients randomized to azithromycin did not show any change in beta diversity at the OTU level when compared to placebo (CAP method, ANOSIM) (Figure S1a). Alpha diversity was not significantly different between the two groups (Figure S1b). Relative abundances showed that both groups had a similar relative abundance of the *Staphylococcus* genus; 50.8% in the placebo group and 46.6% in the azithromycin group (Figure 2). Relative abundances between both groups were also found to be similar for the other genera.

*Microbiome comparison between pre- and post-treatment*

In azithromycin-treated patients, a significant change in beta diversity between was observed between the initiation (pre-treatment visit) and completion of the treatment (post-treatment visit) (CAP method, ANOSIM, P=0.0004). (Figure 3a). There were no significant changes in alpha diversity using Shannon or inverse Simpson indexes at the OTU level (Figure 3b). Relative

abundances showed a gross decrease in the *Staphyloccocus* genus from 45% to 4.7% by the end of the 4-month treatment. (Figure 4a). Differential analysis studies in azithromycin-treated patients demonstrated that 71 *S. aureus* OTUs had a significant log-fold decrease (FDR<0.05 to <0.0001) by the end of the azithromycin treatment. Twelve other species from the *Firmicutes* phylum were also significantly decreased (Figure 5). Additionally, an OTU of *Corynebacterium spp.* and *Enterobacterales spp.* were significantly increased (FDR=0.0120 and FDR=0.0421, respectively) at the end of the azithromycin treatment (Figure 5).

In the placebo group, no changes were observed between pre- and post-treatment visits with respect to alpha and beta diversity. Relative abundances showed almost half of the bacterial community was constituted of the *Staphylococcus* genus at both the pre- (51.1%) and post-treatment (47%) visits (Figure 4b). Differential analysis studies demonstrated a total of 6 OTUs within *Bacteroidetes, Firmicutes* and *Proteobacteria* phyla with a significant log-fold decrease in abundance by the end of the placebo treatment. More specifically, *Bacteroidetes vulgatus, Fusicatenibacter saccharivorans,* 2 OTUs of *Lachnospiraceae spp., Clostridiales spp. and Klebsiella pneumoniae* were included among species and genera that were decreased after a 4-month treatment with placebo (Figure 6). No change was seen in *Staphylococcus* species. Interestingly, a significantly increase in abundance of 3 OTUs of *Streptococcus spp.* was found (Figure 6).

*Microbiome post-treatment comparison*

At the end of the treatments (post-treatment visit), azithromycin- and placebo-treated groups showed two distinct communities suggesting a change in beta diversity (CAP method, ANOSIM, P=0.0958) (Figure 7a). Alpha diversity was significantly decreased in the placebo group compared

to the azithromycin group using the inverse Simpson index of diversity (P=0.0358), but not the Shannon index (P=0.1041) (Figure 7b). Relative abundances showed that the *Staphylococcus* genus was of 46.3% in the placebo group whereas in the azithromycin treated group only 4.9 % (Figure 8). Differential analysis demonstrated that 29 *S. aureus* OTUs presented a significant log-fold decrease (FDR <0.05 to <0.001) in the azithromycin group compared to placebo group (Figure 9). *Haemophilus aegyptius* and *Veillonella spp.* were also significantly decreased (FDR=0.0009 and FDR=0.029, respectively). Interestingly, *Bacteroides acidifaciens* was significantly increased (FDR=0.019) in azithromycin-treated patients along with *Corynebacterium accolens* (FDR=0.012) and an OTU of *Fusobacterium* (FDR=0.010) and *Enterobacteriaceae* (FDR=0.01).

**Discussion**

Chronic rhinosinusitis is a common chronic disease that greatly affects quality of life (QOL)[81,117] and those refractory to medical and surgical treatment are often left with few therapeutic options. It has been suggested that macrolides such as azithromycin can help control the disease through their anti-inflammatory and immunomodulatory effects. However, prior to this study, none have shown its effect on the sinonasal microbiome. Instead, most proposed interpretations come from the gastro-intestinal tract[118,119], lower respiratory tract[120,121], lungs[122] and oropharynx[123]. In this study, we report for the first time the effect of azithromycin on the sinonasal microbiome down to the OTU level of resolution.

To begin, our population demographics were comparable in both groups in all tested parameters, including alpha and beta diversity prior to the randomization process suggesting randomization was well performed.

In patients treated with azithromycin, we report a significant change in the sinonasal microbiome' beta diversity from the start to the end of this treatment. Although we did not see a change in alpha diversity, we do report a significant decrease in 71 OTUs of *S. aureus* associated with the 4-month course of azithromycin. By the end this treatment, other species, members of the *Firmicutes* phylum, were also decreased suggesting an action of azithromycin against Gram positive cocci at large.

Similarly, when comparing azithromycin- with placebo-treated patients at the end of their respective treatments (post-treatment visit), the main change seen in the differential analysis studies was also a significant decrease in 29 OTUs of *S. aureus* in the azithromycin group.

*S. aureus* has often been reported and associated with refractory CRS [60,124,125]. It is interesting to see that azithromycin may play a role in decreasing this pathogen in such a difficult-to-treat population. Its underlying anti-staphylococcal mechanism of action remains to be clarified. It may be expected that low dose macrolides act through their anti-inflammatory properties more so than their antibacterial effects[103,104]. Nevertheless, this anti-staphylococcal effect has been previously reported in low dose azithromycin treated cystic fibrosis patients[126]. One theory may be the fact that azithromycin achieves a much higher tissue than serum concentration due to its increased half-life in the former[105]. This may potentially increase the localized antibacterial effect of macrolides. An alternate theory may be azithromycin's ability to activate macrophages[104] which are known to be effective against gram positive bacteria, more specifically against *S. aureus*[127]. This would also explain the decrease in other Gram positive cocci part of the *Firmicutes* phylum.

Interestingly, *Bacteroides acidifaciens, Corynebacterium accolens,* an OTU of *Fusobacterium* and *Enterobacterales* were significantly increased in azithromycin-treated patients, compared to placebo. These microbes have been often associated to healthy sinonasal microbiomes[128-131],

suggesting azithromycin may also promote repopulation of beneficial/healthy bacteria. This was also observed when comparing the group of patients prior to receiving azithromycin (pre-treatment visit) and after its treatment completion (post-treatment visit) where *Corynebacterium spp.* and *Enterobacterales spp.* were significantly increased. Finally, *Veillonella*, a Gram-positive cocci part of the *Firmicutes* phylum and *Haemophilus spp.,* known sinonasal pathogens, were also decreased in azithromycin treated patient when compared to placebo by the end of the treatment (post-treatment visit). It is important to re-iterate that these differences were not found in placebo treated patients. Instead, an OTU of *Clostridiales*, 2 OTUs of *Lachnospiraceae, Fusicatenibacter saccharivorans*, *Bacteroides vulgatus* and *Klebsiella pneumoniae* were significantly decreased at the end of a 4-month treatment with placebo. *Lachnospiraceae spp.* and *Bacteroides vulgatus* have often been described as part of the healthy microbiome[128,131,132]. *Clostriales spp.* have been associated to maintain mucosal integrity in CRS patients[133]. Interestingly, an OTU of *Streptococcaceae* and *Streptococcus*, along with *Streptococcus anginosus* were significantly increased. All of these are known nasal pathogens[128]. Disturbing the relative abundances of these species may change their interactions and contribute to the persistence of a nasal dysbiosis.

Even though we did obtain the required number of patients to have an standard accepted power in this study, the number of samples with poor DNA quality which were not eligible for analysis was underestimated and may have lowered our statistical power. While our population was primarily composed of CRS patients with nasal polyposis, it is more and more recognized that azithromycin has a greater impact on CRS without nasal polyps than with nasal polyps[134]. Thus, the effect by azithromycin on the microbiome seen in this study may have been even larger with a better representation of CRS patients without nasal polyposis. Though no change was seen in SNOT-22 and Lund-Kennedy scores post-treatment, this must be interpreted with care as microbiome

profiles may not fully correlate with clinical disease status. This is in part due to other independent factors modulating clinical outcome such as immune regulation and barrier function. Furthermore, eight patients from the randomized clinical trial were excluded due to inadequate microbiome samples. However, findings from the completed randomized clinical trial demonstrated that low-dose azithromycin is a treatment option with few side-effects that showed favorable clinical outcomes, especially if patients were AERD-negative[135].

## Conclusion

In this study, we analyzed the microbiome of 36 CRS patients with refractory disease randomized to low-dose azithromycin or placebo for 4 months following ESS with a novel high-resolution ANCHOR pipeline. Results clearly demonstrate that by the end of a treatment with azithromycin there was a significant change in beta diversity. This was driven by a significant log-fold decrease in the abundance of 71 *S. aureus* OTUs. Furthermore, these changes were not seen in placebo-treated patients. Instead, a significant decrease in the abundance of key phyla regulating the healthy sinonasal microbiome was observed, suggesting progression of disease and nasal dysbiosis. Finally, a significant log-fold decrease of abundance in 29 *S. aureus* OTUs was seen between the azithromycin group and the placebo group at the end of their respective treatments (post-treatment visit). Although further studies are warranted to validate our findings, overall, this study reports for the first time the significant impact azithromycin may have on the sinonasal microbiome of refractory CRS patients, and further strengthens its potential as a therapeutic option in this disease.

## Tables

**Table 1. Demographic characteristic of study population**

| | Overall n = 36 | Azithromycin n =19 | Placebo n = 17 | P value |
|---|---|---|---|---|
| Age (SD) | 47.9 (12.1) | 50.4 (12.4) | 45.1 (11.6) | 0.1939[a] |
| Gender | | | | 0.7388[b] |
| Male | 17 (47.2%) | 8 (42.1%) | 9 (52.9%) | |
| Female | 19 (52.8%) | 16 (57.9%) | 14 (47.1%) | |
| Previous ESS | 30 (83.3%) | 28 (84.2%) | 37 (82.4%) | 1.0000[b] |
| Polyposis | 33 (91.7%) | 17 (89.5%) | 16 (94.1%) | 1.0000[b] |
| Asthma | 26 (72.2%) | 16 (84.2%) | 10 (58.8%) | 0.1324[b] |
| Smokers | 6 (16.7%) | 4 (21.1%) | 2 (11.8%) | 0.6617[b] |
| Allergies (all types) | 29 (80.6%) | 16 (84.2%) | 13 (76.5%) | 0.6843[b] |
| Seasonal | 18 (50%) | 8 (42.1%) | 10 (58.8%) | 0.5051[b] |
| ASA hypersensitivity | 11 (30.6%) | 8 (42.1%) | 3 (17.7%) | 0.1560[b] |
| Lund-McKay scores (SD) | | | | |
| Pre-op | 17.4 (4.7) | 17.8 (4.8) | 16.9 (4.7) | 0.5974[a] |
| SNOT-22 scores (SD) | | | | |
| Pre-treatment | 32.1 (22.9) | 34.9 (23.6) | 29.1 (22.4) | 0.4643[a] |
| Post-treatment | 27.2 (18.5) | 28.1 (21.3) | 26.2 (15.7) | 0.7848[a] |
| Lund-Kennedy Score (SD) | | | | |
| Pre-treatment | 6.2 (2.8) | 6.7 (2.9) | 5.6 (2.7) | 0.2644[a] |
| Post-treatment | 5.1 (4.2) | 5.5 (4.3) | 4.6 (4.2) | 0.5646[a] |

SD: standard deviation; ASA: acetyl salicylic acid

SNOT-22: Sino-Nasal Outcome Test

[a] Student t-test performed

[b] Fischer's exact test for independence performed

**Figure legend**

**Figure 1. Data analysis flow chart**

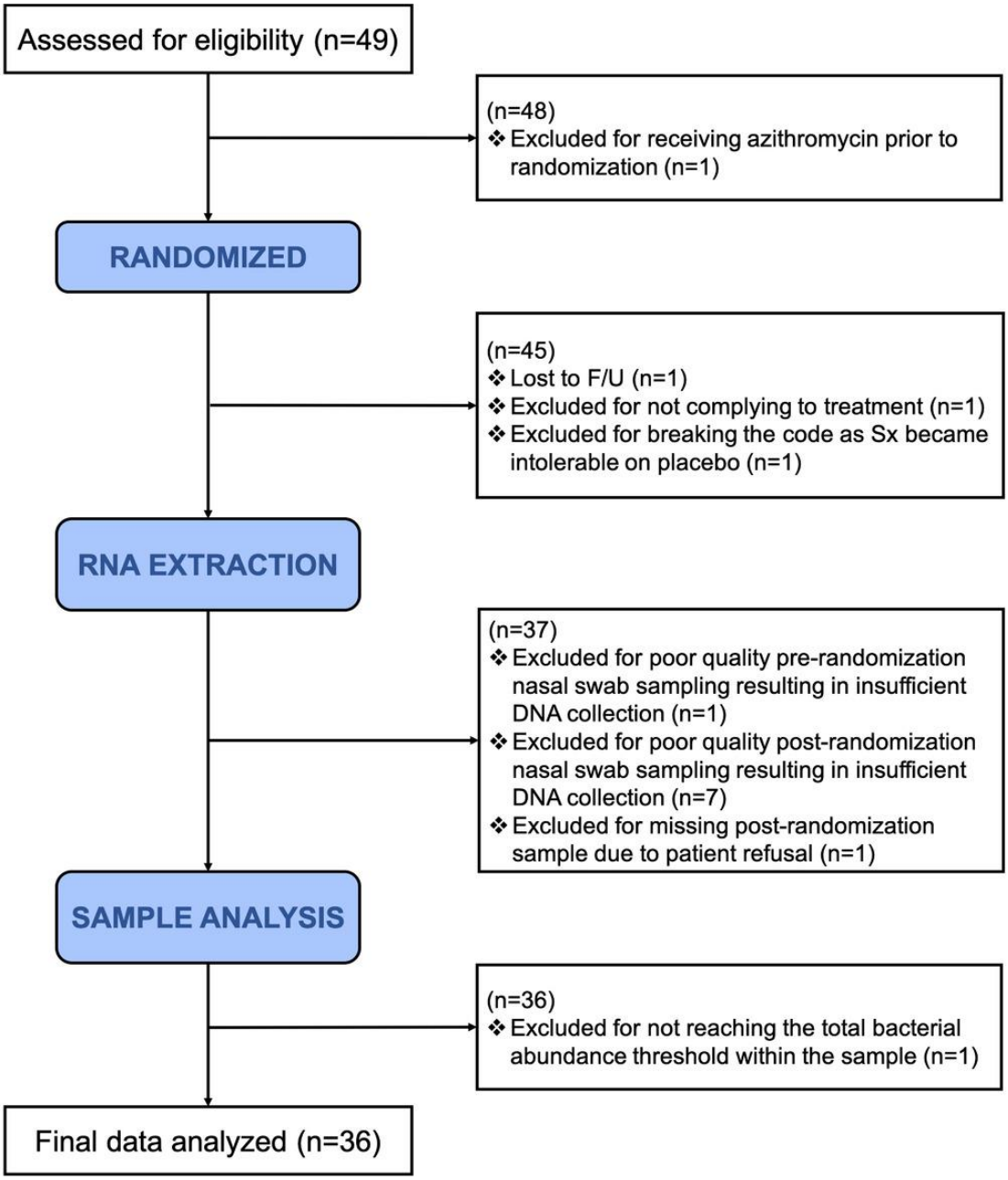


**Figure 2. Relative bacterial abundance at the pre-treatment visit between azithromycin and placebo groups.** Data is presented in percentage. The 21 most abundant genera are presented when available. All the other genera (n=76) were pooled into the "other" group.

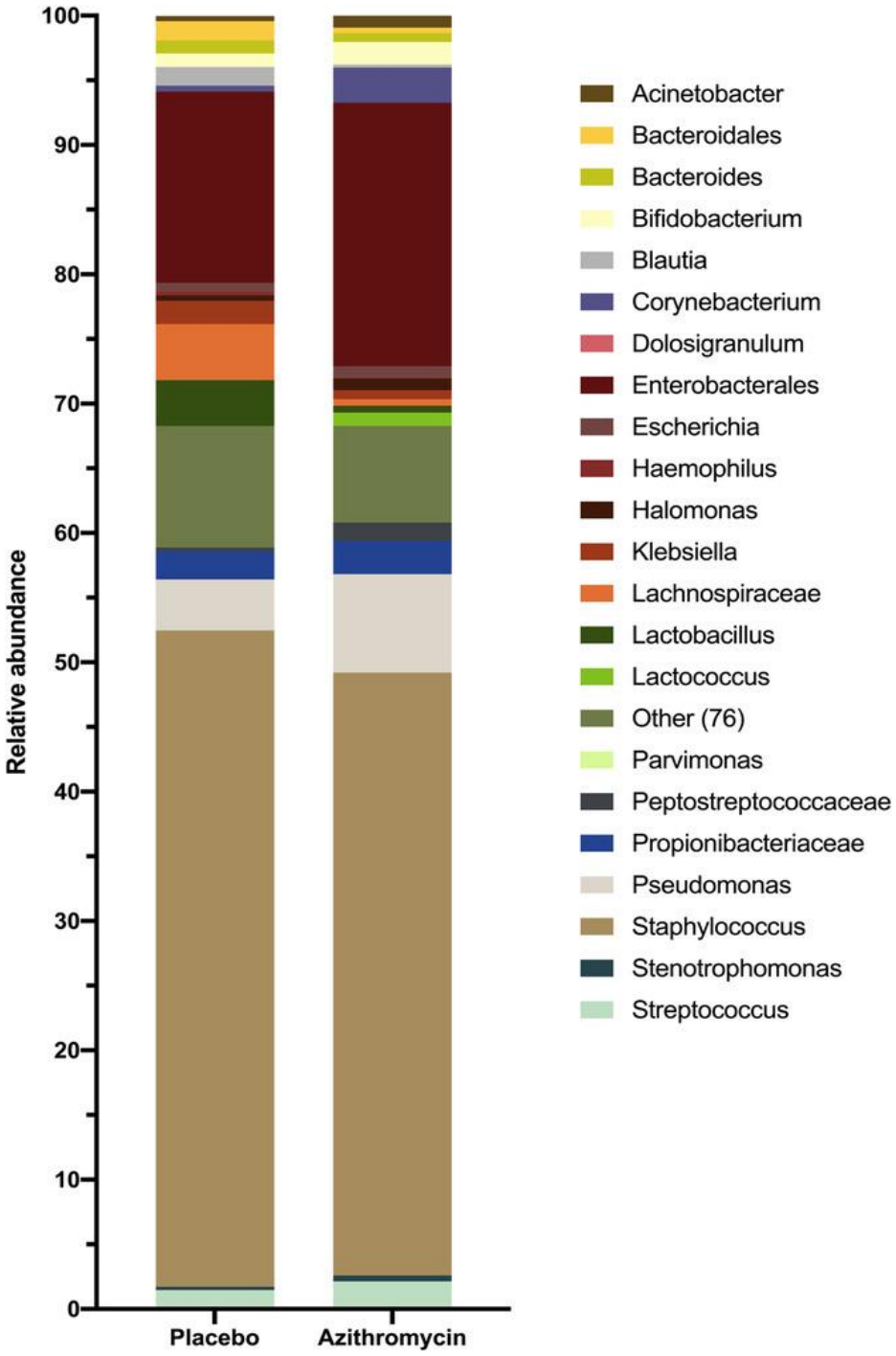


**Figure 3. Microbiome diversity studies at the OTU level between pre- and post-treatment visits in patients receiving azithromycin.** Pre-treatment visit is represented in blue and labelled "prior to treatment" and post-treatment visit in red and labelled "azithromycin" **a)** Estimated beta diversity based on Bray-Curtis dissimilarity plotted in the Constrained Analysis of Principal Coordinates (CAP) ordination plot. Analysis of Group Similarities (ANOSIM) method was used and significance was established at $P<0.05$. The percentage of variation captured in each axis is represented between brackets. Significant difference between pre- and post-treatment visits within azithromycin treated patients was found ($P=0.0004$) with percentage variation captured in axis CAP1 to 6.1% and axis MDS1 to 13.16% **b)** Alpha diversity calculated with the Shannon and inverse Simpson measure of diversity score and significance was established at $P<0.05$. Results are presented as interquartile range (IQR) from first quartile (Q1) to third quartile (Q3) with a median line. Whiskers represent the minimum and maximum, where the minimum is expressed as: Q1 -1.5*IQR and the maximum as: Q3 + 1.5*IQR. No significant change in alpha diversity with both Shannon ($P=0.967$) and the inverse Simpson ($P=0.927$) indexes was seen.

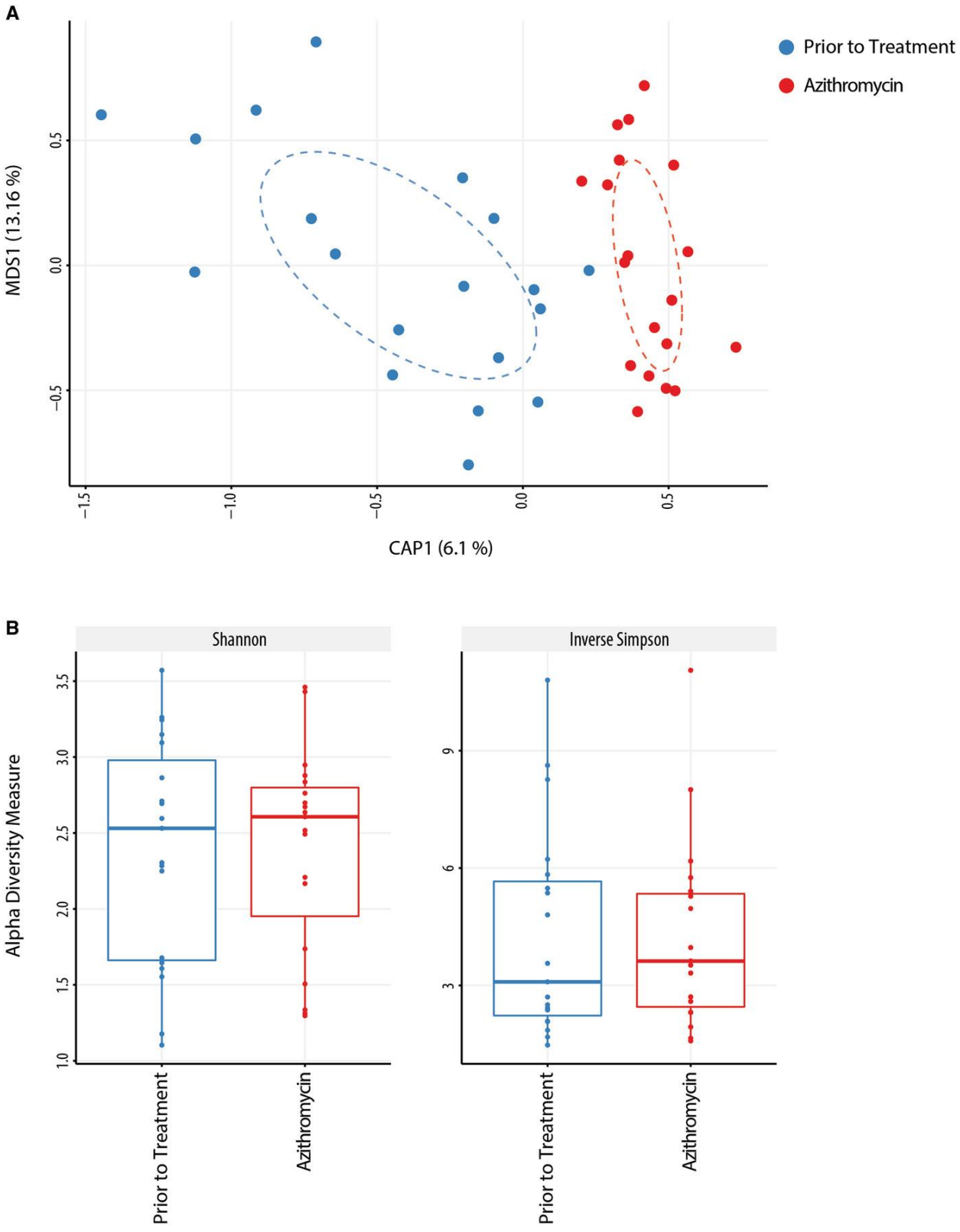

A
Prior to Treatment
Azithromycin
MDS1 (13.16 %)
CAP1 (6.1 %)
B
Shannon
Inverse Simpson
Alpha Diversity Measure
Prior to Treatment
Azithromycin

**Figure 4. Relative bacterial abundance in azithromycin and placebo treated patients.** Data is presented in percentage. Pre-treatment visit is labelled "prior to treatment" and post-treatment is labelled "azithromycin" for "a" and "placebo" for "b". The 21 most abundant genera are presented when available. All the other genera (n=75 for "a" and n=62 for "b" were pooled into the "other" group. **a)** Comparison between pre- and post-treatment visits in patients receiving azithromycin. **b)** Pre- and post-treatment visit comparison between in patients receiving placebo.

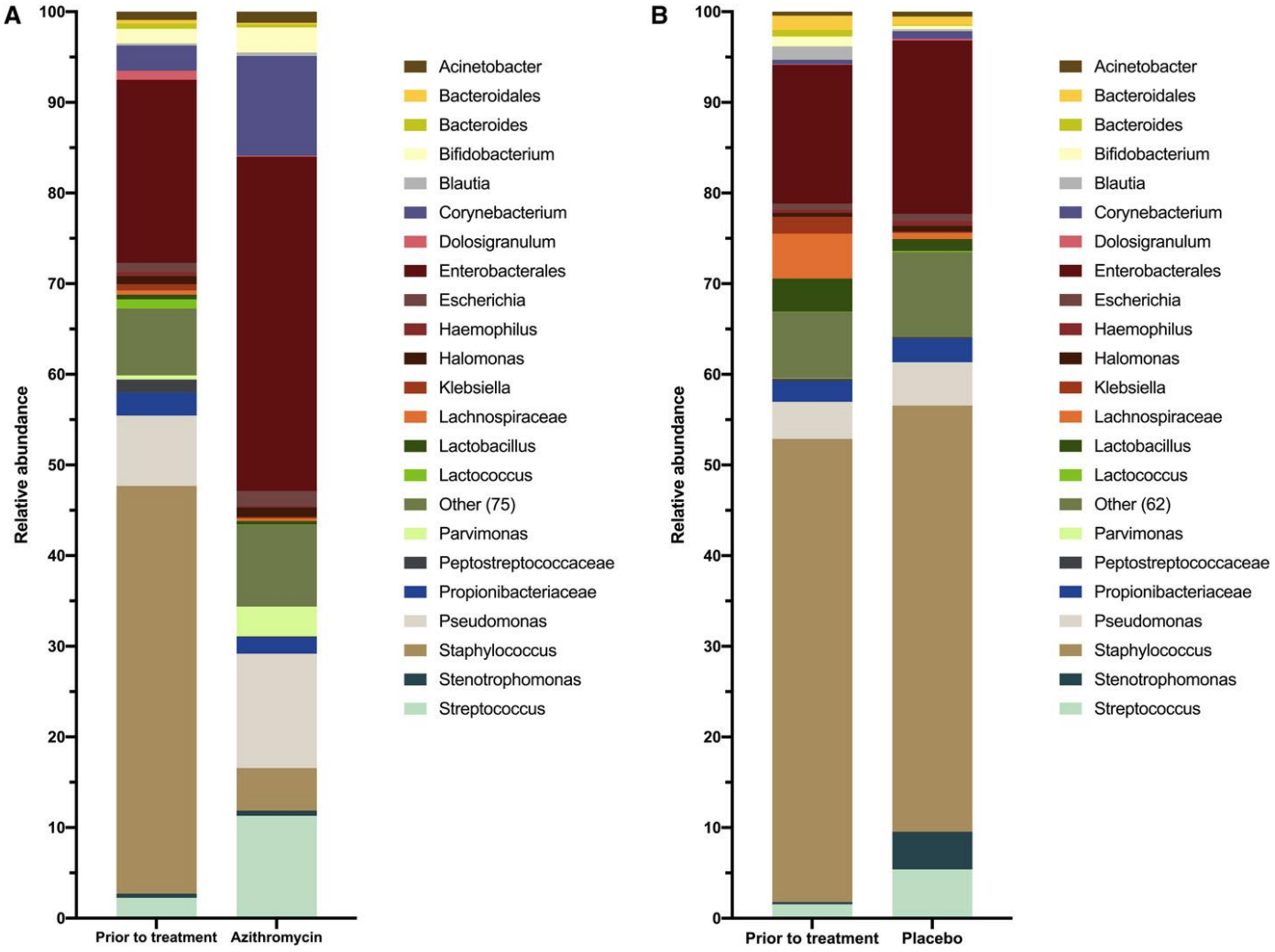


**Figure 5. Differential analysis of significant OTUs between pre- and post-treatment visits in patients receiving azithromycin.** Data is presented as the mean log-fold change between both tested groups with respect to all 110 significant OTUs. Coloured bars represent the range of upper and lower limits of standard error for the log fold change. A negative log-fold change implies a higher abundance in the pre-treatment group whereas a positive log-fold change implies a higher abundance in the post-treatment group. The dotted line separates pre-treatment with post-treatment groups with respect to log-fold abundance change. Significant differences were established at a False Discovery Rate (FDR) < 0.05. Legend represents the phylum to which each OTU belongs to. 71 *S. aureus* OTU were significantly less abundant (FDR <0.05 to < 0.0001) in azithromycin treated patients at the post-treatment visit.

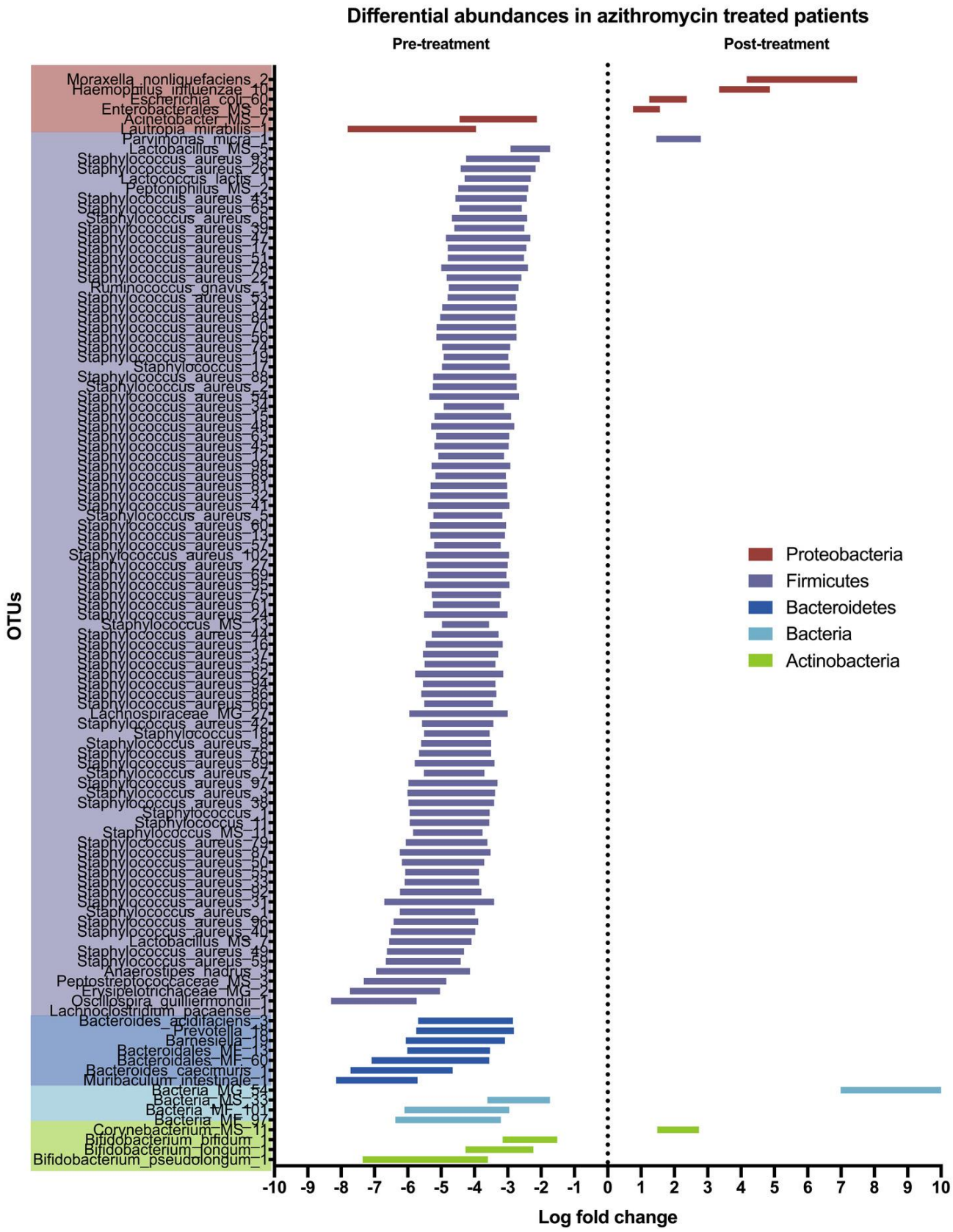

Differential abundances in azithromycin treated patients
Pre-treatment
Post-treatment
OTUs
Moraxella_nonliquefaciens_2
Haemophilus_influenzae_10
Escherichia_coli_60
Enterobacterales_MS_6
Acinetobacter_MS_7
Lautropia_mirabilis_1
Parvimonas_micra_1
Lactobacillus_MS_5
Staphylococcus_aureus_93
Staphylococcus_aureus_26
Lactococcus_lactis_1
Peptoniphilus_MS_2
Staphylococcus_aureus_43
Staphylococcus_aureus_65
Staphylococcus_aureus_6
Staphylococcus_aureus_39
Staphylococcus_aureus_47
Staphylococcus_aureus_17
Staphylococcus_aureus_51
Staphylococcus_aureus_78
Staphylococcus_aureus_22
Ruminococcus_gnavus_1
Staphylococcus_aureus_53
Staphylococcus_aureus_14
Staphylococcus_aureus_84
Staphylococcus_aureus_70
Staphylococcus_aureus_56
Staphylococcus_aureus_74
Staphylococcus_aureus_19
Staphylococcus_aureus_17
Staphylococcus_aureus_88
Staphylococcus_aureus_2
Staphylococcus_aureus_52
Staphylococcus_aureus_34
Staphylococcus_aureus_15
Staphylococcus_aureus_48
Staphylococcus_aureus_63
Staphylococcus_aureus_45
Staphylococcus_aureus_12
Staphylococcus_aureus_98
Staphylococcus_aureus_68
Staphylococcus_aureus_81
Staphylococcus_aureus_32
Staphylococcus_aureus_41
Staphylococcus_aureus_5
Staphylococcus_aureus_60
Staphylococcus_aureus_13
Staphylococcus_aureus_57
Staphylococcus_aureus_102
Staphylococcus_aureus_27
Staphylococcus_aureus_69
Staphylococcus_aureus_95
Staphylococcus_aureus_75
Staphylococcus_aureus_61
Staphylococcus_aureus_24
Staphylococcus_MS_13
Staphylococcus_aureus_44
Staphylococcus_aureus_10
Staphylococcus_aureus_35
Staphylococcus_aureus_82
Staphylococcus_aureus_94
Staphylococcus_aureus_86
Staphylococcus_aureus_66
Lachnospiraceae_MG_27
Staphylococcus_aureus_42
Staphylococcus_16
Staphylococcus_aureus_8
Staphylococcus_aureus_76
Staphylococcus_aureus_89
Staphylococcus_aureus_7
Staphylococcus_aureus_97
Staphylococcus_aureus_3
Staphylococcus_aureus_38
Staphylococcus_1
Staphylococcus_11
Staphylococcus_MS_11
Staphylococcus_aureus_79
Staphylococcus_aureus_87
Staphylococcus_aureus_50
Staphylococcus_aureus_55
Staphylococcus_aureus_33
Staphylococcus_aureus_92
Staphylococcus_aureus_31
Staphylococcus_aureus_9
Staphylococcus_aureus_96
Staphylococcus_aureus_40
Lactobacillus_MS_4
Staphylococcus_aureus_49
Staphylococcus_aureus_59
Anaerostipes_hadrus_3
Peptostreptococcaceae_MS_3
Erysipelotrichaceae_MG_2
Oscillospira_guilliermondii_1
Lachnoclostridium_pacaense_1
Bacteroides_acidifaciens_3
Prevotella_18
Barnesiella_19
Bacteroidales_MF_13
Bacteroidales_MF_60
Bacteroides_caecimuris_1
Muribaculum_intestinale_1
Bacteria_MG_54
Bacteria_MS_33
Bacteria_MF_101
Bacteria_MF_97
Corynebacterium_MS_11
Bifidobacterium_pifidum_1
Bifidobacterium_longum_1
Bifidobacterium_pseudolongum_1
Proteobacteria
Firmicutes
Bacteroidetes
Bacteria
Actinobacteria
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10
Log fold change

**Figure 6. Differential analysis of significant OTUs between pre- and post-treatment visits in patients receiving placebo.** Data is presented as the mean log-fold change between both tested groups with respect to all 9 significant OTUs. Coloured bars represent the range of upper and lower limits of standard error for the log fold change. A negative log-fold change implies a higher abundance in the pre-treatment group whereas a positive log-fold change implies a higher abundance in the post-treatment group. The dotted line separates pre-treatment with post-treatment groups with respect to log-fold abundance change. Significant differences were established at a False Discovery Rate (FDR) < 0.05. Legend represents the phylum to which each OTU belongs to. *Bacteroidetes vulgatus* (FDR=0.025), *Fusicatenibacter saccharivorans* (FDR=0.007), 2 OTUs of *Lachnospiraceae spp.* (FRD<0.004 to <0.008), *Clostridiales spp.* (FDR=0.041) *and Klebsiella pneumoniae* (FDR<0.001) were included among species and genera that were decreased after a 4-month treatment with placebo (Figure 6). No change was seen in *Staphylococcus* species. A significantly increase in abundance of 3 OTUs of *Streptococcus spp.* was found (FDR<0.008 to <0.05).

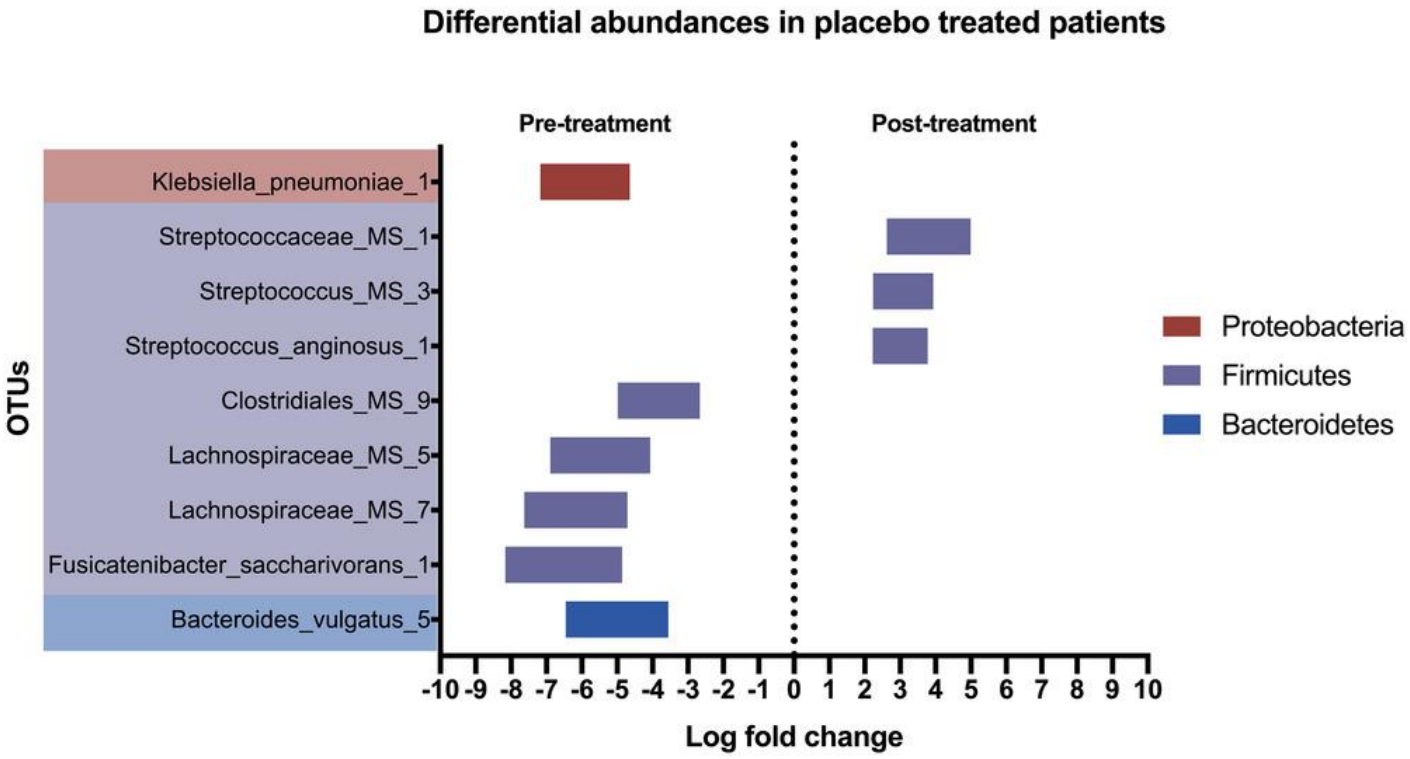
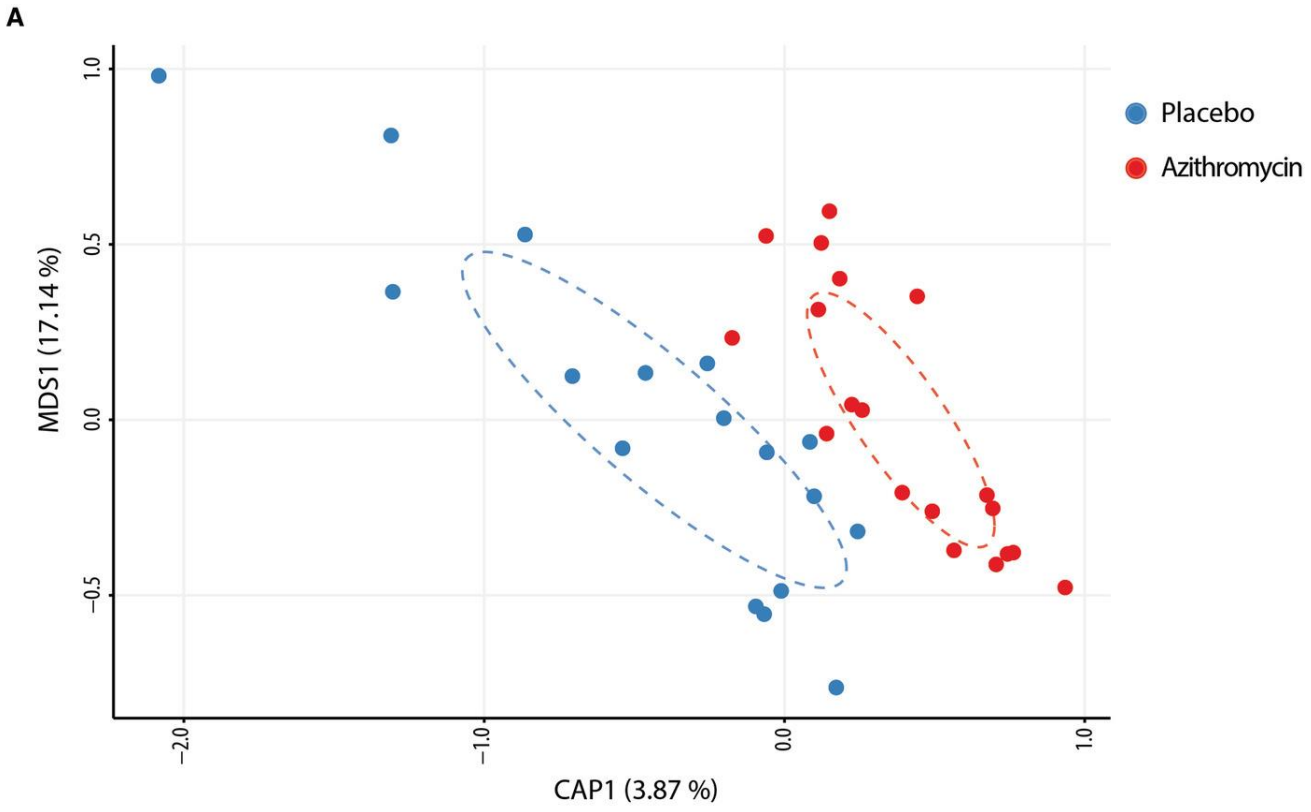

Differential abundances in placebo treated patients
Pre-treatment
Post-treatment
OTUs
Klebsiella_pneumoniae_1
Streptococcaceae_MS_1
Streptococcus_MS_3
Streptococcus_anginosus_1
Clostridiales_MS_9
Lachnospiraceae_MS_5
Lachnospiraceae_MS_7
Fusicatenibacter_saccharivorans_1
Bacteroides_vulgatus_5
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10
Log fold change
Proteobacteria
Firmicutes
Bacteroidetes

**Figure 7. Microbiome diversity studies at the OTU level at the post-treatment visit between azithromycin and placebo groups.** Placebo group is represented in blue and azithromycin in red. **a)** Estimated beta diversity based on Bray-Curtis dissimilarity plotted in the Constrained Analysis of Principal Coordinates (CAP) ordination plot. Analysis of Group Similarities (ANOSIM) method was used and significance was established at $P<0.05$. The percentage of variation captured in each axis is represented between brackets. No significant differences were found in beta diversity ($P=0.096$) **b)** Alpha diversity calculated with the Shannon and inverse Simpson measure of diversity score and significance was established at $P<0.05$. Results are presented as interquartile range (IQR) from first quartile (Q1) to third quartile (Q3) with a median line. Whiskers represent the minimum and maximum, where the minimum is expressed as: Q1 -1.5*IQR and the maximum as: Q3 + 1.5*IQR. A significant decrease in alpha diversity in the placebo group was found with Inverse Simpson index of diversity was alpha diversity ($P=0.039$) but not with the Shannon index ($P=0.104$).


A
Placebo
Azithromycin
MDS1 (17.14 %)
CAP1 (3.87 %)
1.0
0.5
0.0
−0.5
−2.0
−1.0
0.0
1.0

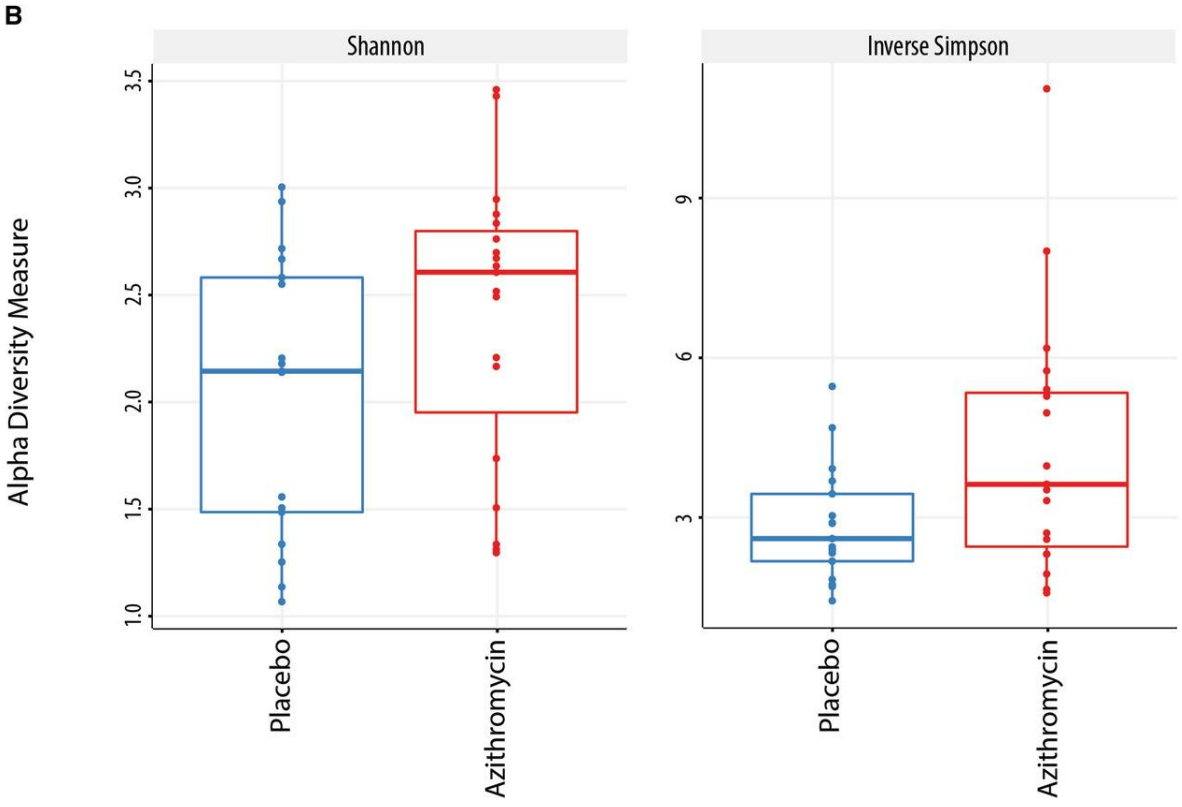

B
Shannon
Inverse Simpson
Alpha Diversity Measure
3.5
3.0
2.5
2.0
1.5
1.0
9
6
3
Placebo
Azithromycin
Placebo
Azithromycin

**Figure 8. Relative bacterial abundance at the post-treatment visit between azithromycin and placebo groups.** Data is presented in percentage. The 21 most abundant genera are presented when available. All the other genera (n=60) were pooled into the "other" group.

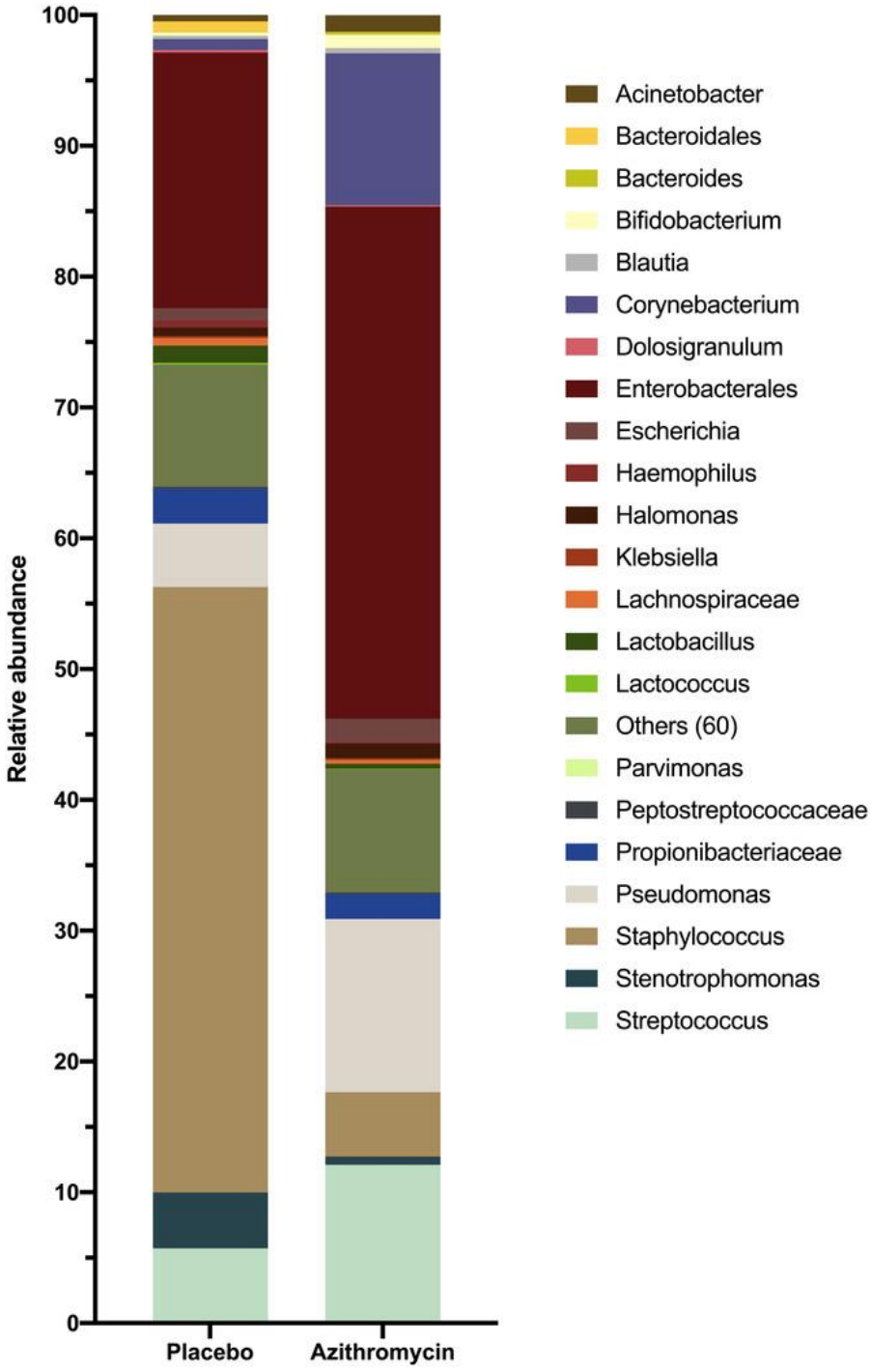


**Figure 9. Differential analysis of significant OTUs between azithromycin and placebo groups at the post-treatment visit.** Data is presented as the mean log-fold change between both tested groups with respect to all 42 significant OTUs. Coloured bars represent the range of upper and lower limits of standard error for the log fold change. A negative log-fold change implies a higher abundance in the azithromycin group whereas a positive log-fold change implies a higher abundance in the placebo group. The dotted line separates azithromycin with placebo groups with respect to log-fold abundance change. Significant differences were established at a False Discovery Rate (FDR) < 0.05. Legend represents the phylum to which each OTU belongs to. 29 *S. aureus* OTU were significantly less abundant (FDR <0.05 to < 0.001) in azithromycin-treated patients compared to placebo.

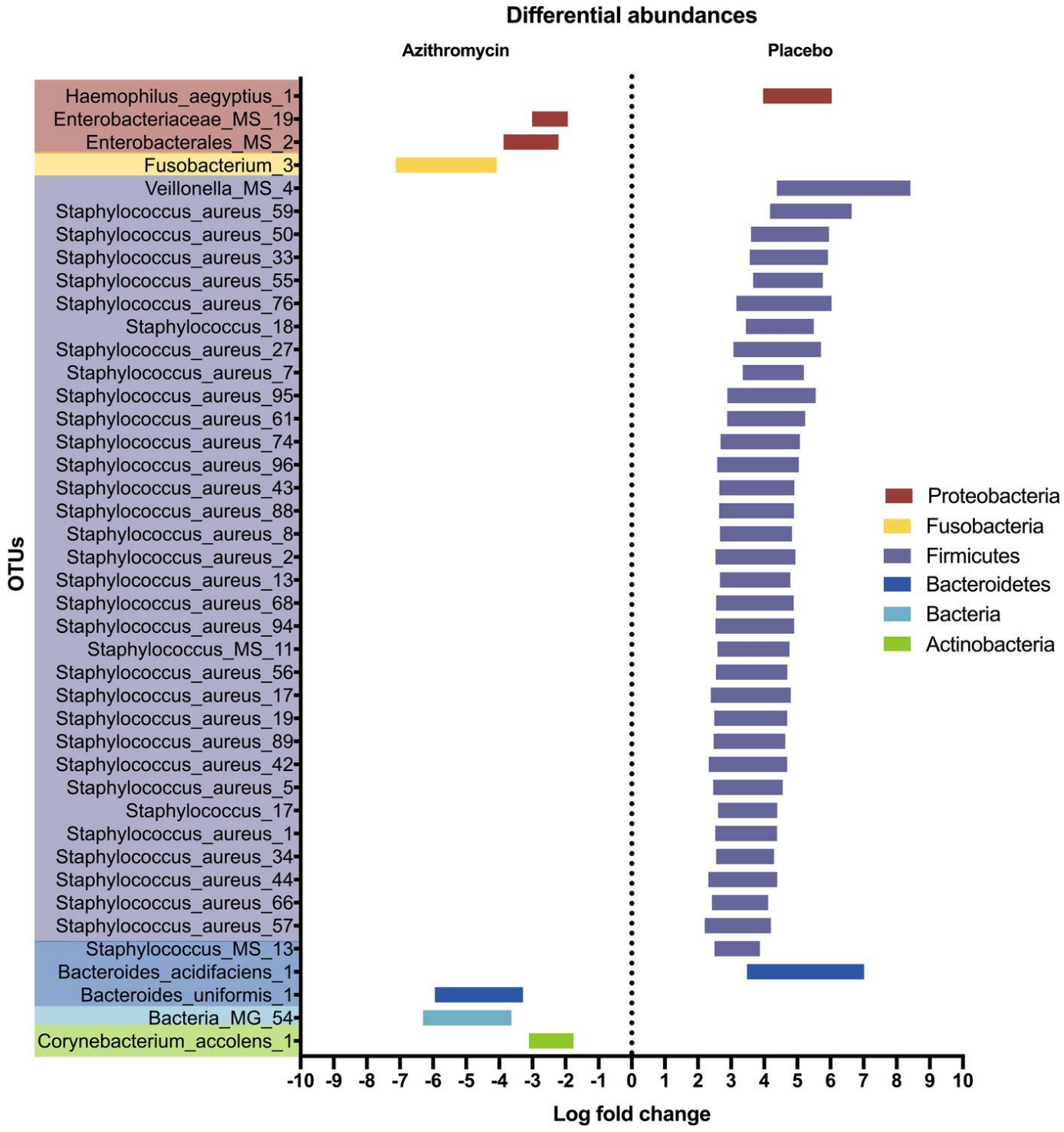
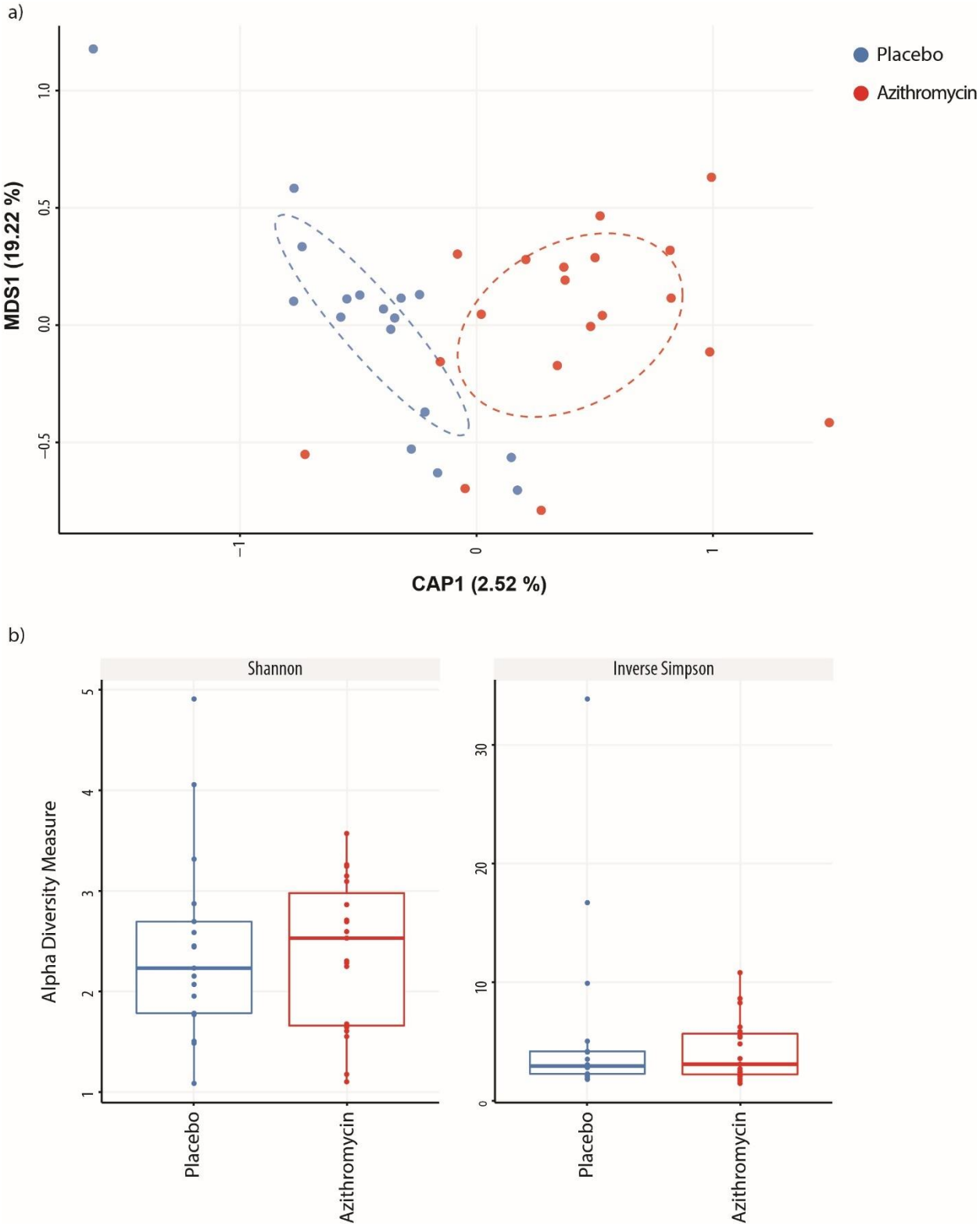

Differential abundances
Azithromycin
Placebo
OTUs
Haemophilus_aegyptius_1
Enterobacteriaceae_MS_19
Enterobacterales_MS_2
Fusobacterium_3
Veillonella_MS_4
Staphylococcus_aureus_59
Staphylococcus_aureus_50
Staphylococcus_aureus_33
Staphylococcus_aureus_55
Staphylococcus_aureus_76
Staphylococcus_18
Staphylococcus_aureus_27
Staphylococcus_aureus_7
Staphylococcus_aureus_95
Staphylococcus_aureus_61
Staphylococcus_aureus_74
Staphylococcus_aureus_96
Staphylococcus_aureus_43
Staphylococcus_aureus_88
Staphylococcus_aureus_8
Staphylococcus_aureus_2
Staphylococcus_aureus_13
Staphylococcus_aureus_68
Staphylococcus_aureus_94
Staphylococcus_MS_11
Staphylococcus_aureus_56
Staphylococcus_aureus_17
Staphylococcus_aureus_19
Staphylococcus_aureus_89
Staphylococcus_aureus_42
Staphylococcus_aureus_5
Staphylococcus_17
Staphylococcus_aureus_1
Staphylococcus_aureus_34
Staphylococcus_aureus_44
Staphylococcus_aureus_66
Staphylococcus_aureus_57
Staphylococcus_MS_13
Bacteroides_acidifaciens_1
Bacteroides_uniformis_1
Bacteria_MG_54
Corynebacterium_accolens_1
Proteobacteria
Fusobacteria
Firmicutes
Bacteroidetes
Bacteria
Actinobacteria
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10
Log fold change

**Figure S1. Microbiome diversity studies at the OTU level at the pre-treatment visit between azithromycin and placebo groups.** Placebo group is represented in blue and azithromycin in red. **a)** Estimated beta diversity based on Bray-Curtis dissimilarity plotted in the Constrained Analysis of Principal Coordinates (CAP) ordination plot. Analysis of Group Similarities (ANOSIM) method was used and significance was established at $P<0.05$. The percentage of variation captured in each axis is represented between brackets. No significant differences were found in beta diversity ($P=0.599$) **b)** Alpha diversity calculated with the Shannon and inverse Simpson measure of diversity score and significance was established at $P<0.05$. Results are presented as interquartile range (IQR) from first quartile (Q1) to third quartile (Q3) with a median line. Whiskers represent the minimum and maximum, where the minimum is expressed as: Q1 -1.5*IQR and the maximum as: Q3 + 1.5*IQR. No significant change in alpha diversity between both groups were found.


a)
Placebo
Azithromycin
MDS1 (19.22 %)
CAP1 (2.52 %)
b)
Shannon
Inverse Simpson
Alpha Diversity Measure
Placebo
Azithromycin

**References**


1. DeConde AS, Soler ZM. Chronic rhinosinusitis: Epidemiology and burden of disease. *American Journal of Rhinology & Allergy.* 2016;30(2):134-139.
2. Macdonald KI, McNally JD, Massoud E. The health and resource utilization of Canadians with chronic rhinosinusitis. *Laryngoscope.* 2009;119(1):184-189.
3. Rudmik L, Smith TL. Quality of life in patients with chronic rhinosinusitis. *Current allergy and asthma reports.* 2011;11(3):247-252.
4. Hopkins C, Slack R, Lund V, Brown P, Copley L, Browne J. Long-term outcomes from the English national comparative audit of surgery for nasal polyposis and chronic rhinosinusitis. *The Laryngoscope.* 2009;119(12):2459-2465.
5. Nader M-E, Abou-Jaoude P, Cabaluna M, Desrosiers M. Using response to a standardized treatment to identify phenotypes for genetic studies of chronic rhinosinusitis. *Journal of Otolaryngology-Head and Neck Surgery.* 2010;39(1):69.
6. DeConde AS, Mace JC, Levy JM, Rudmik L, Alt JA, Smith TL. Prevalence of polyp recurrence after endoscopic sinus surgery for chronic rhinosinusitis with nasal polyposis. *The Laryngoscope.* 2017;127(3):550-555.
7. Mendelsohn D, Jeremic G, Wright ED, Rotenberg BW. Revision rates after endoscopic sinus surgery: a recurrence analysis. *Ann Otol Rhinol Laryngol.* 2011;120(3):162-166.
8. Smith KA, Rudmik L. Medical therapy, refractory chronic rhinosinusitis, and productivity costs. *Current opinion in allergy and clinical immunology.* 2017;17(1):5-11.
9. López-Chacón M, Mullol J, Pujols L. Clinical and biological markers of difficult-to-treat severe chronic rhinosinusitis. *Current allergy and asthma reports.* 2015;15(5):19.

10. Renteria AE, Mfuna Endam L, Desrosiers M. Do Aging Factors Influence the Clinical Presentation and Management of Chronic Rhinosinusitis? *Otolaryngology–Head and Neck Surgery.* 2017;156(4):598-605.
11. Psaltis AJ, Wormald P-J. Therapy of Sinonasal Microbiome in CRS: A Critical Approach. *Current Allergy and Asthma Reports.* 2017;17(9):59.
12. Hand TW, Vujkovic-Cvijin I, Ridaura VK, Belkaid Y. Linking the microbiota, chronic disease, and the immune system. *Trends in Endocrinology & Metabolism.* 2016;27(12):831-843.
13. Wagner Mackenzie B, Waite DW, Hoggard M, Douglas RG, Taylor MW, Biswas K. Bacterial community collapse: a meta-analysis of the sinonasal microbiota in chronic rhinosinusitis. *Environmental microbiology.* 2017;19(1):381-392.
14. Chalermwatanachai T, Vilchez-Vargas R, Holtappels G, et al. Chronic rhinosinusitis with nasal polyps is characterized by dysbacteriosis of the nasal microbiota. *Scientific reports.* 2018;8(1):7926.
15. Byrd AL, Segre JA. Adapting Koch's postulates. *Science.* 2016;351(6270):224-226.
16. Rudmik L, Soler ZM. Medical therapies for adult chronic sinusitis: a systematic review. *JAMA.* 2015;314(9):926-939.
17. Mullol WF-VL-J, Baroody CB-IA-F, Douglas NC-AC-R, et al. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhinology.* 2012;50(1):1-12.
18. Schwartz JS, Tajudeen BA, Cohen NA. Medical management of chronic rhinosinusitis–an update. *Expert review of clinical pharmacology.* 2016:1-10.

19. Maniakas A, Desrosiers M. Azithromycin add-on therapy in high-risk postendoscopic sinus surgery patients failing corticosteroid irrigations: A clinical practice audit. *Am J Rhinol Allergy.* 2014;28(2):151-155.

20. Wallwork B, Coman W, Mackay-Sim A, Greiff L, Cervin A. A double-blind, randomized, placebo-controlled trial of macrolide in the treatment of chronic rhinosinusitis. *The Laryngoscope.* 2006;116(2):189-193.

21. Videler WJ, Badia L, Harvey RJ, et al. Lack of efficacy of long-term, low-dose azithromycin in chronic rhinosinusitis: a randomized controlled trial. *Allergy.* 2011;66(11):1457-1468.

22. Soler ZM, Oyer SL, Kern RC, et al. Antimicrobials and chronic rhinosinusitis with or without polyposis in adults: an evidenced-based review with recommendations. Paper presented at: International forum of allergy & rhinology2013.

23. Meyer M, Huaux F, Gavilanes X, et al. Azithromycin reduces exaggerated cytokine production by M1 alveolar macrophages in cystic fibrosis. *American journal of respiratory cell and molecular biology.* 2009;41(5):590-602.

24. Baines KJ, Wright TK, Gibson PG, Powell H, Hansbro PM, Simpson JL. Azithromycin treatment modifies airway and blood gene expression networks in neutrophilic COPD. *ERJ open research.* 2018;4(4).

25. Parnham MJ, Haber VE, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. Azithromycin: mechanisms of action and their relevance for clinical applications. *Pharmacology & therapeutics.* 2014;143(2):225-245.

26. Zarogoulidis P, Papanas N, Kioumis I, Chatzaki E, Maltezos E, Zarogoulidis K. Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to

clinical practice in respiratory diseases. *European journal of clinical pharmacology.* 2012;68(5):479-503.

27. Foulds G, Shepard R, Johnson R. The pharmacokinetics of azithromycin in human serum and tissues. *Journal of Antimicrobial Chemotherapy.* 1990;25(suppl_A):73-82.

28. Schentag JJ, Ballow CH. Tissue-directed pharmacokinetics. *The American journal of medicine.* 1991;91(3):S5-S11.

29. Psaltis AJ, Li G, Vaezeafshar R, Cho KS, Hwang PH. Modification of the Lund-Kennedy endoscopic scoring system improves its reliability and correlation with patient-reported outcome measures. *The Laryngoscope.* 2014;124(10):2216-2223.

30. Hopkins C, Gillett S, Slack R, Lund VJ, Browne JP. Psychometric validity of the 22-item Sinonasal Outcome Test. *Clin Otolaryngol.* 2009;34(5):447-454.

31. Bartram AK, Lynch MD, Stearns JC, Moreno-Hagelsieb G, Neufeld JD. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Appl Environ Microbiol.* 2011;77(11):3846-3852.

32. Muyzer G, De Waal EC, Uitterlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol.* 1993;59(3):695-700.

33. Martinez JRW, Vargas-Salas S, Gamboa SU, et al. The Combination of RET, BRAF and Demographic Data Identifies Subsets of Patients with Aggressive Papillary Thyroid Cancer. *Hormones & cancer.* 2019;10(2-3):97-106.

34. Gonzalez E, Pitre FE, Page AP, et al. Trees, fungi and bacteria: tripartite metatranscriptomics of a root microbiome responding to soil contamination. *Microbiome.* 2018;6(1):53.

35. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One.* 2013;8(4):e61217.

36. Oksanen J, Blanchet F, Kindt R, et al. Package 'vegan'2016. *Community ecology package, R package version.* 2017:2.4-1.

37. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15(12):550.

38. Minerbi A, Gonzalez E, Brereton NJB, et al. Altered microbiome composition in individuals with fibromyalgia. *Pain.* 2019;160(11):2589-2602.

39. Weiss S, Xu ZZ, Peddada S, et al. Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome.* 2017;5(1):27.

40. Bachert C, Zhang L, Gevaert P. Current and future treatment options for adult chronic rhinosinusitis: Focus on nasal polyposis. *Journal of Allergy and Clinical Immunology.* 2015;136(6):1431-1440.

41. Doan T, Hinterwirth A, Arzika AM, et al. Mass azithromycin distribution and community microbiome: a cluster-randomized trial. Paper presented at: Open forum infectious diseases2018.

42. Doan T, Hinterwirth A, Worden L, et al. Gut microbiome alteration in MORDOR I: a community-randomized trial of mass azithromycin distribution. *Nature medicine.* 2019;25(9):1370-1376.

43. Zhou Y, Bacharier LB, Isaacson-Schmid M, et al. Azithromycin therapy during respiratory syncytial virus bronchiolitis: Upper airway microbiome alterations and subsequent recurrent wheeze. *Journal of Allergy and Clinical Immunology.* 2016;138(4):1215-1219. e1215.

44. Slater M, Rivett DW, Williams L, et al. The impact of azithromycin therapy on the airway microbiota in asthma. *Thorax.* 2014;69(7):673-674.

45. Dickson RP, Morris A. Macrolides, inflammation and the lung microbiome: untangling the web of causality. BMJ Publishing Group Ltd; 2017.

46. dos Santos Santiago GL, Brusselle G, Dauwe K, et al. Influence of chronic azithromycin treatment on the composition of the oropharyngeal microbial community in patients with severe asthma. *BMC microbiology.* 2017;17(1):109.

47. Jervis-Bardy J, Foreman A, Boase S, Valentine R, Wormald PJ. What is the origin of Staphylococcus aureus in the early postoperative sinonasal cavity? Paper presented at: International forum of allergy & rhinology2011.

48. Maniakas A, Asmar MH, Renteria Flores AE, et al. Staphylococcus aureus on Sinus Culture Is Associated With Recurrence of Chronic Rhinosinusitis After Endoscopic Sinus Surgery. *Front Cell Infect Microbiol.* 2018;8:150.

49. Vickery TW, Ramakrishnan VR. Bacterial pathogens and the microbiome. *Otolaryngologic clinics of North America.* 2017;50(1):29.

50. Hansen C, Pressler T, Hoiby N, Johansen H. Long-term, low-dose azithromycin treatment reduces the incidence but increases macrolide resistance in Staphylococcus aureus in Danish CF patients. *Journal of Cystic Fibrosis.* 2009;8(1):58-62.

51. Meyer A, Bril-Bazuin C, Mattie H, Van Den Broek P. Uptake of azithromycin by human monocytes and enhanced intracellular antibacterial activity against Staphylococcus aureus. *Antimicrobial agents and chemotherapy.* 1993;37(11):2318-2322.

52. Wilson MT, Hamilos DL. The nasal and sinus microbiome in health and disease. *Current allergy and asthma reports.* 2014;14(12):485.

53. Liu CM, Price LB, Hungate BA, et al. Staphylococcus aureus and the ecology of the nasal microbiome. *Science advances.* 2015;1(5):e1400216.

54. Biswas K, Hoggard M, Jain R, Taylor MW, Douglas RG. The nasal microbiota in health and disease: variation within and between subjects. *Frontiers in microbiology.* 2015;6:134.

55. Ramakrishnan VR, Feazel LM, Gitomer SA, Ir D, Robertson CE, Frank DN. The microbiome of the middle meatus in healthy adults. *PLoS One.* 2013;8(12):e85507.

56. Bassis CM, Tang AL, Young VB, Pynnonen MA. The nasal cavity microbiota of healthy adults. *Microbiome.* 2014;2(1):1.

57. Kuhar HN, Tajudeen BA, Mahdavinia M, et al. Relative abundance of nasal microbiota in chronic rhinosinusitis by structured histopathology. Paper presented at: International forum of allergy & rhinology2018.

58. Seresirikachorn K, Suwanparin N, Srisunthornphanich C, Chitsuthipakorn W, Kanjanawasee D, Snidvongs K. Factors of success of low-dose macrolides in chronic sinusitis: Systematic review and meta-analysis. *The Laryngoscope.* 2019.

59. Maniakas A, Asmar M, Renteria Flores A, et al. Azithromycin in refractory CRS following ESS and corticosteroid irrigations: double-blind, randomized, placebo-controlled trial (manuscript in preparation). 2020.

## XVI. Discussion

### 1. Rationale

The overarching goal of this PhD thesis was to identify and investigate a novel therapeutic option for refractory CRS patients that would offer favorable outcomes, while remaining low in cost, low in toxicity/morbidity, and easy to administer. To achieve this, we aimed for the highest level of evidence by conducting a double-blind randomized controlled trial. This was a challenging endeavour, especially as it was conducted in a single tertiary care center. Prior to the RCT, a prospective clinical trial was conducted on all patients undergoing ESS for CRS, having failed appropriate medical therapy (BNI). This initial prospective enrollment phase was in an effort to carefully select patients that would fail both medical and surgical therapy, prior to exposing them to our study drug. The methodology and treatment paradigm developed for this study is unique in the literature, and ultimately offers fellow otolaryngologists a systematic approach to CRS management. Furthermore, methodology allowed us to collect numerous data variables at three different time-points along the disease management, offering significant novel data that had yet to be reported in the literature. The breadth of data collected on each patient included demographics, patient-reported outcome measures evaluating symptomatology, endoscopic evaluations using standardized and validated scoring methods, blood and serum sampling, and conventional and 16S ribosomal RNA microbial swabs for microbiome analyses. To date, there has been no study offering such comprehensive reporting on a CRS population and we hope that our work will help set a new standard in clinical trial development and execution in CRS.

## 2. Summary of our findings and clinical recommendations

Phase 1 of this thesis comprised of the patient enrollment phase on the day of surgery and the 16 weeks of post-operative daily BNI. We enrolled a total of 128 patients that met inclusion and exclusion criteria and that were subsequently treated with ESS for CRS following failed appropriate medical therapy.

Key findings from Phase 1 are summarized below:

- The failure rate of ESS+BNI in CRS patients is potentially under-reported in the literature which is primarily populated by retrospective studies. This prospective study demonstrates that the ESS+BNI failure rate may be as high as 50%, especially in a high-risk CRS population.
- Identifying biomarkers and risk factors that may be used as treatment outcome tools are key to any disease management. In the setting of CRS, they can help treating physicians predict which patient may have a higher chance of being refractory to treatment, and this as early as pre-ESS. Through our prospective trial, we demonstrate that patients with a history of prior ESS, regardless of how many or their extent, and/or the presence of *S. aureus* in the sinonasal cavity preoperatively were two independent risk factors for ESS+BNI failure. We therefore suggest that this patient population be followed in a more systematic and close-interval fashion than standard CRS patients. Furthermore, we recommend that all patients undergo microbial swabs prior to their ESS to ensure virulent microbial pathogens are identified and included in the patient's phenotyping and post-operative treatment plan.
- A subgroup analysis was performed on patients having accepted to undergo additional samplings in an effort to identify novel biomarkers of interest. Unfortunately, no serum or

blood cell biomarker showed significant predictive power, although our sample size was small. Additional studies in this field are warranted.

Phase 2 of this thesis comprised of the double-blind, placebo-controlled, randomized clinical trial as well as the extensive microbiome analyses performed on the RCT cohort. Such paired data has never been attempted and/or reported in the literature, making it unique and hopefully a new standard for reporting treatment outcomes in CRS clinical trials.

Key findings from Phase 2 are summarized below:

- Globally, azithromycin did not show a statistically significant improvement in disease clearance rates compared to the placebo
- When excluding patients with AERD, disease clearance rates were significantly improved in patients having received azithromycin. This suggests that AERD patients with refractory CRS failing ESS+BNI are likely a distinct phenotype and may not be good candidates for azithromycin. Therefore, we recommend azithromycin be included in the treatment algorithm for non-AERD patients with CRS who show disease persistence following ESS+BNI

Patients on azithromycin who have disease clearance, not only have healthy sinonasal cavities, but they also show significantly improved symptomatology, which is the main tool in assessing quality of life. This finding was not seen in patients having received placebo, further strengthening the argument in favor of azithromycin administration.

- Patients on azithromycin showing disease clearance were also able to clear their *S. aureus* on conventional microbial swabs, which was further identified in the subsequent more advanced 16S rRNA microbiome analyses. In fact, azithromycin induced a significant

change in the sinonasal microbiome beta diversity, driven by a large log-fold decrease in the abundance *S. aureus*, a finding that was not seen in patients on placebo.

- Azithromycin was not associated to any minor or major side effect and is therefore safe to use at the studied dosage. We however continue to recommend against its use in patients with a high cardiovascular baseline risk, and diligent consideration in patients with low or intermediate cardiovascular baseline risk.

### 3. Limitations

Our studies had certain limitations. Studying a high-risk population known to recur soon after ESS may lead to a strict selection bias, however it does allow us to adequately phenotype CRS patients, identifying the subgroups who will likely respond to azithromycin. Furthermore, using patient-reported outcome measures such as the SNSS and SNOT-22 questionnaires may lead to a recollection bias as they are self-reported, and patients may either not accurately recollect the severity or prevalence of their symptoms. They can also lead to a response bias due to personal perceptive influences on one's symptomatology. Using a strictly objective and reproducible scoring system, such as the Lund-Kennedy endoscopic score, allowed us to minimize the various biases associated with PROMs. Nevertheless, this does decrease the generalizability and comparability of our study and findings to published quality of life driven studies.

Our primary hypothesis for the RCT, aiming to demonstrate a statistically significant improvement in disease clearance rates using azithromycin compared to placebo was not met. This is due to the underpowered nature and the large effect size estimated for our study, but also heavily due to the higher population of AERD patients in the azithromycin group, leading to a potential

Type 2 error. Once the AERD patients were removed from the analyses, azithromycin was shown to have a significant effect on disease outcome, as hypothesized.

Although we had no losses to follow-up in the randomization phase of the study, this did not prohibit us from having cases of inadequate sampling, such as low DNA samples retrieved in the microbiome analyses, inadequate serum volumes, coagulated blood in test tubes rendering them unusable, amongst others. In future studies, we would recommend duplicating all 16S rRNA microbial swab and blood draw samplings.

Real-world generalizability or external validity is always a concern when critically reviewing RCTs, and our cohort of high-risk CRS patients represents a carefully selected group, primarily seen in tertiary care centers. Although community otolaryngologists may not encounter such patients at the same rate, if they follow the treatment-paradigm and administer azithromycin to patients having met all inclusion criteria and demonstrate the simple-to-use biomarkers we have identified, then this may not only minimize this limitation, it will also improve patient care and decrease the healthcare burden on tertiary care centers.

## 4. Future directions

*Clinical trials*

This is the first study in the literature to report a double-blind, placebo-controlled, randomized control study in a high-risk CRS population. Future studies using a similar approach of robust data collection, including 16S rRNA microbiome analyses are needed to validate our findings. Furthermore, investigators should aim for multicenter studies to increase power and external validity, while decreasing the duration of study completion. One such study is the ‘MACRO randomized controlled trial’ which is aiming at enrolling 16 study sites and a total of

600 patients[136]. Although they will be evaluating patients in a pre-operative state, this work will shed additional light on the role of low-dose, long-term macrolides in CRS management.

*Serum biomarkers*

Although our efforts to identify novel serological biomarkers were not successful, we are hopeful that future studies will be able to 1) conduct larger sampled studies, and 2) identify serological markers that will be easy to test for and comport a high predictive power. A recent report has shown an interesting candidate, Pentraxin-3[137], while others are reporting on the serum metabolomics associated with the various phenotypes and endotypes of CRS[138]. Incorporating potential biomarkers in future clinical trials will be of utmost importance.

*Tissue/local biomarkers*

Although sinonasal tissue-directed biomarkers were not analyzed in this trial, identifying local biomarkers can be of interest and allow for a more in depth understanding of the difference between tissue and serum biomarker levels. For example, in our work, we did not demonstrate any difference in serum IgE levels in patients responding or not to azithromycin, a finding that reflects the current literature[139]. Interestingly however, a recent study demonstrated a correlation between low IgE levels in *nasal secretions* and a favorable response to low-dose macrolides[140], a finding that was not seen with *serum* IgE levels in their study cohort. Such findings suggest that tissue-based biomarkers likely represent a distinct clinical entity and may play a more important role in characterizing the disease microenvironment. Future clinical trials should analyze the variability in tissue-based and local biomarkers in the pre vs. post-treatment state, as well as a drug vs. placebo comparison.

*Sinonasal microbiome*

It is evident that as technological advances continue to emerge, so will information on the true role of the microbiome on disease. Until this past decade, microbial research in CRS was almost entirely culture-based, with few to no centers offering 16S rRNA analyses that would then translate to clinical decision-making. Today, there is ever growing data on how microbiomes can have both direct and indirect effects on chronic diseases, cancers, and overall treatment outcomes[141]. One of the most exciting avenues of microbiome research is in its use to modulate treatment outcomes. For example, a recent study demonstrated that fecal microbiota transplantation promoted response to immunotherapy in patients with metastatic (Stage IV) melanoma who had initially failed to respond to treatment[142].

In our work, we demonstrate how therapy with azithromycin modulated the microbiome, and in turn had a positive outcome that is likely to be long-lasting. Future studies should not only confirm our findings, but should also look into potential avenues to modulate the microbiome through microbiota transplantation. This can potentially be achieved by transplanting sinonasal microbiota from patients without CRS and/or CRS patients who have had disease clearance, or through nasal irrigations with probiotics[143]. Findings from such studies may have beneficial outcomes not only for the CRS population, but also for the head and neck cancer population. In a review of the literature published this year with me as senior author, we reported the significant gap in the literature on the role and effect of the microbiome in head and neck cancer outcomes[144]. We recommended that future studies aim to identify whether sinonasal microbiome dysbiosis is accompanied by patient symptomatology and treatment outcome, while investigating potential modes of intervention and microbiome modulation.

## XVII. Conclusion

Chronic rhinosinusitis is a common and debilitating disease which has a high rate of failure to standard medical and surgical therapy. This trial identified prior ESS and *S. aureus* as two independent risk factors for treatment failure. Current treatment options for refractory CRS patients offer high morbidity and possible severe complications, while offering inadequate disease clearance rates. This two-phased clinical trial provides support for the administration of low-dose, long-term azithromycin as a low-morbidity, low-cost, high success rate option in patients with CRS refractory to appropriate surgical and medical therapy. This being said, patient candidates should not have AERD or any high cardiovascular baseline risk. Furthermore, we have demonstrated the favorable effect of azithromycin in two novel manners: first, patients who received azithromycin and had disease clearance had significant improvements in quality-of-life scores, compared to no change, if on placebo, and second, the use of azithromycin demonstrated its ability to modulate the microbiome and significantly lower *S. aureus* prevalence further emphasizing its potential long-term effect.

## XVIII. References


1. Hastan D, Fokkens WJ, Bachert Cet al. Chronic rhinosinusitis in Europe--an underestimated disease. A GA(2)LEN study. Allergy 2011; 66:1216-1223.
2. Nader M-E, Abou-Jaoude P, Cabaluna M, Desrosiers M. Using response to a standardized treatment to identify phenotypes for genetic studies of chronic rhinosinusitis. J Otolaryngol Head Neck Surg 2010; 39:69-75.
3. Chong LY, Piromchai P, Sharp Set al. Biologics for chronic rhinosinusitis. Cochrane Database Syst Rev 2021; 3:Cd013513.
4. Maniakas A, Desrosiers M. Azithromycin add-on therapy in high-risk postendoscopic sinus surgery patients failing corticosteroid irrigations: A clinical practice audit. American journal of rhinology & allergy 2014; 28:151-155.
5. Amali A, Saedi B, Rahavi-Ezabadi S, Ghazavi H, Hassanpoor N. Long-term postoperative azithromycin in patients with chronic rhinosinusitis: A randomized clinical trial. American journal of rhinology & allergy 2015; 29:421-424.
6. Marple BF, Roberts CS, de Caprariis PJ, Reisman A. Onset of symptom resolution in adults with acute bacterial rhinosinusitis treated with a single dose of azithromycin extended release compared with 10 days of levofloxacin: a retrospective analysis of a randomized, double-blind, double-dummy trial. Clin Ther 2007; 29:2690-2698.
7. Videler WJ, Badia L, Harvey RJet al. Lack of efficacy of long-term, low-dose azithromycin in chronic rhinosinusitis: a randomized controlled trial. Allergy 2011; 66:1457-1468.
8. Videler WJM, van Hee K, Reinartz SM, Georgalas C, van der Meulen FW, Fokkens WJ. Long-term low-dose antibiotics in recalcitrant chronic rhinosinusitis: a retrospective analysis. Rhinology 2012; 50:45-55.
9. Haruna S, Shimada C, Ozawa M, Fukami S, Moriyama H. A study of poor responders for long-term, low-dose macrolide administration for chronic sinusitis. Rhinology 2009; 47:66-71.
10. Wallwork B, Coman W, Mackay-Sim A, Greiff L, Cervin A. A double-blind, randomized, placebo-controlled trial of macrolide in the treatment of chronic rhinosinusitis. The Laryngoscope 2006; 116:189-193.
11. Maniakas A. Complex chronic rhinosinusitis management : a prospective trial on refractory patients following maximal medical and surgical therapy and the evaluation of Staphylococcus aureus in disease recurrence. [Montreal]: McGill University Libraries, 2019.
12. Fokkens WJ, Lund VJ, Hopkins Cet al. Executive summary of EPOS 2020 including integrated care pathways. Rhinology 2020; 58:82-111.
13. Alanin MC, Hopkins C. Effect of Functional Endoscopic Sinus Surgery on Outcomes in Chronic Rhinosinusitis. Curr Allergy Asthma Rep 2020; 20:27.
14. Hopkins C. Chronic Rhinosinusitis with Nasal Polyps. New England Journal of Medicine 2019; 381:55-63.
15. Cohen NA, Widelitz JS, Chiu AG, Palmer JN, Kennedy DW. Familial aggregation of sinonasal polyps correlates with severity of disease. Otolaryngol Head Neck Surg 2006; 134:601-604.

16. Chen Y, Dales R, Lin M. The epidemiology of chronic rhinosinusitis in Canadians. The Laryngoscope 2003; 113:1199-1205.
17. Anand VK. Epidemiology and economic impact of rhinosinusitis. The Annals of otology, rhinology & laryngology Supplement 2004; 193:3-5.
18. DeConde AS, Soler ZM. Chronic rhinosinusitis: Epidemiology and burden of disease. American journal of rhinology & allergy 2016; 30:134-139.
19. Rudmik L, Smith TL, Schlosser RJ, Hwang PH, Mace JC, Soler ZM. Productivity costs in patients with refractory chronic rhinosinusitis. The Laryngoscope 2014.
20. Rudmik L. Economics of Chronic Rhinosinusitis. Curr Allergy Asthma Rep 2017; 17:20.
21. Mendelsohn D, Jeremic G, Wright ED, Rotenberg BW. Revision rates after endoscopic sinus surgery: a recurrence analysis. The Annals of otology, rhinology, and laryngology 2011; 120:162-166.
22. Smith TL, Schlosser RJ, Mace JCet al. Long-term outcomes of endoscopic sinus surgery in the management of adult chronic rhinosinusitis. International forum of allergy & rhinology 2019; 9:831-841.
23. Bendouah Z, Barbeau J, Hamad WA, Desrosiers M. Biofilm formation by Staphylococcus aureus and Pseudomonas aeruginosa is associated with an unfavorable evolution after surgery for chronic sinusitis and nasal polyposis. Otolaryngology - Head & Neck Surgery 2006; 134:991-996.
24. Tan NC, Cooksley CM, Roscioli Eet al. Small-colony variants and phenotype switching of intracellular Staphylococcus aureus in chronic rhinosinusitis. Allergy 2014; 69:1364-1371.
25. Ou JJ, Drilling AJ, Cooksley Cet al. Reduced Innate Immune Response to a Staphylococcus aureus Small Colony Variant Compared to Its Wild-Type Parent Strain. Frontiers in cellular and infection microbiology 2016; 6:187.
26. Jackson DJ, Gern JE, Lemanske RF, Jr. Lessons learned from birth cohort studies conducted in diverse environments. The Journal of allergy and clinical immunology 2017; 139:379-386.
27. Jain R, Danziger L. The macrolide antibiotics: a pharmacokinetic and pharmacodynamic overview. Current pharmaceutical design 2004; 10:3045-3053.
28. Menninger JR. Functional consequences of binding macrolides to ribosomes. J Antimicrob Chemother 1985; 16 Suppl A:23-34.
29. Foulds G, Shepard RM, Johnson RB. The pharmacokinetics of azithromycin in human serum and tissues. J Antimicrob Chemother 1990; 25 Suppl A:73-82.
30. Van Bambeke F, Tulkens PM. Macrolides: pharmacokinetics and pharmacodynamics. Int J Antimicrob Agents 2001; 18 Suppl 1:S17-23.
31. Spivey JM. The postantibiotic effect. Clinical pharmacy 1992; 11:865-875.
32. Saiman L, Marshall BC, Mayer-Hamblett Net al. Azithromycin in patients with cystic fibrosis chronically infected with Pseudomonas aeruginosa: a randomized controlled trial. Jama 2003; 290:1749-1756.
33. Wolter J, Seeney S, Bell S, Bowler S, Masel P, McCormack J. Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. Thorax 2002; 57:212-216.
34. Equi AC, Davies JC, Painter Het al. Exploring the mechanisms of macrolides in cystic fibrosis. Respir Med 2006; 100:687-697.

35. Tamaoki J. The effects of macrolides on inflammatory cells. Chest 2004; 125:41S-50S; quiz 51S.
36. Martinez FJ, Curtis JL, Albert R. Role of macrolide therapy in chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2008; 3:331-350.
37. Alsowaida YS, Almulhim AS, Oh M, Erstad B, Abraham I. Sensorineural hearing loss with macrolide antibiotics exposure: a meta-analysis of the association. International Journal of Pharmacy Practice 2020; 29:21-28.
38. Ray WA, Murray KT, Meredith S, Narasimhulu SS, Hall K, Stein CM. Oral Erythromycin and the Risk of Sudden Death from Cardiac Causes. New England Journal of Medicine 2004; 351:1089-1096.
39. Koh TW. Risk of torsades de pointes from oral erythromycin with concomitant carbimazole (methimazole) administration. Pacing and clinical electrophysiology : PACE 2001; 24:1575-1576.
40. Shaffer D, Singer S, Korvick J, Honig P. Concomitant risk factors in reports of torsades de pointes associated with macrolide use: review of the United States Food and Drug Administration Adverse Event Reporting System. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2002; 35:197-200.
41. Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the risk of cardiovascular death. New England Journal of Medicine 2012; 366:1881-1890.
42. Lee JY, Lee SW, Lee JD. Comparison of the surgical outcome between primary and revision endoscopic sinus surgery for chronic rhinosinusitis with nasal polyposis. American journal of otolaryngology 2008; 29:379-384.
43. Philpott C, Hopkins C, Erskine Set al. The burden of revision sinonasal surgery in the UK-data from the Chronic Rhinosinusitis Epidemiology Study (CRES): a cross-sectional study. BMJ open 2015; 5:e006680.
44. Jervis-Bardy J, Foreman A, Boase S, Valentine R, Wormald P-J. What is the origin of Staphylococcus aureus in the early postoperative sinonasal cavity? International forum of allergy & rhinology 2011; 1:308-312.
45. Partyka R, Pałac J, Paluch Zet al. Evaluation of usefulness of hs-CRP and ferritin assays in patients with nasal polyps. Dis Markers 2014; 2014:794060-794060.
46. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 2009; 29:313-326.
47. Shun CT, Lin SK, Hong CYet al. C-C chemokine ligand 2 gene expression in nasal polyp fibroblasts: possible implication in the pathogenesis of nasal polyposis. The Annals of otology, rhinology, and laryngology 2005; 114:879-885.
48. Kuehnemund M, Ismail C, Brieger J, Schaefer D, Mann WJ. Untreated chronic rhinosinusitis: a comparison of symptoms and mediator profiles. The Laryngoscope 2004; 114:561-565.
49. Lin SK, Kok SH, Shun CTet al. Tumor necrosis factor-alpha stimulates the expression of C-C chemokine ligand 2 gene in fibroblasts from the human nasal polyp through the pathways of mitogen-activated protein kinase. Am J Rhinol 2007; 21:251-255.
50. Stevens WW, Ocampo CJ, Berdnikovs Set al. Cytokines in Chronic Rhinosinusitis. Role in Eosinophilia and Aspirin-exacerbated Respiratory Disease. Am J Respir Crit Care Med 2015; 192:682-694.

51. Chan JKL, Yuen D, Too PHet al. Keratin 6a reorganization for ubiquitin-proteasomal processing is a direct antimicrobial response. The Journal of cell biology 2018; 217:731-744.
52. Wang F, Chen S, Liu HB, Parent CA, Coulombe PA. Keratin 6 regulates collective keratinocyte migration by altering cell-cell and cell-matrix adhesion. The Journal of cell biology 2018; 217:4314-4330.
53. Cormier C, Mfuna Endam L, Filali-Mouhim Aet al. A pooling-based genomewide association study identifies genetic variants associated with Staphylococcus aureus colonization in chronic rhinosinusitis patients. International forum of allergy & rhinology 2014; 4:207-215.
54. Schleimer RP. Immunopathogenesis of Chronic Rhinosinusitis and Nasal Polyposis. Annual review of pathology 2017; 12:331-357.
55. Zimmermann N, Doepker MP, Witte DPet al. Expression and regulation of small proline-rich protein 2 in allergic inflammation. American journal of respiratory cell and molecular biology 2005; 32:428-435.
56. Ramakrishnan VR, Gonzalez JR, Cooper SEet al. RNA sequencing and pathway analysis identify tumor necrosis factor alpha driven small proline-rich protein dysregulation in chronic rhinosinusitis. American journal of rhinology & allergy 2017; 31:283-288.
57. Trzeciak M, Sakowicz-Burkiewicz M, Wesserling Met al. Expression of Cornified Envelope Proteins in Skin and Its Relationship with Atopic Dermatitis Phenotype. Acta dermato-venereologica 2017; 97:36-41.
58. Beswick DM, Mace JC, Rudmik L, Soler ZM, DeConde AS, Smith TL. Productivity changes following medical and surgical treatment of chronic rhinosinusitis by symptom domain. International forum of allergy & rhinology 2018; 8:1395-1405.
59. Schlosser RJ, Hyer JM, Smith TLet al. Depression-Specific Outcomes After Treatment of Chronic Rhinosinusitis. JAMA otolaryngology-- head & neck surgery 2016; 142:370-376.
60. Maniakas A, Asmar MH, Renteria Flores AEet al. Staphylococcus aureus on Sinus Culture Is Associated With Recurrence of Chronic Rhinosinusitis After Endoscopic Sinus Surgery. Frontiers in cellular and infection microbiology 2018; 8:150.
61. Valera FCP, Ruffin M, Adam Det al. Staphylococcus aureus impairs sinonasal epithelial repair: Effects in patients with chronic rhinosinusitis with nasal polyps and control subjects. The Journal of allergy and clinical immunology 2019; 143:591-603.e593.
62. Bachert C, Han JK, Desrosiers Met al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. Lancet 2019; 394:1638-1650.
63. Pinto JM, Mehta N, DiTineo M, Wang J, Baroody FM, Naclerio RM. A randomized, double-blind, placebo-controlled trial of anti-IgE for chronic rhinosinusitis. Rhinology 2010; 48:318-324.
64. Gevaert P, Lang-Loidolt D, Lackner Aet al. Nasal IL-5 levels determine the response to anti-IL-5 treatment in patients with nasal polyps. The Journal of allergy and clinical immunology 2006; 118:1133-1141.

65. Bachert C, Sousa AR, Lund VJet al. Reduced need for surgery in severe nasal polyposis with mepolizumab: Randomized trial. The Journal of allergy and clinical immunology 2017; 140:1024-1031.e1014.
66. Edris A, De Feyter S, Maes T, Joos G, Lahousse L. Monoclonal antibodies in type 2 asthma: a systematic review and network meta-analysis. Respiratory research 2019; 20:179.
67. Suzuki H, Ikeda K, Honma Ret al. Prognostic factors of chronic rhinosinusitis under long-term low-dose macrolide therapy. ORL J Otorhinolaryngol Relat Spec 2000; 62:121-127.
68. Cervin A, Kalm O, Sandkull P, Lindberg S. One-year low-dose erythromycin treatment of persistent chronic sinusitis after sinus surgery: clinical outcome and effects on mucociliary parameters and nasal nitric oxide. Otolaryngology - Head & Neck Surgery 2002; 126:481-489.
69. Seresirikachorn K, Suwanparin N, Srisunthornphanich C, Chitsuthipakorn W, Kanjanawasee D, Snidvongs K. Factors of success of low-dose macrolides in chronic sinusitis: Systematic review and meta-analysis. The Laryngoscope 2019; 129:1510-1519.
70. Oakley GM, Christensen JM, Sacks R, Earls P, Harvey RJ. Characteristics of macrolide responders in persistent post-surgical rhinosinusitis. Rhinology 2018; 56:111-117.
71. Piccirillo JF, Merritt MG, Jr., Richards ML. Psychometric and clinimetric validity of the 20-Item Sino-Nasal Outcome Test (SNOT-20). Otolaryngol Head Neck Surg 2002; 126:41-47.
72. Psaltis AJ, Li G, Vaezeafshar R, Cho KS, Hwang PH. Modification of the Lund-Kennedy endoscopic scoring system improves its reliability and correlation with patient-reported outcome measures. The Laryngoscope 2014; 124:2216-2223.
73. Renteria AE, Maniakas A, Mfuna LE, Asmar MH, Gonzalez E, Desrosiers M. Low-dose and long-term azithromycin significantly decreases Staphylococcus aureus in the microbiome of refractory CRS patients. International forum of allergy & rhinology 2020.
74. Desrosiers M, Evans GA, Keith PKet al. Canadian clinical practice guidelines for acute and chronic rhinosinusitis. Allergy Asthma Clin Immunol 2011; 7:2.
75. Hopkins C, Gillett S, Slack R, Lund VJ, Browne JP. Psychometric validity of the 22-item Sinonasal Outcome Test. Clinical otolaryngology : official journal of ENT-UK ; official journal of Netherlands Society for Oto-Rhino-Laryngology & Cervico-Facial Surgery 2009; 34:447-454.
76. Barnes ML, Vaidyanathan S, Williamson PA, Lipworth BJ. The minimal clinically important difference in allergic rhinitis. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology 2010; 40:242-250.
77. Valera FCP, Scrideli C, Queinoz R, Gonzaiga Tone L, Anselmo-Lima WT. NF-kappaB expression predicts clinical outcome for nasal polyposis. Rhinology 2010; 48:408-441.
78. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma : evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest 2001; 119:1329-1336.
79. Moore WC, Meyers DA, Wenzel SEet al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010; 181:315-323.

80. Stevens WW, Peters AT, Hirsch AGet al. Clinical Characteristics of Patients with Chronic Rhinosinusitis with Nasal Polyps, Asthma, and Aspirin-Exacerbated Respiratory Disease. The journal of allergy and clinical immunology In practice 2017; 5:1061-1070.e1063.
81. DeConde AS, Soler ZM. Chronic rhinosinusitis: Epidemiology and burden of disease. American Journal of Rhinology & Allergy 2016; 30:134-139.
82. Macdonald KI, McNally JD, Massoud E. The health and resource utilization of Canadians with chronic rhinosinusitis. Laryngoscope 2009; 119:184-189.
83. Rudmik L, Smith TL. Quality of life in patients with chronic rhinosinusitis. Current allergy and asthma reports 2011; 11:247-252.
84. Hopkins C, Slack R, Lund V, Brown P, Copley L, Browne J. Long-term outcomes from the English national comparative audit of surgery for nasal polyposis and chronic rhinosinusitis. The Laryngoscope 2009; 119:2459-2465.
85. Nader M-E, Abou-Jaoude P, Cabaluna M, Desrosiers M. Using response to a standardized treatment to identify phenotypes for genetic studies of chronic rhinosinusitis. Journal of Otolaryngology-Head and Neck Surgery 2010; 39:69.
86. DeConde AS, Mace JC, Levy JM, Rudmik L, Alt JA, Smith TL. Prevalence of polyp recurrence after endoscopic sinus surgery for chronic rhinosinusitis with nasal polyposis. The Laryngoscope 2017; 127:550-555.
87. Smith KA, Rudmik L. Medical therapy, refractory chronic rhinosinusitis, and productivity costs. Current opinion in allergy and clinical immunology 2017; 17:5-11.
88. López-Chacón M, Mullol J, Pujols L. Clinical and biological markers of difficult-to-treat severe chronic rhinosinusitis. Current allergy and asthma reports 2015; 15:19.
89. Renteria AE, Mfuna Endam L, Desrosiers M. Do Aging Factors Influence the Clinical Presentation and Management of Chronic Rhinosinusitis? Otolaryngology–Head and Neck Surgery 2017; 156:598-605.
90. Psaltis AJ, Wormald P-J. Therapy of Sinonasal Microbiome in CRS: A Critical Approach. Current Allergy and Asthma Reports 2017; 17:59.
91. Hand TW, Vujkovic-Cvijin I, Ridaura VK, Belkaid Y. Linking the microbiota, chronic disease, and the immune system. Trends in Endocrinology & Metabolism 2016; 27:831-843.
92. Wagner Mackenzie B, Waite DW, Hoggard M, Douglas RG, Taylor MW, Biswas K. Bacterial community collapse: a meta-analysis of the sinonasal microbiota in chronic rhinosinusitis. Environmental microbiology 2017; 19:381-392.
93. Chalermwatanachai T, Vilchez-Vargas R, Holtappels Get al. Chronic rhinosinusitis with nasal polyps is characterized by dysbacteriosis of the nasal microbiota. Scientific reports 2018; 8:7926.
94. Byrd AL, Segre JA. Adapting Koch's postulates. Science 2016; 351:224-226.
95. Rudmik L, Soler ZM. Medical therapies for adult chronic sinusitis: a systematic review. JAMA 2015; 314:926-939.
96. Mullol WF-VL-J, Baroody CB-IA-F, Douglas NC-AC-Ret al. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. Rhinology 2012; 50:1-12.
97. Schwartz JS, Tajudeen BA, Cohen NA. Medical management of chronic rhinosinusitis–an update. Expert review of clinical pharmacology 2016:1-10.

98. Wallwork B, Coman W, Mackay-Sim A, Greiff L, Cervin A. A double-blind, randomized, placebo-controlled trial of macrolide in the treatment of chronic rhinosinusitis. The Laryngoscope 2006; 116:189-193.
99. Videler WJ, Badia L, Harvey RJet al. Lack of efficacy of long-term, low-dose azithromycin in chronic rhinosinusitis: a randomized controlled trial. Allergy 2011; 66:1457-1468.
100. Soler ZM, Oyer SL, Kern RCet al. Antimicrobials and chronic rhinosinusitis with or without polyposis in adults: an evidenced-based review with recommendations *International forum of allergy & rhinology*: Wiley Online Library, 2013:31-47.
101. Meyer M, Huaux F, Gavilanes Xet al. Azithromycin reduces exaggerated cytokine production by M1 alveolar macrophages in cystic fibrosis. American journal of respiratory cell and molecular biology 2009; 41:590-602.
102. Baines KJ, Wright TK, Gibson PG, Powell H, Hansbro PM, Simpson JL. Azithromycin treatment modifies airway and blood gene expression networks in neutrophilic COPD. ERJ open research 2018; 4.
103. Parnham MJ, Haber VE, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. Azithromycin: mechanisms of action and their relevance for clinical applications. Pharmacology & therapeutics 2014; 143:225-245.
104. Zarogoulidis P, Papanas N, Kioumis I, Chatzaki E, Maltezos E, Zarogoulidis K. Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases. European journal of clinical pharmacology 2012; 68:479-503.
105. Foulds G, Shepard R, Johnson R. The pharmacokinetics of azithromycin in human serum and tissues. Journal of Antimicrobial Chemotherapy 1990; 25:73-82.
106. Schentag JJ, Ballow CH. Tissue-directed pharmacokinetics. The American journal of medicine 1991; 91:S5-S11.
107. Psaltis AJ, Li G, Vaezeafshar R, Cho KS, Hwang PH. Modification of the Lund-Kennedy endoscopic scoring system improves its reliability and correlation with patient-reported outcome measures. The Laryngoscope 2014; 124:2216-2223.
108. Bartram AK, Lynch MD, Stearns JC, Moreno-Hagelsieb G, Neufeld JD. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. Appl Environ Microbiol 2011; 77:3846-3852.
109. Muyzer G, De Waal EC, Uitterlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 1993; 59:695-700.
110. Martinez JRW, Vargas-Salas S, Gamboa SUet al. The Combination of RET, BRAF and Demographic Data Identifies Subsets of Patients with Aggressive Papillary Thyroid Cancer. Hormones & cancer 2019; 10:97-106.
111. Gonzalez E, Pitre FE, Page APet al. Trees, fungi and bacteria: tripartite metatranscriptomics of a root microbiome responding to soil contamination. Microbiome 2018; 6:53.
112. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013; 8:e61217.
113. Oksanen J, Blanchet F, Kindt Ret al. Package 'vegan'2016. Community ecology package, R package version 2017:2.4-1.

114. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014; 15:550.
115. Minerbi A, Gonzalez E, Brereton NJBet al. Altered microbiome composition in individuals with fibromyalgia. Pain 2019; 160:2589-2602.
116. Weiss S, Xu ZZ, Peddada Set al. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 2017; 5:27.
117. Bachert C, Zhang L, Gevaert P. Current and future treatment options for adult chronic rhinosinusitis: Focus on nasal polyposis. Journal of Allergy and Clinical Immunology 2015; 136:1431-1440.
118. Doan T, Hinterwirth A, Arzika AMet al. Mass azithromycin distribution and community microbiome: a cluster-randomized trial *Open forum infectious diseases*: Oxford University Press US, 2018:ofy182.
119. Doan T, Hinterwirth A, Worden Let al. Gut microbiome alteration in MORDOR I: a community-randomized trial of mass azithromycin distribution. Nature medicine 2019; 25:1370-1376.
120. Zhou Y, Bacharier LB, Isaacson-Schmid Met al. Azithromycin therapy during respiratory syncytial virus bronchiolitis: Upper airway microbiome alterations and subsequent recurrent wheeze. Journal of Allergy and Clinical Immunology 2016; 138:1215-1219. e1215.
121. Slater M, Rivett DW, Williams Let al. The impact of azithromycin therapy on the airway microbiota in asthma. Thorax 2014; 69:673-674.
122. Dickson RP, Morris A. Macrolides, inflammation and the lung microbiome: untangling the web of causality: BMJ Publishing Group Ltd, 2017.
123. dos Santos Santiago GL, Brusselle G, Dauwe Ket al. Influence of chronic azithromycin treatment on the composition of the oropharyngeal microbial community in patients with severe asthma. BMC microbiology 2017; 17:109.
124. Jervis-Bardy J, Foreman A, Boase S, Valentine R, Wormald PJ. What is the origin of Staphylococcus aureus in the early postoperative sinonasal cavity? *International forum of allergy & rhinology*: Wiley Online Library, 2011:308-312.
125. Vickery TW, Ramakrishnan VR. Bacterial pathogens and the microbiome. Otolaryngologic clinics of North America 2017; 50:29.
126. Hansen C, Pressler T, Hoiby N, Johansen H. Long-term, low-dose azithromycin treatment reduces the incidence but increases macrolide resistance in Staphylococcus aureus in Danish CF patients. Journal of Cystic Fibrosis 2009; 8:58-62.
127. Meyer A, Bril-Bazuin C, Mattie H, Van Den Broek P. Uptake of azithromycin by human monocytes and enhanced intracellular antibacterial activity against Staphylococcus aureus. Antimicrobial agents and chemotherapy 1993; 37:2318-2322.
128. Wilson MT, Hamilos DL. The nasal and sinus microbiome in health and disease. Current allergy and asthma reports 2014; 14:485.
129. Liu CM, Price LB, Hungate BAet al. Staphylococcus aureus and the ecology of the nasal microbiome. Science advances 2015; 1:e1400216.
130. Biswas K, Hoggard M, Jain R, Taylor MW, Douglas RG. The nasal microbiota in health and disease: variation within and between subjects. Frontiers in microbiology 2015; 6:134.

131. Ramakrishnan VR, Feazel LM, Gitomer SA, Ir D, Robertson CE, Frank DN. The microbiome of the middle meatus in healthy adults. PLoS One 2013; 8:e85507.
132. Bassis CM, Tang AL, Young VB, Pynnonen MA. The nasal cavity microbiota of healthy adults. Microbiome 2014; 2:1.
133. Kuhar HN, Tajudeen BA, Mahdavinia Met al. Relative abundance of nasal microbiota in chronic rhinosinusitis by structured histopathology *International forum of allergy & rhinology*: Wiley Online Library, 2018:1430-1437.
134. Seresirikachorn K, Suwanparin N, Srisunthornphanich C, Chitsuthipakorn W, Kanjanawasee D, Snidvongs K. Factors of success of low-dose macrolides in chronic sinusitis: Systematic review and meta-analysis. The Laryngoscope 2019.
135. Maniakas A, Asmar M, Renteria Flores Aet al. Azithromycin in refractory CRS following ESS and corticosteroid irrigations: double-blind, randomized, placebo-controlled trial (manuscript in preparation) 2020.
136. Philpott C, le Conte S, Beard Det al. Clarithromycin and endoscopic sinus surgery for adults with chronic rhinosinusitis with and without nasal polyps: study protocol for the MACRO randomised controlled trial. Trials 2019; 20:246.
137. Hussien HA, Habieb MS, Hamdan AM. Evaluation of Serum Total Immunoglobulin E, Interleukin-17 and Pentraxin-3 as Biomarkers for Chronic Rhinosinusitis With Nasal Polyposis. American journal of rhinology & allergy 2021; 35:640-646.
138. Xie S, Zhang H, Liu Yet al. The Role of Serum Metabolomics in Distinguishing Chronic Rhinosinusitis With Nasal Polyp Phenotypes. Frontiers in Molecular Biosciences 2021; 7.
139. Haxel BR, Clemens M, Karaiskaki N, Dippold U, Kettern L, Mann WJ. Controlled trial for long-term low-dose erythromycin after sinus surgery for chronic rhinosinusitis. The Laryngoscope 2015; 125:1048-1055.
140. Seresirikachorn K, Kerr SJ, Aeumjaturapat Set al. Predictive factors for identifying macrolide responder in treating chronic rhinosinusitis. Rhinology 2021; 59:284-291.
141. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. Current opinion in gastroenterology 2015; 31:69-75.
142. Baruch EN, Youngster I, Ben-Betzalel Get al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science 2021; 371:602-609.
143. Endam LM, Alromaih S, Gonzalez Eet al. Intranasal Application of Lactococcus lactis W136 Is Safe in Chronic Rhinosinusitis Patients With Previous Sinus Surgery. Frontiers in cellular and infection microbiology 2020; 10:440.
144. Zagury-Orly I, Khaouam N, Noujaim J, Desrosiers MY, Maniakas A. The Effect of Radiation and Chemoradiation Therapy on the Head and Neck Mucosal Microbiome: A Review. Frontiers in Oncology 2021; 11.

## XIX. Additional Figures

**Fig. 1.** Sino-nasal symptom score (scored 0 to 3, 0 being none and 3 being severe, maximum score of 15)

| | **None** | **Light** Occasional episode that is limited | **Moderate** Symptoms are present but tolerable | **Severe** Symptoms are difficult to tolerate and can interfere with everyday activities and sleep |
|---|---|---|---|---|
| Nasal congestion | | | | |
| Facial pain | | | | |
| Headache | | | | |
| Need to blow nose | | | | |
| Post-nasal drip | | | | |

**Fig. 2.** Sino-Nasal Outcome Test – 22 item (maximum score of 110)

## SINO-NASAL OUTCOME TEST

Date……..

Below you will find a list of symptoms and social/emotional consequences of your nasal disorder. We would like to know more about these problems and would appreciate your answering the following questions to the best of your ability. There are no right or wrong answers, and only you can provide us with this information. Please rate your problems as they have been over the past two weeks. Thank you for your participation.

| A. Considering how severe the problem is when you experience it and how frequently it happens, please rate each item below on how "bad" it is by circling the number that corresponds with how you feel using this scale → | No problem | Very mild problem | Mild or slight problem | Moderate problem | Severe problem | Problem as bad as it can be | Most Important Items (5) |
|---|---|---|---|---|---|---|---|
| 1. Need to blow nose | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 2. Sneezing | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 3. Runny nose | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 4. Nasal obstruction | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 5. Loss of smell or taste | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 6. Cough | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 7. Post-nasal discharge | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 8. Thick nasal discharge | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 9. Ear fullness | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 10. Dizziness | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 11. Ear pain | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 12. Facial pain/pressure | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 13. Difficulty falling asleep | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 14. Wake up at night | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 15. Lack of a good night's sleep | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 16. Wake up tired | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 17. Fatigue | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 18. Reduced productivity | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 19. Reduced concentration | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 20. Frustrated/restless/irritable | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 21. Sad | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |
| 22. Embarrassed | 0 | 1 | 2 | 3 | 4 | 5 | ☐ |

B. Please tick the most important items affecting your health (maximum of 5 items)……↑

**Fig. 3.** Lund-Kennedy endoscopic scoring system (maximum score of 12)

**ENDOSCOPIC EVALUATION SCORE – LUND-KENNEDY**

**Note: Record score for both left and right**

| **0 Points** | | | | **1 Point** | | | **2 Points** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Polyps** | Absent | **(L)** | **(R)** | Only in the middle meatus | **(L)** | **(R)** | Beyond the Middle meatus | **(L)** | **(R)** |
| **Oedema** | Absent | | | Mild | | | Severe | | |
| **Discharge** | Absent | | | Clear, thin discharge | | | Thick purulent discharge | | |
| **Total:** | | | | | | | | | |

## XX. Ethics Approval

Comité d'éthique de la recherche du CHUM
Pavillon R, 900 rue St-Denis, 3e étage
Montréal (Québec) H2X 0A9

Le 29 octobre 2014

Docteur Martin-Yvon Desrosiers
Axe de recherche : insulte tissulaire, infection, immunité et inflammation

a/s : Mme Leandra Mfuna Endam
courriel : leandra_mfuna@yahoo.ca

| Objet : | 14.140 – Approbation FINALE CÉR |
|---|---|
| | **L'Azithromycine comme thérapie complémentaire pour les patients ayant un échec au traitement médico-chirurgical standard pour la rhinosinusite chronique: un essai clinique randomisé, contrôlé par placebo, à double-insu** |

Docteur,

Nous accusons réception des documents, précisions et corrections demandées ainsi que des documents suivants en vue de l'approbation finale du projet mentionné en rubrique :

- formulaire d'information et de consentement français et anglais modifié – principal - version du 29 octobre 2014--
- formulaire 20 complété

Le tout étant jugé satisfaisant, vous retrouverez dans Nagano une copie du formulaire de consentement portant l'estampille d'approbation du comité. Seule cette version finale devra être utilisée pour signature par les sujets.

La présente constitue l'approbation finale, **valide pour un an à compter du 29 octobre 2014**. Vous devrez compléter le formulaire de renouvellement que nous vous ferons parvenir annuellement. De même, vous devrez soumettre pour approbation préalable, toute demande de modification ou document de suivi requis par le comité d'éthique conformément à ses Statuts et Règlements et ce via Nagano.

Lorsque cela s'applique à votre situation, veuillez noter que le projet ne peut débuter tant que le contrat n'est pas finalisé et dûment signé. De même, vous ne pouvez commencer votre projet avant d'avoir fait parvenir votre "NOL" (lettre de non objection) de Santé Canada pour ce projet au CÉR du CHUM.

Le comité suit les règles de constitution et de fonctionnement de l'Énoncé de Politique des trois Conseils (ÉPTC 2) et des Bonnes pratiques cliniques de la CIH.

**Attestation du CÉR (REBA)**
La composition du comité d'éthique de la recherche du CHUM est conforme aux exigences réglementaires de la partie C, Division 5 du Food and Drug regulations de Santé Canada ;
Le comité exerce ses fonctions conformément aux exigences des Bonnes pratiques cliniques ;
Le comité d'éthique de la recherche du CHUM a révisé et approuvé le protocole et le formulaire d'information et de consentement pour l'essai clinique mentionné en titre, qui sera réalisé au CHUM par l'investigateur qualifié nommé ci-haut. Cette approbation et les exigences du comité d'éthique ont été documentées par écrit.

**Pour toute question relative à cette correspondance**, veuillez communiquer avec la personne soussignée via NAGANO, ou avec sa collaboratrice Mme Lynda Ferlatte, par courriel ou téléphone : **lynda.ferlatte.chum@ssss.gouv.qc.ca – 514 890-8000 poste 14030.**

Vous souhaitant la meilleure des chances dans la poursuite de vos travaux, nous vous prions d'accepter, nos salutations distinguées.

Me Marie-Josée Bernardi, avocate
Vice-présidente
Comité d'éthique de la recherche du CHUM